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Conference Organizers

Editor-in-chief and responsible compiler of Conference Proceedings –
leader researcher Evita Straumite
professor Ruta Galoburda

Dear participants,

I am proud and honoured that 11th Baltic Conference on Food Science and Technology „Food Science and Technology in a changing world" (FOODBALT 2017) takes place from 27th to 28th April, 2017 in the Latvia University of Agriculture. The conference aim is to bring together leading food scientists and new researchers, as well as doctoral students from European countries to advance food science globally.

The conference plans to attract more than 110 delegates from 10 countries. The conference programme covers 4 key lectures and 36 oral presentations over 7 sessions. Additionally a total of 71 posters will be presented. The conference Organising Committee had received 42 full paper (3 – Reviews, 36 – Original Papers and 3 – Short Communications) submissions from 6 countries. A peer review process was enforced with the contribution of experts and researchers from Conference Scientific Committee and Latvia University of Agriculture, all of them internationally recognized in one of the conference topic areas.

On behalf of the Organising Committee I welcome you at FOODBALT 2017 conference and hope that conference will provide a venue for scientific discussion and exchange of the information, for the development of common ideas and the initiation of international cooperation among young researchers, doctoral students and established experts in the area of Food Science.

Inga Ciprovica

The chair of the 11th Baltic conference of Food Science and Technology

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REVIEWS

GLYCOALKALOIDS IN POTATOES: A REVIEW

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Abstract

Potatoes (*Solanum tuberosum* L.) are one of the most consumed and nutritionally important vegetables in the world, which is why its quality and safety are very important. Potential risk factors for potato consumers are some toxins called glycoalkaloids, which naturally accumulates in potatoes during growth, harvesting, transportation and storage. It is stated that safe glycoalkaloid concentration level into fresh potato tubers with skin is not higher than 200 mg kg^{-1}. Figures above this concentration might affect the health of consumers, causing, for example, digestive troubles and nerve system damage. The objective of this review is to outline the glycoalkaloid classification, the factors affecting the glycoalkaloid accumulation process, and the effect on technological processes of the glycoalkaloid level in potato tubers. The goal of this review is to highlight the importance of this subject and to encourage the widening of the glycoalkaloid research horizon. Different research shows that the most significant glycoalkaloid concentration raise happens during potato tuber exposure to direct sunlight. If for a few hours under direct sunlight, the glycoalkaloid concentration rises considerably. Prolonged potato storage under bulb light, for example, on shop counters, also raises the glycoalkaloid concentration. Peeling significantly reduces glycoalkaloid concentration, because glycoalkaloids are partly removed from potato tubers with the skin. Also, boiling potatoes in water and also the frying process remove partly the glycoalkaloids. Cutting, slicing, rinsing with water, baking, cooking, blanching, pulsed electric field do not significantly affect the glycoalkaloid concentration in potatoes tubers.

Keywords: potatoes, glycoalkaloids, solanine, chaconine, technological processes.

Introduction

Potatoes are important vegetables and are widely grown and consumed due to the fact that they yield good crops under different growing conditions, while containing many valuable nutritional compounds, such as proteins, carbohydrates, vitamins, minerals (Lisińska et al., 2009; Rytel et al., 2011). Potatoes yield also has more calories per acre than other widely grown crops (Navarre et al., 2009).

Apart from nutritional compounds, potatoes also contain glycoalkaloids, viz. naturally accumulated toxins during potato growth and storage. Glycoalkaloid accumulation is triggered by environmental (natural and human stimulated) stress (Papathanasiou et al., 1999; Sharma, Salunkhe, 1989). Major glycoalkaloids in potatoes are α-solanine and α-chaconine (Sotelo, Serrano, 2000; Friedman, Levin, 2009; Omayio et al., 2016). As stated by the World Health Organization, the safe level of glycoalkaloids in fresh potato tubers is considered to be from 20 to 100 mg kg^{-1} (Food and Agriculture Organization / World Health Organization, Joint Expert Committee on Food Additives, 1992). However, other studies state that even up to 200 mg of glycoalkaloids kg^{-1} of fresh potatoes is still safe (Jansky, 2010; Friedman et al., 1997; Karim et al., 1997). Glycoalkaloid intoxication might cause digestive troubles, diarrhoea and vomiting (Hellenas et al., 1992), but higher doses can cause nerve system damage, coma and even death (Friedman, 2006; Langkildea et al., 2009).

The bitter taste of potatoes is one of the indicators of increased glycoalkaloid level presence and is common in potatoes (fresh weight) which have more than 140 mg per kg of glycoalkaloids (Sinden et al., 1976; Zitnak, Filadelfi, 1985; Johns, Keen, 1986).

Some in vitro studies show certain beneficial effects of glycoalkaloids, for example, anticancer effect (Friedman et al., 2005; Lee et al., 2004), but this possibly positive value of glycoalkaloids still need to be well studied and admission of glycoalkaloids for medical purposes cannot be done directly from fresh or processed potatoes.

It is important to avoid potatoes exposure to direct sunlight during harvesting and storage, as this can drastically and fast raise the glycoalkaloid level, forcing its concentration above the maximum safe limit (Kirui et al., 2009).

In the storage process, for example, in shop counters usually potatoes are affected by two negative factors, i.e., too high (room) temperature and bulb light; after prolonged influence of those factors, potatoes start to sprout and/or turn green, while also accumulating glycoalkaloids (Cantwell, 1996).

Basically any light source can increase the glycoalkaloid level during storage and even during potatoes growth, when some potatoes are not properly covered with soil (Dimenstein et al., 1997).

Slicing, bruising and cutting are human-stimulated stress factors which also raise the glycoalkaloids level (Mondy et al., 1987; Mondy, Gosselin, 1988).

Potatoes are quite different genetically in their ability to produce glycoalkaloids (Dale et al., 1993), which is why it would be advisable to find and use potatoes varieties which have lower glycoalkaloid accumulation tendency.

Some technological processes might help to reduce the glycoalkaloid level. It is important to understand the conditions in which glycoalkaloid level raises, so as to try to avoid such conditions, while it is vital to ascertain technological processes that reduce the glycoalkaloid level.

A great deal of research has been made worldwide on glycoalkaloids. The aim of this article is to summarize the key points on glycoalkaloid classification, accumulation and reduction. The objective of this

review is to outline the classification of glycoalkaloids, the factors affecting the glycoalkaloid accumulation process, and the effect of technological processes on glycoalkaloid level in potatoes tubers.

Materials and Methods

In this review, a monographic method has been used, i.e., studying scientific papers on the classification of glycoalkaloids, the factors affecting the glycoalkaloid accumulation process, and the technological process effect on glycoalkaloid level.

Results and Discussion

Chemical structure of glycoalkaloids

Glycoalkaloids are secondary metabolites and the most common alkaloids within the *Solanaceae* family. These compounds are biosynthesised from cholesterol – the same precursors as steroids. In *Solanum* species, the main glycoalkaloids are solanidanes and spirosolanes. In potatoes, two most important glycoalkaloids are α-solanine and α-chaconine (Figure 1), consisting of solanidine as an aglycone, but bound with different sugar moieties (Chowanski et al., 2016; Wang et al., 2013). Generally, glycoalkaloids α-solanine and α-chaconine are present in plants together, especially in *S.tuberosum* (Vaananen, 2007).

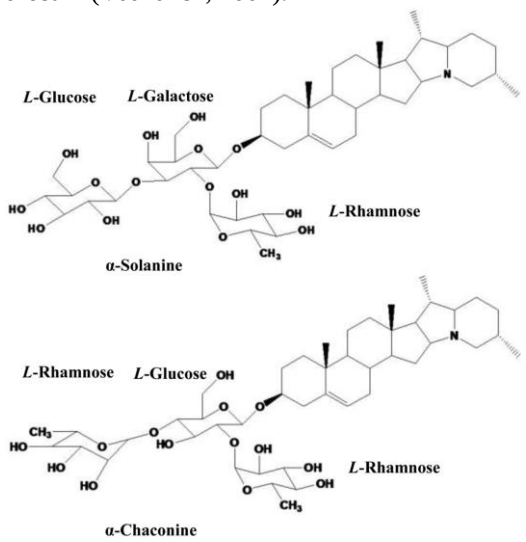

Figure 1. Structures of α-solanine and α-chaconine (Wang et al., 2013)

The trisaccharide sugar moieties of α-solanine is called a solatriose, consisting of D-glucose, D-galactose, L-rhamnose, whereas α-chaconine is called a chacotriose, consisting of D-glucose and two L-rhamnose molecules (Vaananen, 2007). Other glycoalkaloids (β-solanine, γ-solanine, β-chaconine, γ-chaconine, α-solamarine, β-solamarine, 5-β-solanidan-3-aol and demissidine) are in very low concentrations (Friedman, McDonald, 1997). α-chaconine is more toxic than α-solanine and in several studies the ratio between α-solanine and α-chaconine is compared.

Biological activity of glycoalkaloids

Glycoalkaloids are present in potatoes and many other members of *Solanaceae*, including eggplants and tomatoes, and they are secondary metabolites, whose main role is to protect potatoes from environmental stress, for example, pathogens and injuries (Navarre et al., 2009). The biological activity of alkaloids for protecting plants comes in a broad range: redox imbalance, disruption of biological membranes, disturbed metabolism, inhibition of cholinesterase, reproductive toxicity, disturbed development, etc. (Chowanski et al., 2016).

Based on insecticidal activities, different studies are performed for glycoalkaloids application as sources of new insecticides (Chowanski et al., 2016; Nenaah, 2011). There are many glycoalkaloid poisoning cases, but it is hard to identify all of them, as such poisoning symptoms are similar to bacterial food poisoning (Smith et al., 1996; Mensinga et al., 2005).

Content of glycoalkaloids in potatoes

No maximum levels for glycoalkaloids in potatoes have been established at EU level. Some Member States have a national maximum level of 200 mg per kg[1]. Glycoalkaloids are located in the whole potatoes plant, but the highest concentration is in unripe fruit, sprouts, flowers and potato tuber skin (Friedman, 2005).

The glycoalkaloid content of potato tubers depends on the potato cultivar and ranges from 22.4 mg to 208.9 mg per kg of fresh potato tubers (Friedman, McDonald, 1999).

In Latvia, grown potatoes total glycoalkaloid level ranges from 12.9 mg to 28 mg per kg fresh potato weight (whole potato with skin) and from 1.9 to 21.5 mg per kg fresh potato weight (potato without skin) (Saleniece et al., 2011).

Examples of distribution of glycoalkaloids in white, yellow, red and blue flesh potatoes are presented in Table 1.

Table 1

Glycoalkaloids in white, yellow, red and blue flesh potatoes (Rytel et al., 2013; Friedman, 2005)

Sample	Total glycoalkaloids mg kg^{-1} fresh weight
Whole tuber	
White-flesh potatoes	approx. up to 629
Blue-flesh potatoes	54–59
Red-flesh potatoes	51–55
Yellow-flesh potatoes	approx. up to 100
Peels	
White-flesh potatoes	approx. up to 3526
Blue-flesh potatoes	181–245
Red-flesh potatoes	approx. up to 1264
Yellow-flesh potatoes	approx. up to 425

The glycoalkaloid level can depend on specific potatoes genetics and geographical factors (Friedman, 2006). The level can raise during harvesting

[1] https://ec.europa.eu/food/sites/food/files/safety/docs/reg-com_toxic_20150623_sum.pdf

process, transportation and storage and it is provoked by tuber damage, exposure to pathogens, direct light from sun or bulb, heat (Friedman, 2006; Kirui at al., 2009). If prolonged exposure to direct sunlight is avoided during harvesting, then such exposure might take place when potato tubers are sold in fairs, as in such cases tubers are temporarily stored in uncovered counters whole day under clear sky and there is a high risk of buying high glycoalkaloid level affected potato tubers (Kirui et al., 2009).

To present the ideological distribution of glycoalkaloids in different potato parts, there is an example below, in Table 2. These data were established in the 20th century and are still valid under specific research conditions and are provided here to generally show the significant difference of glycoalkaloids in different potato parts.

Table 2

Glycoalkaloids in potatoes (Wood, Young, 1974)

Potato part	Total glycoalkaloids, mg per kg fresh weight
Tuber with skin	75
Tuber with skin (bitter taste)	250–800
Peel (skin)	150–600
Peel (skin) from bitter tuber	1500–2200
Tuber without skin	12–50
Sprouts	2000–4000
Flowers	3000–5000
Stems	30
Leaves	400–1000

Glycoalkaloids accumulate twice as fast at 24 °C than 7 °C in dark room, but in light they can develop even up to nine times faster already after 24 hours at 24 °C when also exposed to bulb light (Cantwell, 1996).

Table 3 shows examples of glycoalkaloid accumulation amount in different light and temperature conditions. Direct sunlight, brighter artificial light, less glycoalkaloid accumulation resistant potato varieties and the simultaneous influence of several negative conditions (i.e., direct light plus high temperature) might result in faster glycoalkaloid accumulation.

Table 3

Glycoalkaloid accumulation in different light and temperature conditions in whole tuber (Machado, 2007)

Condition	Days	Total glycoalkaloids, mg per kg fresh weight
Indirect sunlight exposure	0 / 3	51.4 / 96.9
Fluorescent light exposure (lamps of 40 W)	0 / 3	51.4 / 59.9
Storage in darkness under refrigeration temperature (7–8 °C)	0 / 3	51.4 / 75
Storage in darkness under room temperature (19–26 °C)	0 / 3	51.4 / 76.5

Influence of technological processes to glycoalkaloids content

The influence on glycoalkaloids of the technological processes carried out on potatoes are presented in Table 4. Depending on the glycoalkaloid overall content in specific cases, potato tubers peeling can reduce the glycoalkaloid level up to 58% (Czopek et al., 2008; Czopek et al., 2012).

Cutting, slicing and rinsing with water barely reduces the level of glycoalkaloids. Blanching potatoes has a slightly higher effect compared to the previously mentioned processes and removes a small part of glycoalkaloids, because, since they are water soluble, a part of them is removed during this process; however, the removed amount is insignificant (Rytel et al., 2005; Peksa et al., 2006).

Boiling in water reduces glycoalkaloids in whole potato tubers by 22% in average, due to the glycoalkaloid solubility in water, hence some part of glycoalkaloids leak from tubers in that process (Czopek et al., 2008). Comparing frying, baking and cooking, only frying reduces glycoalkaloids due to high working temperature (Friedman, McDonald, 1997; Rytel et al., 2005; Peksa et al., 2006).

Table 4

Influence of technological processes to glycoalkaloids

Process	Reduction of total glycoalkaloids	Reference
Frying	approx. up to 94%	Peksa et al., 2006; Czopek et al., 2012; Rytel et al., 2011
Blanching	Insignificant effect	Rytel et al., 2005; Peksa et al., 2006
Dehydration	78–90%	Rytel et al., 2013
Boiling	22%	Czopek et al., 2008
Potato chips	approx. up to 82%	Czopek et al., 2012
Peeling	approx. up to 58%	Czopek et al., 2008; Czopek et al., 2012
Granulation	approx. up to 90%	Ji et al., 2012
Cutting, slicing, rinsing with water	No effect	Rytel et al., 2005; Peksa et al., 2006
Baking, cooking	Insignificant effect	Friedman, McDonald, 1997; Rytel et al., 2005; Peksa et al., 2006
Pulsed electric field	Insignificant effect	Hossain et al., 2015

Other study shows similar result when the frying temperature was higher than 170 °C (Friedman, McDonald, 1997). However, some studies present even better results, decreasing total glycoalkaloids by up to 94% during the frying process (Czopek et al., 2012).
Pulsed electric field does not significantly reduce glycoalkaloid level in potatoes tubers after treatment (Hossain et al., 2015).

Conclusions

Generally, the glycoalkaloid level in potato tubers depends on genetics, environmental and physical stresses.
Damage during harvesting, transportation or storage raises glycoalkaloid level.
Prolonged storage of potatoes should be done at temperature of 7 °C, since higher temperature can stimulate accumulation of glycoalkaloids level.
Room temperature plus bulb light in potato tubers particularly raises glycoalkaloid level.
Direct sunlight gives fastest glycoalkaloid accumulation in potato tubers, while direct bulb light influence to tubers takes a bit longer time for glycoalkaloids to accumulate.
Significant glycoalkaloid level reduction can be achieved by peeling, sliced tubers boiling in water or frying, dehydration and granulation.
Insignificant glycoalkaloid level reduction results from cutting, slicing, rinsing with water, baking, cooking and pulsed electric field.
Bitter taste, sprouts and green skin of potatoes are indicators which consumers can observe, to avoid intoxication with glycoalkaloids.

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REVIEWS

MICROBIOLOGICAL SAFETY OF ORGANIC AND CONVENTIONAL FOODS

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Abstract

Occasionally, concerns are expressed that use of organic manure in fertilisation, and free-ranging of livestock may result in higher loads of (mainly) enteric pathogens in organic food, and that avoiding the use of synthetic fungicides may increase the risk of mycotoxin contamination. Therefore, we compared published data on the levels of these hazards on some organically and conventionally produced foods. The results can be summarised as follows:

- There is no evidence that use of organic fertilizer in organic crops increases the prevalence of bacterial pathogens and/or of antibiotic-resistant bacteria on fresh produce.
- The prevalence of *Campylobacter* in live free-ranging poultry and of *Toxoplasma* in live free-ranging pigs tends to be somewhat higher than in animals kept conventionally indoors. However, this difference is hardly seen any more if carcasses and meat are analysed.
- Methicillin-resistant *Staphylococcus aureus* (MRSA) strains were found much more frequently in conventionally-produced raw food while the levels of extended-spectrum-β-lactamase-positive *Escherichia coli* (ESBL-*E. coli*) were reported as similar on somewhat lower on organic raw food.
- Most studies found lower levels of *Fusarium* toxins in organic grains and cereal products, if compared to conventional products.

In conclusion, common practice in the organic sector (in particular, allowing sufficient time for transformation of manure; crop rotation) leads to food with similar or even lower levels of microbiological hazards and mycotoxins.

Keywords: organic food; food safety; enteric pathogens; antibiotic resistance; mycotoxins.

Introduction

In the last years, the organic food sector grew continuously in the European Union and many other parts of the world. Big retail chains and even discounters entered the organic market. As a consequence, quality managers of these businesses and of major suppliers representing strong but "vulnerable" brands – as represented in the "Consumer Goods Forum" of the Global Food Safety Initiative – have a strong interest to know whether organic products are as safe as conventional foods or whether special preventive measures should be taken to ensure their safety. Especially in U. S. and Canadian media, it has been speculated that keeping livestock outdoors, and the use of animal excreta for fertilization may result in elevated levels of microbiological hazards on organic foods. Therefore, the author of this article was requested to summarize current data on the safety of organic foods as compared to conventional foods. The present paper focuses on microbiological hazards including mycotoxins.

Materials and Methods

A literature survey was carried out by using literature databases (SCOPUS, Google Scholar), other reviews and data from official food inspection. Special attention was paid to peer-reviewed papers comparing organic with conventional foods.

Results and Discussion

Bacterial pathogens on fresh produce

In recent years, there is much concern about contamination of vegetables by zoonotic agents that may be present in organic fertilizers. Without doubt, manure (or human excreta) not stabilized by composting or anaerobic fermentation is a reservoir for enteric pathogens (FDA, 1998; Alsanius, 2014; Strawn et al. 2013). However, no increased levels of enteric pathogens or index organisms have been reported on organic produce (Table 1). This statement also applied to the prevalence of antibiotic resistance in plant-associated *Enterobacteriaceae* (van Hoek et al., 2013; Ruimy et al., 2010; Kim, Woo, 2014). Use of organic fertilizers according to good agricultural practice (GAP) does not increase the rate of contamination of fresh produce by enteric pathogens (Alsanius, 2014) or *Escherichia coli* (literature data summarized by Smith-Spangler et al., 2012). As learned from outbreaks and field studies, the main risk factors are use of irrigation water that had been contaminated by human or livestock excreta (Park et al., 2012), and handling of produce by carriers of pathogens, but not the farming system (Marine et al., 2015).

Some outbreaks have been reported in which leafy vegetables were shown or suspected to be involved (CDC, 2016). The 2006 outbreak of *E. coli* O157:H7 infections was linked to the consumption of bagged spinach. According to USDA documents (2007), the most likely cause was use of river water for irrigation that had been contaminated by excreta from cattle of a nearby farm, and of wildlife, with no evidence for the involvement of organic fertilizers.

Table 1

Zoonotic agents in organic and conventional livestock and food of animal origin

Hazard and sampling site	Prevalence organic vs. conventional	References
Salmonella in pig faeces at slaughter	Equal or lower	Bonde, Sørensen, 2007
Salmonella in poultry faeces at slaughter	Equal or lower	Van Loo et al., 2012
Campylobacter in live poultry	Somewhat higher	Heuer et al. 2001; Hoogenblom et al., 2008
Campylobacter on poultry meat	About equal	Van Loo et al., 2012
E. coli on beef carcasses	About equal	Smith-Spangler et al., 2012
Toxoplasma gondii in live pigs	Higher	Schulzig, Fehlhaber, 2006

The *E. coli* O157:H7 outbreak due to Romaine lettuce, in 2013, was linked to the vicinity of cattle operations, too (Jung et al., 2014). The more recent outbreak of STEC (*E. coli* O26) infections at the *Chipotle Mexican Grill Restaurants* could not be linked to a particular food (CDC, 2016). Previously, this company made strong statements about health and sustainability. However, a view to the menu shown the *Chipotle* website shows that only few minor meal components (jalapeno, cilantro) were organic, and there is no indication for the involvement of fresh produce fertilized with fresh manure shortly prior to harvest.

Nevertheless, appropriate time intervals between application of manure and harvest are necessary (Mukherjee et al., 2007; Leifert et al., 2008; de Quadros Rodrigues et al., 2014; Park et al., 2014). Unstabilized (fresh, not composted or fermented) manure should (and is normally) only be applied in the year before planting vegetables (and other crops). The USDA Organic Rule requires 120 days between manure application and harvest, and standards issued by organisations such as the UK Soil Association are similar (Leifert et al., 2008). It should be remembered that use of farmyard manure is by no means restricted to organic systems, and properly managed soil and compost is a very hostile environment to enteric pathogens (Diez-Gonzalez, Mukherjee, 2009). This applies, in particular, to soils managed organically (Franz et al., 2008). Use of fresh manure only a few days before harvesting fresh produce is a serious violation of "good agricultural practice" but is very unlikely to happen in (organic or conventional) commercial practice. Such a treatment will not result in better yields, anyway, since time is missing to turn organic nitrogen into plant nutrients.

Other risk factors identified from outbreaks and field studies include irrigation with water contaminated with animal excreta, the presence of wildlife, weather (e.g. heavy rainfall and flooding) and handling of food by human carriers of pathogens. To quote from Marine et al. (2015), the farming system is not a food safety risk determinant for leafy greens.

Bacterial pathogens in free-ranging livestock

In organic agriculture, livestock has, within limits, access to "fresh air" outside the housing. Pigs and poultry kept outdoors may face a higher risk of exposure to zoonotic agents from the environment. Uncontrolled exposure to wildlife may result in serious losses due to parasites, other animal pathogens, and predators, and modern organic husbandry systems minimize these risks, also for economic reasons. Table 1 summarizes data on the prevalence of zoonotic agents in livestock and food of animal origin. The percentage of organic pigs carrying antibodies against *Toxoplasma gondii* tends to be higher in organic systems, with 9 and 2.5% of the animals (n=200 each) found positive, respectively (Schulzig, Fehlhaber 2006). On the other hand, the prevalence of live salmonellae in fecal samples of pig and poultry at slaughter tends to be lower in organic pigs, indicating that organic pigs are less prone to be colonized by salmonellae (Leifert et al., 2008; Bonde, Sørensen, 2007). *Campylobacter* tends to be present more frequently in free-range chicken (Heuer et al., 2001, Hoogenbloom et al., 2008; Luangtongkum et al., 2006) but contamination rates on chicken meat were found to be similar, indicating the key role of cross-contamination during slaughter (van Loo et al., 2012). With respect to STEC, little if any difference in prevalence has been reported but there is some evidence that feeding more roughage (such as in organic farming) may reduce the prevalence of Shiga toxin forming *E. coli* (STEC) in cattle colons (Diez-Gonzalez, 2007). Generally speaking, however, contamination of carcasses and raw milk is mostly depending on the slaughtering and milking practice, respectively.

Antibiotic-resistant bacteria

Selection of antibiotic-resistant bacteria strains is one of the greatest problems in public health. Therefore, the levels of methicillin-resistant *Staphylococcus aureus* (MRSA) and of *Enterobacteriaceae* (in particular, *E. coli*) containing and expressing genes for extended-spectrum β-lactamases (ESBL) in raw materials and foods are monitored. The majority of studies indicate a lower prevalence of MRSA in organically reared pigs and on organic farms in general (Table 2). Organic poultry meat carried MRSA much less frequently than conventional poultry meat (Teramoto et al., 2016) whereas the contamination rates for ESBL-*E. coli* on organic poultry meat were reported to be similar (Kola et al., 2012; Cohen Stuart et al., 2012) or lower (Smith-Spangler et al., 2012). The lower prevalence of antibiotic-resistant bacteria on organic meat may be due to restrictions in the (therapeutic) use of antibiotics, of certain disinfectants (quaternary ammonium compounds, QAC), zinc, and to a higher resistance of the animals against colonization and infection

(Slifierz et al., 2015). Again, the degree of contamination of meat by antibiotic-resistant bacteria strongly depends on slaughtering and butchering hygiene.

Table 2

Antibiotic-resistant bacteria in organic and conventional livestock and food of animal origin

Hazard and sampling site	Prevalence organic vs. conventional	References
MRSA[1] in live pigs and farm environment	Lower	Meemken, Blaha, 2009, van de Vijver et al., 2014
MRSA on poultry meat	Lower	Teramoto et al., 2016
ESBL[2] *E. coli* on poultry meat	Equal or lower	Smith-Spangler et al., 2012; Kola et al., 2012; Cohen Stuart et al., 2012
Antibiotic-resistant *Campylobacter* on poultry at slaughter	Lower	Luangtongkum et al., 2006

[1]Methicillin-resistant *Staphylococcus aureus*
[2]Extended-spectrum-β-lactamase-positive

With regard to the presence of antibiotic resistance genes in plant-associated *Enterobacteriaceae*, little differences between organically and conventionally grown vegetables were reported (van Hoek et al., 2013; Ruimy et al., 2010; Kim, Woo, 2014).

***Fusarium* toxins in grains and cereal products**

Fusarium toxins are, in low concentrations, frequently found in cereal products. Surveys clearly showed that restrictions in use of fungicides and insecticides do not lead to a higher prevalence of these toxins in cereal products (Edwards, 2009; Tangni et al., 2009). Seven studies analysed by Smith-Spangler et al. (2012) indicated lower contamination levels of these toxins in organic wheat while the study by Edwards (2009) did not report significant differences between levels in conventionally and organically grown wheat. When German-style bread (made from wheat and rye flour), sampled in 1999, was analysed for *Fusarium* toxins, levels were found to be lower in organic bread (Schollenberger et al., 2005).

Table 3 shows the results of some studies in Norway and Poland on *Fusarium* toxins in oats and rye. In Norway, toxin levels were significantly lower in organic oats while in Poland, only small differences were observed. Organic rye was found to contain lower levels of deoxynivalenol (DON) than did conventionally grown rye.

Various factors reduce the levels of *Fusarium* toxins in organically-growing grains (summarized by Benbrook, 2006): (i) Infection of the oars by *Fusarium* is difficult to control with fungicides, (ii) in organic agriculture, crop rotation reduces the level of inoculants, and (iii) restriction of nitrogenous fertilizers strengthens the defense mechanisms of the plants and improves the microclimate within the field. Moreover, cropping wheat immediately after maize, which, at least in Germany, is common practice in conventional but not in organic agriculture, favours infestation by *Fusarium*, resulting in higher levels of *Fusarium* toxins (Döll et al., 2002).

Table 3

***Fusarium* toxins in oats and rye[1]**

Cereal and origin	n		Myco-toxin	Results of comparison	Refe-rence
	conv	org			
Oats, Norway, 2002-04	101	101	DON	conv > org	(3)
			NIV	conv > org	
			HT-2	conv >org	
Oats, Poland, 2006-2008	22	36	DON	n. s.	(20)
			NIV	n. s.	
			HT-2	n. s.	
Oats, Poland, 2009-11	24	34	DON	n. s.	(36)
			NIV	n. s.	
			HT-2	conv > org	
Rye, Poland, 2009-12	42	75	DON	conv > org	(4)
			NIV	n. s.	
			HT-2	conv > org	

[1]Abbreviations: DON, deoxynivalenol; NIV, nivalenol; HT-2, HT-2 toxin; conv > org, significantly higher levels in conventional grains that in organic grains.

Conclusions

Peer-reviewed studies do not support allegations that organic foods are more hazardous than conventional foods.

Modern outdoor systems for pigs and poultry, and organic fertilization using good agricultural practice do not increase risk.

However, the organic sector is advised not to raise unrealistic expectations among consumers in terms of safety, keeping in mind the (ever increasing) sensitivity of analytical methods, and communication should not raise.

Good management / manufacturing practice on farms and in processing is crucial for food safety, especially to avoid contamination of vegetables by enteric pathogens, and grains by toxigenic *Fusarium* strains.

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REVIEWS

APPLICATION OF HIGH-PRESSURE PROCESSING FOR SAFETY AND SHELF-LIFE QUALITY OF MEAT – A REVIEW

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Abstract

In recent years, there has been growing consumer demand for the minimally processed, free from chemical additives and healthier meat, which has led to the development of alternative technologies to conventional heat treatments. High pressure processing (HPP) is known as a non-thermal intervention for extending the shelf-life and safety of meat. The aim of this review is to analyse the scientific literature about the changes that occur in meat after application of the HPP.

The HPP is effective in controlling microflora in meat and meat products at 400–600 MPa, thus extending its shelf-life. The inactivation efficiency of the HPP mainly depends on treatment conditions, type of microorganisms, and food matrix characteristics. However, the HPP may negatively affect meat quality attributes, such as colour and texture. Meat processed at pressures above 300 MPa has cooked like appearance, which might be unacceptable to consumers. The HPP increases meat toughness, but when processed at relatively low pressures (100–250 MPa) in combination with increased temperature (above 60 °C) meat becomes more tender. Pre-rigor muscle treatment proved meat tenderization after cooking. It has been revealed that the HPP above 400 MPa makes the polyunsaturated fatty acids more susceptible to oxidation, which may have negative effect on stored meat flavour. Another factor affecting meat flavour may be activity of enzymes. Processing of meat at 150 MPa decreases cook loss and increases water holding capacity. Several researchers suggest using multi hurdle approach (use of antimicrobials and antioxidants) for processing at lower pressures reducing negative effect on quality characteristics.

Keywords: microbial inactivation, meat colour, meat texture, shelf-life.

Introduction

Nowadays consumers more often pay attention to nutrition information and list of ingredients on labels of food packaging and there is a high demand for less salty, less acidified, less chemical preserved, minimally processed, but at the same time very safe products with high nutritional value, and extended shelf life (Campus, 2010; Hygreeva, Pandey, 2016). By applying proper technological treatment it is possible to eliminate a number of additives used for product preservation maintaining nutritional and organoleptic properties of the food similar or identical to the raw or fresh form (Heinz, Buckow, 2010). During classical thermal treatments natural flavour and quality of the food product are affected, therefore the necessity for effective non-thermal treatment emerges. Some promising non-thermal technologies are applied at industrial level for decontamination of meat products, gamma, electron and X-ray irradiation, high hydrostatic pressure, natural antimicrobials, active packaging, and radiofrequency, to name just some of them (Campus, 2010). These technologies have high acceptability for use in food processing as they preserve natural appearance of treated food product while eliminating the pathogens and spoilage microoorganisms, moreover, they are energy saving and environmentally friendly what is not of less importance for producers (Aymerich et al., 2008). High pressure processing (HPP), also known as cold pasteurization of food is a non-thermal technology and at present moment is one of the most successful alternatives for thermal food preservation technologies adopted by the food industry and commonly used for treating meat and meat products as approximately 29% of the industrial HP equipment is used in the meat industry (Aymerich et al., 2008; Campus, 2010). As a commercially viable technology for the pasteurization it is used for both liquid and solid products of diverse origin such as meat, seafood, beverages, dairy, fruits, and vegetables (Tonello, 2011). By using HPP, both spoilage and pathogenic microorganisms in meat are inactivated, shelf life is extended, while the characteristics and the quality of meat and meat products are maintained (Balasubramaniam, Farkas, 2008; Jofré, Serra, 2016). High pressure might be transferred instantly throughout a flexible package regardless of size, shape, or composition of the food. HPP offers the possibility to have mildly processed, wholesome, fresh-tasting product with minimal to no preservatives to satisfy health conscious consumers (Jofré, Serra, 2016). Furthermore, HPP has big potential for the innovative development of new products with relatively low energy consumption as well as can be combined with existing trends in the food sector to boost the development of the food industry (Hugas et al., 2002).

The present review covers high pressure processing effects on quality attributes of meat regarding its safety and shelf-life.

1. General principles of high pressure application on foods

Pressure is an important thermodynamic parameter with unique effects on biological systems (Aertsen et al., 2009). During HPP, the food is placed in the pressure vessel and submitted to pressures from 100 to 900 MPa. The pressure applied is isostatically transmitted inside the pressure vessel. The technology is based on the *Pascal's* law and *Le Chatellier* principle, so high pressure is transmitted in a uniform

and instantaneous manner and the product is compressed, independently of its size and geometry, or its constituents (Balasubramaniam et al., 2016). The temperature of the food subjected to high-pressure treatment is usually increased by approximately 3 °C per each 100 MPa increase when applied at ambient temperatures (~25 °C) (Aymerich et al., 2008). This temperature increase is known as "heat of compression" which is generated within the material due to compressive work against intermolecular forces. If the food contains a significant amount of fat the temperature increase can reach up to 9 °C per 100 MPa increase (Rasanayagam et al., 2003). The efficacy of the treatment depends on the achieved pressure, the treatment temperature and the exposure time. In industrial applications of high pressure food processing pressures of up to 800 MPa may be applied.

High pressure effects on foods are highly dependent on the primary effects of pressure and temperature on the relevant thermodynamic and transport properties of food systems such as density, viscosity, thermal conductivity, compressibility, heat capacity, diffusivity, phase transition properties (e.g., melting point), and solubility. Pressure drastically influences the values of those properties. Biochemical transformations under high pressure can be irreversible or reversible, depending on involved substances, environmental conditions, and the combination of pressure, temperature, and exposure time (Buckow et al., 2013).

2. Influence of high pressure on meat

HPP affects quality parameters of fresh meat and thus typical characteristic associated with fresh meat like texture and especially colour can be remarkably modified (Bajovic et al., 2012; Hughes et al., 2014). As a consequence of the le Chatelier principle, during high pressure, depending on its level, occurs degradation or modification of the meat proteins, inactivation of enzymes, changes in the substrate–enzyme interactions and in carbohydrates and fats (Butz, Tauscher, 2002). However, the nutritional value, vitamins and the majority of small substances responsible for the flavours of the products are preserved (Schindler et al., 2010).

2.1. Effect of HPP on meat proteins

High pressure application to muscle proteins alters their properties as they undergo physiochemical changes such as denaturation, dissociation, solubilisation, aggregation, and gelation. These factors strongly depend on pressure level, temperature, pH and ionic strength (Jofré, Serra, 2016). In meat, the most significant effect of pressure is detected for sarcoplasmic and myofibrillar proteins. Sarcoplasmic meat proteins (mainly enzymes and heme pigments) are very susceptible to denaturation when undergo HPP at pressure level above 200 MPa during which water holding capacity and colour of the meat changes (Marcos et al., 2010). Myofibrillar proteins, are related to the meat structure and are unfolded if pressure is 300 MPa and higher. As a result, occurs denaturation, agglomeration, and gel formation (Sun, Holley, 2010; Chan et al., 2011; Grossi et al., 2016). Thus, apart from its food preservation capabilities, HPP also has potential to manipulate the texture of foods and, hence, has been suggested as a physical and additive-free process to tenderize and soften meat and meat products. Such structural modifications of meat proteins also are used by the food industry in new product development (Sun, Holley, 2010; Sikes et al., 2010; Buckow et al., 2013).

2.2. Effect of HPP on the colour of meat

Colour is one of the most important quality attributes for the consumer when purchasing meat (Cheftel, Culioli, 1997). Meat colour is determined by the amount and chemical state of the hemoproteins present as well as by the structure of the meat. Studies indicate that HPP provokes drastic changes in fresh meat colour, while the changes in cured meat products are acceptable and depending on the water content and water activity (a_W) value (Bajovic et al., 2012; Ferrini et al., 2012). Colour changes due to oxidation of ferrous myoglobin into ferric metmyoglobin. To reach microbial inactivation in meat, usually pressure above 400 MPa is applied, as the result of such pressure increase, meat discoloration occurs due to the protein denaturation (Wackerbarth et al., 2009) It has often been reported that even by application of pressure above 200 MPa drastically changes the appearance of red meat within a few minutes of treatment at low temperatures (Tintchev et al., 2010; Buckow, 2013). Most studies report increase of lightness (L*) in the pressure range 200–350 MPa turning red colour of meat into a paler pink, redness (a*) is observed to decrease in values at 400–500 MPa, resulting in a grey-brown meat with a cooked-like appearance, however, this is the more variable parameter and dependent on experimental design (i.e., type of meat, minced or whole muscle, and HPP conditions), and yellowness (b*) either increases or is not affected (Jung et al., 2003; Morales et al., 2008; Tintchev et al., 2010; Souza et al., 2011; Ferrini et al., 2012; Jofré, Serra, 2016). The changes of colour of high pressure treated pork meat at 200 to 800 MPa at 5 and 20 °C for 10 min was shown to depend mostly on pressure level and to a lesser degree on applied temperature (Bak et al., 2012).

2.3. Effect of HPP on lipid oxidation of meat

Pressure levels between 300 and 600 MPa are critical for inducing lipid oxidation in fresh pork, beef and poultry meat as well as in meat products, which may lead to significant changes in the lipid content and fatty acid composition of phospholipids and free fatty acids (Marcos, 2010; Fuentes et al., 2010; Huang et al., 2015). HPP induced lipid oxidation mechanisms are not fully understood, it has been suggested that HPP can promote lipid oxidation as it increases accessibility for iron from hemoproteins and disrupts membranes (Bajovic et al., 2012). Oxidation is one of the most important factors in the non-microbial

degradation of meat (Guyon et al., 2016). Lipid oxidation is not usually evident immediately after HPP but may become evident during chilled storage (Tume et al., 2010). Such oxidation affects quality through flavour deterioration (rancidity), colour changes, loss of nutritive value, and alterations of textural and functional properties through associated protein denaturation (Fuentes et al., 2010; Buckow, 2013). There are suggested possibilities to inhibit the lipid oxidation by limiting oxygen availability in the packaging, use of antioxidant active packaging or use of different antioxidants derived from natural by-products and their combination (Mariutti et al., 2008; Bolumar et al., 2011; Alves et al., 2012).

3. Microbiological Aspects

Meat is nutritionally rich product and serves as suitable media for growth of meat spoilage microorganisms and common food-borne pathogens, therefore, it is of high importance to choose and apply proper preservation technologies (Aymerich et al., 2008). HPP as a non-thermal food preservation technique has proved to be effective in inactivating variety of food-borne pathogens and spoilage-causing organisms (vegetative cells, yeasts, moulds, and viruses) (Considine et al., 2008; Tonello, 2011).

Inactivation of microorganisms by HPP is a combination of factors affecting physical properties of cell membrane, proteins and enzymes, and genetic mechanisms. Cell membrane is known to be the primary site of a pressure damage with consequent changes in the permeability of the cells, transport systems, loss of the osmotic responsiveness and incapacity to keep ΔpH, as well as changes in the rate of specific physiological functions that cause irreversible or lethal damage on bacteria cells (Ritz et al., 2001; Molina-Guitierrez et al., 2002). Surface dwelling microorganisms live at atmospheric pressure (0.1 MPa) and progressively stop growing at 40–50 MPa (Simonato et al., 2006). For the majority of microorganisms, the highest pressure tolerance is found between 20 and 30 °C. It is possible to decrease the stability of these microorganisms if the lower temperatures are applied during high pressure treatments (Buckow, Heinz, 2008). However, there are an increasing number of mesophilic microorganisms with achieved significant improvement of growth under high pressure or resistance to high pressure directed by evolution (Hauben et al., 1997; Karatzas, Bennik, 2002; Pavlovic et al., 2008; Aertsen et al., 2009).

Ability to resist pressure treatment varies considerably among different microorganisms' type, form (vegetative cells or spores, Gram-positive or Gram negative), genus, species, and strain. With some exceptions, the pressure resistance of bacteria depends on the morphology and size of the cells, as it is observed the most sensitive bacteria are rod-shaped whereas the spherical-shaped are more resistant (Ludwig, Schreck, 1997). Gram-positive bacteria are generally more resistant than Gram-negative bacteria, and spores are more resistant than vegetative cells to pressure treatment (Patterson, 2005; Jofré et al., 2010). Strains within the same species can have wide range of sensitivity against physical stresses caused by HPP (Liu et al., 2012), therefore, identification of target microorganism is crucial for the process validation studies. Moreover, higher pressure resistance is reported for microbial cells in stationary growth phase comparing to those at exponential conditions (Manas, Mackey, 2004; Manas, Pagan, 2005).

The response to HPP of microorganisms in meat and meat products is variable and depends on process parameters such as pressure, temperature, and processing time as well as on product parameters such as pH, a_W, salt content, and the presence of antimicrobials (Töpfl, Heinz, 2009; Rendueles et al., 2011; Bajovic et al., 2012). Rich nutrient media such as meat reinforce the resistance of the microorganisms to HPP due to the protective effect of carbohydrates, proteins and lipids in meat (Simpson, Gilmour, 1997).

A great number of studies have shown HHP efficiency to control microorganisms in meat and meat products (Hugas et al., 2002; Aymerich et al., 2005; Lindsay et al., 2006; Morales et al., 2006; Rubio et al., 2007; Campus, 2010; Bajovic et al., 2012 Jofré, Serra, 2016). Pressure levels applied for the pasteurization of meats and meat products, range in an area of 400–600 MPa for a short processing time, from seconds to several minutes at room temperature. Eukaryote vegetative forms from fungi and moulds are inactivated with pressure of 200–300 MPa while their spores need a 400 MPa treatment. The majority of pressure-sensitive bacteria begin to lose viability at approximately 180 MPa. In a pressure range of about 200−400 MPa occur irreversible changes such as cell leakage leading to cell death (Lado, Yousef, 2002). These treatments lead in most cases to an inactivation of > 4 log units for the most common vegetative pathogenic and spoilage microorganisms resulting in an increased shelf-life and improved safety (Rubio et al., 2007; Bajovic et al., 2012).

The cell death increases with pressure but does not follow a first order kinetics and a tail of inactivation is sometimes present (Garriga et al., 2002). These resistant or sublethally injured cells could be able to grow during storage (Bozoglu et al., 2004), therefore, HPP cannot be used as single technology for meat preserving as products still require refrigerated storage during subsequent handling and distribution (Carlez et al., 1994; Chen, Hoover, 2003).

4. Hurdles technology

Hurdles technology describes application of two or more preservation techniques combined to establish a series of preservative factors (hurdles), as the result, microbial stability, sensory quality, and nutritional properties of food products improve (Leistner, 2000; Zhou et al., 2010).

However, more than 60 potential hurdles for foods, which improve the stability and / or quality of the

products, have been described, and the list of possible hurdles for food preservation is by no means complete (Leistner, Gorris, 1995; Zhou et al., 2010; Rodriguez et al., 2016). Combination of hurdles together with HPP increases the antimicrobial effect of low pressure processes and minimize the unwanted changes induced by ultra-high pressures (above 400 MPa) (Bajovic et al., 2012). As hurdles may be used temperature (high or low), water activity, redox potential, vegetable extracts, organic acids, carbon dioxide, bacteriocins, osmotic dehydration, pulsed electric field, ohmic heating, and others. The combination of hurdles may be positive if the effect of preservation factors summarizes. In a highly effective case may be observed synergistic effect when both preservation factors together enhance the effect of each factor. For meat and meat products synergistic effects with HPP have been described with antimicrobials, low pH, carbon dioxide, vacuum packaging and chilled storage (Garriga, Aymerich, 2009; Jofré et al., 2010). Moreover, additional hurdles or processes are useful to avoid the recovery of injured cells (Liu et al., 2012).

Bacterial spores may be extremely resistant if pressure treatment is applied at ambient temperatures, therefore a different approach to the method is required. Application of very high pressure (1 GPa) or very long holding times (from 30 min to hours) is effective but not suitable for industrial applications due to high energy consumption. In such cases it is necessary to apply pressure in combination with high temperature. This method is called pressure assisted thermal sterilization (PATS) and inactivates even the most heat-resistant spores. By increasing temperature during HPP lethality of total microorganisms enhances, however, it leads to a higher degree of protein denaturation and, as a result, it affects the fresh-like characteristics of the meat (Balasubramaniam et al., 2016).

5. Packaging

During storage time packaging protects products against deteriorative effects such as discolouration, off-flavour and off-odour development, nutrient loss, texture changes, pathogenicity and other factors (Zhou et al., 2010). High pressure treatment commonly is combined with vacuum packaging. If the meat is subjected to HPP after packaging it is possible to reduce secondary contamination simultaneously maintaining the freshness of the meat and extending its shelf life (Huang et al., 2017). The lack of O_2 in packages may minimise the oxidative deteriorative reactions, and reduce aerobic bacteria growth. Low O_2 vacuum packages for retail meat cuts are usually vacuum skin packaging systems with vacuum sealing barrier films that are heat shrunk to conform to the shape of the product (Belcher, 2006).

Conclusions

HPP is an alternative technology to preserve foods with reduced thermal requirement and commonly is used for meat and various meat products treatment. As the result, the nutritional value, of the products is preserved and shelf-life extended without the use of preservatives or additives. However, depending on the pressure level applied, HPP affects quality parameters like texture and colour typically associated with fresh meat − the meat becomes more gel-structured and paler. HPP is an effective method to enable the control of pathogenic and spoilage microorganisms in meat products. The response to HPP of microorganisms in meat and meat products is variable and depends on process parameters and on product parameters. Commercially applied pressure levels range in an area of 400–600 MPa with short processing times at ambient temperatures resulting in inactivated majority of pathogenic and spoilage microorganisms. If additional hurdles technologies are applied in combination with HPP it is possible to increase shelf-life and improve safety of the meat. Lately HPP gains its popularity on commercial scale as a low-temperature treatment, environmentally friendly and waste-free technology.

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MORPHOMETRIC AND BIOCHEMICAL ANALYSIS OF YEAST CELLS UNDER LOW TEMPERATURE STORAGE

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Abstract

The aim of the research is to measure thiol compounds as well as glycogen content in yeast cells of different sizes during long-term storage at low temperature. The size, SH-groups and glycogen content changes in the *S. cerevisiae* cells were studied over the period of 35 days storage at 0±5 °C. The new morphometric method used has showed that the total amount of cells and number of large cells (7–8 × 12–15 μm) remained unchanged when compared with control under model conditions; the number of budding yeasts decreased 4 times, and the number of dead cells increased 2 times during the storage period. The number of middle size cells (5–7 × 10–12 μm) decreased 1.2 times, while the number of small cells (4–5 × 7–9 μm) increased 9.5 times. Under the same conditions the quantity of low molecular SH-groups increased 2 times, while protein-containing SH-groups dropped down by 31%. The content of glycogen decreased 1.2 times. The results elaborated revealed that generally yeasts under unfavorable conditions lose the deposition nutrients such as glycogen and store up the adaptive SH-containing substances. The cells of a large size probably have more high adaptation capacity to long-term storage at low temperature and in the absence of a nutrient medium. The rising amount of small cells and of low molecular SH-groups evidently indicates the development of adaptation state in the yeast population. Future investigations have to find out the range correlations between morphologic parameters of yeast cells and the content of biochemical substances.

Keywords: yeast, size of cells, thiols.

Introduction

Yeast of *Saccharomyces cerevisiae* species are a source of low-molecular and high-molecular biologically active substances and find varied commercial technological applications. Yeast cultures are also used for modeling physical and chemical processes (Coelho et al., 2013; Schreuder et al., 1996), medical and biological investigations (Ksenzhek, Petrova, 1984; Leung-Toung et al., 2002). Among intracellular yeasts metabolites, thiol containing compounds have a special attention of researchers, especially the water-soluble antioxidant – tripeptide glutathione which accounts for about 90% of intracellular low-molecular thiols. Glutathione participates in red/ox homeostasis, regulation of enzyme activity and immune responses (Liu et al., 1996; Schafer, Buettner, 2001), protects cell structures against the damaging effects of reactive oxygen species (Müller, Lösche, 2004; Schafer, Buettner, 2001. Thiol-disulfide cellular system is known to be involved in the response of living systems to geophysical, biological and manmade environmental factors (Sokolovskij, 2008). Low-molecular thiols are likely to be cellular detectors with a non-specific reaction to external stimuli by a change in the ratio of reduced to oxidized sulfhydryl groups. Thiol-disulfide exchange reactions of low molecular thiol substances with sulfhydryl groups of protein triggers provide some conformational changes in their three-dimensional structures, which initiates biochemical reactions of adaptation mechanisms (Müller, Lösche, 2004). The paradigm of thiols significance understanding in living systems has recently changed from antioxidant defense to redox regulation of cell functions (Flohe, 2010). Thioredoxins are the most likely key reactive proteins in mechanisms of red / ox cell signaling (Schreuder et al., 1996; Baronian, Gurazada, 2007).

Depletion of reduced glutathione and protein thiol oxidation is known to result in an activation of transcription of heart shock protein genes (Petit et al., 1996). In 1950s it was found that aging and stressful situations result in antioxidant depletion in cells and tissues of multicellular organisms, which affects the amount of glutathione, the main thiol intracellular antioxidant, and leads to a decrease of the organism resistance damaging factors (Ksenzhek, Petrova, 1984). Under unfavorable conditions the amount of reduced glutathione in yeasts increases, which makes these organisms different from other cells (Munday, Winterbourn, 1989). An investigation of the accumulation dynamics of reduced low-molecular thiols (mainly, glutathione) as thiol enzyme activators is very interesting for microbiologists, biochemists, enzymologists and biotechnologists. The use of yeast storage methods at low temperatures 0–4 °C results in a significant change of biochemical characteristics of cultures, which is important for their further research and commercial applications. The changes of antioxidant status in yeast cells used as biosensors may have two implications. Firstly, they can determine the strength of the acting factor, for instance, for environmental monitoring. On the other hand, cells need to be standardized to control their uniformity for obtaining reproducible biosensor signals. This can be achieved by some biochemical tests as well as by some morphological methods due to development of high-performance equipment for cell sorting. The capability of the equipment to analyze a large number of individual cells for several parameters simultaneously has changed the understanding of the behavior of cells in culture and of the population dynamics (Deere et al., 1998, Mattanovich,

Borth, 2006). The potential of this method for biotechnological research is invaluable.

Thus, the aim of the present study was to observe the ratio of changes of high to low molecular thiols and glycogen content in yeast cultures during long-term storage at low temperatures.

Materials and Methods

The yeast culture *S. cerevisiae*, species LV-7 was obtained at St. Petersburg food processing plant. The yeasts were stored for 1 day at 0–4 °C. Aliquots of yeast cells were selected every week for microscopic and biochemical investigations. Computer cytophotometry was used for morphometric analysis (Zhilinskaia et al., 2016). To prepare the culture dilutions, yeasts were suspended in a 0.9% NaCl solution in the ratios of 1 : 10, 1 : 100 and 1 : 1000. No nutrients were added to yeast suspension. The living cells were prepared for microscopic analysis using crushed drops method. The presence of glycogen granules in cells was determined by staining the diluted yeast suspension with a Lugol aqueous solution according to the Gram staining method. Microscopic analysis of samples was carried out in visible spectral region using microscope Leica DM LB2 (Germany) with a Leica DFC 320 digital camera providing a total 1000-fold magnification. Images were recorded by video system and displayed on a Pentium 4 computer screen with Leica YM - 1000 software for Windows. Morphometric measurements of yeast cells were carried out using staining samples with Lugol solutions for 36 randomly selected fields of view. The number of cells was counted in Gorjaev chamber, the total number of analyzed cells was more than 1 000. The criteria for morphometric analysis of yeast cells were as follows: the size and shape of cells, budding, the number of dead and budding cells, cytoplasm and organelles, presence of glycogen inclusions. The biochemical analysis included the following procedures: yeast cells walls were frozen at -20 °C and quickly unfrozen, homogenized for 5 min in a Potter-Elvehjem glass homogenizer with distilled water (with yeasts: water ratio of 1 : 10). The obtained water suspensions were pelleted in a centrifuge for 30 min at 13000 g and +4 °C. The total protein content was determined using Lowry method (Lowry et al., 1951). The low-molecular sulfhydryl (SH-) and disulfide (SS-) groups were determined using TDA-02 amperometric titrator of the Institute for Analytical Instrumentation of the Russian Academy of Sciences (Sokolovskij, 1962). The concentration of protein SH- and SS-groups in the samples was measured in $\mu mol \cdot g^{-1}$, that of low-molecular SH- and SS-groups – in $\mu mol \cdot L^{-1}$. Microsoft Excel-2000 was a standard method for statistical data analysis. The difference between the values compared was found to be significant at $p<0.05$.

Results and Discussion

The microscopic analysis has shown a heterogeneous yeast cell population. There were revealed large, medium, small and budding living cells in the view field. For a correct analysis, we divided them into three categories. According to their geometrical size the yeast cells were classified into these groups:

1. The large cells (size is 7–8 × 12–15 μm) were lemon-shaped and had a granular cytoplasm, plenty of vacuoles, thickened cell wall, a clearly contoured large nucleus and a lot of stained glycogen granules.
2. The medium cells (size 5–7 × 10–12 μm) were round or lemon-shaped, characterized by a granular cytoplasm and the presence of single stained glycogen granules. Around 47–50% cells of this group have a thickened cell wall.
3. The small cells (4–5 × 7–9 μm) had ellipsoid shape, a homogeneous cytoplasm, a thin cell wall and small vacuoles without any glycogen granules.

The size of dead yeast cells was smaller than 2–3 × 5–6 μm. They were irregularly shaped cells with a condensation of the cytoplasm and the nucleus and vacuoles and no glycogen granules. Some results of morphometric analysis of yeast cells during their storage are presented in the Figure 1.

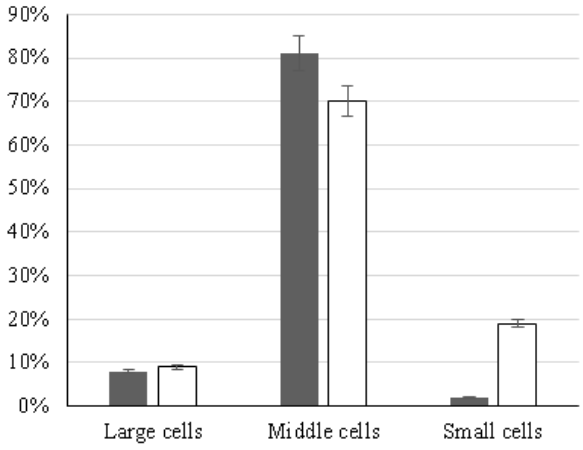

Figure 1. The ratio of the number of large *S. cerevisiae* cells to the number of medium and small cells during storage at low temperatures

■ – the number of fresh yeast cells after 1 day of storage at 2±2 ºC;

□ – the number of yeast cells after 21 days of storage at 2±2 ºC.

The morphometric analysis of fresh yeast showed that medium cells accounted for 81%, large cells made 8%; small cells took 2% of the examined yeast population (Fig. 1). 87% of large cells and 36% of medium cells contained glycogen granules in cytoplasm. The number of yeast cells in the samples made 2%. The data obtained has shown that there were no dead cells in the samples. Medium sized cells were predominant (70% of the total) after 21 days of storage at low temperatures. However, the amount of medium cells after 21 days decreased 1.2 times compared to that in fresh yeast. The number of large cells increased 1.13 times during their storage and made 9% out of the total cell number. The amount of small cells increased

9.5 times during their storage and they accounted for 19% of the total cell number. The presence of glycogen granules in the cytoplasm of 70% large cells and 30% medium cells indicated that nutrient reserves of yeast cells decreased 1.2 times during storage (Fig. 2). The small cells had no glycogen granules. The number of budding yeast cells decreased 4 times and made 0.5%: the number of dead cells doubled and was equal to 2%.

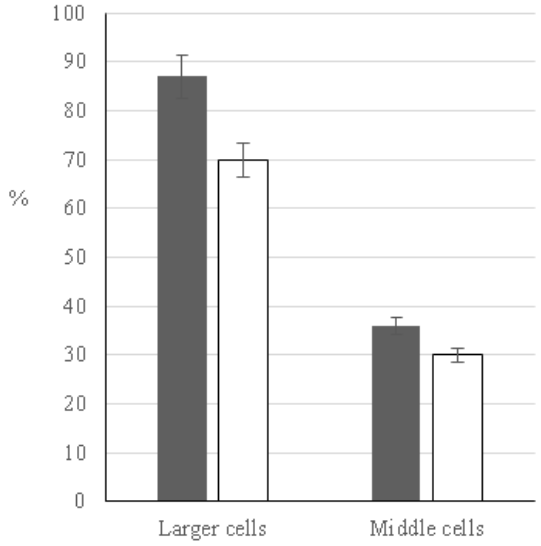

Figure 2. The number of yeast cells *S.cerevisiae* containing granules of glycogen, for different periods of storage at low temperature

■ – the number of fresh yeast cells after 1 day of storage at 2±2 ºC;

□ – the number of yeast cells after 21 days of storage at 2±2 ºC.

No significant differences have been revealed in protein SH- and SS- groups in the supernatant liquid obtained from the fresh yeast and after 7 days of storage and also within intervals of 14–21 and 28–35 days of storage. The amount of protein in the supernatant liquid of fresh yeasts was of 1.04±0.06 g L^{-1} and decreased to 0.81±0.03 g L^{-1} after 35 days of storage. The average concentrations of SH- and SS- groups in water-soluble proteins (μmol g^{-1}) and in low-molecular substances (μmol L^{-1}) for different periods of the storage was as follows: 1 after 0–7 days; 2 after 14–21days; 3 after 28–35 days.

Table 1

Concentrations of reduced (SH) and oxidized (SS) thiol groups in water extracts of yeast

Thiol compounds		Time of storage, days		
		0–7	14–21	28–35
Protein's, μmol·g^{-1}	SH	353±22	352±23	243±31
	SS	147±10	139±15	129±19
Low-molecular, μmol L^{-1}	SH	60.0±4.3	78.3±9.1	120±13
	SS	36.1±2.3	37.5±3.5	39.0±4.4

According to the data obtained, the amount of SH- groups in the proteins of yeast water extracts decreased by 31% after 35 days of storage (from 353.41 to 243.15 μmol g^{-1}), and the amount of SS-groups did not significantly change (147.52 and 129.36 μmol g^{-1} respectively). Under the same conditions, the amount of low-molecular SH-groups doubled (from 60.29 to 120.45 μmol L^{-1}) and the amount of SS- groups did not have any significant changes (36.18 and 39.29 μmol L^{-1}, respectively). There were proposed several possible reasons for an increase of the amount of low-molecular thiol compounds in yeast cells under low-temperature storage when cannot be an efficient anabolism. The first reason could be a decrease in utilization of reduced glutathione for intracellular metabolic reactions which go slower due to a low temperature. The second one is a possible activation of partial proteolysis of intracellular proteins that create low-molecular peptides with a high concentration of cysteine containing SH- groups. An intense expression of metallothioneins in yeast S. cerevisiae DV 747 including a high amount of sulfur containing amino acids, up to 30% per mol of protein, and resistance to active oxygen, appears to confirm the latter mechanism (Nakamura, 2004). It can be assumed that yeast cells were under a starvation stress, which leads to activation of free radical oxidation processes and antioxidant defense mechanisms. The obtained increase of water-soluble low-molecular thiols during the storage of yeast at low temperatures can occur due to the response of cells to the unfavorable environmental factors. Further research is planned to investigate the mechanism of accumulating reduced low-molecular thiol compounds in yeast cells during the storage and to take an attempt of revealing the factors which affect this mechanism.

Conclusions

A close connection is known to exist between morphologic and physiologic states of cells. The cell size and cell shape are determined by genetic and physiological properties of biological objects and also by environmental conditions. The cell ability to react to different external factors makes it possible to use these cells as biosensors. The experiment results have shown a long-term effect of low temperature on *S. cerevisiae* yeast population adaptive morphological and biochemical modifications in the cells. The most important result of the present study was a small decrease (by 20%) in the amount of medium yeast cells (5–7×10–12 μm) at low temperatures. A 4-time increase in the amount of budding cells by and an increase in the amount of dead cells demonstrated a decrease in the physiological activity of yeast cells. A decrease in the amount of protein thiol compounds (by 31%) and a simultaneous 2-fold increase in the number of low-molecular thiols were the most significant changes in yeast cells. The results obtained may need some further research and some theoretical analysis in order to fully explore the possibilities of their prospective practical applications.

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THE IMPACT OF CALCIUM IONS ON COMMERCIALLY AVAILABLE β-GALACTOSIDASE

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Abstract

Commercial lactose hydrolysing enzymes producers provide purchasers with information as optimal enzyme temperature and pH, as well recommended amount of enzyme. However, there are also other indices in food substrate which might have impact on enzyme activity. The cations concentration in the substrate is the one of those. Whey is a dairy by-product with a relatively high Ca^{2+}, K^{+} and Na^{+} ions concentration. These cations (especially Ca^{2+}) are added in cheese making and remain in whey in comparatively large amounts. Moreover, Na^{+} also remains in whey since salt is added for cheese grains salting. The aim of the study is to determine the impact of Ca^{2+} ions on commercially available β-galactosidase. The effect of Ca^{2+} ions on commercial β-galactosidase (NOLA Fit5500, Ha-Lactase 5200, Chr. HANSEN, Denmark; Lactozym® Pure 6500 L, Novozymes, Denmark) were tested. For investigation of the effect of Ca^{2+} ions on the β-galactosidase activity, chromogenic lactose substare – oNPG (2-nitrophenyl β-D-galactopyranoside; Sigma, Germany) was used, reactions were carried out at pH 6.6, using 0.1 M Tris-HCl buffer, and Ca^{2+} ions concentration range 5–50 mM. The enzymatic reactions were measured spectrophotometrically at 410 nm using Tecan 96-well plate reader (Tecan Group, Switzerland).The results imply, that Ca^{2+} ions alone do not have effect on lactose hydrolysis, moreover, in some they even stimulate it (NOLA Fit5500). The study results will help to precisely adjust the amount of commercially available enzymes to dairy substrates with high cations content.

Keywords: commercial β-galactosidase, lactose hydrolysis, calcium ions, enzyme kinetics.

Introduction

β-galactosidase (β-D-galactoside, galactohydrolases, EC 3.2.1.23) catalyses the cleavage of terminal galactosyl groups from the non-reducing ends of galactose containing carbohydrates. β-galactosidase is commercially relevant enzyme that is prevalently used for lactose hydrolysis in dairy industry (Carević et al., 2015).

Commercial β-galactosidase preparates are produced from bacterial and eukaryotic hosts: bacteria, yeasts, moulds, (*Bacillus* spp., *Kluyveromyces* spp., *Aspergillus* spp.) (Dagbagli, Goksungur, 2008; Panesar et al., 2006). Enzyme characteristics depends on the source of amino-acids composition, active site and presence or absence of allosteric regulatory sites, pH- and thermal- optimum and stability (Mlichová, Rosenberg, 2006).

Various substances can alter enzyme catalytic activity activating or inhibiting it; metal ions and their complexes are play important role in enzyme structure stabilization and / or activation or inhibition of the reaction. If metal ions are required by enzyme to maintain its stable, native state, it is called as metalloenzyme whereas if metal ions require only during catalytic activity enzymes are called "metal activated enzymes" (Zohra et al., 2016).

Calcium ions (Ca^{2+}) are known as inhibitor for many β-galactosidases, but for some β-galactosidase act also as activator when added in concentrations of 1–10 mM, which also conform to concentrations of free calcium in milk or whey. This property can be advantageous for applications in lactose hydrolysis processes directly in milk or when using lactose-rich substrates based on whey with high level of Ca^{2+} in solution. (Juajun et al., 2011).

The aim of this paper is to determine the impact of Ca^{2+} ions on commercially available β-galactosidase.

Materials and Methods

Chemicals and enzymes

Three commercial preparates of β-galactosidase were used in the study: NOLA Fit5500 and Ha-Lactase 5200 (Chr.HANSEN, Denmark) and Lactozym® Pure 6500 L (Novozymes, Denmark), NOLA Fit5500 is derived from *Bacillus licheniformis*, other two enzymes are of *Kluyveromyces lactis* origin. All enzymes were stored at 4 °C and remained fully active throughout the study All reagents used: $MgCl_2$, $CaCl_2$, p-nitrophenol, 2-nitrophenyl-galactoside (o-NPG) were purchased from Sigma (Germany).

Research was carried out to determinate Ca^{2+} influence at different concentrations (5–50 mM) on β-galactosidase activity of three different, commercially available, enzyme preparates.

Enzymatic assay

β-galactosidase activity was determined using chromogenic substrate o-NPG by monitoring the increasing of absorbance at 410 nm.

Enzyme. Each commercial β-galactosidase were diluted with distillate water for optimal concentration: NOLA Fit5500 with average activity 5500 $BLUg^{-1}$ (1:16); Ha-Lactase 5200, with average activity 5200 NLU g^{-1} (1:2); and Lactozym® Pure6500 L with average activity 6500 LAU g^{-1} (1:4). Activity units of commercial β-galactosidases are defined differently by each producer.

Substrate. All reactions were performed in 0.1 M Tris-HCl buffer, pH 6.6, containing 1 mM $MgCl_2$ and o-NPG concentration in the range from 1.25 to 7.25 mM. To asses Ca^{+2} effect on enzyme activity we used $CaCl_2$ in concentrations 5–50 mM. The measured activities were compared with the activity of the enzyme without added Ca^{+2} under the same conditions.

Enzymatic reactions were measured using 96-well plate in multimode plate reader Infinite 200 M Pro (Tecan Group, Switzerland). Total reaction volume was 200 µL per well, all reactions were started by adding 10 µL of enzyme.

Calibration

o-nitrophenol is hydrolysis product of oNPG. To quantify its production in multimode reader was set up calibration curve by using pure o-nitrophenol solution in the range 0–3.15 mM.

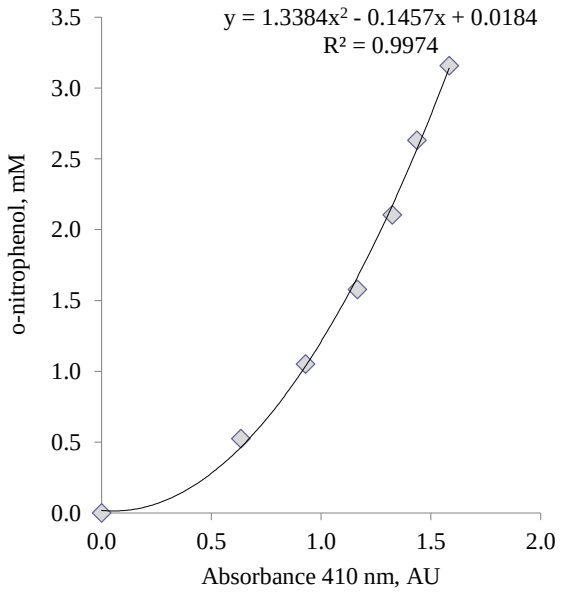

Figure 1. Calibration curve of o-nitrophenol

We found 2nd order polynome approximation to fit our data. By the calibration curve of o-nitrophenol (see Figure 1), were calculated reaction kinetics.

Data analysis

Data were treated by Microsoft Office 2016 Excel.

Results and Discussion

Ca^{+2} is the major cation in bovine milk with a total concentration up to 25 mM (Tanaka et al., 2011). For cheese production, Ca^{+2} is added in concentrations 0.2–0.4 M (Abdalla, Ahmed, 2010; Landfeld et al., 2002; Mehaia, 2006). In whey, cheese by-product, Ca^{+2} concentration can reach 2.0–6.0 mM (Hill et al., 1985; Theoleyre, Gula, 2004; Wong et al. 1978). Due to Ca^{+2} impact potential and its wide range of concentrations in milk and by-products, it is of practical importance to evaluate Ca^{+2} impact on β-galactosidases activity at concentrations up to 50 mM.

Enzyme kinetic constants

To assess Ca^{+2} impact on lactose hydrolysis we studied changes of enzymatic kinetic parameters V_{max} (velocity) and K_M (Michaelis–Menten constant). We used classical Michaelis-Menten type kinetics to describe enzyme activity. For K_M and V_{max} measurement, we used reciprocal graphical representation of enzymatic kinetics (Lineweaver-Burk plots) (Güleç et al., 2010). K_M is equal to the substrate concentration at which the reaction rate is half its maximal value (Atrooz et al., 2016).

To compare the results, all enzyme V_{max} and K_M values were expressed as fold change calculated against respective enzyme kinetic values without Ca^{+2} addition. Results are presented in Table 1.

Table 1

Ca^{2+} effect on commercial β-galactosidase V_{max} and K_m

Ca^{+2}, mM	Lactozym® Pure 6500 L		NOLA Fit5500		Ha-Lactase 5200	
	V_{max}, %	K_M, %	V_{max}, %	K_M, %	V_{max}, %	K_M, %
0	1.000	1.000	1.000	1.000	1.000	1.000
5	1.022	1.421	0.847	0.889	0.011	95.071
10	0.777	*ND	0.836	0.896	0.011	88.906
15	0.834	*ND	*ND	0.782	0.011	91.792
30	0.776	*ND	0.916	0.926	0.011	89.913
50	0.791	*ND	0.859	0.909	0.011	99.612

*ND – not determined

V_{max} and K_M values are presented as fold change when normalised to reaction without Ca^{2+}.

As seen in Table 1, the results showed that commercial β-galactosidases preparates NOLA Fit5500 and Lactozym® Pure 6500 L were rather insensitive to increasing Ca^{2+} concentration, whereas Ha-Lactase 5200 exhibit strong inhibition. Analysing NOLA Fit5500 enzyme activity by changing Ca^{2+} concentration, results showed that oNPG hydrolysis stayed almost the same as it was for the reaction without Ca^{2+} addition. Enzyme kinetic parameters – K_M and V_{max} was the same up to 30 mM of Ca^{2+}. Lactozym® Pure 6500 L showed the highest results at Ca^{2+} concentration 5mM where K_M (1.421%) and V_{max} (1.022%). While at Ca^{2+} concentration up to 10 mM enzyme V_{max} decreased to 0.777% and till 50 mM stayed in similar condition.

Several researches have been made to analyse various monovalent and divalent cations impact on β-galactosidase activity. In the case of β-galactosidase from *Bacillus licheniformis*, results show that Ca^{2+} at concentrations of 1–10 mM together with Na^{+} (1–10 mM) can activate β-galactosidase (Juajun et al., 2011).

Banerjee and co-authors (1982) examined the effect of different metal ion concentrations (Mg^{2+}, Ca^{2+}) on native and immobilized cells of β-galactosidase producing by *Saccharomyces anamensis*, the highest activity was at 2.35 mM concentration. Furthermore it was established that at higher Ca^{2+} concentration the enzyme activity remains unchanged (Banerjee et al., 1982).

We have summarised information from some literature sources on cationic effects on β-galactosidases from various organisms (Table 2). As seen in Table 2, Ca^{2+} can be as β-galactosidase activator or inhibitor.

A wide range of metal ions are known to influence the activity of β-galactosidase (Pandey et al., 2017). The bacterial cell wall contains many types of cations including Mg^{2+}, Ca^{2+}, Na^{+}, and K^{+} (Sahalan et al., 2013).

Table 2

Cation effects on β-galactosidases

Source of enzyme	Activator	Inhibitor	References
Aeromonas caviae	Ca^{2+}; Mg^{2+}	–	(Karunakaran & Devi, 1994)
Kluyveromyces lactis	K^{+}; Mg^{2+}	Ca^{2+}; Na^{+}	(Otieno, 2010)
Lactobacillus reuteri	K^{+}; Na^{+}; Mn^{2+}	Fe^{2+}; Ca^{2+}; Cu^{2+}	(Nguyen et al., 2006)
Bacillus licheniformis	Ca^{2+}; Mn^{2+}; Mg^{2+}	Cu^{2+};Zn^{2+}; Fe^{2+}	(Akcan, 2011)
Kluyveromyces fragilis	Mn^{2+}, Mg^{2+}, K^{+}	Ca^{2+}	(Mlichová & Rosenberg, 2006)
Amygdalus communis	Ca^{2+}; Mn^{2+}	K^{+}; Na^{+}	(Pal et al., 2013)
Saccharomyces anamensis	Ca^{2+}; Mn^{2+}	–	(Banerjee et al., 1982)

Bacterial β-galactosidase can be activated by Ca^{2+}, while yeast or mould β-galactosidase for most of the cases – inhibited (see Table 2) (Mlichová, Rosenberg, 2006). In some cases, Ca^{+2} and Mg^{+2} is absolute necessity for β-galactosidase activity of *Aeromonas cauiae* (Karunakaran, Devi, 1994). Instead Kumar and co-authors (2015) stated that metal ion Ca^{2+} and Na^{+} in concentration of 10–30 mM did not affected the *Serratia quinivorans* β-galactosidase activity (Kumar et al., 2015).

It should be noted that nowadays the commercial β-galactosidase is mainly produced by *Kluyveromyces lactis* (You et al., 2017) such as Lactozyme 2600 L, GODO-YNL2, Ha-Lactase 5200, Lactozym® Pure 6500 L and Maxilact® LX5000 which is used in industrial field.

Conclusions

The results imply, that Ca^{2+} alone does not have effect on lactose hydrolysis by NOLA Fit5500 and Lactozym® Pure 6500 L β galactosidases, enzyme kinetic parameters – K_m and V_{max} at different Ca^{2+} concentrations stayed almost the same as it is for the reaction without Ca^{2+} addition. Ha-Lactase 5200 β-galactosidase is strongly inhibited by Ca^{2+}, this preparate would not be recommended to use for lactose hydrolysis in media with a high concentration of Ca^{2+}. Future research should be done to find out if Ca^{2+} together with other cations present in whey has additive effects on β-galactosidase activity.

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INFLUENCE OF SELENIUM, COPPER AND ZINC ON PHENOLIC COMPOUNDS IN RYE MALT

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Abstract

The natural phenolic compounds have received increasing interest in the last years due to their role in plants. The aim of this study was to determine the influence of microelements (selenium, copper and zinc) on content of phenolic compounds in rye malt. To obtain rye malt, grains were soaked in water with addition of three mineral salts – sodium selenate (Na_2SeO_4), copper sulphate ($CuSO_4$ $5H_2O$) and zinc sulphate ($ZnSO_4$ $7H_2O$) at different concentrations of salts. Total and individual phenolic compounds in rye malt were determined by spectrophotometric and HPLC methods. It was identified 19 phenol-type compounds, of which 14 were phenolic acids (7 benzoic acid, 6 cinnamic acid and 1 phenylacetic acid derivatives), four flavonoids, and one catechin. Results showed that the majority of determined phenolic compounds were in amount less than 0.1 mg 100 g^{-1} DM and only four of them (α-resorcylic acid, protocatechuic acid, catechin and kaempferol) were higher content than 1 mg 100 g^{-1} DM. Individual and total phenolic content in rye malt was higher than in non-germinated rye grain. Protocatechuic acid and catechin were not observed in rye samples, but they were present in all malt samples, indicating formation of mentioned substances in germination process.

Keywords: microelements, HPLC, spectrophotometric.

Introduction

Rye (*Secale cereale*) is a cereal commonly grown in Central and Eastern Europe, especially Poland and Germany (Edney, Izydorczyk 2003). Rye malt is the dried product of rye germinated under controlled conditions and is widely used in the production of bread, food flavouring, as ingredient for bakery products and as colour additive in the preparation of caramel (Antonenko et al., 2013).

Phenolics are the products of secondary metabolism in plants, providing essential function in the reproduction and growth of the plant, acting as defense mechanisms against pathogens and parasites (Gani et al., 2012; Reis Giada, 2013). In food, phenolics may contribute to the bitterness, astringency, colour, flavour, odour, and oxidative stability of products (Naczk, Shahidi 2014). Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups and generally are categorized as phenolic acids, flavonoids, stilbenes, coumarins and tannins (Gani et al., 2012). The most common phenolic compounds found in wholegrain cereals are phenolic acids and flavonoids (Gani et al., 2012; Amarowicz, Weidner 2012). The majority of phenolic compounds are located in the bran (Ondrejovič et al., 2014). Phenolic acids are derivatives of benzoic and cinnamic acids (Gani et al., 2012). Phenolic acids consist of two subgroups: the hydroxybenzoic and hydroxycinnamic acids. Hydroxybenzoic acids include gallic, *p*-hydroxybenzoic, protocatechuic, vanillic and syringic acids. Hydroxycinnamic acids include caffeic, ferulic, *p*-coumaric and sinapic acids (Ozcan et al., 2014). Plant genetics and cultivar, soil composition and growing conditions, maturity state and post-harvest conditions are effective on the quantity and quality of the polyphenols present in plant foods (Ozcan et al., 2014; Gani et al., 2012; Ondrejovič et al., 2014),

Messias (2012), reported that mineral fertilization during the growing season increase the contents of some phenolic compounds, as maintenance of mineral levels is a prerequisite to provide co-factors for many enzymes in the phenylpropanoid pathway. Mineral micronutrients, such as zinc (Zn), and copper (Cu), as well as trace elements, including selenium (Se), have important metabolic functions, acting as cofactors for a number of antioxidant enzymes (Messias et al., 2013). Cu is known as an essential micronutrient for the function of copper-zinc superoxide dismutases (SOD) and catalase (CAT) which are the most important reactive oxygen species scavenger enzymes. Copper plays an important role in the synthesis of phenolic compounds and its deficiency can decrease phenolics in the plants (Mehrizi et al., 2012). Similarly, Se, for which cereals are an excellent source, is an essential trace element for the regulation of antioxidant metabolism in plants and animals. It functions as a cofactor for glutathione peroxidase, an enzyme that protects tissues against oxidative damage and has a suppressive action on cell proliferation (Messias et al., 2012).

The aim of this study was to determine the influence of microelements: selenium, copper and zinc on phenol content in rye malt.

Materials and Methods

Plant material

Experiments were carried out in Department of Food Technology at the Latvia University of Agriculture. The research object was rye grain (variety ´Kaupo´) from Ltd. Naukseni (Latvia). Rye grains of 96% viability were soaked for 48 h at 8.5 °C and there after germinated for 72 h at 12 °C. Twelve samples of rye malt were prepared. To obtain rye malt, 3 kg of grains were soaked in 10 L of water with addition of three mineral salts – sodium selenate (Na_2SeO_4), copper sulphate ($CuSO_4$ $5H_2O$) and zinc sulphate ($ZnSO_4$ $7H_2O$) at different concentrations of salts (Table 1). As a control the samples without microelement additives were used in all experimental series. After germination grains were dried in the oven for 12 hours at temperature of 60 °C and 12 hours at temperature of 112 °C. Ground in a

laboratory mill fitted with a 0.4 mm sieve. Moisture of malt samples ranged from 7.2% till 8.9%.

Table 1

The content of microelements in water

Used mineral salts	The content of microelements additives in water, (mg L^{-1})			
Se (Na_2SeO_4)	0	5.0	8.5	17.0
Cu ($CuSO_45H_2O$)	0	10.0	20.0	50.0
Zn ($ZnSO_47H_2O$)	0	10.0	50.0	100.0

Determination of total phenolic content (TPC)

Total phenol determination started with preparation of extracts from rye malt. Rye malt was finely ground in the laboratory mill CIATRONIC KSW 2669. Four grams from each sample were extracted for10 min in the ultrasound bath (ULTRASONS, SELECTA P) with 40 mL of solvents ethanol / acetone / water mixture. After centrifugation at 3000 min^{-1} for 10 min using the centrifuge MEDITRONIC BL-C, the supernatant was removed and the extraction was repeated once more. The supernatant was collected in a 50 mL volumetric flask and refilled by solvent till mark. The TPC of the malt extract was determined according to the Folin-Ciocalteu spectrophotometric method with some modifications. First, 0.25 mL of sample was transferred to a 25.0 mL volumetric flask containing 6 mL of H_2O, to which 1.25 mL of undiluted Folin-Ciocalteu reagent was subsequently added. After 1 min, 3.75 mL of 20% aqueous Na_2CO_3 was added, and the volume was made up to 25.0 mL with H_2O. The control sample contained all the reaction reagents except the extract. After 2 h of incubation at 25 °C, the absorbance was measured at 760 nm wavelength using the spectrophotometer JENWAY 6300 (Singleton et al., 1999). Results were expressed as milligram gallic acid equivalent per 100 gram dry weight (mg GAE 100 g^{-1} DW).

Determination of individual phenolic compounds (HPLC)

Content of phenolic compounds was determined with High Performance Liquid Chromatography (HPLC) Schimadzu LC–20 Prominence (Shimadzu USA Manufacturing Inc, USA), detector DAD SPD-M20A, Solvent Delivery Unit LC-20AD, Column Oven CTO-20A, Autosampler SIL-20A, System Controller CBM-20A and data system LC solution software.

Preparation of calibration solution: weight in 100 mL volumetric flask with narrow neck 6.8±0.1 mg gallic acid, 7.4±0.1 mg 3.5-dihydroxybenzoic acid, 11.4±0.1 mg 3.4-dihydroxybenzoic acid, 12.0±0.1 mg catechin, 12.8±0.1 mg 4-hydroxybenzoic acid, 13.1±0.1 mg chlorogenic acid, 12.1±0.1 mg homovanillic acid, 14.5±0.1 mg vanillic acid, 13.8±0.1 mg caffeic acid, 16.0±0.1 mg epicatechin, 18.8±0.1 mg syringic acid, 9.8±0.1 mg vanillin, 12.1±0.1 mg *p*-coumaric acid, 88.1±0.1 mg sinapinic acid, 9.2±0.1 mg ferulic acid, 11.2±0.1 mg 2-hydroxycinnamic acid, 6.1±0.1 mg rutin, 10.3±0.1 mg trans-4-hydroxycinnamic acid, 4.3±0.1 mg quercetin, 9.1±0.1 mg luteolin and 9.6±0.1 mg kaempherol and fill with HPLC grade *CHROMASOLV*® methanol till mark and mix.

Parameters of chromatography: the analytical column Perkin Elmer C18, 4.6 × 250 mm, 5 µm and temperature of column +30 °C were used for separation of polyphenols in wavelength at 278 nm. Injection volume of sample 100 µL. Mobile phase: A (*deionized water), B* (HPLC grade *CHROMASOLV*® methanol) and C (Acetic acid solution for HPLC) in the gradient conditions was used. Start flow rate is 1.0 mL min^{-1}.

The obtained calibration chromatogram is given in Figure 1.

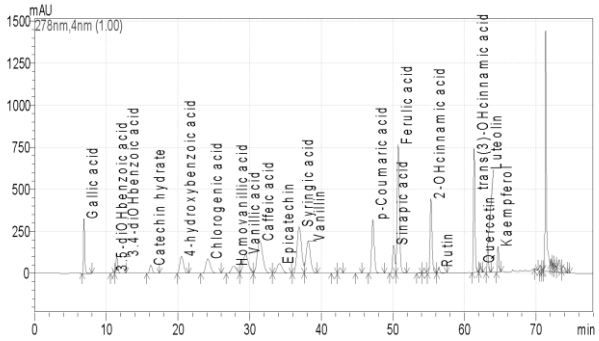

Figure 1. Calibration chromatogram of phenols

Statistical analysis

The statistical analyses of data were carried out using Microsoft Excel for Windows 7.0 (Microsoft Corporation, Redmond, WA). Mean value, standard deviations and significant value were calculated for total phenols and individual phenolic compounds. p-values<0.05 were regarded as significant.

Results and Discussion

To determine content of phenolic compounds in rye malt, rye grain was soaked in varying concentrations of selenium, copper and zinc salt solutions (Table 1).

The content of the total phenolic was higher almost 30 times compared with amount of individual phenolic compounds. It could be explained with spectrophotometric method disadvantages, because using this method we determined not only phenolic compounds but also others compounds containing phenolic groups. The purpose of this article is to give an overview about the influence of different microelements on the tendency of phenolic compounds change in rye malt.

The influence of selenium additives on total and individual phenolic content is presented in Table 2.

The obtained results (Table 2) show that all analysed selenium additives have positive influence on the content of phenolic compounds in rye malt. The highest content of total phenolic was determined at 17.0 mg L^{-1} of selenium and this was 37% more than in the control sample.

The content of individual phenolic compounds was increased about two times. Similar results were found by other authors, suggesting that selenium increased the polyphenol content in plants (Motomura et al., 2008).

Table 2

The influence of selenium on total and individual phenolic content in rye malt

Compounds	The content of selenium additive in water, mg L^{-1}			
	0	5.0	8.5	17.0
Total phenolic compounds, mg 100 g^{-1}	313.00±12.00	332.00±17.00	357.00±14.00	363.00±11.00
Individual phenolic compounds, mg 100 g^{-1}	6.21±0.54	9.52±0.72	10.82±0.42	11.3±0.68

Table 3

The influence of copper on total and individual phenolic content in rye malt

Compounds	The content of copper additive in water, mg L^{-1}			
	0	10	20	50
Total phenolic compounds, mg 100 g^{-1}	231.00±13.00	221.00±15.00	288.00±18.00	311.00±11.00
Individual phenolic compounds, mg 100 g^{-1}	5.48±0.23	7.18±0.42	8.18±0.48	9.99±0.61

Table 4

The influence of zinc on total and individual phenolic content in rye malt

Compounds	The content of zinc additive in water, mg L^{-1}			
	0	10	50	100
Total phenolic compounds, mg 100 g^{-1}	297.00±18.00	288.00±14.00	233.00±17.00	275.00±16.00
Individual phenolic compounds, mg 100 g^{-1}	8.33±0.38	6.98±0.56	7.74±0.49	9.09±0.41

The use of copper additives had also positive influence on the content of phenolic compounds in rye malt (Table 3). Obtained results showed that at copper concentration in solution 50 mg L^{-1} an increase in total phenolic content was 26% comparing with the control sample. The increase of individual phenolic content was significant ($p<0.05$) at all analysed copper concentrations comparing to the control, and the highest phenolic content was observed when the copper concentration in the solution was 50 mg L^{-1}.

Analysing obtained results regarding the zinc additives it is not possible to make common conclusion about its influence on forming of phenols. Experimental results showed (Table 4) that all analysed zinc additives decreased total phenol content in rye malt compared with control sample. On the other hand the highest amount of individual phenolic compounds was observed when the zinc concentration in the solution was 100 mg L^{-1}. At lower concentrations, amount of individual phenolic compounds decreased, compared with control sample. Our results are similar with the results of other studies who found that phenolic content of plants were increasing with increasing levels of microelements (Cu and Zn) (Hamid et al., 2010; Ganeva, Zozikova 2007; Vinod et al., 2012).

It was identified 19 of phenol-type compounds, of which 14 were phenolic acids: 7 benzoic acid derivatives (gallic acid, α-resorcylic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, syringic acid, p-coumaric acid), 6 cinnamic acid derivatives (chlorogenic acid, caffeic acid, sinapic acid, ferulic acid, o-hydroxycinnamic acid, m-hydroxycinnamic acid) and one phenylacetic acid derivative (homovanillic acid). There was also identified four flavonoids (rutin, quercetin, luteolin, kaemferol) and one flavonoid (catechin).

The majority of determined phenolic compounds were less than 0.1 mg 100 g^{-1}, moreover, in some samples it was not possible to determine all individual phenols, because their content was under the detection limit.

The content of only four of phenolic compounds (α-resorcylic acid, protocatechuic acid, catechin and kaempferol) were higher than 1 mg 100 g^{-1}.

The influence of selenium, copper and zinc additives on α-resorcylic acid, protocatechuic acid, catechin and kaempferol content in rye malt are shown in Figures 2, 3 and 4.

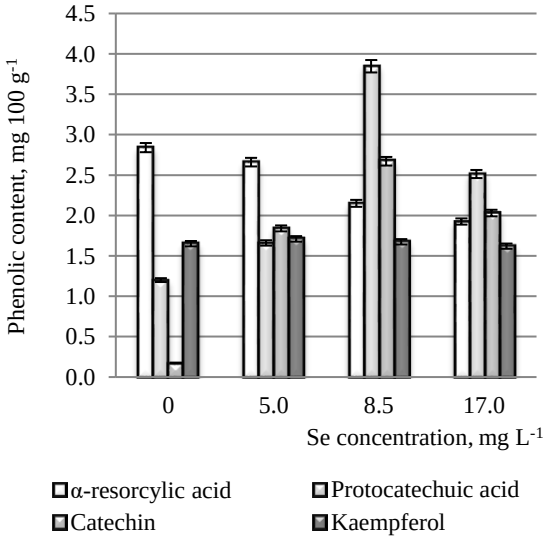

Figure 2. The influence of different Se concentration on α-resorcylic acid, protocatechuic acid, catechin, and kaempferol content in rye malt

The obtained results (Figure 2) showed that content of α-resorcylic acid, protocatechuic acid, catechin and kaemferol in rye malt depends on the Se concentration in solution. Content of protocatechuic acid and catechin increases with increasing Se concentration in solution. At the Se concentration 8.5 mg L^{-1} the content of protocatechuic acid and catechin acids were the highest 3.81 and 2.67 mg 100 g^{-1}. On the other hand, content of

α-resorcylic acid decreased with increasing of Se concentration in solution.

The content of α-resorcylic acid, protocatechuic acid, catechin and kaemferol in rye malt depended on the Cu concentration in solution (Figure 3). Content of α-resorcylic acid and protocatechuic acids increased with increasing of Cu concentration in solution. The highest catechin content (2.79 mg 100 g^{-1}) in rye malt was determined at copper concentration 20 mg L^{-1}.

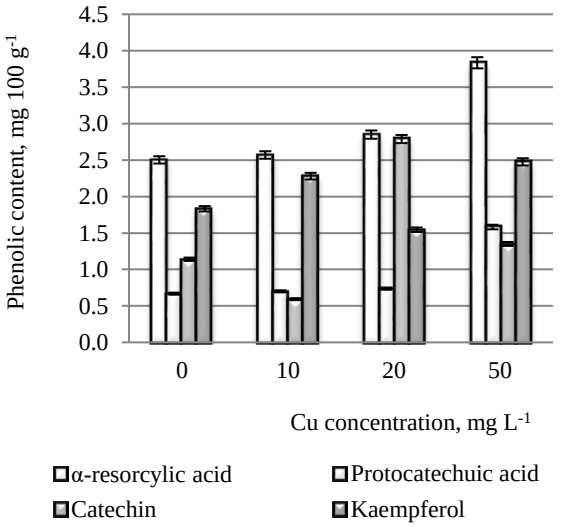

Figure 3. The influence of different Cu concentration on α-resorcylic acid, protocatechuic acid, catechin and kaempferol content in rye malt

The obtained results (Figure 4) showed that Zn affected the content of α-resorcylic acid, protocatechuic acid, catechin and kaemferolin rye malt like other two microelements (Se and Cu) and that degree of influence depended on the Zn concentration in solution. Content of α-resorcylic acid, catechin and kaempherol increased with the increase of Zn concentration in solution. At the highest Zn concentration (50 mg L^{-1}) the content of α-resorcylic acid, catechin and kaempherol was 3.99, 0.35 and 3.43 mg 100 g^{-1}, respectively. On the other hand, content of protocatechuic acid decreased with the increase of Zn concentration in solution.

The amount of individual phenolic in rye grain was 2.92 mg 100 g^{-1} on average. The amount of identified phenolic compounds in malt control samples ranged from 5.48 mg 100 g^{-1} to 8.33 mg 100 g^{-1}wich was almost two or three times higher, compared with ungerminated rye grain. Two phenolic compounds (protocatechuic acid and catechin) were not identified in rye grain. The content of kaemferol in rye grain (1.73 mg 100 g^{-1}) was similar with rye malt control samples (1.65–1.83 mg 100 g^{-1}), but content of α-resorcylic acid (0.59 mg 100 g^{-1}) was from 4.2 to 4.8 times lower compared with rye malt control samples.

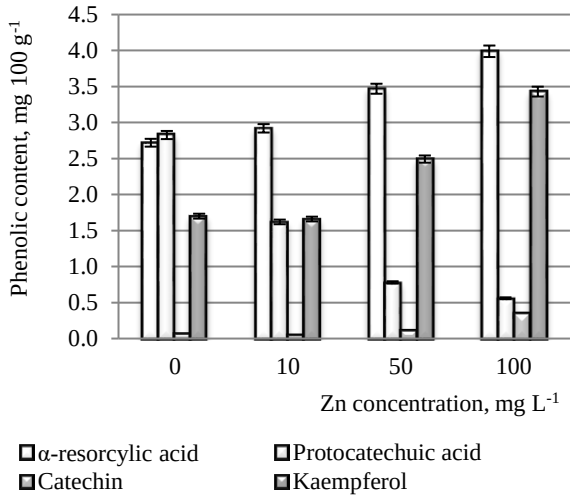

Figure 4. The influence of different Zn concentration on α-resorcylic acid, protocatechuic acid, catechin and kaempferol content in rye malt

This can be explained by the enzymatic release of the bound antioxidants of grain as well as glycosylation reactions during malting and non-enzymatic changes during kilning. Therefore, the levels of extractable phenolic compounds are usually reported to be higher| in malt than in grain. During kilning, the level of flavan-3-ols (catechins) is decreasing, whereas the content of extractable phenolic acids increases (Čechovská et al, 2012).

Conclusions

Selenium and copper additives had positive influence on the increase of phenolic content in malt. Regarding zinc salt additives we could not make such conclusion. It was identified 19 of phenol-type compounds, of which 14 were phenolic acids, four flavonoids and one flavanoid (catechin). The majority of determined phenolic compounds were in amount less than 0.1 mg 100 g^{-1}. Only four of phenolic compounds (α-resorcylic acid, protocatechuic acid, catechin, and kaempferol) were higher than 1 mg 100 g^{-1}. The content of α-resorcylic acid was higher comparing with other determined phenolic compounds both in control samples and samples with Se, Cu and Zn additives (1.95–2.92 mg 100 g^{-1}). In control samples and samples with Zn additives catehin content was the lowest (0.06–0.17 mg 100 g^{-1}). Selenium and copper additives promoted formation of catehin. The amount of individual phenolic compounds in rye grain was almost two or three times lower, compared with control samples of rye malt. Protocatechuic acid and catechin were not detected in rye grain samples. The content of kaemferol in rye grain was similar with rye malt control samples, but content of α-resorcylic acid was more than 4 times lower comparing to rye malt control samples.

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PHENOLIC PROFILE OF FRESH AND FROZEN NETTLE, GOUTWEED, DANDELION AND CHICKWEED LEAVES

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Abstract

Worldwide it is of great interest to find new and safe antioxidants from natural sources. Green leafy vegetables and wild plant leaves are healthy nutrients, containing vitamins, minerals and biological active compounds, therefore these plants provide beneficial health effects due to the presence of antioxidant compounds. It is useful and popular to supplement human diets with fresh or frozen edible plants. It is known that freezing may help to preserve the quality of plants, and is superior to other preservation methods. The aim of research was to compare the phenolic compounds content of fresh and frozen edible wild plants leaves grown in Latvia. The samples were processed using freezing -20 °C and for a comparison fresh samples were analysed. The leaves of stinging nettle (*Urtica dioica*), common goutweed (*Aegopodium podagraria*), dandelion (*Taraxacum officinale*) and chickweed (*Stellaria media*) were collected in May 2016 in Latvia. In the current research the content of total phenolics was determined in four types of fresh and frozen plant leaves and they can be arranged as follows (starting from plant with less phenolics content as gallic acid equivalent): dandelion<chickweed<goutweed<nettle. The freezing process affected the values of extracted phenolics and results in an increase in the extractability of the polyphenols for about 9.3%. Using HPLC analysis, we quantified 21 different individual phenols. The eight most abundant phenolics were: catechin hydrate, protocatechnic acid, α-resorcylic acid, vanillic acid, sinapic acid, routine, quercetin, luteolin, kaempferol. Experimentally it was ascertained, that in the frozen plant samples contents of some polyphenols increased ($p=0.007$), compared to the fresh samples.

Keywords: edible wild plants, phenolic compounds, HPLC.

Introduction

Wild medicinal plants are used by the majority of the world's population because these plants provide beneficial health effects due to the presence of antioxidant compounds (Dias et al., 2014). Leafy vegetables are rich in natural antioxidants which can neutralize free radicals in the human body. It is generally accepted that therapeutic effects of many plant species including spring wild plants are attributed to the presence of antioxidative phenolics in their tissues. In plants they are involved in numerous roles from structural to protective (Vajic et al., 2015). Beneficial effects of phenolics on human health have been extensively examined and there are studies that prove their protective role in cases of chronic cardiovascular diseases, cancer, and aging (Del Rio et al., 2013). Phenolic compounds are plants secondary metabolites and they are structurally diverse with in excess of 8000 structures having been reported, and many are found in only a limited number of species (Del Rio et al., 2013).

Goutweed *(Aegopodium podagraria)* is a genus of the *Apiaceae* family growing in Europe and Asia. The plant is a common weed. Its leaves are used as a spring vegetable like spinach (Orav et al., 2010).

Across the world, there are known more than 1000 plant species of the nettle family *(Urticaceae)*. In Latvia, only two nettle species are found: *Urtica dioica* L. often called common nettle or stinging nettle, and *Urtica urens* L. known as annual nettle. Stinging nettle is a perennial herbaceous cosmopolitan plant with long history of usage in treatment of different kinds of health problems (Nencu et al., 2013). Moreover, numerous researches confirmed antiinflammatory, analgesic, antiplatelet, positive cardiovascular and smooth–muscle activity, as well as hypotensive effect of stinging nettles (Upton, 2013). Di Virgilio et al. (2015) reports that nettle can be used as leafy vegetable, it has been used for centuries in salads, pies and soups. Stinging nettle leaves can be used in early spring as a leafy vegetable in salads and soups also in Latvia (Zeipiņa et al., 2015).

Dandelion *Taraxacum officinale* L. leaves and roots have been used for medicinal infusions (Ivanov, 2015). Leaves help prevent anemia, because they are rich in iron and helps the fetus to develop a strong liver of its own. Extracts from dandelion possess anti-influenza virus, anti–fertility and strong anti–HIV–1 retrovirus activity, antioxidant and hepatoprotective effects (Ivanov, 2015). Dandelion is important source of cichoric acid with potential application as radical scavengers and metal reducing activity. Therefore, this complex of biologically active substance offers many future applications in field of herbal medicine and nutrition for production of healthy food with well–pronounced healthy effect (Ivanov, 2015).

Chickweed (*Stelleria media)* is a plant of Eurasian origin which belongs to the order *Caryophyllale*s which comprises several families of herbs and shrubs with simple leaves. These kinds of plants and herbs are of nutritional and medicinal importance (Singh, Yadav, 2010).

Phenolic composition of plants is affected by different factors – variety, genotype, climate, soil, vegetative stage of the plant, harvest time, storage, processing and treatment (Marrelli et al., 2012; Angela, Meireles, 2009). Changes of the content of biologically active compounds in plants can cause climate, and also technological processes applied (Angela, Meireles, 2009). Freezing methods are easily applied and therefore are wildly used, because this method allows the preservation of taste, texture and nutritional

value of plants. Frozen products are very similar to the original fresh product.

There is insufficient information to conclude that freezing has negative effects on the qualities of plants. The aim of research was to compare the phenolic compounds content of fresh and frozen edible wild plants leaves grown in Latvia.

Materials and Methods

Investigations were carried out at the Latvia University of Agriculture, Department of Chemistry. Samples of stinging nettle *(Urtica dioica)*, common goutweed *(Aegopodium podagraria)*, dandelion *(Taraxacum officinale)* and chickweed *(Stellaria media)* were grown in Latvia, Jelgava region, harvested in April 2016 and stored in polyethylene bags until analysis. In the same day the fresh samples were prepared for analysis. The samples were processed by freezing for seven days at –20 °C.

Determination of individual polyphenols

The analysis was performed with Shimadzu HPLC system LC–20 Prominence including photo–diode array detector SPD–M20A, solvent delivery unit LC–20AD, column oven CTO–20A, autosampler SIL–20A, system controller CBM–20A and data system LC solution software.

The analytical column PerkinElmer C18, 4.6 mm×250 mm, 5 µm and temperature of column 30 °C was used for separation of polyphenols in wavelength of 278 nm. Injection volume of sample 100 µL. Mobile phase: A (deionized water), B (HPLC grade CHROMASOLV® methanol) and C (Acetic acid solution for HPLC) in the gradient conditions was used. Start flow rate is 1.0 mL min^{-1}.

Determination of total phenols

For total phenols extraction 1.0±0.001 g of finely ground plant samples was weighed into volumetric flasks, 10 mL of extractant, a mixture of methanol, distilled water and hydrochloric acid (79:20:1 v/v/v) was added. The vials were shaked at 20 °C for 60 min in the dark, then centrifuged for 10 min at 5000 rpm. The total phenols content of the samples was determined using the Folin–Ciocalteu reagent. To 0.5 mL of extract 2.5 mL of Folin–Ciocalteu reagent (diluted 10 times with water) and, after 3 minutes 2 mL of sodium carbonate Na_2CO_3 (75 g L^{-1}) was added. The sample was mixed. After 1 hour of incubation at room temperature, the absorbance was measured at 765 nm. Total phenols were expressed as gallic acid equivalents (GAE) 100 g^{-1} FW of plant samples.

Results and Discussion

The presence of phenolic compounds in plant extracts is an important for characterization of plants biological properties. Rappoport (2003) reported that the potency of antioxidants is determined by many factors – the chemical reactivity towards radicals, localization of antioxidants, concentration and mobility at the microenvironment, fate of antioxidant–derived radical, interaction with other antioxidants, and absorption, distribution, retention, metabolism, and safety.

The experimental data showed that content of total phenols in fresh plant samples varied from 510.6±25.5 mg GAE 100 g^{-1} (dandelion) till 743.4±96.3 mg GAE 100 g^{-1} (stinging nettle) (Figure 1). Our results are in accordance with findings of Vajic et al. (2015), Ivanov (2015) and Pinelli et al. (2008), but less than results reported by scientists from University of Baghdad, Iraq (Ghaima et al., 2013). These differences could be explained with other climate conditions.

The freezing process affected the values of extracted phenols and results in an increase in the extractability of the total phenols for about 9.3%. Similar influence of freezing was reported by Tomsone and Kruma (2014).

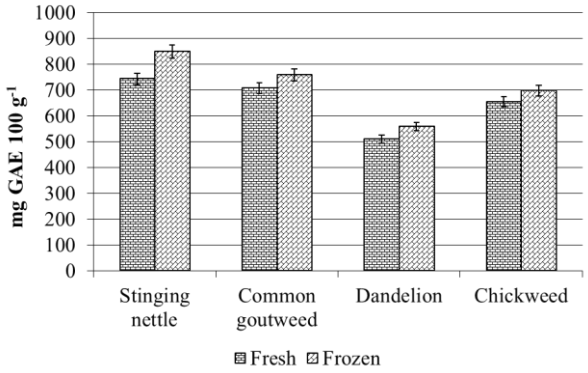

Figure 1. Content of total phenols in plants

The chemical composition of polyphenols compounds in different plants was determined by HPLC. The main polyphenols (Figure 2) in dandelion were sinapic acid 0.97±0.09 mg 100 g^{-1} and kaempferol 0.76±0.08 mg 100 g^{-1}, in common goutweed vanillic acid 3.56±0.15 mg 100 g^{-1} and catechin hydrate 1.96±0.08 mg 100 g^{-1}, but in chickweed luteolin 0.32±0.05 mg 100 g^{-1}.

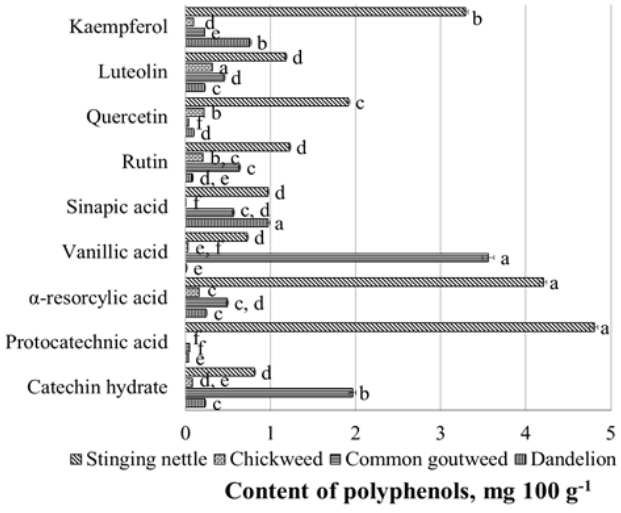

Figure 2. Content of polyphenols in fresh plants

* mean values within the same column followed by different letters significantly differ according to the LSD test ($p<0.05$)

The other compounds were found to be present in small quantities. Experimental results showed that stinging nettle samples are the richest in individual polyphenols compared with other analysed plants. Vajic et al. (2015) reported that predominant phenolic compound in stinging nettle leaves was rutin, but Ghaima et al. (2013) confirmed that the content of phenolic compounds in stinging nettle leaves significantly exceeds those of dandelion leaves.

Freezing is one of the oldest and most widely used method of food preservation, which allows preservation of taste, texture, and nutritional value in foods better than any other method. The freezing process is a combination of the beneficial effects of low temperatures, chemical reactions are reduced, and cellular metabolic reactions are delayed (Delgado, Sun, 2000).

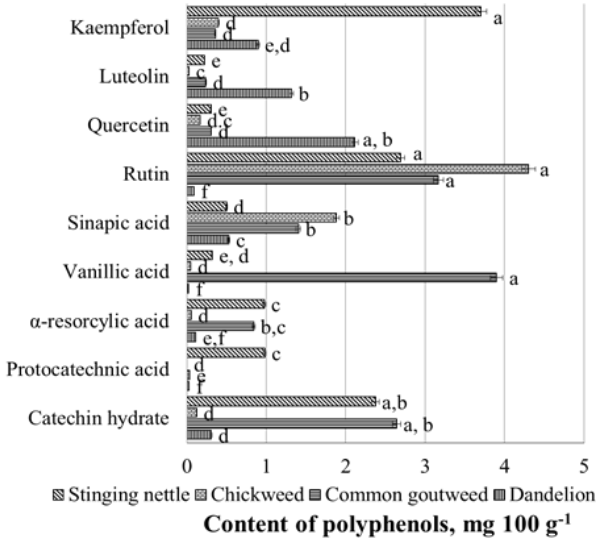

Figure 3. Content of polyphenols in frozen plants

* mean values within the same column followed by different letters significantly differ according to the LSD test ($p<0.05$)

Analyzing the content of catechin hydrate in frozen stinging nettle (Figure 3) we can conclude that it increased by 66% and rutin by 54%. The highest increase of quercetin, rutin, sinapic acid, α–resorcylic acid and kaempferol content was observed in frozen common goutweed samples – for 88%, 80%, 60%, 41% and 38%, respectively. After freezing the higher content of sinapic acid 97%, rutin 95% and kaempferol 76% were determined in chickweed samples. The increase of quercetin 99%, luteolin 83% and catechin hydrate 24% were observed in dandelion leaves comparing to fresh material.

Also literature studies showed that each phenolic compound behave different depending on treatment method. Content of polyphenols after freezing increased (Mulinaccia et al., 2011).

Conclusions

The content of total phenols in fresh plant samples could varied from 510.6±25.5 mg GAE 100 g^{-1} (dandelion) till 743.4±96.3 mg GAE 100 g^{-1} (stinging nettle). The freezing process affected the values of extracted phenols and extractability of the polyphenols increases for about 9.3%.

The predominant phenolic acid in plant samples were protocatechnic acid, α–resorcylic acid, vanillic acid and kaempferol. Experimentally it was ascertained, that in the frozen plant samples contents of some polyphenols significantly increased ($p=0.007$), compared to the fresh samples.

Research on the total phenols and individual polyphenols content showed that freezing is suitable method not only for preserving, but also for more quantitative extraction of these compounds.

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EFFECT OF EXTRACTION CONDITIONS ON PHENOLIC COMPOUNDS FROM BLACKBERRY LEAVES EXTRACTS

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Abstract

Blackberry leaves have been used as a tea substituent in many herbal mixtures. Medicinal properties of this plant material are related to a high level of components with antioxidant activity, such as phenolic compounds. The aim of the research was to evaluate the effect of different extraction conditions on the content of phenolic compounds and antioxidant activity of blackberry leaves extracts. In this study, blackberry leaves extracts were produced by an aqueous extraction procedure. Different extraction conditions: water temperature (40 and 80 °C) and extraction time (15 and 30 min) were investigated. The blackberry leaves extract prepared by applying higher temperature (80 °C) and longer time (30 minutes) was characterized by the highest contents of total phenolic compounds (1534.15 mg gallic acid equivalents L^{-1}), flavonoids (715 mg quercetin equivalents L^{-1}) and flavan-3-ols (28.21 mg (+)-catechin L^{-1}). Also, this extract expressed the highest antioxidant activity in terms of the ferric reducing ability of plasma (27.33 mmol Trolox equivalents L^{-1}) and generation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (1.47 mmol Trolox equivalents L^{-1}). The obtained results indicated that the produced extracts are a rich source of phenolic compounds with high antioxidant activity. Among investigated conditions, the use of water heated to higher temperature (80 °C) during prolonged time (30 min) is the most optimal procedure for the extraction of phenolic compounds with antioxidant activity from blackberry leaves. Further research is needed to determine the exact phenolic profile and their bioavailability, as well as to develop new functional food ingredients or nutraceuticals containing blackberry leaves extracts.

Keywords: blackberry leaves, phenolic compounds, antioxidant activity, flavonoids, flavan-3-ols.

Introduction

The use of herbal remedies is widespread among different patient groups and in the general population to promote health (Holst et al, 2009). The trend of wide using and applications of herbs as herbal infusions or tea, as the common name, is preserved from traditional medicinal system to this day owing to the rich and diverse phytochemicals composition (Komes et al., 2014). The investigation of unconventional raw materials for tea preparation has gained particular interest (Melkadze et al., 2008).

Blackberry (*Rubus fruticosus* L.) is widely grown and processed due to its fruits with pleasant organoleptic characteristics and a high content of compounds with health beneficial effects (Mikulic-Petkovsek et al., 2017). In addition to fruits, blackberry leaves represent a rich complex of biologically active substances (Nikitina et al., 2000). Blackberry leaves have been used as a tea substituent or constituent of herbal mixtures for many therapeutic purposes (Melkadze et al., 2008). Namely, tea made from the blackberry leaves has been used in folk medicine for their anti-inflammatory, antiviral and antimicrobial properties, as well as antiproliferative activity (Martini et al., 2009).

Health beneficial effects are mainly attributed to phenolic compounds (Nile, Park, 2014). Furthermore, sensory properties of tea, such as taste and smell, depend on the composition and content of phenolic compounds in the raw material and the degree of their changes during tea preparation (Melkadze et al., 2008).

Blackberry leaves are known to contain high content of phenolic compounds such as ellagic acid, quercetin, kaempherol, rutin, procyanidins, (-)-catechin, caffeic acid, as well as their derivates such as ellagitannins and quercetin 3-O-β-d-glukopyranoside (Buricova et al., 2011; Gudej, Tomczyk 2004; Martini et al., 2009, Oszmiański et al., 2015, Pavlović et al., 2016). Phenolic compounds, depending on the quantitative and qualitative composition, contribute to a high antioxidant activity of blackberry leaves (Wang, Lin, 2000). The ability to subdue free radicals contributes to an important role of phenolic compounds in the prevention or delay of cancer, heart diseases and diseases of the aging process (Nile, Park, 2014). Therefore, these phytochemicals offer numerous opportunities to be used as health beneficial agents for development of new functional food products (Nile, Park, 2014). Additionally, blackberry leaves contain significant amounts of triterpenes, mineral salts and vitamin C (Gudej, Tomczyk, 2004).

Due to the potential health beneficial effects related to tea drinking, it is important to determine the optimal extraction conditions to obtain tea with high content of biologically active compounds and strong antioxidant activity. Therefore, the aim of this study was to evaluate the effect of different extraction conditions (water temperature and extraction time) on the content of phenolic compounds and antioxidant activity of blackberry leaves extracts.

Materials and Methods

Chemicals and Materials

All the chemicals used in this study were of analytical grade and used as such without further purification. Folin-Ciocalteu reagent and gallic acid were purchased from Merck (Darmstadt, Germany). Sodium carbonate, sodium nitrite, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium acetate, iron (III) chloride and aluminium chloride were supplied by Centrohem

(Belgrade, Serbia). Hydrochloric acid and acetic acid were procured from Zorka (Šabas, Serbia). DPPH (2,2-dyphenyl-1-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), TPTZ (2,4,6-tri(2-pyridyl)-s-triazine), ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)), potassium persulfate, sodium hydroxide, quercetin and vanillin were provided by Aldrich (Sigma-Aldrich Chemie Steinheim, Germany). Ethanol was procured from Vrenje Spiritana (Belgrade, Serbia).

Blackberry leaves (Čačanska Bestrna variety) were collected in Arilje (Western Serbia) during the full ripening stage of the fruits (July, 2015). Fully ripened leaves are more desirable concerning the phenolic compounds, since their content and antioxidant activity are decreased in old leaves (Wang, Lin, 2000). Collected leaves were healthy, uniform in the size and at the same senescence stage.

Sample preparation

Harvested leaves were air-dried at room temperature in dark. Moisture content was determined (92.3%) according to a method approved by AOAC (Association of Official Analytical Chemists) International (AOAC, 1997). Afterwards, the dried material was ground in a laboratory mill to fine powder.

The aqueous extraction procedure was applied. The blackberry leaves (0.5 g) were extracted in 50 mL of distilled water on a magnetic stirrer. The effect of different extraction conditions: water temperature (40 and 80 °C) and extraction time (15 and 30 min) on the content of phenolic compounds, as well as antioxidant activity of extracts was studied. After extraction, the samples were centrifuged (centrifuge model: Boeco U-320 Hamburg, Germany) at 6000 rpm during 4 minutes.

Determination of total phenolic content (TPC)

TPC was determined according to a method with Folin-Ciocalteu's reagent (Singleton, Rossi, 1965). An aliquot of each extract (0.5 mL) was mixed with 10-fold diluted Folin-Ciocalteu's phenol reagent (2.5 mL) and allowed to react for 5 minutes. The sodium carbonate solution (75 g L^{-1}, 2 mL) was added to the mixture and shaken. After 2 h of reaction at room temperature in dark, the absorbance of blue coloration was measured at 760 nm. Gallic acid was used as the standard and the results were expressed as mg gallic acid equivalents (GAE) L^{-1}.

Determination of flavonoids content (TFC)

TFC was assessed spectrophotometrically according to a previously published method with some modifications (Zhishen et al., 1999). The volume of 2.5 mL of extracts was mixed with 150 μL 5% $NaNO_2$ solution and allowed to react during 6 minutes. 10% $AlCl_3$ (150 μL) was added and left to react for 5 minutes. Afterwards, 1 mL of 1 mol L^{-1} NaOH solution and 1.2 mL of distilled water were added and absorbance was measured at 510 nm. Quercetin was used as the standard and the results were expressed as mg quercetin equivalents (QE) L^{-1}.

Determination of flavan-3-ols content

The content of flavan-3-ols was estimated using the vanillin assay (Di Stefano et al., 1989). 500 μL of each extract was mixed with 3 mL of 4% vanillin solution and allowed to react for 5 minutes. 35% chloric acid (1.5 mL) was added to mixture and vigorously shaken. After 15 minutes of reaction at room temperature in dark, the absorbance of red coloration was measured at 500 nm. The amount of flavan-3-ols was calculated according to the corresponding formula based on difference between absorbance of vanillin-containing sample and blank. The results were expressed as mg (+)-catechin equivalents (CE) L^{-1}.

Determination of DPPH radical-scavenging activity

The samples were analysed according to the slightly modified previously published procedure (Sharma, Bhat, 2009). An aliquot of each extract (0.2 mL) was mixed with 2.8 mL of DPPH (2,2-diphenyl-1-picrylhydrazyl) solution (mixture of $1.86^{x}10^{-4}$ mol L^{-1} $DPPH^{\cdot}$ in ethanol and 0.1 mol L^{-1} acetate buffer (pH 4.3) in volume ratio 2:1). Free radical scavenging activity was determined by measuring the absorbance of solution at 525 nm after 30 minutes of reaction at room temperature in dark. Trolox was used as standard and the results were expressed as mmol Trolox equivalents (TE) L^{-1}.

Determination of ferric reducing/antioxidant power (FRAP assay)

The FRAP assay was carried out according to the published procedure with some modifications (Benzie, Strain, 1996). The FRAP solution was prepared by mixing acetate buffer (pH 3.6), TPTZ (2,4,6-tri(2-pyridiyl)-s-triazine) (10 mmol L^{-1} TPTZ solution in 40 mmol L^{-1} HCl) and $FeCl_3 \times 6H_2O$ (5.41 g L^{-1}) in volume ratio 10:1:1, respectively. Each extract (0.1 mL) was mixed with distilled water (0.3 mL) and FRAP reagent (3 mL). After the reaction at 37 °C for 40 min, the absorbance of blue coloration was measured at 593 nm. Trolox was used as standard and the results were expressed as mmol Trolox equivalents (TE) L^{-1}.

Determination of ABTS radical-scavenging ability

The Trolox equivalent antioxidant capacity (TEAC) of extracts was evaluated by the $ABTS^{\cdot+}$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical cation decolorization assay (Re et al., 1999). The volume of 30 μL of each sample was mixed with 3 mL of ABTS solution. After reaction for 6 minutes the absorbance of green coloration was measured at 734 nm. Trolox was used as standard and the results were expressed as mmol Trolox equivalents (TE) L^{-1}.

Statistical analysis

All results were obtained in three independent measurements and expressed as mean ± standard deviation. The experimental data were tested using One-way analysis of variance (ANOVA) and Tukey's test in order to detect significant difference ($p<0.05$) between the mean values. Additionally, Pearson correlation was determined between content of phenolic compounds and

antioxidant activity. Statistical analysis was performed using the statistical program Statgraphics Centurion XV (Statpoint technologies, Virginia, USA).

Results and Discussion

The effect of different extraction conditions (water temperature 40 and 80 °C and extraction time 15 and 30 min) on the content of phenolic compounds and antioxidant activity of the blackberry leaves extracts was studied.

The contents of total phenolic compounds (TPC), flavonoids (TFC) and flavan-3-ols of produced extracts are shown in Table 1. As can be seen, the highest contents of these compounds were determined when the water heated to higher temperature (80 °C) was used for the extraction during prolonged time (30 min) (1534.15 mg GAE L^{-1}, 715.00 mg QE L^{-1} and 28.21 mg+(-)-catechin L^{-1}, respectively). Significantly higher content of total phenolic compounds in the extracts was achieved using water heated to 80 °C. At this temperature the extraction time had a significant influence on the content of these compounds which was increased with prolongation of the extraction time. Extraction of increased contents of phenolic compounds using water heated at higher temperature is in the agreement with the studies for extracts of green tea (Komes et al., 2010), raspberry leaves (Salević et al, 2016) and herbal mixture (Veljović et al., 2015). The produced extracts have higher content of total phenolic compounds compared to blackberry leaves extract prepared with phosphate buffer by centrifugation (Wang, Lin, 2000) or in deionised water at 98 °C during 20 min (Buricova et al., 2011), but lower than blackberry leaves extract prepared with a mixture of methanol / water during 1 h in an ultrasonic bath (Pavlović et al., 2016). Generally, the content of extracted phenolic compounds is greatly influenced by the solvent nature and numerous extraction conditions, such as pH, temperature, solvent to solid ratio and particle size (Nour et al., 2014).

The results of this study point out that blackberry leaves extracts are a source of total flavonoids and flavan-3-ols. Content of total flavonoids was significantly increased in the extract produced in water at 80 °C during 30 min. In the extracts prepared using water at higher temperature significantly higher content of flavan-3-ols was determined, while extraction time did not have statistically significant influence on the content of those compounds.

Generally, the chemical properties of extracts depend on the solvent type, the applied temperature and time, as well as the extraction technique (Ong, 2004). According to the results of this study, the extraction conditions have to be chosen concerning the phenolic class of interest.

The values of antioxidant activity of the blackberry leaves extracts determined by different methods are shown in Table 2. All analysed extracts expressed good ferric reducing / antioxidant power and DPPH radical scavenging activity, but weak ABTS radical scavenging activity. It has been reported that various methods for evaluation of antioxidant activity could give widely divergent results since they are based on different mechanisms (Tabart et al., 2009).

DPPH radical scavenging ability was a significantly lower in the extract prepared in the water heated to 40 °C during 15 min compared to the other extracts. Significantly stronger ferric reducing / antioxidant power was determined in the extracts prepared using water heated to 80 °C. At this temperature the extraction time had no a significant influence on antioxidant activity determined by FRAP assay. Therefore, the results point out to fluctuations in the antioxidant activity of all prepared extracts depending on the extraction conditions and applied assay. The results published by Wang, Lin (2000) indicated that leaf age could affect the antioxidant activity. It was also found that *Rubus* L. leaves have high antioxidant capacity and total phenolic content compared to their fruit tissues (Wang, Lin, 2000).

Table 1

Contents of total phenolic compounds (TPC), flavonoids (TFC) and flavan-3-ols in blackberry leaves extracts depending on extraction conditions

Extraction conditions	TPC, mg GAE L^{-1}	TFC, mg QE L^{-1}	Flavan-3-ols, mg +(-) catechin L^{-1}
40 °C / 15 min	1515.85 ± 24.89^{bc}	449.00 ± 31.84^{a}	12.80 ± 0.70^{a}
40 °C / 30 min	1487.40 ± 4.98^{ab}	621.00 ± 2.45^{b}	13.67 ± 0.63^{a}
80 °C / 15 min	1458.94 ± 18.26^{a}	616.00 ± 26.13^{b}	26.75 ± 0.96^{b}
80 °C / 30 min	1534.15 ± 13.28^{c}	715.00 ± 15.51^{c}	28.21 ± 1.17^{b}

Data represent mean ± standard deviation.

Within the same column, values followed by different letters are significantly different (Tukey's test, $p < 0.05$).

Table 2

Antioxidant activity of blackberry leaves extracts depending on extraction conditions determined by DPPH, FRAP and ABTS assays

Extraction conditions	DPPH, mmol TE L^{-1}	FRAP, mmol TE L^{-1}	ABTS, mmol TE L^{-1}
40 °C / 15 min	14.29 ± 0.00^{a}	23.26 ± 0.08^{a}	1.47 ± 0.09^{c}
40 °C / 30 min	15.05 ± 0.13^{b}	26.31 ± 0.26^{b}	1.15 ± 0.07^{a}
80 °C / 15 min	14.93 ± 0.06^{b}	27.04 ± 0.45^{c}	1.30 ± 0.06^{ab}
80 °C / 30 min	14.91 ± 0.10^{b}	27.33 ± 0.16^{c}	1.47 ± 0.08^{bc}

Data represent mean ± standard deviation.

Within the same column, values followed by different letters are significantly different (Tukey's test, $p < 0.05$).

Significant positive correlations were found between the results of antioxidant activity (DPPH radical scavenging activity and ferric reducing / antioxidant power) and total flavonoids (Pearson correlation = 0.8171 and 0.8981, respectively). Study published by Oszmiański et al. (2015) showed that phenolic compounds of high molecular weight, primarily ellagitannins, are major contributors to antioxidant activity in leaves of *Rubus* L. species.

According to the results of this study investigated conditions are suitable for production of extracts with high content of phenolic antioxidants. Using water as a solvent is acceptable since aqueous extraction simulates tea production and there is no need for evaporation and removing of potentially harmful solvents. Further research is needed in order to evaluate the effect of extraction conditions on the qualitative and quantitative composition of bioactive compounds.

Conclusions

The different extraction conditions (water temperature: 40 and 80 °C and extraction time: 15 and 30 min) were compared on the basis of the content of phenolic compounds and antioxidant activity of blackberry leaves aqueous extracts. This study indicates that blackberry leaves extracts are the rich source of phenolic antioxidants. The highest phenolic content and antioxidant activity were obtained in the extract prepared using water at higher temperature during prolonged time.

Among investigated conditions, the use of water heated to higher temperature (80 °C) during prolonged time (30 min) is the most optimal procedure for the extraction of phenolic compounds with antioxidant activity from blackberry leaves.

It is very important to determine the content and pharmacological properties of each phenolic compound in the prepared extracts since individual compounds have different antioxidant and health properties. Therefore, further research is needed to determine the exact phenolic profile, their bioavailability and health benefits of the extracts. Also, protective mechanisms should be investigated in order to maintain those compounds active during food processing and storage and enable their delivery to the target site in an organism.

Acknowledgment

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INFLUENCE OF HARVEST TIME ON THE PHENOLIC CONTENT OF HORSERADISH LEAVES

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Abstract

A perennial herb horseradish (*Armoracia rusticana* L.) contains biologically active substances and cultivated in temperate regions of the world. The aim of the current research was to determine the content of phenolic compounds and antioxidant properties of horseradish leaves depending on the harvest time. For experiments horseradish leaves three years at six different times (during the period from May to October) were collected. Fresh plant material was extracted with ethanol using conventional extraction. For all extracts total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (DPPH$^{\bullet}$, ABTS$^{\bullet+}$ scavenging activity, reducing power) were determined using a spectrophotometric methods. Results showed that content of phenolic compounds and antioxidant activity of horseradish leaves were significantly affected by harvest time. The highest TPC and ABTS$^{\bullet+}$ scavenging activity was observed in the horseradish leaves collected in May, but the highest TFC and antioxidant activity (DPPH radical scavenging activity and reducing power) was observed in June. The content of phenolics compounds and antioxidant activity significantly decreased during post flowering stage and continues till October. Horseradish leaves contained considerable amount of phenolics compounds and natural antioxidants. In further experiments, use of horseradish leaves as natural antioxidants in different food matrixes should be studied.

Keywords: horseradish, harvest time, phenolic, antioxidant.

Introduction

Plants are rich in natural antioxidants, which are very important for human health (Naczk, Shahidi, 2006). The antioxidant characteristics of plant raw materials can be attributed to their phenolic compounds. Chemically phenolic compounds are highly active substances and over the past twenty years their popularity as natural antioxidants has grown significantly (Kammerer, Carle, 2012; Naczk, Shahidi, 2003).

Biologically active substances, especially phenolic compounds, composition, content and function in plants is affected by different factors – harvest time, various external factors (cultivation, storage conditions, processing, climate), genetic background (variety, genotype) (Angela, Meireles, 2009; Marrelli et al., 2012). Plants phenolic compounds are synthesized in a normal stage of development, but they increasingly can be synthesized by biotic or abiotic stress conditions (ultra-violet (UV) radiation, mechanical injury or microbial infection) (Naczk, Shahidi, 2006). Different plant enzymatic systems, which are involved in the biosynthesis of phenols, vary the profiles significantly (Hilt et al., 2003), thereby the phenols have wide variations in function (Kammerer, Carle, 2012).

The plants development stage at harvest time is an important factor of product quality. Several authors have reported that antioxidant activity (AA) and chemical composition are influenced by the harvest time, as well as the content of compounds and properties vary from plant development stages (Imene et al., 2012; Tomsone et al., 2012; Marrelli et al., 2012). The content and composition of phenolic compounds and antioxidant potential of areal parts of *Calamintha nepeta* L. Savi (*Lamiaceae*) was significantly affected by ontogenic growth stages (Pacifico et al., 2015). Tunisian researchers have found that for some plants (*O. ficus-indica* (L.) Mill. and *O. stricta* (Haw.) Haworth) significantly higher total phenolics content (TPC) and AA are directly during flowering (Imene et al., 2012), similar trend also was observed for phenolic compounds in horseradish roots (Tomsone et al., 2012). Whereas the lowest TPC of *Ficus caricacv.* 'Dottato' fruit was at the beginning of maturation and gradually increased with the pulp ripening stage (Marrelli et al., 2012). Such differences might be related to changes in the secondary metabolism (Imene et al., 2012).

Horseradish (*Armoracia rusticana* L.) belong to *Brassicaceae* family. There is few found data about phenolic quantitative content of horseradish in the literature. One of the studies showed that TPC of horseradish leaves ranged from 256 mg GAE 100 g^{-1} DW to 385 mg GAE 100 g^{-1} DW (Calabrone et al., 2015). That is significantly more than the kale (*Lathyrus* L.), spinach (*Spinacia* L.) and broccoli (*Bromelia* L.), but less than the potato (*Kochia scoparia* L.) and carrot (*Bromelia* L.) (Zhou et al., 2006). The plants of *Brassicaceae* contain a number of compounds that can act as natural antioxidants (Raghavan, 2000). Several authors reported that also the chemical composition of *Brassicaceae* plants varies depending on the harvest time, growing conditions (Kusznierewicz et al., 2008) and stage of development (Björkman et al., 2011).

The aim of the current research was to determine the content of phenolic compounds and antioxidant properties of horseradish leaves depending on harvest time.

Materials and Methods

Chemicals

Gallic acid, Folin-Ciocalteu phenol reagent, and 2,2-diphenyl-1-picrylhydraziyl (DPPH$^{\bullet}$) were purchased from Sigma-Aldrich (Switzerland). All other chemicals

(Na_2CO_3, ethanol) used in the research were obtained from Acros Organic (USA).

Sample preparation

Fresh samples were collected from May to October in Jelgava Latvia during three-year period (2014–2016). Average temperature and precipitation during study in harvest place are shown in Figure 1.

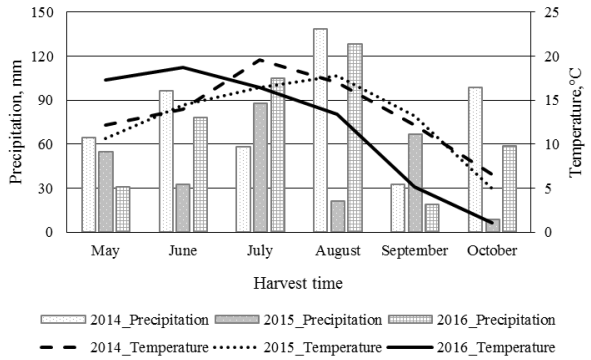

Figure 1. Average temperature and precipitation

Characterization of collected horseradish leaves are shown in Table 1.

Table 1

Height of collected horseradish plants, cm

Harvest times	2014	2015	2016
May	10–15	10–14	9–14
June	14–20	12–21	13–20
July	15–28	17–30	13–26
August	17–33	17–38	18–33
September	20–45	18–44	22–40
October	24–50	27–53	27–52

Extraction procedure

Extraction procedure was applied as outlined by Tomsone et al. (2012). The extraction process was done in triplicate.

Analytical methods

Determination of total phenolic content (TPC) and total flavonoid content (TFC). The TPC of the plant extract was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999). TPC was expressed as the gallic acid equivalents (GAE) 100 g^{-1} dry weight (DW) of plant material. The TFC was measured by a colorimetric method (Kim et al., 2003). TFC was expressed as the catehin equivalents (CE) 100 g^{-1} DW of plant material.

Determination of antioxidant activity (AA). AA of the plant extracts was measured on the basis of scavenging activities of the stable 2,2-diphenyl-1-picrylhydraziyl (DPPH$^{\bullet}$) radical as outlined by Yu et al. (2003). The radical scavenging activity of extract was also measured by 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic) acid (ABTS$^{\bullet+}$) cation assay (Re et al., 1999). The radical scavenging activity was expressed as TE 100 g^{-1} DW of plant material. The higher the Trolox equivalent antioxidant capacity (TEAC) of a sample, the stronger the antioxidant activity. The reducing power was determined by the method of Athukorala et al. (2006) and reducing power was expressed as the ascorbic acid equivalents (AAE) 100 g^{-1} DW of plant material. Additionally for all horseradish leaves samples the moisture content was determined according to the standard ISO 6496:1999 and all results were expressed on dry basis.

Statistical analysis

Experimental results are 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. A linear correlation analysis was performed in order to determine relationship between TPC, TFC, antioxidant activity such as DPPH$^{\bullet}$, ABTS$^{\bullet+}$ and reducing power. Differences were considered as significant at $p < 0.05$.

Results and Discussion

Total phenolics and flavonoids content

The TPC determined in horseradish leaves depending on harvest time are shown in Table 2. ANOVA analysis of variance showed that TPC was significantly affected ($p<0.05$) by harvest time but not by harvest year.

Table 2

Total phenolic content of horseradish leaves at different harvest times, mg GAE 100g^{-1} DW

Harvest times	2014	2015	2016
May	2634±57 ^{a*,A**}	2705±59^{a,A}	2590±55^{a,A}
June	2368±52^{b,A}	2351±53^{b,A}	2339±50^{b,A}
July	1853±56^{c,A}	1834±50^{c,A}	1814±54^{c,A}
August	1568±50^{d,A}	1616±52^{d,A}	1642±48^{d,A}
September	1339±59^{e,A}	1380±51^{e,A}	1368±58^{e,A}
October	1279±54^{e,A}	1235±51^{f,A}	1252±50^{e,A}

* Different small letters in the same column represents significant differences between values (Tukey's test, $p<0.05$).
** Different capital letters in the same row represents significant differences between values (Tukey's test, $p<0.05$).

Comparing harvest years similar tendency in all analysed samples was observed - the highest TPC was in May and by October it gradually decreased by 52% on average. No significant differences between TPC of horseradish leaves collected in May 2014, 2015 and 2016 were determined while comparing weather conditions – in May 2015 there was the lowest average air temperature (+10.6 °C), compared with 2014 (+12.2 °C) and 2016 (+17.3 °C). Environmental stress conditions (heat, cold, ozone, drought, intense light before the harvest) have a positive impact on the TPC in fruit and vegetables – lettuce (*Leucojum vernum* L.), for sweet potatoes (*Salix* L.), strawberries (*Zantedeschia aethiopica* L.), tomatoes and maize (*Kniphofiauvaria* L.) (Capanoglu, 2010).

A group of researchers found that content of phenolic acids (for example caffeic acid) of areal parts of *Calamintha nepeta* L. Savi was significantly higher at the flowering stage than at the post flowering stage (Pacifico et al., 2015).There is no general tendency for differences of TPC in horseradish roots depending on genotype and harvest time (Tomsone et al., 2012).

The highest TFC (Table 3) was determined in June 2016, when the average air temperature was higher (+18.7 °C) compared to 2014 (+14.0 °C) and 2015 (+14.4 °C). The TFC in July decreased on average by 37%. This may be due to the intensity of UV radiation, influenced by the daylight hours and ontogenic growth stages of plants. There was a tendency, that after June to October (during post flowering stage) TFC in horseradish leaves gradually decreased by an average of 76% in three analysed years.

Flavones and flavonols are highly UV absorbers, which accumulates mainly in the cells of the epidermis. Consequently, in different parts of one plant is significantly different flavonoid content and leaves contain more flavonoids than roots (Gould, Lister, 2006; Naczk, Shahidi, 2003).

The obtained results showed that the largest content of phenolic compounds of horseradish leaves was in the period from May to June, which coincides with the horseradish development period until the flowering and the flowering period (Raghavan, 2000). A similar trend was also observed in *W. somnifera* leaves (Fernando et al., 2013). The phenolic compounds synthetic reinforced surface plant parts until flowering period.

Perennial plants post flowering period, virtually all biological processes are allocated to the root development and nutrient accumulation in roots. The same tendency can be observed in certain genotypes of horseradish roots (Tomsone et al., 2012), where a higher content of phenolic compounds were directly in the post flowering stage. Pacifico et al. (2015) reported that quercetin derivative of areal parts of *Calamintha nepeta* L. Savi was significantly higher at the flowering stage than at the post flowering stage.

Antioxidant activity (AA)

Phenolic compounds have been reported to have strong AA (Li et al., 2006). The antioxidant potential of these biological active compounds is dependent on the structural conformation (Elzaawely et al., 2007). Results of multivariate dispersion analyses showed that harvest time is significant ($p<0.05$) factor affecting AA.

Scavenging activity of DPPH radicals for all samples has similar tendencies that the higher activity was in June, and during post flowering stage by October it gradually decreased by an average of 45%. The highest $DPPH^{\cdot}$ scavenging activity was determined in horseradish leaves in June 2016 (Table 4) similar to TFC. It allows thinking that exactly flavonoids of horseradish leaves have a high $DPPH^{\cdot}$ scavenging activity.

Table 3

Total flavonoid content of horseradish leaves at different harvest times, mg CE 100 g^{-1} DW

Harvest times	2014	2015	2016
May	$8515\pm223^{b*,A**}$	$9192\pm237^{b,B}$	$9087\pm229^{b,A,B}$
June	$11136\pm233^{a,A}$	$11099\pm261^{a,A}$	$11697\pm257^{a,A}$
July	$7036\pm183^{c,A,B}$	$7409\pm245^{c,B}$	$6873\pm199^{c,A}$
August	$5486\pm211^{d,A}$	$5038\pm253^{d,A}$	$5070\pm226^{d,A}$
September	$3921\pm230^{e,A}$	$3518\pm245^{e,A}$	$776\pm219^{e,A}$
October	$2486\pm220^{f,A}$	$2970\pm252^{e,A}$	$2784\pm231^{f,A}$

Table 4

$DPPH^{\cdot}$ scavenging activity of horseradish leaves at different harvest times, mM TE 100 g^{-1} DW

Harvest times	2014	2015	2016
May	$52.84\pm2.18^{b*,A,B**}$	$50.92\pm2.02^{b,A}$	$57.41\pm2.31^{b,B}$
June	$69.33\pm1.92^{a,A}$	$71.09\pm2.11^{a,A}$	$73.38\pm2.07^{a,A}$
July	$51.69\pm2.27^{c,A}$	$57.30\pm2.40^{c,A,B}$	$61.50\pm2.35^{b,c,B}$
August	$48.03\pm2.09^{c,A}$	$49.66\pm1.89^{c,A}$	$53.04\pm2.12^{c,A}$
September	$43.74\pm1.95^{d,A}$	$40.97\pm2.30^{d,A}$	$45.53\pm2.28^{d,A}$
October	$39.18\pm1.83^{d,A}$	$39.51\pm1.75^{d,A}$	$37.13\pm1.99^{e,A}$

Table 5

$ABTS^{\cdot+}$ scavenging activity of horseradish leaves at different harvest times, mM TE 100 g^{-1} DW

Harvest times	2014	2015	2016
May	$148.92\pm5.74^{a*,A**}$	$150.83\pm5.44^{a,A}$	$146.71\pm6.3^{a,A}$
June	$140.08\pm4.99^{a,A}$	$143.13\pm5.16^{a,A}$	$142.52\pm3.69^{a,A}$
July	$125.50\pm3.93^{b,A}$	$127.70\pm3.65^{b,A}$	$126.24\pm4.03^{b,A}$
August	$121.44\pm4.34^{b,c,A}$	$122.20\pm4.47^{b,c,A}$	$124.24\pm5.25^{b,A}$
September	$112.04\pm3.62^{c,A}$	$111.63\pm3.03^{c,A}$	$111.48\pm3.23^{c,A}$
October	$91.76\pm2.98^{e,B}$	$88.07\pm2.48^{d,A,B}$	$81.42\pm2.68^{d,A}$

* Different small letters in the same column represents significant differences between values (Tukey's test, $p<0.05$).

** Different capital letters in the same row represents significant differences between values (Tukey's test, $p<0.05$).

Table 6

Reducing power of horseradish leaves at different harvest times, mg AAE 100 g^{-1} DW

Harvest times	2014	2015	2016
May	8548±210^{b*,A**}	7834±223^{b,B}	7639±198^{b,B}
June	9000±207^{a,A}	9135±190^{a,A,B}	9573±192^{a,B}
July	7990±187^{c,B}	7437±216^{b,A}	7576±205^{b,A,B}
August	5708±150^{d,A}	5532±178^{c,A}	5457±161^{c,A}
September	4621±197^{e,B}	3691±209^{d,A}	3981±176^{d,A}
October	2873±165^{f,A}	2638±176^{e,A}	2998±190^{e,A}

* Different small letters in the same column represents significant differences between values (Tukey's test, p<0.05).
** Different capital letters in the same row represents significant differences between values (Tukey's test, p<0.05).

The same tendency was observed with *Calamintha nepeta* L. Savi where an antioxidant potential (DPPH$^{\cdot}$) decreased during the post flowering stage (Pacifico et al., 2015).

The highest $ABTS^{\cdot+}$ scavenging activity was determined in horseradish leaves in May 2015 (Table 5), similar to TPC. Apparently other phenolic compounds fractions (not flavonoids) have a way of better scavenging activity of $ABTS^{\cdot+}$. The highest $ABTS^{\cdot+}$ scavenging activity was in May for all analysed samples and by October it gradually decreased on average by 41%.

Imene et al. (2012) reported about scavenging activity depending on the harvest time – as chemical composition, amounts and nature of compounds vary within development stages and species; it can be influenced by changes in secondary metabolism. AA shows a marked variation with ontogenic growth stages and the maximum of AA is observed during post flowering stage for the two species – *Opuntia ficus-indica* (L.) Mill. and *O. stricta* (Haw.) Haworth (Imene et al., 2012). It is the opposite trend by horseradish leaves AA and areal parts of *Calamintha nepeta* L. Savi (Pacifico et al., 2015).

Reducing power (RP) associated with indirect antioxidants and it can serve as a significant reflection of activities (Oktay et al., 2003). Literature reports show that antioxidant activity of plant and herb is related to the reducing power that interrupts radical chain reactions (Singh, Rajini, 2004). The existence of reductants is key to reducing power, which shows the antioxidant activity because donate hydrogen atoms and interrupt free radical chain reactions (Xing et al., 2005).

The experiments showed that reducing power is dependent on the plant harvest time. The highest reducing power was determined in June 2016 (Table 6), similar to TFC. It is considered that in plant material poor in vitamin C, main antioxidants are flavonoids and phenolic acids (Igual et al., 2012). Apparently horseradish leaves flavonoids are able to donate hydrogen atoms by interrupting free radical chain reactions.

As mentioned above, higher reducing power was in June, and by October (during post flowering stage) it gradually decreased by an average of 69%. It can be explained by the modification of chemical composition of horseradish leaves in the post flowering stage. The antioxidant potential (reducing power) of areal parts of *Calamintha nepeta* L. Savi was significantly higher at the flowering stage than at the post flowering stage (in October) (Pacifico et al., 2015).

The results show that pre-flowering and flowering stages corresponds to the maximum accumulation of phenolic compounds and antioxidant activities.

Correlation between phenolic content and antioxidant activity (AA)

The AA is influenced by the phenolic composition. A various correlation coefficients were obtained by analysing relationship between phenolics compounds and AA in. Overall, positive strong correlation between TPC, TFC, DPPH$^{\cdot}$, $ABTS^{\cdot+}$ and reducing power was determined. This can be explained by the fact that phenolic compounds are the most important antioxidant of horseradish leaves. Further research is necessary to identify individual phenolic compounds and analyse their influence on the overall free radical scavenging activity.

Prasad et al. (2009) found, that statistical correlations between TPC and total antioxidant capacity of litchi seed were strong (r=0.98), that is similar to results of the current research. But Pacifico et al. (2015) found that correlation between TPC and $ABTS^{\cdot+}$ scavenging activity of areal parts of *Calamintha nepeta* L. Savi were moderate (r=0.64). Whereas stronger correlation between TFC – DPPH$^{\cdot}$ and TFC – reducing power was observed in June (r=0.861 and r=0.853, respectively). Strong correlation between phenolic compounds and AA was also found in seabuckthorn (*Hippohae rhamnoides* L.) leaves (Kumar et al., 2011), canola meal (Hassas-Roudsari et al., 2009) and lychee (*L. chinenesis* Sonn.) flowers (Liu et al., 2009). It coincides with the highest concentration and activity of these compounds. The correlation ranged from medium to weak at the others harvest times. Fresh *L. lucidus* Turcz. roots were harvested at three different times and there was significant correlation between phenolic content and different antioxidant assays (Lu et al., 2015).

Conclusions

This research is contribution to the determination of the content of phenolic compounds in horseradish leaves and its variability depending on the harvest time. Results showed that content of phenolic compounds and antioxidant activity was significantly affected both

by harvest time and year. The highest TPC and $ABTS^{\cdot+}$ was observed in the horseradish leaves collected in the May, but TFC, $DPPH^{\cdot}$ and reducing power – in the plants collected in June. The content of phenolics compounds and antioxidant activity significantly decreased during post flowering stage and continued till October. Horseradish leaves contained considerable content of phenolics compounds and natural antioxidants. Also a strong correlation between the TPC and $ABTS^{\cdot+}$ as well as TFC and $DPPH^{\cdot}$, reducing power at harvest time in May was detected. Further experiments are necessary to evaluate antioxidant activity of horseradish leaves extracts in food matrixes.

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COMPARISON OF VOLATILE AND PHENOLIC COMPOSITION OF COMMERCIAL AND EXPERIMENTAL CIDERS

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Abstract

In the study five ciders produced in Latvia, three in France, and one sample from each – Ireland, Finland, South Africa were tested. The best 10 samples differing by raw materials, fermentation and maturation technology were selected from experimentally produced ciders. The total phenolic content was determined spectrophotometrically, but volatile compounds were analysed using SPME followed by GC/MS. The total phenolic content in commercial ciders is from 324. mg L^{-1} to 3831.33 mg L^{-1} and significantly higher content in ciders produced in France. In the experimental ciders the total phenolic content was similar: from 793 to 3399 mg L^{-1}. A lower content of volatile substances was identified in French ciders, while the highest content in the cider produced in Latvia. The main classes of identified volatiles were alcohols (6.53–74.05%), and esters (18.81–73.61%), with the most characteristic ones being ethyl carpylate, hexyl acetate, ethyl hexanoate, 3-methylbutyl acetate and ethyl decanoate. Experimental ciders matured with lactic acid bacteria *Oenococcus oeni* and oak chips showed a content of volatile compounds similar to that of French ciders. Experimental cider sample made from the crab variety 'Kerr', in its turn, showed a profile of volatile compounds similar to Latvian ciders.

Keywords: experimental cider, commercial cider, total phenolic, volatile compounds.

Introduction

Nowadays in Latvia small wine and cider producing companies are developing rapidly and it is important to develop best technological conditions. Cider is low – alcoholic drink made by fermentation of apple juices. In Latvia there are no long cider making traditions and consumers are likely to recognise it by drink produced in large companies being heavily carbonated and having sweet taste. For successful acceptation of drink from consumers it is necessary to develop cider with good sensory characteristics. Odour and flavour are the most important quality attributes of alcoholic beverages, parameters determining the preferences of potential consumers. The development of these attributes throughout the technological process is influenced by the different and complex steps: selection of raw material, fermentation process and maturation or ageing (Antón-Díaz et al., 2016) Mastering the quality and the repeatability of production needs a better understanding of the way cider components interact to construct the final sensory characteristics of cider flavour (Symoneaux, 2015). For evaluation it is necessary to compare the quality parameters of experimental and commercial ciders available in retailing. Cider is produced from sharp, bitter sharp, bittersweet and sweet cider apples, and classification is based on apple sensory properties, acid and phenolic content and different apple varieties blends made perfect balance between sweetness and tartness (Lea, Drileau, 2003). Traditionally, cider makers have a preference for specific cider apple varieties –'crab apples', popular in western England, north-western France and northern Spain. Latvian producers mostly produce cider from culinary and / or dessert apples. In cider production, regardless the selected variety of apples or blends, it is more important to select the most suitable technological methods of cider fermentation, including the selection of the best pure yeast culture and maturation conditions, which ensures a basis for a high-quality end product with the best sensory characteristics.

Polyphenols play important role in the cider quality as they are related to the colour, bitterness and astringency, whose balance defines the overall mouthfeel of the beverage (Lea, Drilleau, 2003; Alonso-Salces et al., 2004) and the highest content is in ciders made from crab apples varieties (Riekstina et al., 2014). Research into the use of dessert apples alone for cider production has suggested they are less suitable due to their chemical attributes (Kuhn, 1994), yet these varieties fill an important volume gap due to their availability compared to traditional cider apple varieties (Girschik et al., 2017). Composition of volatile compounds of cider depends from many technological aspects. The study of volatile compounds can provide significant information about the raw materials and technological processes employed in the fermentation to guarantee cider quality and avoid financial losses (Pizarro et al., 2009). Volatiles identified in wines are usually dominated by fermentation products, the flavour compounds underlying the so-called 'yeast bouquet'; ethyl esters, acetate esters, fusel alcohols, carbonyls, and volatile fatty acids, are secondary metabolites synthesized by a wide range of microbial species and upon winemaking practices contribute to wine flavour (Romano et al., 2003). Malolactic fermentation significantly affects the sensory characteristics of cider and *Oenococcus oeni* can contribute significantly to the formation of volatile aroma compounds in white wine (Knoll et al., 2011). The aroma compounds of cider can be increased by maturation with oak chips (Fan et al., 2006) and chip roasting in various ways positively affects the amount of volatile substances in cider and oak phenolic may provide enhancement effects on wine aging systems with a significant improvement in the wine's colour, aroma and taste (Zhang et al., 2015). The aim of the current research was to compare the qualitative and quantitative content of

phenolic and volatile compounds in commercial and experimental ciders.

Materials and Methods

Cider samples

Ten experimental ciders were selected from the series of studies for cider quality improvement (Table 1) and 11 commercial ciders from five different countries (Table 2) were analysed in the study.

The experimental ciders differed by used apple varieties, yeast strains, blending and maturation conditions. All the cider samples were stored in a refrigerator at 4±1 °C for providing constant conditions.

Determination of total phenolic content

The total polyphenol concentration was determined spectrophotometrically according to the Folin-Ciocalteu colometric method (Singleton et al., 1999).

Cider was diluted with ethanol / acetic acid solution in proportion 1:20 (v/v). Ethanol / acetic acid solution was prepared using ethanol (98% vol.) and acetic acid water solution (2.5%) in ratio 10:90 (v/v). 0.5 mL of aliquot was mixed with 0.25 mL Folin-Ciocalteu reagents, after 3 minutes 1 mL 20% Na_2CO_3 and 3.25 mL distilled water were added. Samples were heated for 10 min at 70 °C and kept for 30 minutes at 18±2 °C temperature. The absorbance was measured at 765 nm using a spectrophotometer JENWAY 6300. Total phenols were expressed as gallic acid equivalents (mg L^{-1}).

Determination of volatile aroma compounds

Volatiles from ciders were extracted using solid phase microextraction (SPME). 5 g of sample were weighed in a 20 mL headspace vial and capped with a septum. A divinylbenzene / carboxen / polydimethylsiloxane (DVB / Car / PDMS) fiber (Supelco Inc., Bellefonte, PA, USA) was used for headspace SPME sampling. SPME parameters were: incubation time 30 min, extraction temperature 22±2 °C, extraction duration 30 min, desorption 15 min, 250 °C. For the analysis of the SPME extracts, a Perkin Elmer Clarus 500 GC / MS and an Elite-Wax ETR (60 m × 0.25 mm i.d.; DF 0.25 μm) was used. Working conditions were as follows: injector 250 °C; transfer line to MSD 260 °C; oven temperature start 50 °C, hold 2 min, programmed from 50 to 100 °C at 5 °C min^{-1} hold 5 min, and from 100 to 210 °C at 5 °C min^{-1}, hold 15 min; carrier gas (He) 1 mL min^{-1}; split ratio 2:1; ionization EI+; acquisition parameters in full scan mode: scanned m / z 40–300. Compounds were identified by comparison of their mass spectra with mass spectral libraries (Nist98), and by calculation of linear retention indexes and comparison with literature data. All analyses were performed in triplicate. As a quantitative measure, the share in the total GC peak area for each compound is given.

Statistical analysis

Each determination was performed in triplicate and results are expressed as mean ± SD. The calculations were carried out using software MS Excel, SPSS 17.0 and statistical software programme Multibase 2014.

Table 1

Characterization of experimental cider samples

Abbrev.	Characterization	Alc., vol. %
A	Made from variety ‘Auksis’ apples	5.5±0.2
K	Made from variety ‘Kerr’ apples	5.1±0.2
71B-112	Fermented with 71B-112 yeast strains	5.4±0.1
EC-1118	Fermented with EC-1118 yeast strains	5.9±0.1
LP_2_BF	Blended–fermented var. – ‘Auksis’ : ‘Lietuvas Pepins’ : ‘Kerr’ (2 : 1 : 2)	5.4±0.1
R_2_BF	Blended–fermented var. – ‘Auksis’ : ‘Remo’ : ‘Kerr’ (2 : 1 : 2)	5.3±0.1
DI_1_BF	Blended–fermented var. – ‘Auksis’ : DI-9-4-14 :‘Kerr’ (1 : 1 : 1)	5.6±0.2
M	Cider with added lactic acid bacteria	5.6±0.2
Ch	Matured with unroasted oak chips	5.5±0.3
RCh	Matured with medium roasted chips	5.5±0.2

Table 2

Characterization of commercial cider samples

Abbrev.	Name / Producer / Country	Alc., vol. %
C1	Cidre Bouche Brut De Normandie / Calvados Christian Drouin Ltd / France	4.5
C2	Cidre Bouche Doux Lieblich / Cidrerie du Val de Vire Ltd / France	2.0
C3	Cidre Bouche Pierre Huet / Calvados Pierre Huet Ltd / France	4.0
C4	Bulmers Original Irish Cider / Bulmers Ltd / Ireland	4.5
C5	Upcider apple cider / Harvall Ltd natural / Finland	4.7
C6	Savanna dry premium cider / Distell Group Ltd/ South Africa	6.0
C7	Abavas sidrs / Milli Ltd / Latvia	6.5
C8	Abavas sidrs / Milli Ltd / Latvia	6.5
C9	Sabiles sidrs / Kroņstrauts Ltd / Latvia	8.0
C10	Lucky Dog Apple / Latvijas balzams JSC / Latvia	5.0

Results and Discussion

Phenolic compounds

The total phenolic content in commercial ciders varied from 324. mg L^{-1} (sample C10) to 3831. mg L^{-1} with the highest content in the sample C2 (Fig. 1). The total phenolic content in experimental ciders was similar ranging from 793 mg L^{-1} in sample fermented with *Saccharomyces bayanus* yeast strains (EC-1118) to 3399 mg L^{-1} in sample produced from crab variety 'Kerr' apple (sample K).

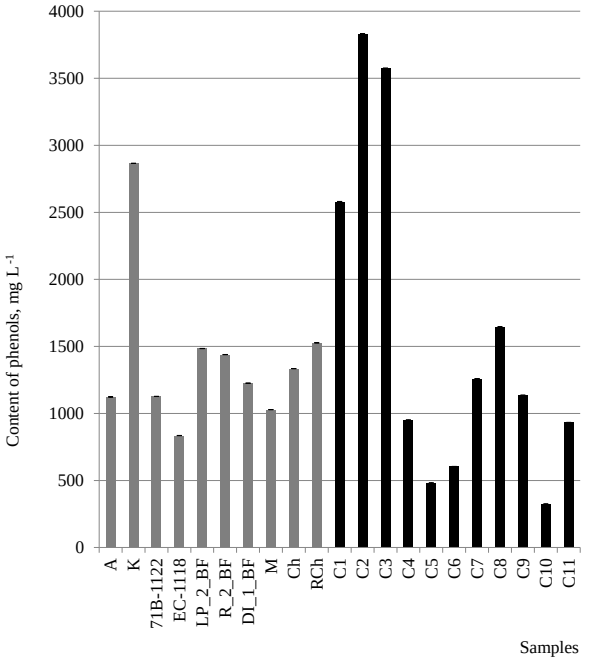

Figure 1. Total phenolic content in commercial and experimental ciders

The total phenolic content of ciders produced in France (samples C1, C2, C3) was higher that corresponded to the findings of Alonso-Salcese et al. (2005), where the total phenolic content in French ciders was 143–2488 mg $L^{-1;}$, it was much less in Basque ciders, namely 24–331 mg L^{-1}. Phenolic content of cider is mainly dependent on the used apple varieties and cider making technology. Cider making process (added enzyme, centrifugation, filtration, and clarification) as a result of the French cider is partially reduced procianidins content, because of their ability to precipitate proteins and interact with the cell wall polysaccharides (Alonso-Salce et al., 2004).

Comparing the phenolic content of ciders was carried out by cluster analysis. The first clusters comprise ciders produced in France C2 ("Cidre Bouche Doux Lieblich") and C3 ("Cidre Bouche Pierre Huet") with the highest total phenolic content, followed by the second cluster, which comprises C1 ("Cidre Bouche Brut De Normandie") and experimental cider of apple variety 'Kerr'. The third cluster includes several commercial ciders from big manufactories and all other experimental ciders and ciders made in small Latvian wineries providing similar phenolic content. The fourth cluster includes commercial ciders C5 ("Upcider apple cider natural"), C6 ("Savanna dry Premium cider") and C10 ("Lucky Dog Apple") that are known as sparkling ciders with distinct aroma and sweet taste.

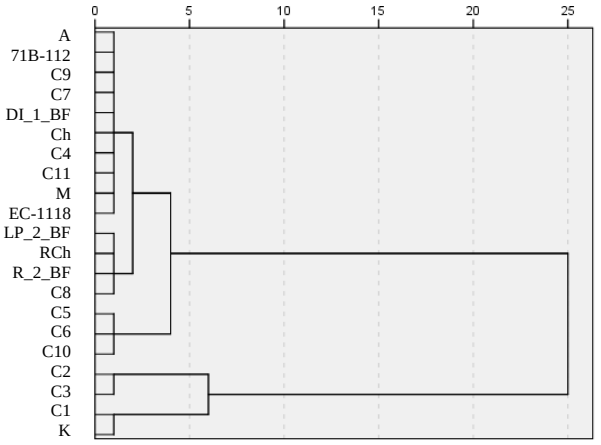

Figure 2. Division of the commercial and experimental ciders in clusters based on phenolic content

The fourth cluster comprises C1 cider and cider of experimental apple variety 'Kerr'. It is possible to conclude that phenolic contents both of commercial ciders and experimental ciders were similar due to the used apple varieties and producing technologies. The ciders contained higher phenolic contents and ciders made of crab variety apples contained a similar amount of phenolic.

Volatile compounds

The analysis of the content of volatile compounds in commercial ciders identified 33 volatile compounds and 21 in experimental ciders.

In French ciders there was identified the smallest peak area of volatile compounds with a total of the 1400 10^5 PAU in sample C2 and 2147 × 10^5 PAU in sample C1. The highest volatile content was in the sample LP_2_BF with total of the 19710. 43×10^5 PAU and R_2_BF with total of the 18750.3 × 10^5 PAU. These samples were made from three apple varieties, where the largest proportion was of apple varieties 'Auksis' and 'Kerr'.

The major volatile compounds in all samples are alcohols and esters. Volatile alcohol percentage differed from 6.5% (sample C10) to 74.1% (sample C9). Ethanol, 3-metibutan-1-ol and phenylethylalcohol are typical volatile alcohols in commercial ciders, and similar compounds have also been identified in the experimental ciders. 3-methylbutane-1-ol is a typical volatile alcohol in cider produced in China. In turn, hexane-1-ol is a compound with fruit characteristic and formed from linoleic acid oxidation (Ledauphin et al., 2003). The ester compound content as a percentage of the total volatile compounds cider samples ranged from 18.8% (sample C3) to 73.6% (sample C5). Typical cider esters are acetates and ethyl esters that make up the fruity cider flavoured. Ethylcapriolate, hexylacetate, octoate, 3-metilbutilacetate and ethyldecanoate are the main compounds in the typical commercial ciders and specific to the experimental ciders.

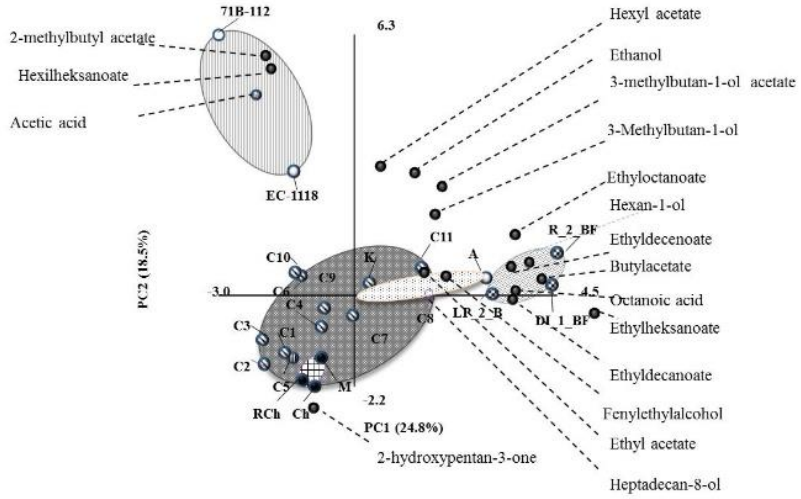

Figure 3. Principal component analysis of volatile compounds of experimental and commercial ciders

In commercial and experimental ciders were identified four acids, namely acetic acid in samples C3 and C6, sorbic acid in samples C5 and C10, decanoic acid in samples C1, C2, C5 and C10, while octanoic acid, characterized by a weak, fruity aroma (Villiere et al., 2012) was identified in all samples, except for samples C3. Sorbic acid is an organic acid, for use in foods and beverages as a preservative, which is characterized by mildly spicy flavour. Sorbic acid is widely used as a mould inhibitor, a variety of food and flavour formation of sorbic acid catabolism with sorbate resistant yeast and mould strains (Gürbüz et al., 2011).

Factor analysis following summative dispersion results suggested that the first two factors explained 24.8%, and 18.5%, of total set of variables (Fig. 3). The high dispersion of volatiles in experimental and commercial ciders represents differences in raw materials and technologies. Ciders matured with lactic acid bacteria *Oenococcus oeni* and oak chips had similar content of volatile substances with those of French ciders. French ciders are characterized by lactic acid fermentation that occurs during maturing in oak barrels (Swaffield et al., 1997). The sample of crab variety 'Kerr' presented a similar profile of volatile compounds with that of the samples from ciders produced in Latvia – C7 ("Sabiles sidrs") and C9 ("Abavas sidrs").

Conclusions

The results showed that in terms of phenolic content and the content of volatile compounds, experimental ciders and commercial ciders were similar. The highest phenolic content was in French ciders and ciders of 'Kerr' variety apples. Ciders matured by adding lactic acid bacteria *Oenococcus oeni* and oak chips revealed similar content of volatile compounds as French ciders. But the sample of crab variety 'Kerr' ciders had a similar profile of volatile compounds with ciders C7 ("Abavas sidrs") and C9 ("Sabiles sidrs") produced in Latvia.

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DIETARY MICRONUTRIENT CONTENT IN PEA (*PISUM SATIVUM* L.) AND BUCKWHEAT (*FAGOPYRUM ESCULENTUM* M.) FLOUR

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Abstract

Micronutrient (Fe, Zn etc.) malnutrition is a major public health problem in the most parts of the world. The attempt to solve micronutrient malnutrition could be to increase the consumption of nutri-dense products, like pseudo-cereals or legumes. This study was carried out to determine the mineral (P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo and B) and vitamin (B_1 and B_2) content of pea (conventional and organic) and buckwheat (raw, roasted, white and dark) flour. Conventional and organic pea flour (PF) was naturally rich in Fe (36.0 mg kg^{-1} and 64.0 mg kg^{-1}, respectively). Iron content in buckwheat flour (BF) ranged from 7.2 mg kg^{-1} (white-BF) to 260 mg kg^{-1} (dark-BF). Zinc content of BF was between 7.0 mg kg^{-1} (white-BF) and 24 mg kg^{-1} (raw- and roasted-BF) while that of pea flour ranged from 20.0 mg kg^{-1} in organic-PF to 24.0 mg kg^{-1} in conventional-PF. There were small differences in the content of P, K, Ca, Mg, S, Fe, Mn, Cu, Mo and B between raw- and roasted-BF. Ca : P ratio in PF and BF revealed a high concentration of phosphorus compared to calcium. This ratio was less than 1.0. The pea and buckwheat flour showed a good content of vitamins B_1 and B_2. The highest quantity of vitamins B_1 and B_2 was observed in roasted-BF under buckwheat flour samples (1.39 mg 100 g^{-1} and 1.35 mg 100 g^{-1}, respectively) and in conventional-PF under pea flour samples (1.11 mg 100 g^{-1} and 0.71 mg 100 g^{-1}, respectively).

Keywords: minerals, vitamins, pea, buckwheat, flour

Introduction

Micronutrient (Fe, Zn etc.) malnutrition is a major public health problem in the most parts of the world. The attempt to solve micronutrient malnutrition could be to increase the consumption of nutri-dense products, like pseudo-cereals or legumes.

Vitamins and minerals are required in small amounts but they are essential micronutrients for regulation of physiological functions in the body. According to World Health Organization, 2 billion people suffer from anaemia of various types where iron deficiency anaemia is the most prevalent type (McLean et al., 2008). Iron is an essential trace element which is involved in metabolic functions by being an important component of hemoglobin, myoglobin and cytochromes (Hemalatha et al., 2007).

Buckwheat is a nutritional food product rich in vitamins B_1 and B_2 and good source of minerals (Préstamo et al., 2003). Buckwheat contains more minerals except calcium than many cereals and is rich source of zinc, copper, manganese, magnesium, potassium and phosphorus (Steadman et al., 2001; Ikeda et al., 2001).

Legumes are good sources of protein, carbohydrates, dietary fibre, vitamins, carotenoids, macronutrients, micronutrients and phytochemicals (Zia-ul-haq et al., 2011; Kotlarz et al., 2011). Iqbal et al. (2006) indicated that legumes may provide sufficient amounts of minerals to meet the human mineral requirement. Field peas are good source of iron, zinc and magnesium where iron content ranged from 46 mg kg^{-1} to 54 mg kg^{-1}, zinc: 39-63 mg kg^{-1} and magnesium: 1350-1427 mg kg^{-1} (Amarakoon et al., 2012). The vitamins present in appreciable quantities in legumes are thiamin, riboflavin, pyridoxine, niacin and folic acid (Sulibursk a, Krejpcio, 2014; Ofuya, Akhidue, 2005).

The use of buckwheat and pea flours as ingredients in gluten-free products could improve the mineral and vitamin profile of these speciality products and of gluten-free diet in general (Alvarez-Jubete et al., 2009). The purpose of this research was to determine and compare mineral (P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Mo and B) and vitamin (B_1 and B_2) contents of buckwheat (raw, roasted, white, and dark) and pea flours (conventional and organic).

Materials and Methods

Materials

Two pea (*Pisum sativum* L.) flours: conventional (Fasma, Lithuania) and organic (Farm "Kaņepītes", Latvia) and four buckwheat (*Fagopyrum esculentum*) flours: raw, roasted, white and dark (Farm "Bebri", Latvia) were analysed (Table 1). Fine wheat flour as control was purchased from "Dobeles Dzirnavnieks", Latvia.

Table 1

Description of flour

Code	Sample
WF	Wheat flour
Conventional-PF	Conventional pea flour
Organic-PF	Organic pea flour
Raw-BF	Raw buckwheat flour
Roasted-BF	Roasted buckwheat flour
White-BF	White buckwheat flour
Dark-BF	Dark buckwheat flour

Mineral analysis

Pea and buckwheat flour samples were dry-ashed in concentrated HNO_3 vapours and re-dissolved in 3% HCl for K, P, Ca, Mg, Fe, Cu, Zn, Mn and Mo detection.Ca, Mg, Fe, Cu, Zn and Mn contents were measured by Atomic Absorption Spectrophotometry (AAS) AAnalyst 700 (Perkin-Elmer, Singapore) and

acetylene-air flame (Methods of Soil Analysis, 1982). K was detected with the flame photometer JENWAY PFPJ. The contents of P, Mo, N, S and B were determined with a spectrophotometer JENWAY 6300 (Rinkis et al, 1987). Each sample was analysed thrice.

Vitamin analysis

Vitamin B_1 content was determined according to AOAC Official Method 986.27; vitamin B_2 was measured by AOAC Official Method 970.65.

Statistical analysis

The results were analysed using the analysis of variance (ANOVA). T-test was applied to compare the mean values, and p-value at 0.05 was used to determine the significant differences.

Results and Discussion

Table 2 shows the composition of five macro-elements, i.e., calcium, phosphorus, potassium, magnesium and sulphur, and calcium phosphorus rate in pea and buckwheat flour.

There were variations in the contents of some minerals between pea and buckwheat flour, and among the varieties of buckwheat flour. A relatively higher content of macro-elements, except magnesium and sulphur, was found in pea flour than in buckwheat flour. These conclusions are confirmed by Suliburska and Krejpcio (2014) that the best sources of bioaccessible minerals seem to be leguminous grains. However the results of macro-elements in pea flours were lower than those reported by Iqbal et al. (2006) and Amarakoon et al. (2012). Generally the highest contents of these minerals were determined in organic-PF, followed by conventional-PF, roasted-BF, raw-BF, WF, dark-BF, while they were the lowest in white-BF.

Within buckwheat flour samples the concentrations of Ca, P, K, Mg and S showed a wide range of value, reflecting the influence of processing conditions applied during the production of flour. Roasted-BF had the highest content of phosphorus, potassium, magnesium and sulphur while dark-BF – calcium. However, the concentrations of calcium in buckwheat flour were insignificant. Research data confirmed the results of Ikeda et al. (2001) that buckwheat flour was poor in calcium. Acquired data for Ca, P, K and Mg in buckwheat flour were lower than those given in literature (Mota et al., 2016; Ikeda et al., 2005; Ikeda et al., 2001). It could be explained by conclusions reported by Suliburska and Krejpcio (2014) and by Koplík et al. (2004) that minerals content of cereals and leguminous grain products depend on a particular plant variety, cultivars, agriculture practices, soil, climatic conditions, and technological practices applied.

Ca : P ratios in PF and BF were greatly low (from 0.06 for raw-BF to 0.19 for white-BF) except dark-BF (0.69). It revealed a high concentration of phosphorus compared to calcium in PF and BF. Ca : P ratio should not be less than 1.0.

Evaluating the contents of trace elements in pea and buckwheat flour (Figure 1) the highest sums of trace elements were determined for raw-BF and roasted-BF.

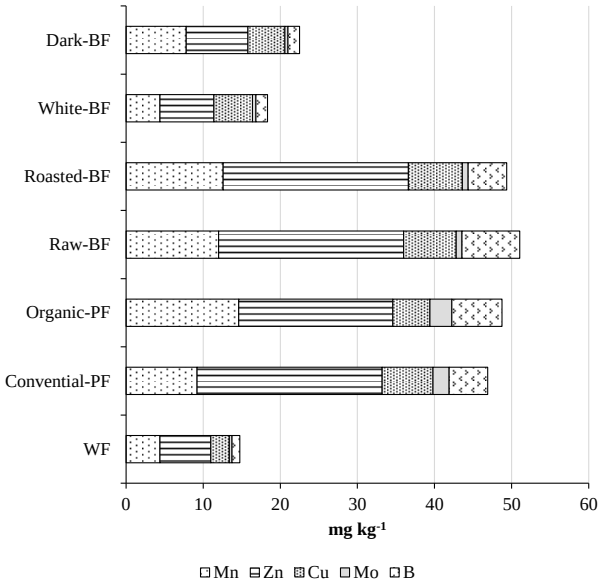

Figure 1. Content of trace elements in pea and buckwheat flour

Manganese content in pea flour ranged between 9.2 mg kg^{-1} for conventional-PF and 14.6 mg kg^{-1} for organic-PF, in buckwheat flour – 4.4 mg kg^{-1} for white-BF and 12.6 mg kg^{-1} for roasted-BF. The results for raw-BF and roasted-BF were similar to those reported by Mota et al. (2016) for raw buckwheat, whereas the results for pea flour were lower than those reported by Iqbal et al. (2006) for peas.

Table 2

Contents of certain macro-elements in pea and buckwheat flour

Samples	Ca	P	K	Mg	S	Ca:P ratio
	mg 100 g^{-1}					
WF	0.15	0.65	1.80	0.26	0.75	0.23
Conventional-PF	0.45	4.01	12.20	1.20	0.94	0.11
Organic-PF	0.58	3.23	12.40	1.26	0.81	0.18
Raw-BF	0.13	2.35	5.80	1.98	1.25	0.06
Roasted-BF	0.16	2.44	6.00	2.18	1.31	0.07
White-BF	0.10	0.54	1.76	0.46	0.63	0.19
Dark-BF	0.35	0.51	1.78	0.48	0.56	0.69

Zinc content in pea flour ranged between 20.0 mg kg[1]for organic-PF and 24.0 mg kg^{-1} for conventional-PF, while in buckwheat flour – 7.0 mg kg^{-1} for white-BF and 24.0 mg kg^{-1} for raw- and roasted-BF. Comparing zinc content in PF in this research with literature data, these results showed lower contents than those mentioned by Amarakoon et al. (2012) – 32–35 mg $kg^{-1}$for zinc in field peas. Results of raw- and roasted-BF were higher than those reported by Mota et al. (2016) but similar to those indicated by Ikeda et al. (2001).

Copper content in pea flour ranged from 4.8 mg kg^{-1} in organic-PF to 6.6 mg kg^{-1}in conventional-PF, while in buckwheat flour – from 4.8 mg kg^{-1} in dark-BF to 7.0 mg kg^{-1} in roasted-BF. These results for pea flour were lower compared to literature (Iqbal et al., 2006) whereas results for buckwheat flour were similar to literature data (Mota et al., 2016).

Molybdenum concentrations in pea flour were 2.1 mg kg^{-1} for conventional-PF and 2.85 mg kg^{-1} for organic-PF, while values of buckwheat flour ranged between 0.4 mg kg^{-1} for dark-BF and 0.75 mg kg^{-1} for raw- and roasted-BF. Results of molybdenum content in pea flour were similar to those given in literature by Koplík et al. (2004) for peas.

Boron content in pea flour was determined 5.0 mg kg^{-1} for conventional-PF and 6.5 mg kg^{-1} for organic-PF, while in buckwheat flour it ranged from 1.5 mg kg^{-1} for white- and dark-BF to 7.5 mg kg^{-1} for raw-BF.

Pea and buckwheat flour showed significantly higher content of trace elements compared to wheat flour, except white-BF for Mn, Zn, Mo, B, and dark-BF for Mo and B.

The highest sum of trace elements among pea flour was determined for organic-PF. However organic-PF had significant higher content of manganese compared to conventional-PF ($p<0.05$).

Statistical analysis showed that there were insignificant ($p>0.05$) differences in the contents of Mn, Zn, Cu, Mo and B between raw- and roasted-BF. However they were rich in above mentioned trace elements compared to white- and dark-BF.

Comparing Latvian recommended dietary intakes (RDI) for essential minerals (established by Latvia Ministry of Health) with trace element content in raw- and roasted-BF it could be concluded that 100 g of buckwheat flour can provide about 40 to 42% for manganese, about 17% for zinc, about 23% for cooper and about 30% for molybdenum of RDI for adults. Evaluating organic- and conventional-PF 100 g of them can provide about 31 to 49% for manganese, about 14 to 17% for zinc, about 16 to 23% for cooper and about 84 to 114% for molybdenum of RDI.

Iron content in pea and buckwheat flour is presented in Figure 2.

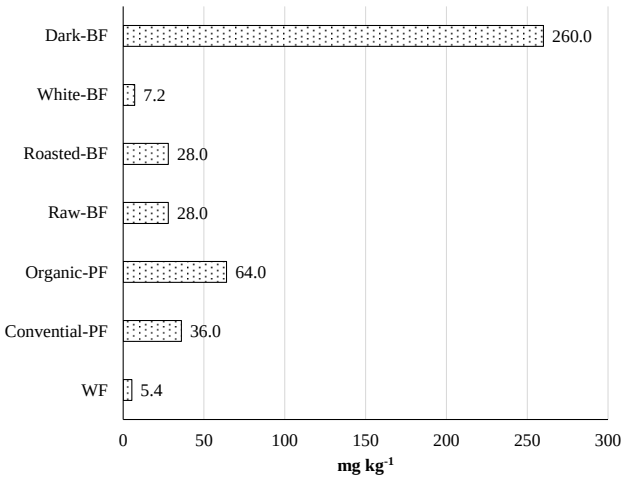

Figure 2. Iron content of pea and buckwheat flour

Iron content for pea flour ranged between 36.0 mg kg[1] for conventional-PF and 64.0 mg $kg^{-1}$for organic-PF while for buckwheat flour – between 7.2 mg kg^{-1} for white-BF and 260.0 mg kg^{-1} for dark-BF. Results of iron content in pea flour were close to those reported by Amarakoon et al. (2012). These data clearly showed that pea flour is substantial source of iron and 100 g of organic-PF can provide 64% for iron of Latvian RDI for males and 36% for females. Similar conclusions are indicated by Amarakoon et al. (2012).

Evaluating iron content among buckwheat flour there were determined significant ($p<0.05$) differences between raw-, roasted-BF and white-BF as well as raw-, roasted-, white-BF and dark-BF. Iron content of raw- and roasted-BF (28.0 mg kg^{-1}) was close to those reported by Mota et al. (2016) and Suliburska and Krejpcio (2014) for raw buckwheat. Surprising result was showed by dark-BF with iron content 260.0 mg kg[1]. It could be explained by the presence of bran in buckwheat flour. Bonafaccia et al. (2003) indicated that buckwheat bran exhibited the properties of an excellent food material.

Results indicated that buckwheat flour could be good source of iron, especially dark-BF. 100 g of raw- and roasted-BF can provide 28% of iron of Latvian RDI for males and 16% for females whereas 100 g of dark-BF – 260% for males and 144% for females.

Figure 3 shows B group vitamin concentration in pea and buckwheat flour.

B_1 vitamin content in pea flour was 0.80 mg 100 $g^{-1}$for organic-PF and 1.11 mg 100 $g^{-1}$for conventional-PF while the highest content of B_1 vitamin among buckwheat flour was determined in roasted-BF followed by raw-BF, white- and dark-BF.

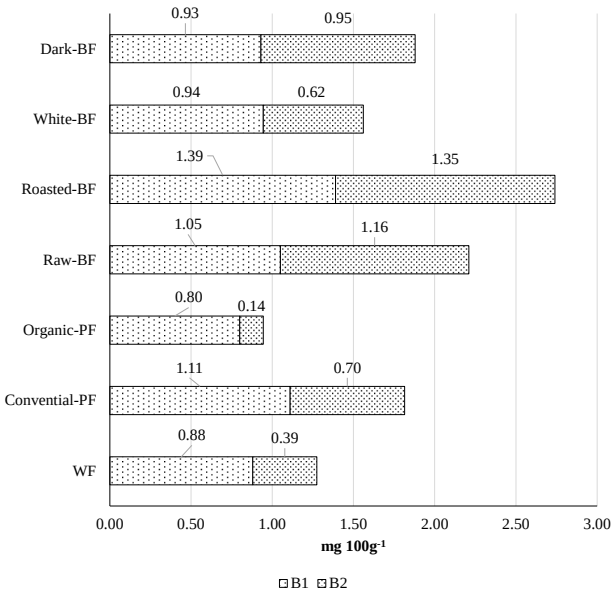

Figure 3. Contents of vitamins B_1 and B_2 in pea and buckwheat flour

All studied samples showed higher concentration of vitamin B_1 except organic-PF compared to wheat flour (0.88 mg 100 g^{-1}). It could be concluded that buckwheat flour and conventional-PF are excellent source of vitamin B_1. Latvian RDI for vitamin B_1 is 1.2 mg per day therefore 100 g of raw-BF can provide 87.5%, roasted-BF – 116%, white-BF – 78%, dark-BF – 77.5% and conventional-PF – 92.5%.

Evaluating vitamin B_2 content there was determined significantly higher ($p<0.05$) content of this vitamins in pea and buckwheat flour except organic-PF compared to wheat flour. In addition roasted-BF showed the highest content ($p<0.05$) similar to vitamin B_1. Buckwheat flour except white-BF showed higher concentration of vitamin B_2 compared to pea flour. Latvian RDI for vitamin B_2 is 1.6 mg per day therefore 100 g of raw-BF can provide 72.5%, roasted-BF – 84%, dark-BF – 59%.

Results of both vitamins in buckwheat flour were higher than those reported by Bonafaccia et al. (2003) for common and tartary buckwheat flour.

Conclusions

Pea flour had low macro-element content but they are rich in trace elements and can provide substantial part of recommended di intake for iron, manganese, copper and molybdenum. Conventional-PF was a good source of vitamins B_1 and B_2 compared to wheat flour.

Substantial indicator which influences mineral and vitamin content in buckwheat flour was the type of flour (raw, roasted, white or dark). Buckwheat flour was poor in content of macro-elements but rich in trace elements. Dark-BF was excellent source of iron and roasted-BF – of vitamins B_1 and B_2.

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NUTRITIONAL VALUE, VITAMINS, SUGARS AND AROMA VOLATILES IN NATURALLY FERMENTED AND DRY KVASS

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Abstract

Naturally fermented rye bread kvass is a seasonal product with a pronounced rye bread flavour having the highest demand during hot summer days. However, non-pasteurised and non-filtered kvass has a very short shelf-life. There are numerous benefits of drying to extend kvass shelf-life, however it can have a significant influence on the product composition and quality. The aim of this research was to assess and compare nutritional value, vitamins, sugars and aroma volatiles in naturally fermented and spray dried kvass. Naturally fermented non-pasteurised, non-filtered bread kvass was used to produce dry kvass at the University of Warmia and Mazury in Olsztyn, Poland. Maltodextrin was used in 25% quantity to kvass dry matter in order to aid the spray drying process. Nutritional value of liquid kvass (7% solids) and dry kvass (powder, 93% solids) was determined according to EU Regulation 1169/2011, B vitamins – according to AOAC 986.27 (B_1), AOAC 970.65 (B_2) and AOAC 961.14 (B_3). Content of sugars was determined using high performance liquid chromatography, whereas aroma volatiles were assessed using solid phase microextraction in combination with gas chromatography/mass spectrometry. Drying process had a significant influence on the content of B vitamins in kvass; the highest decrease was observed for niacin (vitamin B_3). The content of major sugars was lower in dry kvass based on the dilution by the addition of maltodextrin. Totally 26 different volatile compounds were detected in liquid and dry kvass, total values of peak areas were significantly lower in dry kvass ($p<0.05$).

Keywords: kvass, spray drying, micronutrients, volatile compounds.

Introduction

Soft drinks are in the diet of consumers throughout their lives, and the choice of the product depends on the taste of the drink, its impact on the health, national traditions and market trends. Kvass is a non-alcoholic beverage that can be used without restriction, as its effects on the human body are similar to kefir; furthermore, the energy value of naturally fermented kvass is approx. ½ less than of typical non-alcoholic beverages (Lidums et al., 2014). Due to the favourable microflora composition (lactic acid bacteria, yeast), kvass is enriched with B vitamins, lactic acid and carbon dioxide which is a product of incomplete alcoholic and lactic acid fermentation (Omasheva et al., 2015).

Naturally fermented non-pasteurised and non-filtered rye bread kvass is a seasonal product with a pronounced rye bread flavour and a very short shelf-life. Similar to dry juices (juice powders), the benefits of drying to extend kvass shelf-life are reduced volume or weight, less packaging, easier handling and transport. Therefore, dry naturally fermented kvass could be a valuable contribution in comparison with liquid kvass. There are several drying methods, but spray drying is one of the techniques used to produce dry powders. Spray drying is the transformation of the substance of the liquid or slurry to dry powdery substance. The liquid product is atomized into a chamber where the resulting spray mixes with hot gas, which evaporates the liquid component of the spray leaving dried particles (Goula, Adamopoulos, 2010). Drying, however, can have a significant influence on the product composition and quality, thus, it is important to investigate the effect of drying technological processes.

Recently Lidums and Karklina (2016) investigated the possibilities of dry kvass application in food flavour enrichment and concluded that milk candy 'Gotiņa', ice-cream 'Plombir', biscuits, meringue cookies, éclair filling and cupcakes can be supplemented with dry kvass with good sensory and physico-chemical outcomes.

The aim of this research was to assess and compare nutritional value, vitamins, sugars and aroma volatiles in naturally fermented and spray dried kvass.

Materials and Methods

Experimental design

The object of the research was liquid and dry kvass. Kvass samples were analysed at:

- Latvian Certification centre, Ltd. – nutritional value of kvass,
- Institute of Biology, University of Latvia – content of B vitamins,
- Department of Chemistry, Latvia University of Agriculture – content of sugars,
- Department of Food Technology, Latvia University of Agriculture – aroma volatiles.

Naturally fermented non-pasteurised, non-filtered bread kvass from Liepzeme Ltd. (water, rye bread rusks 10% (rye flour, wheat flour, sugar, rye malt, salt, yeast, barley malt extract, caraway), sugar, barley malt, wheat malt, acidifier: citric acid, yeast) was used to produce dry kvass by spray drying as described by Lidums et al. (2016). Dry kvass was obtained at the Institute of Process Engineering and Equipment, The University of Warmia and Mazury in Olsztyn, Poland.

Kvass was atomized from a rotary atomizer (disk speed 11 000 rpm) into a vertical co-current drying chamber with inlet and outlet air temperatures of 170 °C and 103 °C, respectively; temperature inside the drying chamber was 75–80 °C. Maltodextrin was used in 25% quantity to kvass dry matter in order to aid the spray drying process.

Determination of nutritional value and calculation of energy value

Nutritional composition of liquid and dry kvass samples was determined according to standard methods: protein content (ISO 5983-1:2005), fat and saturated fatty acid content (ISO 12966-4:2015), sugar content by Bertrand's method (Chidan et al., 2011), sodium content (ISO 7485:2000), ethanol content (ГОСТ 6687.7-88) and moisture content (ISO 5537:2004). Carbohydrates (%) were determined by difference (FAO, 2003) according to formula:

$$C = 100 - (m + p + l + a), \qquad (1)$$

where C – carbohydrates, %, m – moisture content, p – protein content, %, l – lipid content, %, a – ash content, %.

Energy value of liquid and dry kvass samples was calculated according to coefficients described in EU Regulation No 1169/2011: carbohydrates 17 kJ g^{-1}; protein 17 kJ g^{-1}, fat 37 kJ g^{-1} and ethanol 29 kJ g^{-1}.

Determination of B vitamins

Vitamin B_1 was determined by the fluorometric method (AOAC 986.27), which is based on the oxidation of thiamine to thiochrome, followed by measurement of fluorescence intensity. Vitamin B_2 was determined by the fluorometric method (AOAC 970.65), which is based on fluorescence measurement of riboflavin after acid and enzymatic hydrolysis. Vitamin B_3 was determined by the colorimetric method (AOAC 961.14), which is based on the König reaction with cyanogen bromide.

Determination of sugar content

The content of fructose, glucose and maltose was determined using high performance liquid chromatography (HPLC) method (Shimadzu LC 20 Prominence) according to Lidums et al. (2016). Identification of sugars in liquid and dry kvass was done by comparing retention times of individual sugars in the reference vs. tested solution (qualitative analysis).

Detection of aroma volatiles

Volatile compounds were determined in liquid and dry kvass samples using solid phase micro-extraction (SPME) in combination with gas chromatography/mass spectrometry (GC/MS) according to the method described by Lidums et al. (2015). The SPME fibre was coated with a thin bipolar polymer film – Carboxen/Polydimethylsiloxane (CAR/PDMS). The film thickness was 85 μm with bipolar polarity (Supelco, Inc., USA). The process consisted of heating the samples to release volatile compounds above the liquid phase and absorb them onto the CAR/PDMS fibre. Then volatile compounds from the fibre were thermally desorbed in GC/MS injector and transferred to the capillary column for separation. Compounds were identified by comparison of their mass spectra with mass spectral library Nist98 and the amount of compounds was measured as peak area units (PAU).

Data analysis

The obtained data processing was performed with the Microsoft Excel 13 for Windows; mean values and standard deviations were calculated. t-test and Tukey's test were used for data cross-comparison. For the interpretation of the results it was assumed that α=0.05 with 95% confidence.

Results and Discussion

The characteristics of naturally fermented non-pasteurised and non-filtered rye bread kvass were evaluated in two products – liquid and dry form of kvass. Kvass and similar products have been produced before, whereas dry kvass was produced and investigated for the first time. The comparison was carried out on dry weight basis, except for nutritional composition and aroma volatiles.

Nutritional value of naturally fermented and dry kvass

Dry kvass had a higher energy value than naturally fermented kvass on fresh weight basis (Table 1). Dry kvass contains only traces of ethanol contrary to kvass, as alcohol evaporates during drying process (USDA Table of Nutrient Retention Factors).

Table 1

Nutritional and energy value of naturally fermented and dry kvass

Parameters	Liquid kvass	Dry kvass
Dry matter, %	7.00±0.02	93.00±0.04
Protein content, g 100 g^{-1}	0.15±0.02	1.90±0.10
Fat content, g 100 g^{-1}	<0.10	<0.10
of which saturates, g 100 g^{-1}	<0.01	<0.10
Carbohydrate content, g 100 g^{-1}	5.90±0.02	75.20±0.21
of which sugars, g 100 g^{-1}	4.70±0.02	61.30 ±0.15
Sodium content, mg 100 g^{-1}	0.16±0.01	2.10±0.05
Ethanol content, vol %	1.20±0.03	<0.10
Energy value, kJ 100 g^{-1}	130.40	1285.77

B vitamins in naturally fermented and dry kvass

Drying process had a significant influence on the content of B vitamins in kvass (Table 2); the highest decrease was observed for niacin (vitamin B_3).

Table 2

Content of B vitamins in kvass samples, mg 100 g^{-1} DW

Vitamins	Liquid kvass	Dry kvass
Thiamine (B_1)	0.71±0.09	0.25±0.02
Riboflavin (B_2)	1.28±0.12	0.48±0.02
Niacin (B_3)	18.14±0.48	4.36±0.12

A kinetic analysis of the thermodegradation process on B vitamins showed that these vitamins are thermolabile (Fuliaş et al., 2014), and the decrease after thermal processing can account to 60% (Asadullah et al., 2010). Several authors have reported the reduction of water-soluble vitamins after spray drying (Grabowskia et al., 2008; Abubakar, Jega, 2010), besides the addition of maltodextrin decreased the

overall kvass solids together with the amount of B vitamins in dry kvass.

Sugar content in naturally fermented and dry kvass

Major sugars in kvass were fructose and glucose, both kvass samples also contained significant amount of maltose (Table 3). The content of major sugars was higher in liquid kvass. Barba et al. (2014) showed that drying process caused a significant decrease in the reducing sugars content which was associated with Maillard's reactions. However, Grabowskia et al. (2008) reported that spray dried sweet potato powder with the addition of maltodextrin showed lower content of sugars compared to sweet potato puree, based on the dilution by the addition of maltodextrin.

Table 3

Sugars in kvass samples, g 100 g^{-1} DW

Sugars	Liquid kvass	Dry kvass
Fructose	25.13±0.19	15.83±0.12
Glucose	21.76±0.14	14.31±0.17
Maltose	8.26±0.11	6.12±0.07

Therefore, we conclude that the decrease of fructose, glucose and maltose in dry kvass can be accounted to the addition of maltodextrin as drying aid, which increases glass transition temperature and improves product stability (Tonon et al., 2011; Oberoi, Sogi, 2015).

Aroma volatiles in naturally fermented and dry kvass

A total of 26 different aroma volatile compounds were isolated and characterized by GC–MS analysis. The identified volatile compounds belong to esters, alcohols, acids, aldehydes and ketones. A more various volatile compound profile was found for naturally fermented kvass. 19 volatile compounds were identified in naturally fermented kvass with the total sum of peak area – 19.16×10^7 PAU. The total sum of peak area (11.81×10^7 PAU) in dry kvass was approx. 40% lower than in liquid kvass ($p<0.05$), a total of 15 volatile compounds were present in dry kvass (Table 4). The highest value of peak area (10.05×10^7) among all detected volatile compounds was detected for 4-penten-2-ol (alcohol) in naturally fermented kvass, which gives fruity aroma, which is in agreement with a previous research by Lidums et al. (2015), whereas in dry kvass the amount of 4-penten-2-ol was about 7 times lower. Carvone had the second highest peak area (2.28×10^7) in naturally fermented kvass, but it was not present in dry kvass. According to Sedláková et al. (2003), carvone and limonene form the main portion of essential oils in caraway fruits, which are an ingredient in the rye bread used for naturally fermented kvass production. Salim et al. (2015) reported that drying affected the reduction of carvone in spearmint.

A significant amount of ethyl octanoate (1.03×10^7) was found in naturally fermented kvass, resulting in fruit and fat odour. The three aroma volatiles with the highest peak area values in dry kvass were hexanal, 4-penten-2-ol and benzaldehyde, forming green, fruity, bitter and almond odours, respectively.

Volatile compounds found in both liquid and dry kvass samples form base aroma profile which includes fruity (4-penten-2-ol), green (hexanal), sour (acetic acid), bread, almond, sweet (furfural), burnt (furfuryl alcohol), fatty type (hexanoic acid), floral type (benzylalcohol), rose (phenylethylalcohol), sweat, cheese (octanoic acid) aroma.

Table 4

Volatile compounds (PAU×10^7) in liquid and dry kvass samples

Volatile compounds	Odour*	Liquid kvass	Dry kvass
4-penten-2-ol	fruity	**10.05**	**1.39**
Hexanal	green	0.59	**3.04**
3-methyl-butanal	chocolate, peach, fatty	–	0.10
2-pentylfuran	fruity	–	0.68
1-pentanol	fermented	–	0.17
3-hydroxy-2-butanone	butter, cream	–	0.22
1-octen-3-ol	mushroom	–	0.50
Acetic acid	sour	0.58	0.98
Furfural	bread, almond, sweet	0.03	0.62
Benzaldehyde	bitter, almond	–	**1.56**
Furfuryl alcohol	burnt	0.17	0.72
Hexanoic acid	fatty type	0.07	0.41
Benzylalcohol	floral type	0.55	0.66
Phenylethyl alcohol	rose	0.26	0.35
Maltol	caramel	–	0.21
Octanoic acid	sweat, cheese	0.30	0.21
Carvone	caraway	**2.28**	–
Ethyl decanoate	waxy type	0.60	–
Decanoic acid	rancid, fat	0.19	–
2-phenylethyl acetate	rose, honey, tobacco	0.59	–
3-methyl-1-butanol	whiskey, malt, burnt	0.70	–
Isoamyl acetate	banana	0.63	–
Ethyl caproate	fruity, green, pineapple, sweet	0.28	–
Hexyl acetate	sweet, fruity	0.12	–
Heptyl acetate	green	0.05	–
2-ethyl-1-decanol	–	0.08	–
Ethyl octanoate	fruit, fat	**1.03**	–
	The sum of peak areas	**19.16**	**11.81**

*Gas chromatography - olfactometry of natural products (2004); Odor Descriptors (2015)

Volatile compounds found in kvass samples are associated with the roasting of geminated grains (malt production), bread baking and the metabolism of yeast cells during fermentation process as argued by

Hazelwood et al. (2008), Purlis (2010), Birch et al. (2013) and Riu-Aumatell et al. (2014).

Conclusions

Dry kvass had a higher energy value than naturally fermented kvass. Spray drying had a significant influence on the decrease of B vitamins in dry kvass (p<0.05); the highest decrease was observed for niacin (B_3). The content of major sugars was lower in dry kvass based on the dilution by the addition of maltodextrin. 26 different volatile compounds were detected in liquid and dry kvass, total values of peak areas were significantly lower in dry kvass (p<0.05). However, the profile of aroma volatiles in dry kvass demonstrates that it can be used for food flavour enrichment.

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THE CHEMICAL COMPOSITION OF TWO COMMERCIAL FISH SPECIES – PIKEPERCH (*SANDER LUCIOPERCA*) AND RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) CULTIVATED IN ARTIFICIAL CONDITIONS

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Abstract

Fish is a nutrient rich food and possesses many components that have positive impact on human health. Nowadays, the fish come to the market from wild-capture fisheries and aquaculture. Latter becomes increasingly global for human consumption, thus total finfish aquaculture includes over 300 species farmed in ponds, floating net cages and recirculating aquaculture systems (RAS). It is reasonable to assume that the chemical composition of farmed fish will vary compared to wild-caught ones due to the different rearing conditions and artificial diets used for feeding the fish.

This study gives an overview of basic chemical composition of pikeperch (*Sander lucioperca*) and rainbow trout (*Oncorhynchus mykiss*). We analysed the muscle flesh composition of fresh and frozen pikeperch obtained from the local commercial tank-based recirculating aquaculture system together with rainbow trout obtained from river-based net cage farming system. We determined that fresh pikeperch contained 78.27±0.34% moisture, 18.95±2.45% protein, 1.34±0.18% fat and 1.62±0.21% ash, while frozen ones had 78.26±0.44, 20.91±0.62, 0.77±0.15 and 0.99±0.09% respectively. Statistically significant differences were found for fat and ash content ($p<0.05$). In both cases, artificially cultivated pikeperch has high nutritional value and fit for human consumption.

The chemical composition of trout was the following: 69.61±1.33% moisture, 17.78±0.48% protein, 11.41±2.24% fat and 0.88±0.01% ash. After reviewing the literature, we found that farmed trout has considerably higher fat content than wild trout. This primarily can be explained by use of lipid-rich diets and lower mobility in cages.

Keywords: aquaculture, fish species, body composition, pikeperch, rainbow trout.

Introduction

Fish is a nutrient rich food and possesses many components that have positive impact on human health since it is a complete protein source that contains all essential amino acids as well as particular vitamins and minerals.

A sizeable share of fish comes to the market from aquaculture. More and more species are being raised in ponds, floating net cages and recirculating aquaculture systems (RAS). In recent years, pikeperch (*Sander lucioperca)* and rainbow trout (*Oncorhynchus mykiss*) are becoming increasingly popular for aquaculture production in Europe together with most commonly farmed species such as carp, tilapia and salmon.

In Russia, pikeperch is completely new farming species since it's widely available on the market are usually caught by fishermen from inland waters. According to FAO statistics, the Russian catch of pikeperch in 2013 was estimated in 6256 tons (more than 30% of the global catch) making the Russian Federation one of the main exporters of this species (FAO, 2015).

In case of rainbow trout, culture of this species in Russia takes place on small-scale local fish farms. However, production volume of trout is low and its mainly imported from Chile, Turkey and Denmark (Villegas, 2015).

At the same time, the potential of pikeperch cultivation might be grounded on its taste qualities, good growth rates and high price for fillets, whereas farming of trout along with the above advantages can reduce the pressure on wild caught stocks. These both species provide a supply of healthy food source and have low body lipid content of fish meat and highly digestible protein.

Based on the above, our interest was directed to the quality of domestic aquaculture products. It's generally known that consumption of farmed fish demands the certain requirements to the quality, food safety and nutritional value (Josupeit et al., 2001). Due to specific conditions, nutritional properties of fish are influenced by artificial diets, water quality, stocking density, rearing technology and many other factors (Crețu et al., 2014; Siemianowska et al., 2016). Thus, the aim of this study was to investigate the chemical composition of farmed pikeperch and rainbow trout. Our selection of these species based on their popularity on the market and similar amino acid profile (Jarmołowicz, Zakęś, 2014). The obtained results were compared with data from various literature sources.

Materials and Methods

Sampling

Pikeperch samples were collected from the local commercial RAS consisted of tanks (volume 7000 L), biofilters (loaded by pelleted polyethylene), mechanical filters, UV lamps and air compressor. This commercial company supply fish to the market both in fresh and frozen state.

Pikeperch were stocked in tanks at density of 13.7 $kg \cdot m^{-3}$. The water temperature remained constant at 20.2±0.1 °C, oxygen level was maintained above 125% of saturation. Due to the lack of specific diets for pikeperch, fish were fed by commercial sturgeon diets consisted of feather meal, fish meal, poultry meal, rapeseed, rapeseed oil, soy, soy protein concentrate,

vitamins, minerals and premix. The nutrient composition according to producer was the following: 45% crude protein; 15% crude fat; 6.9% crude ash; 23.8% NFE; 3.3% fibre; 0.9% phosphorous and 21.2 MJ gross energy.

Two batches of pikeperch consisting of 6 individuals each were analysed. The first batch of product was fresh fish, while the second one was frozen: pikeperch were taken from the tank, killed and subsequently placed in a freezer at -20 °C for 3 days prior to realization.

In both cases, proactive and clinically healthy pikeperch were taken for analyses. All specimens have the age of 23 months and mean body mass of 727.7±12.7 g.

Rainbow trout fresh individuals (n=6) were collected from the local cage-based fish farm. The squared shape cages were 3.8 × 2.3 × 4 m with synthetic nets and with an average stocking density of 45 $kg \cdot m^{-3}$. During the rearing period the water temperature ranged from 1.2 to 26 °C. Fish were fed by commercial trout diets consisted of feather meal, fish meal, fish oil, krill meal, poultry meal, rapeseed oil, soy, soy protein concentrate, sunflower protein concentrate, wheat, wheat gluten, vitamins, minerals and premix. The nutrient composition of the diet is 43% crude protein; 29% crude fat; 7% ash; 14% NFE; 1% fibre; 0.9% phosphorous and 24.2 MJ gross energy. It should be noted that feeding regime was influenced by water temperature and concentration of dissolved oxygen. When the rearing conditions were unfavorable, the feeding was usually suspended for a few days. All trout specimens were taken from cages in December, when the water temperature was 4.3 °C. The mean body weight of fish was 1549.0±21.4 g.

The fish transportation to the laboratory was carried out in plastic containers with dry ice as a refrigerant.

Proximate composition

All laboratory tests were performed at the Department of Food Biotechnology of Kaliningrad State Technical University. All samples for proximate composition were analysed in accordance with the Russian state standard GOST 7636-85 "Fish, marine mammals, invertebrates and products of their processing. Methods for analysis". The frozen pikeperch specimens were thawed in the refrigerator overnight. The fish were gutted and filleted. The fillets were homogenized and used in subsequent analyses. Chemical tests of the homogenates were done in triplicate. Water content was determined after dryness in desiccator at 105 °C until constant weight was reached. Ash content was measured via ashing the samples in a muffle oven. Lipid content was measured with the Soxhlet method based on the fat extraction from a dry sample by an organic solvent (anhydrous sodium sulfate). Dietary crude protein levels were determined according to the methods of Kjeldahl via distillation and titration using a nitrogen to protein coefficient of 6.25.

Statistical analysis

All data were statistical analysed by one-way ANOVA test using R Software version 3.2.3. The variability of the mean values is represented by the standard error. Significant differences were defined at $P < 0.05$.

Results and Discussion

The obtained results of proximate composition of fresh and frozen pikeperch and fresh rainbow trout assumed in Table 1.

Table 1

Proximate composition of fish (n = 6)

Pikeperch (*Sander lucioperca*)		
Component	**Fresh**	**Frozen**
Moisture, %	78.27±0.34	78.26±0.44
CV, %	0.76	0.96
Protein (N total × 6.25), %	18.95±2.45	20.91±0.62
CV, %	22.42	5.12
Fat, % *	1.34±0.18	0.77±0.15
CV, %	23.49	34.10
Ash, % *	1.62±0.21	0.99±0.09
CV, %	22.29	16.65
Rainbow trout (*Oncorhynchus mykiss*)		
Component	**Fresh**	
Moisture, %	69.61±1.33	
CV, %	3.31	
Protein (N total × 6.25), %	17.78±0.48	
CV, %	4.73	
Fat, %	11.41±2.24	
CV, %	33.97	
Ash, %	0.88±0.01	
CV, %	1.21	

* Means in the row differ significantly ($p<0.05$);
CV – coefficient of variation

The fat content of frozen pikeperch (0.77%) was significantly lower than that of fresh one (1.34%) as well as ash content (0.99 and 1.62%, respectively) ($p<0.05$). Thus, the reduced fat content in frozen fish is may be due to the microbial load occurred after refrigerator thawing. Since at the initial stage of microbial spoilage, large amount of lipases enzyme can be produced, which breaks down lipids to form fatty acids (Latip et al., 2013) However, why is protein wasn't affected is a matter of consideration and further investigations are necessary.

Another reason is maybe be due to the differences in the fish individuals initially, since even under the same conditions of fish cultivation, there are still insignificant differences in the chemical composition can occur.

Protein is one of the most important nutrients from fish, therefore the farmed fish should not be compromised in this respect to wild-caught ones. According to several studies, the average protein content in fresh water pikeperch is ranged approximately from 16.9 to 23.7% (Molnár et al., 2006; Schulz et al., 2006; Skurihin, 2007; Özyurt et al., 2009). Thereby, on the basis of our findings, pikeperch farmed in the RAS have similar amount of protein (18.95%) and corresponds to the previous range.

Usually, the feeding diets with increasing lipid content resulted in significantly higher lipid, that is why farmed fish are frequently fatter than their wild counterparts (Schulz et al., 2006). Jankowska et al. (2003) established that intensive cultivation of pikeperch led to three-fold increase in fat content than that of wild specimens,

where amount of lipid typically does not exceed 1.2% (Skurihin, 2007). In our case, farmed pikeperch were characterized by low fat content (1.34%), which can be explained by many factors such as relatively low stocking density for this species, composition of the diet and feed ration. Moreover, closely related to pikeperch species the Eurasian perch had similar lipid content (from 1.23% to 1.35%) when fed with diets contained the fat range of 11.9–22.2% (Mathis et al., 2003).

It's opposite, however, to farmed rainbow trout. In according with data from Table 1 the content of fresh trout meat in lipids is 11.41%. This explains the relatively reduced moisture (69.61%), since it is well known that quantity of water is inversely proportional to the quantity of fat (Shafi, 2003). One of the factors responsible for the increase in fat is the rise of temperature (Martinez et al., 1992). As mentioned above, in the different periods of cultivation the water temperature in cages rose up to 26 °C exceeded the thermal optimum for this species. As a result it had an impact on higher lipid content, which is corresponds with the data obtained by Martinez et al. (1992), when cultivation under the temperature of 20.7 °C led to increase a lipid content in trout that ranged from 10.79 to 13.97%, while the water content fluctuated between 67.50–70.05%. Another reason is the high stocking density (45 $kg \cdot m^{-3}$) and consequently, the lower mobility in limited volume cages. Thus, Creţu et al. (2014) indicated that the cultivation of trout at higher stocking densities may lead to higher degree of fat retention. Just as the use of lipid-rich diets for feeding is also can be reflected on higher lipid content in the muscle flesh composition (Vranić et al., 2013).

The amount of the chemical composition of fish is also depends on age. Lieb at al. (1974) established that lipid content of rainbow trout doubled from 4.4 to 8.4% over the rearing period from the age of 14 to 32 weeks which corresponds with our results. In our case, farmed trout were approximately 96 weeks-old.

At the same time, the protein content (17.78%) are in accordance with the results obtained before by various scientists, where the values of this component varied from 15.60 to 19.40% (Martinez et al., 1992; Vranić et al., 2013; Creţu et al., 2014; Beličovska et al., 2015).

Based on the foregoing, we can say that both farmed species had high nutritional value and concede nothing to wild specimens or a previous cultivation experience.

Conclusions

This study provides a brief overview of proximate composition of farmed pikeperch and rainbow trout. The results obtained show that both species are generally fit for human consumption which may indicate the feasibility of local cultivation. However, the further research are necessary to investigate a mineral content as well as the fatty acid and amino acid composition to gain a more accurate understanding of chemical changes during the different stages of individual growth.

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POTENTIAL APPLICATION OF PULSED ELECTRIC FIELDS TO IMPROVE THE RECOVERY OF BIOACTIVE COMPOUNDS FROM SOUR CHERRIES AND THEIR BY-PRODUCTS

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Abstract

Pulsed electric fields (PEF) treatment induces transmembrane potential by means of an externally applied electric field of sufficient intensity, which causes an increase in the permeability of a cell membrane. PEF can be applied for plant cell disruption as upstream step to enhance the efficiency of mass transfer in further processing such as hydraulic pressing or extraction. The objective of the study was to evaluate the potential of using PEF technology for the increase of the juice yield and improved extraction of bioactive compounds from sour cherries (*Prunus cerasus* L.) and their by-products. PEF treatments (1–5 kV cm^{-1} at 10 kJ kg^{-1}) were applied to cherries before the juice pressing. The by-products (press cake) generated were extracted with aqueous methanol. The samples from PEF pre-treated cherries and their by-products were compared to both untreated (control) samples and samples obtained from freeze-thawed (PEF untreated) cherries. The highest increase in juice yield by 45% was obtained at PEF intensity of E=3 kV cm^{-1}, showing similar results to freeze-thawed sample (41%). The application of PEF significantly increased the release of total anthocyanins (48.3–53.3 mg 100 mL^{-1}) and total phenolics (126.6–133.9 mg 100 mL^{-1}) into juice as compared with the control (33.8 and 112.9 mg 100 mL^{-1}, respectively), whereas juice from freeze-thawed sample exhibited the highest value only for total phenolics content (164.4 mg 100 mL^{-1}). The extracts from by-products obtained after PEF assisted juice pressing of cherries showed significantly higher contents of bioactive compounds and higher antioxidant power as compared with the control extract and the extract of freeze-thawed sample. Overall, the results of this work demonstrated promising use of PEF technology in sour cherry processing.

Keywords: PEF, extraction, juice pressing, by-products, phenolics.

Introduction

Sour cherries (*Prunus cerasus* L) (also called tart cherries) are an important source of anthocyanins and other phytochemicals that possess many potential biological properties (Damar et al., 2012; Šarić et al., 2009). Studies have demonstrated that sour cherries exhibit anticarcinogenic effect in various colon cancer models (Ferreti et al., 2010). Furthermore, anthocyanins were identified as anti-inflammatory agents, which are major contributors to the antioxidant activity in cherries (Ferreti et al., 2010; Repajić et al., 2015).

The total anthocyanin content of sour cherries is reported to range from 28 to 80 mg 100 g^{-1} (Blando et al., 2004). However, the total anthocyanin content and composition differ depending on sour cherry genotype (Blando et al., 2004; Damar, Eksi, 2012).

Processing of fruits and vegetables affects their phytochemical and nutrient content, which may also influence their related health benefits (Ou et al., 2012). Therefore, there is a need to study the potential of new, non-thermal processing technologies, such as pulsed electric fields (PEF) that can enhance the recovery of high-added value components from plant materials without having negative effect on nutritional or sensorial attributes of the final product. PEF processing involves the application of a high intensity electric fields in the form of short pulses to a food placed between two electrodes. PEF induces permeabilization of cell membranes (electroporation), which facilitates the release of intracellular substances. It has been demonstrated that PEF pre-treatment can enhance hydraulic pressing efficiency, diffusion rate and increase the yield in production of several fruit and vegetable juices (Mahnic-Kalamiza et al., 2014). However, to date, no work dealt with the use of PEF to assist expression of juice from sour cherries and the subsequent valorization of cherries by-product (press cake). Therefore, the objective of the study was to evaluate the potential of using PEF technology for the increase of the juice yield and bioactive compounds extraction from sour cherries (*Prunus cerasus* L.) and their by-products.

Materials and Methods

A pulsed electric fields treatment was applied using an electric field generator (Modulator PG, ScandiNova, Uppsala, Sweden) able to deliver monopolar square pulses. The PEF treatment of sour cherries was carried out in a cylindrical batch treatment chamber with uniformly pierced (d = 0.5 mm) lateral surface, closed at both ends by two stainless steel electrodes (3.4 cm in diameter).

During each experiment, 10 g of sour cherries (de-stoned and cut into 4 pieces) were loaded into the treatment chamber and subjected to PEF treatments at different electric field strength (E=1, 3, and 5 kV cm^{-1}), constant total specific energy input (W_T=10 kJ kg^{-1}), constant frequency (10 Hz) and pulse width of 20 µs. PEF-treated samples were then pressed in the treatment chamber by loading a weight on the top of upper electrode (10 kg weight for 9 minutes). The expressed liquid was centrifuged to obtained clear juice, which was subjected to further analysis. The subsequent extraction of press cakes left after juice pressing was performed with acidified aqueous methanol

(70% MeOH and 0.5% HCl, v / v). The solvent to press cake ratio was 10 : 1 (v / w). The extraction was carried out for 24 hours at ambient temperature with constant shaking.

Total anthocyanins content was measured by the pH differential method previously described by Lee et al. (2005). Juice and press cake extracts were added to pH 1.0 and pH 4.5 buffers and after an equilibration period (20 min), the absorbance was measured at 520 and 700 nm using a V-650 UV-vis spectrophotometer (Jasco Inc., Easton, USA). The corrected absorbance values were calculated as follows:

$$A = (A_{520nm} - A_{700nm})pH\ 1.0 - (A_{520nm} - A_{700nm})pH\ 4.5$$

The total anthocyanin content was calculated using the molar absorptivity (ϵ=26,900) and molecular weight (MW=449.2) of cyanidin 3-glucoside. Results were expressed in mg of cyanidin 3-glucoside equivalents per 100 mL of juice or 100 g of press cake.

Total phenolics content was determined with the Folin-Ciocalteau reagent, following the procedure previously described by Bobinaite et al. (2012). After 1 h incubation of the reaction mixture, the absorbance of the samples was measured at 765 nm using a spectrophotometer. Gallic acid was used as a standard for the calibration curve and results were expressed in mg of gallic acid equivalents per 100 mL of juice or 100 g of press cake.

Ferric reducing absorbance power (*FRAP)* was determined following a slightly modified method of Benzie and Strain (1996). To conduct the assay, 2 mL of freshly prepared FRAP working solution was combined with 20 μL of diluted juice or extract sample. After incubation for 30 min at ambient temperature, the absorbance of the samples was determined at 593 nm using a spectrophotometer. Trolox was used as a standard for the calibration curve and results were expressed as μmol of Trolox equivalents (TE) per mL of juice or g of press cake.

Each experiment and analyses was repeated, at least, three times. Mean values and standard deviations of data were calculated. Significant differences between the results were calculated by analyses of variance (ANOVA). Mean values were further compared, using Turkey's test, and differences were considered to be statistically significant when $p \leq 0.05$. SPSS software, version 20 was used for statistical analysis (SPSS Inc., Chicago, USA).

Results and Discussion

The juice yield obtained from untreated and PEF treated sour cherries is shown in Figure 1. The juice yield was defined as the amount of juice (g) obtained from 100 g of cherries. PEF assisted pressing led to significant increase in juice yield from 26.0 g 100 g^{-1} (untreated cherries) to 37.7 g 100 g^{-1}. The highest increase in juice yield (by 45%) was achieved at the electric field strength of 3 kV cm^{-1}, while higher values (up to 5 kV cm^{-1}) did not lead to a further increase in juice yield. The results obtained were compared with other cell disintegration method, based on the freeze-thawing process. The formation of ice crystals during freezing cause damage (increase permeabilization) of plant cells thus can enhance the yield of fruit juice. In the present work, sour cherries were frozen to -20 °C for 24 h and prior to juice pressing, they were thawed in the refrigerator. The juice yield obtained from freeze-thawed cherries was 36.7 g 100 g^{-1}, which is comparable to that of PEF-assisted pressing (when E was 1 and 3 kV cm^{-1}) (Fig. 1).

Efficacy of the PEF treatment was also previously demonstrated for expression of juices from grapes, apples, carrots, and blueberries (Mahnic-Kalamiza et al., 2014; Bobinaite et al., 2015). Moreover, it has been previously stated that the application of PEF can possibly replace or allow the reduction of the amount of pectolytic enzymes that are traditionally used in fruit juice production (Korma et al., 2016).

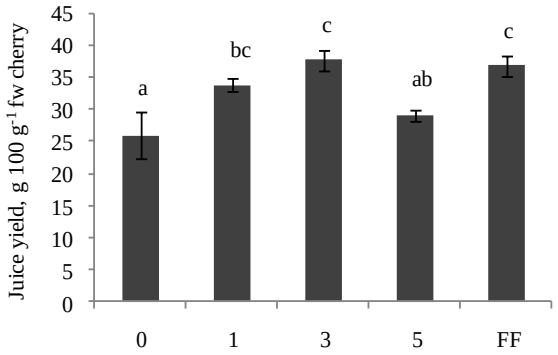

Figure 1. Juice yield obtained after pressing untreated, PEF-treated and frozen-thawed sour cherries

0 – untreated sample (0 kV cm^{-1}), 1 – PEF treated sample (1 kV cm^{-1}), 3 – PEF treated sample (3 kV cm^{-1}), 5 – PEF treated sample (5 kV cm^{-1}), FF – frozen and thawed sample. *different letters above bars indicate significant differences between the samples ($p \leq 0.05$).

Phenolic compounds, and in particular anthocyanins, are among the main components that determine berry juice quality and the efficiency of the pressing process. The total phenolics and anthocyanins content of PEF treated cherry juice and control juice is shown in Table 1. The total phenolics content of sour cherry juice after PEF application ranged between 126.6 and 133.9 mg 100 mL^{-1}, corresponding to 12–19% increment, compared to the untreated sample. Mechanical pressing of frozen and thawed sour cherries led to the highest release of phenolic compounds into juice, which corresponds to a 46% increase compared to the untreated sample.

The total monomeric anthocyanin content of sour cherry juices after PEF application was by 43 to 58% higher, compared to control sample. Interestingly, PEF assisted pressing of sour cherries led to the similar extraction yield of monomeric anthocyanins compared with that obtained from freeze thawed sample (Table 1). The lower content of monomeric

anthocyanins in polyphenolic fraction of frozen-thawed cherry juice was most probably due to the enzymatic reactions that could take place in damaged sour cherry tissues during the thawing process.

Anthocyanins, in fact, are degraded by oxidative mechanisms involving the enzyme polyphenol oxidase (PPO). PPO is found in cherries and plays an important role in the browning of their fruit juices (Welch et al., 2008). Degraded anthocyanins are resistant to color changes regardless the pH of the media, therefore they are not included in the calculation applying pH differential method (Lee et al., 2005).

The increase in PEF treatment intensity to 3 and 5 kV cm^{-1} did not contribute to a more significant increase in the content of total phenolics and total anthocyanins in the juice (Table 1). These results are similar to those previously observed for blueberries, where it was noted that electric field strength higher than 1 kV cm^{-1} might not be necessary to facilitate the release of phenolic compounds into the juice (Bobinaite et al., 2015). A positive impact of PEF on extraction of polyphenols was also observed in juices obtained after PEF treatment of grapes (Grimi et al., 2009), blueberries (Bobinaite et al., 2015), and apples (Korma et al., 2016).

The juices obtained from PEF-treated cherries showed 25–36% higher FRAP values, while the control (untreated) sample displayed the minimum one (Table 1). The antioxidant activity of the juice from frozen and thawed cherries was by 51% higher compared to control sample. The increased ferric reducing absorbance power of the juices may be ascribed to an enhanced release of matrix-bound phenolic antioxidants.

PEF pre-treatment of plant tissues has also been reported to facilitate the diffusion of intracellular compounds during the subsequent extraction with solvent process (Mahnic-Kalamiza et al., 2014). Interestingly, in a previous work (Bobinaite et al., 2015), it has been shown that press cake extracts obtained after PEF-assisted pressing of blueberry fruits contained a higher amount of antioxidant compounds as compared with those of the extracts obtained without the application of PEF.

A similar approach was used in the present work. The results in terms of total phenolics and total anthocyanins content detected in the extracts obtained from the by-products (press cakes) of untreated, PEF treated and frozen-thawed cherries are shown in Table 2. The extraction yield of total phenolics and total anthocyanins obtained from the press cake of frozen-thawed cherries was comparable to that obtained from the control press cake sample, whereas the amount of total phenolics extracted from the press cake of PEF treated sour cherries was by 22–33% higher and the amount of total anthocyanins – by 44–54% higher compared to control extraction. A possible explanation is that pores formed during PEF treatment of sour cherries were still open after the juice pressing, thus leading to higher recovery yield of phenolic compounds.

Table 1

Total phenolics, total anthocyanins and FRAP of sour cherry juice

Samples	Phenolics	Anthocyanins	FRAP
	mg 100 mL^{-1} juice		µmol TE mL^{-1}
0 kV cm^{-1}	112.90±4.22^{a}	33.80±6.83^{a}	4.90±0.22^{a}
1 kVcm^{-1}	126.60±2.87^{b}	48.30±3.87^{b}	6.10±0.66^{ab}
3 kVcm^{-1}	132.80±6.48^{b}	49.90±3.85^{b}	6.40±1.07^{b}
5 kVcm^{-1}	133.90±2.67^{b}	53.30±0.97^{b}	6.60±0.13^{b}
FF	164.40±8.12^{c}	56.60±2.00^{b}	7.40±0.42^{b}

0 kV cm^{-1} – untreated (control) sample, FF – frozen and thawed sample.
*Different letters in the same column indicate significant differences between the samples ($p \leq 0.05$).

Table 2

Total phenolics, total anthocyanins and FRAP of extracts obtained from sour cherry press cake

Samples	Phenolics	Anthocyanins	FRAP
	mg 100 g^{-1} press cake		µmol TE g^{-1}
0 kV cm^{-1}	304.90±13.18^{a}	109.40±6.66^{a}	45.30±3.76^{b}
1 kVcm^{-1}	388.50±16.83^{b}	164.10±12.33^{b}	52.70±1.33^{bc}
3 kVcm^{-1}	372.80±13.92^{b}	158.00±4.18^{b}	55.70±0.66^{c}
5 kVcm^{-1}	407.90±18.54^{b}	168.50±3.99^{b}	56.10±1.25c
FF	307.20±15.38^{a}	123.20±7.92^{a}	36.00±3.08^{a}

0 kV cm^{-1} – untreated (control) sample, FF – frozen and thawed sample.
*Different letters in the same column indicate significant differences between the samples ($p \leq 0.05$).

When PEF are applied in such way that irreversible permeabilization is achieved, some pores would never reseal, thus cell membrane is permanently damaged and therefore, the extraction process is facilitated.

Similarly to the results observed for sour cherry juice, electric field strength did not have significant ($p \leq 0.05$) influence on the extraction yield of total phenolics and total anthocyanins, i.e. higher electric field strength applied did not further enhance the extraction of phenolic compounds from sour cherry by-products (Table 2). Interestingly, in our previous work, the extractability of phenolic compounds from blueberry by-products was shown to be dependent on the applied electric field strength (Bobinaitė et al., 2015). As it was previously documented, the influence of PEF treatment on extractability of phenolic compounds depends on the location of these compounds in the plant cell and also on the cell size distribution between berry skin and flesh (Corrales et al., 2008; Barba et al., 2015). In addition, it has been demonstrated previously that the effect of PEF might be very distinct even when applied to different fruit varieties of the same plant species (Donsi et al., 2010). PEF treatment of *Aglianico* grapes prior to the fermentation/maceration step induced a significantly higher release of polyphenols (+20%) and anthocyanins (+75%), whereas the same PEF treatment had only minor impact on the polyphenolic release from *Piedirosso* grapes (Donsi et al., 2010).

The extracts obtained from the by-products of PEF treated cherries possessed 16–24% higher FRAP values with respect to control extract (obtained from untreated cherry press cake), while the extract obtained from freeze-thawed cherry by-products showed the lowest FRAP value. Moreover, FRAP of the extract obtained from press cake of frozen-thawed cherries was by 21% lower compared with the control.

It has been shown previously that PEF-assisted extraction of grape (Corrales et al., 2008), blueberry (Bobinaite et al., 2015), and raspberry (Lamanauskas et al., 2016) by-products increased the antioxidant activity of the extracts.

Conclusions

This study suggests that application of PEF in sour cherry juice production can enhance the juice yield and quality, as well as improve extraction efficiency of press cake residues.

The electric field strength of 1 kV cm^{-1} at the total specific energy input of 10 kJ kg^{-1} were shown to be sufficient for optimum PEF-assisted pre-treatment for sour cherry juice production and for further enhancement of extractability of anthocyanins and other phenolic compounds from the by-products (press cake).

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ENRICHMENT OF FRUIT LEATHERS WITH BERRY PRESS CAKE POWDER INCREASE PRODUCT FUNCTIONALITY

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Abstract

Fruit leathers are tasty, chewy, dehydrated fruit products which are eaten as snack or dessert. Dried berry press cake powders could be a promising ingredient for enhancing nutritional value of such products. The aim of this work was to enrich apple and black currant fruit leather with dried blackcurrant and raspberry press cake powders, and to evaluate the effect of the press cake additives on the content of total phenolics, total anthocyanins, antioxidant activity and textural properties of the product (fruit leathers). In this study, fruit leather made of apple and black currant puree was enriched with two different berry press cake powders (1) freeze-dried raspberry press cake powder (dry-matter content 97.3%) (2) conventionally dried black currant press cake powder (dry-matter content 95.4%). The contents of total phenolics, total anthocyanins, antioxidant activity, colour and textural properties of the test samples was evaluated. The content of phenolic compounds in raspberry and black currant press cake powders was 28.5 and 25.4 mg g^{-1} respectively. The content of anthocyanins in black currant press cake powder was more than 19-fold higher than in raspberry press cake powder. The addition of berry press cake powder reduced hardness of the fruit leathers and samples with black currant press cake powder was two times softer than the control. Raspberry press cake additive increased the lightness (L* = 24.0) and the redness (a* = 3.4), whereas black currant press cake powder reduced the lightness (L* = 22.5) and the redness (a* = 1.5) of the product. The content of anthocyanins in the fruit leathers enriched with black currant press cake powder was significantly higher than in control sample (116.4 and 74.1 mg 100 g^{-1}, respectively). Berry press cake powder also increased antioxidant capacity of the fruit leathers. The results of the study can be useful for health-conscious food producers and consumers.

Keywords: fruit leathers, berry press cakes, phenolic, products, texture.

Introduction

Fruit leather is a dehydrated, dietary fruit product which is often eaten as snack or dessert. Fruit leathers are restructured fruit made from fresh fruit pulp or a mixture of fruit juice and other ingredients that involves a dehydration step (Huang, Hsieh, 2005). During manufacturing, fruit pulps are mixed with sugar, pectin, acid, and colourants and then dried into sheet-shaped products. Most fruit leathers are dried at 30 to 80 °C for up to 24 hours until the target moisture content (12–20%) is reached (Quintero Ruiz et al., 2012; Diamante et al., 2014). Fruit leathers are nutritious, tasty and retain substantial quantities of minerals, vitamins, and phenolic phytochemicals that are initially present in raw materials (fruits, berries and vegetables) (Diamante et al., 2014).

An important portion of fruits and berries are processed into juice, which generates high amounts of by-product (press cake residues). Berry press cake (pomace) comprises berry skins and seeds, which contain fibres and various bioactive substances (Kruczek et al., 2016). The levels of phenolic antioxidants in fruit and berry processing wastes are usually found to be higher than in the actual product itself (Tian, 2016). Epidemiological studies confirmed that consumption of phenolic phytochemicals reduces the risk of cardiovascular diseases, cancer, and other degenerative diseases (Shahidi, 1997).

Nowadays, food is not merely viewed as a source of essential nutrients to ensure proper growth and development, but as a route to optimal wellness. Because of the rising interest in functional foods, scientists and food producers are looking for new sources of bioactive substances and also carriers of those substances. One of the possibilities to increase the content of natural antioxidants in the diet is to enrich food products with concentrated fractions of plant polyphenolic compounds. For instance, it was reported that grape seed and peel extract additive improved biochemical composition and functional properties of the grape juice (Ghafoor et al., 2011). Furthermore, it was suggested that raspberry seed extract can be suitable antioxidant added to muesli and cereal products (Klensporf, Jelen, 2008). In the study reported by Gailite et al. (2006) raspberry marc was used to improve the nutritional value of wheat bread. The results showed that the contents of fibre, carotenoids and vitamin E were higher in wheat bread with berry marc additive (Gailite et al., 2006). Partial replacement of wheat flour with the raspberry and black currants press cake powders increased the amount antioxidants and dietary fibre in the cookies (Górecka et al., 2010; Molnar et al., 2015). The antioxidant rich raspberry marc extract added to different fruit purees increased their functionality (Bobinaitė et al., 2016).

The aim of this work was to enrich apple and black currant fruit leather with dried black currant and raspberry press cake powders, and to evaluate the effect of the press cake additives on the content of total phenolics, total anthocyanins, antioxidant activity and textural properties of the product (fruit leathers).

Materials and Methods

Fruit puree preparation

Fresh fruits and berries were blanched and their edible part was separated and pureed using machine EP1000 (Voran Maschinen GmbH, Pichl bei Wels, Austria). Fruit purees were prepared using standardized recipes developed in biochemistry and technology laboratory of Institute of Horticulture LRCAF.

Apple / black currant puree was prepared by blending 78.0% of apple puree and 16.5% of black currant puree,

and adding 5.5% of sugar (control sample). Prepared puree was then enriched with either 1% of milled, freeze-dried raspberry press cake powder or 2% milled, conventionally (convection drying) dried black currant press cake powder.

Drying of fruit puree

Apple / black currant leathers were made by pouring pureed fruit onto a flat surface for drying. Fruit puree was dried using a convection drying method and performed in a UDS-150/1 hot-air laboratory dryer (Utena, Lithuania) at a temperature of 55±2 °C and an air-flow rate of 1.5 m s^{-1}. After drying fruit leathers were cut into strips.

Colour and texture measurement

The colour of raspberry and black currant press cake powders and fruit leathers was measured with a spectrophotometer MiniScan XE Plus (Hunter Associates Laboratory, Inc., Reston, Virginia, USA). In the reflectance mode, CIE L*a*b* colour parameters were recorded as L* (lightness), a* (+ redness), and b* (+ yellowness). The chroma ($C = (a^{*2} + b^{*2})^{1/2}$) and hue angle ($h° = \arctan(b^*/a^*)$) were also calculated (McGuiere, 1992). The total colour differences (ΔE) between dried fruit leather without additives and with berry press cake powder additive were calculated using following formula:

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

Data was presented as the average of three measurements. Colour coordinates were processed with the program Universal Software V.4-10.

The texture of fruit leathers was measured using a TA.*XTplus* Texture Analyzer (Stable Micro Systems, Godalming, England), with 2 mm-diameter flat head probe. The analysis data was processed with "Texture Exponent" program.

Extract preparation

Dried berry press cake was ground in an ultra-centrifugal rotor mill Retsch ZM200 (Retsch, Haan, Germany) using 0.2 mm particle size sieve, then 5 grams of powder was extracted with 50 mL of 70% methanol at room temperature for 60 min under constant shaking (Sklo Union LT, Teplice, Czech Republic). After decantation the residue was re-extracted second time under the same conditions. The combined extracts were filtered and subjected to further analysis.

Dried fruit leather (5 g) was added to 50 mL of 70% methanol and homogenized using Polytron PT 1200E homogenizer (Kinematica, Luzern, Switzerland). The extraction was carried out at room temperature for 120 min under constant shaking. The extract was then filtered and analysed.

Analysis of total anthocyanins, total phenolics and antioxidant activity

The content of total phenolic compounds in the extracts of dried berry press cake powders and dried leathers was measured using the Folin-Ciocalteu procedure (Slinkard, Singleton, 1977). The total anthocyanins content in the extracts of dried berry press cake powders and dried leathers was determined using the pH differential method (Giusti, Wrolstad, 2003). The concentration of anthocyanins was expressed in mg of cyanidin-3-glucoside in 100 g of press cake powder or fruit leather (dry weight, d.w.).

Three different methods were used to test the antioxidant activity of the methanolic fruit leather extracts: FRAP assay (Ferric reducing antioxidant power) (Benzie, Strain, 1996), DPPH radical scavenging assay (1,1-diphenyl-2-picryl hydrazyl radical reducing power) (Brand-Williams, 1995), and ABTS (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) assay (Re et al., 1999).

Antioxidant activity of the samples was expressed in micromoles (μmol) of Trolox equivalents (TE) per one gram of dried (d.w.) fruit leather.

Statistical analysis

All measurements were performed in triplicate and data was reported as mean ± standard deviation. Mean values were further compared using Turkey's test, and differences were considered to be statistically significant when $p<0.05$.

Results and Discussion

The chemical composition of dried berry press cake powders is presented in Table 1. The content of phenolic compounds was extremely high in both investigated press cake powders. The content of total phenolics in freeze-dried raspberry press cake powder was only slightly higher than in dried black currant press cake powder. However, the amount of total anthocyanins in raspberry and black currant press cake powder was significantly different. The concentration of anthocyanins in the dried black currant press cake powder was almost 19 times higher than in freeze-dried raspberry press cake powder. These findings are not surprising since anthocyanins account for up to 80% of the total phenolic compounds present in black currants (Borges et al., 2010; Anttonen, Karjalainen, 2006), whereas the major phenolic compounds found in red raspberries are ellagitannins (Määttä-Riihinen et al., 2004; Vrhovsek et al., 2008). The strong positive correlation between total phenolics content and ellagitannins of raspberries was reported in different studies (Anttonen, Karjalainen, 2005; Bobinaite et al., 2012).

It is worth noting that anthocyanins in the food industry are increasingly utilized not only as natural colorants (E163) substituting synthetic colorants, but also because of their biofunctional properties.

The chemical composition of dried fruit leathers is presented in Table 2. The dry matter content of fruit leathers was very similar. As it was mentioned in the introduction the moisture content of fruit leathers should not be higher than 20% (Diamante et al., 2014) since the preservation of fruit leathers depends on residual moisture content. The moisture content of the investigated fruit leathers varied from 16.1 to 17.8%.

Table 1

Total phenolics, anthocyanins and dry-matter content of berry press cake powders

Examples	Anthocyanins, mg 100 g^{-1} (d.w.)	Phenolics, mg 100 g^{-1} (d.w.)	Dry-matter content, %
Raspberry press cake powder	134.02±5.39^{b}	2846.9±145^{a}	97.3±0.05^{ba}
Black currant press cake powder	2539.4±19.09^{a}	2428.2±114^{b}	95.4±0.06^{ba}

Different letters in the same column indicates significant differences between the means ($p \leq 0.05$).

Table 2

Total phenolics, total anthocyanins and dry-matter content of dried fruit leathers

Samples	Anthocyanins, mg 100 g^{-1} (d.w.)	Phenolics, mg 100 g^{-1} (d.w.)	Dry-matter content, %
Control	74.1±2.21^{c}	949.36±11.0^{bc}	83.7±0.06^{bc}
RP	80.5±4.10^{b}	961.76±15.41^{b}	83.9±0.05^{bc}
BC	156.37±7.39^{a}	1001.98±25.74^{a}	82.2±0.02^{b}

RP – fruit leather with raspberry press cake powder; BC – fruit leather with black currant press cake powder.
Different letters in the same column indicates significant differences between the means ($p \leq 0.05$).

Table 3

Antioxidant activity of fruit leathers, µmol TE g^{-1} (d.w.)

Samples	DPPH	FRAP	ABTS
Control	19.0±0.57^{b}	33.3±0.17^{c}	72.9±0.92^{c}
RP	20.1±0.97^{a}	36.9±1.20^{b}	74.9±0.23^{b}
BC	21.3±0.33^{a}	38.0±0.50^{a}	80.0±1.31^{a}

RP – fruit leather with raspberry press cake powder; BC – fruit leather with black currant press cake powder. Different letters in the same column indicates significant differences between the means ($p \leq 0.05$).

Table 4

CIEL*a*b* colour parameters of dried fruit leathers

Samples	L*	a*	b*	h°	ΔE
Control	23.9±0.79^{a}	2.1±0.17^{b}	0.4±0.18^{b}	349.7±4.67^{a}	–
RP	24.0±0.97^{a}	3.4±0.84^{a}	0.1±0.32^{a}	0.8±4.63^{c}	1.33^{b}
BC	22.5±1.95^{b}	1.5±0.31^{c}	0.6±0.27^{b}	337.3±10.1^{b}	1.82^{a}

RP – fruit leather with raspberry press cake powder; BC – fruit leather with black currant press cake powder. Different letters in the same column indicates significant differences between the means ($p \leq 0.05$).

Higher contents of total phenolics and total anthocyanins were detected in the fruit leathers enriched with dried berry press cake powders. Higher content of anthocyanins and phenolics compounds was found in the fruit leather with dried black currant press cake powder. The content of total phenolics was by 1.3 and 5.5% higher in the fruit leathers enriched with raspberry and black currant press cake powder, respectively (Table 2). The content of total anthocyanins in the fruit leather enriched with dried black currant press cake powder was more than 2 times higher compared to control sample (fruit leather without berry press cake additive). Compared to control sample, the content of total anthocyanins in the fruit leather enriched with freeze-dried raspberry press cake powder was by 8.6% higher.

The berry press cake powders also increased antioxidant activity of the investigated fruit leathers (Table 3). The highest antioxidant activity possessed fruit leathers enriched with dried black currant press cake powder. The antioxidant activity of fruit leathers enriched with dried black currant press cake powder was by 9.7, 12.1 and 14.1% higher (ABTS, DPPH and FRAP assay, respectively) compared to the control sample. Compared to control sample, the antioxidant activity of fruit leathers enriched with raspberry press cake powder was by 2.7, 5.8 and 10.8% higher (determined by ABTS, DPPH and FRAP assay, respectively).

The lower antioxidant activity of fruit leathers with raspberry press cake powder compared to black currant press cake powder enriched product most probably is only due to the lower percentage of raspberry press cake powder used. Anthocyanins appear to be the main contributors to the antioxidant potential of black currants (Bordonaba, Terry, 2012; Borges et al., 2010). As reported previously, although raspberries had a lower content of anthocyanins than black currants there was only a slight difference in the antioxidant capacities of those two berries (Borges et al., 2010). The authors concluded that high antioxidant capacity of raspberries is because of the presence of the ellagitannins sanguine H-6 and lambertianin C, which were responsible for 58% of the total antioxidant capacity of raspberries (Borges et al., 2010). Furthermore, it has been shown that the content of ellagitannins was considerably higher in raspberry press cake extracts than in fruit or fruit pulp extracts (Bobinaitė et al., 2013).

The colour measurement results of dried fruit leathers are presented in Table 4. The raspberry press cake powder additive increased the lightness (L*) and redness (a*) of fruit leather, whereas the black currant press cake powder reduced the lightness (L*) and redness (a*) of the product. Furthermore, black currant press cake

powder shifted the colour of the fruit leather slightly more to the blue (reduced b* value). The fruit leather with black currant press cake powder additive was the darkest.

The colour difference (ΔE), between control fruit leather and fruit leathers enriched with berry press cake powders, shows that black currant press cake powder additive changed the colour of the control (apple/black currant) fruit leather more noticeably than freeze-dried raspberry press cake powder (Table 4).

The press cake powder additive had significant influence on the texture properties of dried fruit leathers (Figure 1).

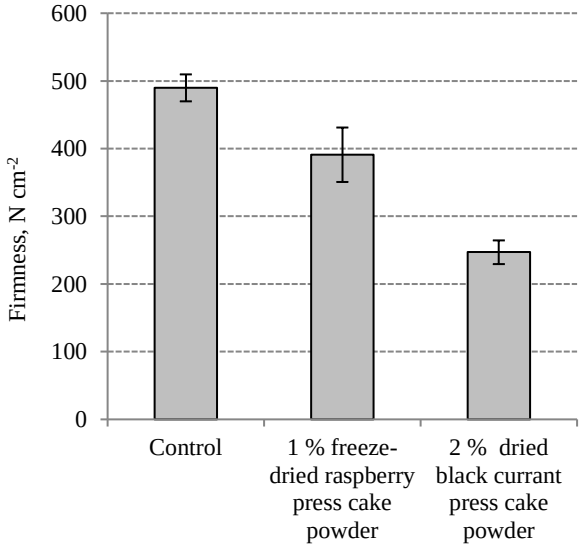

Figure 1. Firmness of dried fruit leathers

The addition of freeze-dried raspberry press cake powder reduced firmness of the fruit leather from 489.78 to 390.9 N cm^{-2} (product was 1.2 times softer), whereas 2% of dried black currant press cake powder reduces firmness of the product to 247.4 N cm^{-2} (fruit leather was 2 times softer).

Conclusions

Freeze-dried raspberry and dried black currant press cake powders contain high amounts of phenolic compounds (2846.9 mg 100 g^{-1} and 2428.2 mg 100 g^{-1}, respectively). The concentration of anthocyanins in the dried black currant press cake powder was 19 times higher than in the freeze-dried raspberry press cake powder.

The addition of berry press cake powders during the manufacturing of dried fruit leather significantly enhanced its biochemical composition and reduced firmness of the final product.

The highest content of anthocyanins and phenolics compounds was found in the apple / black currant leather with dried black currant press cake powder. The firmness of fruit leather with dried black currant press cake powder was 2 times lower compared to the control sample (fruit leather without press cake additive).

Enrichment of fruit leathers with dried raspberry and black currant press cake powders effectively enhance their polyphenolic composition and increase their antioxidant activity, thus improving functional properties of the product.

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THE EFFECT OF HIGH-PRESSURE PROCESSING ON ENTERAL FOOD MADE FROM FRESH OR SEMI-FINISHED INGREDIENTS

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Abstract

The enteral feeding can be defined as a delivery of nutrients directly into the stomach, called also enteral nutrition. Dietetic products for enteral nutrition are a specific group of products designed to provide nutrients to the human body in case of various diseases and after surgery, when the daily intake of the product is affected. Today market offers special dietetic products, which are supplemented with synthetic vitamins and minerals, which bioavailability in the body is lower than that of natural organic complexes. Therefore it is important to develop special dietetic products from natural raw materials. The aim of this study was to analyse the effect of high-pressure processing on bioactive compounds in the enteral products. For this research enteral food was made using fresh or semi-finished fruit and vegetable juices. Products were processed applying high pressure, namely 400 MPa, 500 MPa and 600 MPa for 5 minutes at room temperature. All samples were tested for their content of vitamin C, total carotenes, anthocyanins, total phenols and antioxidant activity, as the control untreated enteral food samples were used. The obtained data showed that samples made from semi-finished juices have higher contents of vitamin C and total anthocyanins than samples prepared from fresh juices. Similarly this was observed with total phenol content where after high-pressure treatment in samples made from heated juices it was more stable and had in higher amounts than in samples from fresh ingredients. There were no significant differences in the content of bioactive compounds between products treated at different pressures.

Keywords: vitamin C, total carotene, total phenols, antioxidant activity.

Introduction

Enteral nutrition (EN) by means of oral nutrition supplements (ONS) and if necessary tube feeding (TF) offers the possibility of increasing or ensuring nutrient intake in cases where food intake is inadequate (Weimann et al., 2006). EN is only used for patients with a sufficient digestion where the food can be digested and nutrients assimilated in the body of a specially prepared diet (Rozenbergs, 2011). Enteral feeding is a method of supplying nutrients (oral food and fluids) using nasogastric, gastrostomy or jejunostomy feeding, which are sometimes referred to as enteral tube feeding (Jones et al., 2011).

There can be several types of EN, depending on the intended use and the specific needs of the patient, so creating foodstuff from natural ingredients can be challenging when it comes to producing microbially safe and stable products with necessary health attributes. Taking all of this into consideration high pressure processing (HPP) has emerged as a novel, additive-free food preservation technology that has been scientifically and commercially proven to be very useful (Barba et al., 2015).

High-pressure processing (HPP) is a method where very high pressure from 100 to 1000 MPa is used to process packaged food using liquids a medium to transmit pressure. Using HPP it is possible to process food in a wide range of temperaturesfrom-20 ºC to 100 °C for a certain time (typically from few seconds to 30 minutes). This allows eliminating harmful pathogens and microorganisms that are responsible for vegetative spoilage and to inactivate enzymes with minimal modifications in nutritional and sensory quality (Andrés et al., 2016, 2016b; Carbonell-Capella et al., 2013).

Aroma compounds, vitamins and minerals are rarely affected by HPP, because of their low molecular weight and low compressibility of covalent bonds, however this doesn't apply to macromolecules such as proteins and starch which can change their native structure during HPP similarly to thermal treatments (Barba et al., 2015). The aim of this study was to analyse the effect of high pressure processing on the shelf life of enteral products made from fresh and heated (semi-finished) fruit and vegetable juices.

Materials and Methods

Sample preparation

For the purpose of this research samples of the same recipe enteral food was prepared ensuring 100 kcal intake per 161.25 g of product by using juices from blackcurrant, beet, pumpkin, cabbage, Jerusalem artichoke and whey protein, canola oil, cod liver oil, iodized salt. For one part of the experiment fresh juices (obtained from raw fruits and vegetables grown in organic management system), for the other part juices previously vacuum cooked (prepared by Ltd 'KEEFA' 'Natural Food manufacturer' from the same raw material) were used. One average sample of each set of ingredients was made to be divided between 18 individually packaged 100 ml PP bottles (Kartell, Italy) for the HPP. Before applying the HPP each bottle was vacuum packaged in a polymer film (PA/PE) to prevent any product leakage during processing as a result of applied pressure.

High-pressure processing

The HPP was carried out using ISO-Lab High Pressure Pilot Food Processor (S-FL-100-250-09-W, Stansted Fluid Power Ltd., Essex, UK) in a 2.0 L pressure vessel. A propylene glycol, water mix (1:2 v / v) was used as the pressure transmitting liquid (Kirse et al., 2015).

Both of the experimental groups were subjected to high pressure processing under 400 MPa, 500 MPa and 600 MPa for 5 minutes at room temperature. Due to pressure increase the product temperature increased by

15 °C during pressurization at 400 MPa, by 17 °C at 500 MPa and by 20 ºC at 600 MPa.

After product processing, samples (Table 1) were stored at room temperature in direct light to observe the changes of bioactive compound content during storage. Obtained results were compared depending on the type of used ingredients for the preparation of products and the selected HPP modes. For initial comparison of the HPP impact on both group samples control tests were done with samples without the use of the HPP.

Table 1

Abbreviations used in sample identification

Sample abbreviation	Type of juices	Applied HPP
Fresh	Fresh	not applied
Heated	Heated	not applied
I AS	Fresh	400
II AS	Fresh	500
III AS	Fresh	600
I AP	Heated	400
II AP	Heated	500
III AP	Heated	600

The quality changes of the samples during the storage were evaluated by detection of vitamin C, soluble solids content, pH value, total carotenes, total anthocyanins, total phenols, and antiradical activity. The microbial safety was tested with the detection of total plate count, coliforms, molds, and yeasts.

Microbiological analyses

Microbiological testing of enteral food was completed using 90 ml of 0.5% sterile peptone water solution to which 10 ml of enteral food was added and mixed. The mixture was pour-plated in duplicate for determination of total plate count (TPC) according to standard LVS EN ISO 4833:2003 (Ref. No. 01-14, Sharlau, nutrient agar, incubation at 30 °C for 72 h); Coliforms according to standard LVS ISO 7251:2005 (Ref. No. 401460, Sharlau ENDO agar, incubation at 37 ºC for 24 h); mold fungi and yeast cells according to standard ISO 21257-2:2008 (Ref.No.01-111, MRS agar, incubation at 27 ºC for 48 h (yeast cells) and 5 to 7 days (mold fungi).

Microbiological safety of enteral food was evaluated according to the guidelines by Cabinet of Ministers, Latvia regulation No 441/2016 for Vegetable jams, purees and similar products which sets allowed limits for TPC at $5 \cdot 10^3$ CFU g^{-1}; presence of Coliforms per 1 g of product is not allowed; Mold fungi and yeast cells no more than 50 CFU g^{-1}.

Soluble solids content

The soluble solids content (Brix%) was measured with digital refractometer Refracto 30GS (Mettler Toledo, Japan) using standard method ISO 2173:2003 Fruit and vegetable products - Determination of soluble solids - Refractometric method. Measurements were carried out in five replications.

pH

pH was measured by pH-meter (Lutron electronic enterprise CO., Ltd., UK) using standard method LVS ISO 5542:2010. Measurements were carried out in two replications.

Vitamin C

Content of vitamin C was determined according to iodine method as described by (Kerch et al., 2011). This method determines L-ascorbic acid, which is the reduced form of ascorbic acid. Measurements were carried out in four replications.

Total carotenes

Total carotenes were analysed by spectrophotometric method using UV/VIS spectrophotometer Jenway 6705 (Bibby Scientific Ltd., UK), at 440 nm described by Kampuse et al. (2015). The content of carotenes (mg 100 g^{-1}) was calculated in four replications.

Total anthocyanins

Total anthocyanin content was determined by spectrophotometric method according to (Moor et al, 2005), detected on spectrophotometer Jenway 6705 at wavelength of 540 nm. Measurements were carried out in two replications.

Total phenol content

Total Phenol content was determined according to the Folin-Ciocalteu method (Yu et al., 2003) with modifications: to 0.5 mL of extracted sample add 2.5 mL of 0.2N Folin-Ciocalteu reagent, that has been diluted ten times with distilled water; after 5 minutes 2.0 mL of 7.5% $NaCO_3$was added; the resulting solution was mixed and allowed to stand for 30 minutes at 18±1 °C in a dark place; absorption was read at 760 nm using JENWAY 6300 (Banoworld Scientific Ltf., UK) spectrophotometer (Priecina et al., 2014).

Measurements were carried out in six replications from two separately weighed samples.

Antiradical scavenging activity (DPPH)

The antiradical scavenging activity of extracts was determined on the radical scavenging ability in reacting with stable 2,2-diphenil-1-picrylhydrazyl (DPPH) free radical according to researchers group (Yu et al., 2003) with modifications: to 0.5 mL of extracted sample 3.5 mL freshly made DPPH solution was added (4 mg of DPPH reagent was dissolved in 100 ml pure ethanol);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 (Priecina et al., 2014).

Measurements were carried out in six replications from two separately weighed samples.

Statistical analysis

The obtained data was processed using 'Microsoft Office Excel' 2007 version, differences between the results were analysed using ANOVA: Two-factor with replication. The obtained results are presented as their mean with standard deviations. Differences among results were considered to be significant if p-value$<\alpha_{0.05}$.

Results and Discussion

Effect of the HPP and storage on microbial safety

Microbial counts were evaluated for both types of ingredients high pressure processed samples and also to control samples without HPP.

No coliforms were found in the evaluated samples, also mold fungi where only detected in control samples made from fresh juices (2 CUF g^{-1}) before storage.

Control samples weren't tested during storage, only their initial results were used for evaluation of the efficiency of the HPP. The total plate count is shown in Table 2 where the mean value of untreated fresh juice samples was $3.3 \cdot 10^2$ CUF g^{-1} and for semi-finished juices 5 CUF g^{-1}. The testing showed that after the HPP all samples were microbiologically safe and with both types of used ingredients (fresh and semi-finished) the applied pressures (400 MPa, 500 MPa, 600 MPa) were sufficient for reduction of microbial activity. This coincides with findings of Andrés et al. (2016) on microbial shelf life on refrigerated milk- and soy-smoothies. However during the four weeks of storage only samples made from semi-finishedingredients stayed microbiologically safe, but sampeles from fresh juices after the week 1 started to show contamination, which gradually grew and at the week 2 exceeded the allowed yeast cell count 50 CUF g^{-1} and became unsuitable for further testing Table 2.

The highest TPC after the week 1 was in the sample I AS treated at 400 MPa – 62 CUF g^{-1} and the lowest in sample III AS (500 MPa pressure) 36 CUF g^{-1} but no significant difference was found between the applied pressure effect on TPC.

Table 2

Total plate count, CUF g^{-1}

Sample	Storage time				
	Before storage	week 1	week 2	week 3	week 4
Fresh	$3.3 \cdot 10^2$	NA	NA	NA	NA
Heated	5	NA	NA	NA	NA
I AS	ND	62	$1.1 \cdot 10^2$	NA	NA
II AS	ND	59	95	NA	NA
III AS	ND	36	$1.02 \cdot 10^2$	NA	NA
I AP	ND	ND	ND	ND	ND
II AP	ND	ND	ND	ND	ND
IIIAP	ND	ND	ND	ND	ND

ND – not detected, NA – not analysed

As mentioned before only the growth of yeast cells (Table 3) was observed in enteral food samples during storage where the mean value of untreated fresh ingredient EN food was $8.4 \cdot 10^2$ CUF g^{-1} and for heated ingredient EN food 8 CUF g^{-1}. The highest yeast cell count was in the sample I AS (HPP at 400 MPa) after week 1 it was 39 CUF g^{-1}, after the week 2 cell count grew up to $1.09 \cdot 10^2$ CUF g^{-1}. A similar change was detected with the rest of the samples.

Table 3

Yeast cell count, CUF g^{-1}

Sample	Storage time				
	Before storage	week 1	week 2	week 3	week 4
Fresh	$8.4 \cdot 10^2$	NA	NA	NA	NA
Heated	8	NA	NA	NA	NA
I AS	ND	39	$1.09 \cdot 10^2$	NA	NA
II AS	ND	37.5	95	NA	NA
III AS	ND	36	98.5	NA	NA
I AP	ND	ND	ND	ND	ND
II AP	ND	ND	ND	ND	ND
IIIAP	ND	ND	ND	ND	ND

ND – not detected, NA – not analysed

The effect of the HPP and storage on soluble solids and pH

Both soluble solids and pH showed no significant change after the HPP or storage, however enteral food made from fresh juices had higher content of soluble solids 12 Brix% on average than those made from semi-finished juices, which on average was 11 Brix%. Similarly, enteral food from fresh juices had pH 5, but from semi-finished juices pH 4.5. These findings coincide with other researcher findings (Andrés et al., 2016; Landl et al., 2010) of no significant change in pH and soluble solids during refrigerated storage of the HPP treated samples.

Effect of the HPP and storage on vitamin C content

The content of vitamin C (Table 4) in the sample without high pressure processing made from fresh ingredients was 25±3.46 mg 100 g^{-1}, but in sample made from semi-finished ingredients 28.2±1.37 mg 100 g^{-1}.

The high pressure processing initially shows better vitamin C retention in the samples made from semi-finished ingredients but after 2 weeks of storage the loss of vitamin C on average was 50%. However samples made from fresh ingredients showed a 33 to 65% decrease right after applying HPP. This could be explained with the enzymatic activity in fresh juices where enzymes keep deteriorate ascorbic acid contrary to semi-finished ingredients where enzymatic inactivation was achieved by heat treatment. The statistical analysis showed a significant difference in content of vitamin C between samples made from different groups of ingredients ($p<0.05$) and a slight difference between the applied pressure ($p=0.003$) for samples before storage.

Table 4

Content of vitamin C, mg 100 g^{-1}

Sample	Storage time				
	Before storage	week 1	week 2	week 3	week 4
Fresh	25.0±3.5	NA	NA	NA	NA
Heated	28.2±1.4	NA	NA	NA	NA
I AS	17.4±1.3	20.5±2.9	11.4±1.5	NA	NA
II AS	15.9±2.8	19.2±1.1	13.7±1.4	NA	NA
III AS	11.1±1.8	16.2±2.5	14.5±1.4	NA	NA
I AP	29.8±1.0	25.5±1.1	14.6±1.4	14.6±1.4	13.4±1.4
II AP	33.3±2.2	27.4±2.5	13.3±1.4	13.3±1.4	12.8±2.1
IIIAP	30.8±0.1	29.5±1.3	13.5±1.4	14.5±1.4	13.3±1.2

NA – not analysed

The highest vitamin C content was 33.3±2.24 mg 100 g^{1} in the sample II AP (made from semi-finished ingredients processed at 500 MPa) but the lowest in the sample III AS (fresh ingredients, processed at 600 MPa) 11.1±1.83 mg 100 g^{-1}. It is partially possible to link the obtained data to other studies on this subject however the storage in those was mostly refrigerated. For example Andrés et al. (2016) also show a more rapid loss of vitamin C, but on the day 30 (32% for sample processed at 450 MPa and 36% for 600 MPa). The statistical analysis showed a significant loss of vitamin C during storage for samples from both ingredient groups ($p<0.05$) and also between applied pressures for samples made from fresh ingredients (I AS, II AS, III AS) $p=0.006$, these results are consistent with Andrés et al. (2016), Oey et al. (2008) who also reported the enhanced degradation rate of vitamin C by increased pressure.

The effect of the HPP and storage on total carotene content

Similarly to the content of vitamin C, also content of total carotenes (TC) show a significant difference between enteral food samples made from fresh juices which is lower and samples made from semi-finished juices. The lowest amount of TC (Table 5) was in I AS (0.15±0.01 mg 100 g^{-1}), but the highest in the sample processed at the same pressure made from semi-finished ingredients I AP (0.44±0.01 mg 100 g^{-1}) immediately after processing. No significant difference was found between samples of fresh juice enteral food and semi-finished ingredient EN food without the HPP treatment, but such was found in all samples after the HPP ($p<0.05$). EN food made from fresh juices showed a significant degradation of TC which isn't supported by other author findings, but the samples made from semi-finished ingredients didn't show such degradation.

Evaluating the obtained data no direct coherence of total carotenes change during storage can be lined, but it does show a tendency of degradation with every week of storage and increasing pressure for semi-finished ingredient EN food samples. The uneven data from this study could be indicative of an uneven distribution of oil and oil-soluble ingredients during filling. Barba et al. (2015) also reported that the HPP treatment can increase extractable carotenoid amount in plant-based products explaining it with the permeabilization of the plasma membrane cell and denaturation of the carotenoid-binding protein induced by the HPP (400-600 MPa/20-25ºC/2–5 min).

The effect of the HPP and storage on total anthocyanin content

Evaluating total anthocyanin content a significant difference ($p<0.05$) between fresh and semi-finished ingredient enteral food samples and a slight difference between the applied pressure ($p=0.03$) was detected before storage and also during storage for all samples ($p<0.05$). This however does not apply to semi-finished ingredient enteral foods that show the highest total anthocyanin content from 2.25±0.02 mg 100 g^{-1} (II AP) to 2.38±0.01 mg 100 g^{-1} (III AP) which in all samples similarly decreased during storage Figure 1.

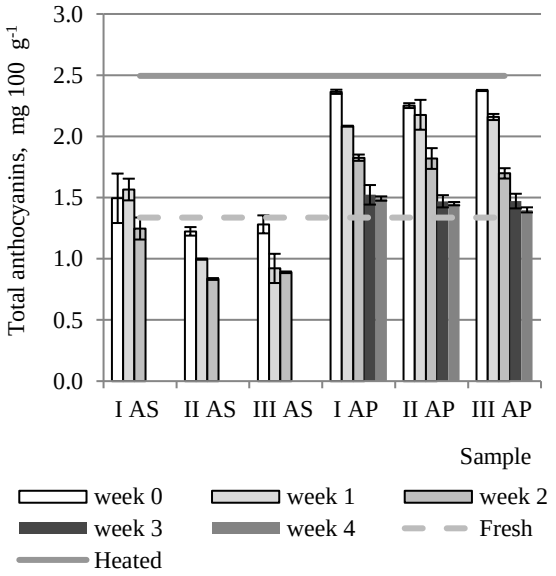

Figure 1. Content of total anthocyanins, mg 100 g^{-1}

There was a significant difference between processing pressures on fresh juice samples. The more divers' changes with these samples could also be explained with one medium weight difference and some authors do mention that some enzymatic activity is still present in products even after the HPP treatment which makes these samples unstable during storage (Denoya et al., 2016).

Table 5

Content of total carotene, mg 100 g^{-1}

Sample	Storage time				
	Before storage	week 1	week 2	week 3	week 4
Fresh	0.44±0.00	NA	NA	NA	NA
Heated	0.44±0.02	NA	NA	NA	NA
I AS	0.15±0.01	0.20±0.03	0.16±0.02	NA	NA
II AS	0.18±0.04	0.16±0.01	0.19±0.02	NA	NA
III AS	0.21±0.02	0.13±0.00	0.16±0.02	NA	NA
I AP	0.42±0.02	0.44±0.01	0.37±0.01	0.37±0.02	0.24±0.01
II AP	0.25±0.01	0.38±0.03	0.35±0.01	0.38±0.01	0.32±0.04
IIIAP	0.35±0.00	0.32±0.00	0.34±0.01	0.37±0.00	0.33±0.00

NA – not analysed

Table 6

Content of total phenols, mg GAE 100 g^{-1}

Sample	Storage time				
	Before storage	week 1	week 2	week 3	week 4
Fresh	52.15±4.16	NA	NA	NA	NA
Heated	49.62±3.15	NA	NA	NA	NA
I AS	36.08±6.37	48.57±7.45	38.91±3.51	NA	NA
II AS	34.56±5.20	50.82±6.98	47.33±0.98	NA	NA
III AS	33.52±4.34	46.76±3.13	50.40±4.33	NA	NA
I AP	49.201±2.29	55.95±4.47	39.87±4.01	36.61±2.49	31.07±3.50
II AP	45.981±2.84	54.06±4.13	48.70±3.48	33.03±3.91	28.07±3.23
IIIAP	46.492±2.33	51.28±4.29	47.59±4.87	32.78±3.92	28.61±3.93

NA – not analysed

The effect of the HPP and storage on total phenol content

The total phenol content (Table 6) after HPP showed a significant difference between used ingredient groups, but an increase of total phenols content was detected during storage, which has been reported also by Andrés et al. (2016), however samples made from fresh juices do not show the same tendency. Barba et al. (2015) on extraction of polyphenols using the HPP treatment similarly to total carotene and also anthocyanin content suggests, that this type of treatment compared to conventional methods can be able to enhance mass transfer processes within plant cellular tissues, as the permeability of cytoplasmatic membranes can be affected. Having said that the initial analyses of bioactive compounds could show less total phenols content, because they have not been fully extracted, compared to storing them for a week (Barba et al. 2015).

The effect of the HPP and storage on antiradical activity (DPPH)

Similarly to total phenol content DPPH appears to be stable up until the second week when its activity starts to drop. The obtained data is shown in Figure 2.

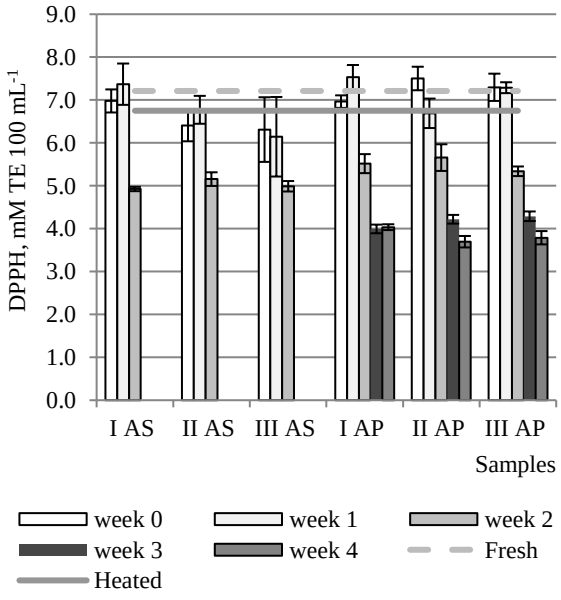

Figure 2. Antiradical activity (DPPH) of EN foods during storage, mM TE 100 mL^{-1}

The antiradical activity of EN foods after the HPP isn't significantly different from samples without applied HPP and no significant difference was found between the applied pressures ($p<0.05$). Although a significant change was observed during storage where the DPPH gradually decreased which coincides with findings of Andrés et al. (2016) and Oey et al. (2008). The highest mean of DPPH was found in the semi-finished ingredient sample processed at 500 MPa before storage II AP 7.50±0.27 mM TE 100 mL^{-1}, which also had the lowest antiradical activity after storing the sample for 4 weeks (3.70±0.13 mM TE 100 mL^{-1}).

The data analysis of several bioactive compounds to some enteral food samples showed a higher content after storing samples for one week than it was determined before storage. These findings do not fully coincide with other author findings on bioactive compound changes during storage and does suggest the need of additional testing. The sample preparation technique of one average sample volume and division between separate packages could cause an uneven distribution of ingredients that can impact the outcome of tested compounds. In literature it has also been mentioned that HPP improves the extraction of bioactive compounds from plant cellular tissues, as the permeability of cytoplasmatic membranes are affected, as this isn't an instant occurrence it may take some time to be fully detectable (Barba et al. 2015).

Conclusions

After this preliminary research it can be concluded that additional research needs to be done, to provide more data on the HPP treatment impact on enteral foods made from different ingredients and the shelf life of these products. The obtained data showed different results for the tested bioactive compounds which not always were compatible with findings of other authors. For the further research it can be suggested to evaluate and make some additional changes in the sample preparation to ensure greater reliability on the obtained data.

Over all the research showed that samples prepared from vacuum heated (semi-finished) ingredients were more stable after high pressure processing and were microbiologically safe for 4 weeks when they were stored at room temperature in direct light. Samples made

from fresh juices showed bigger variation in contents of bioactive compounds during storage, but similarly to semi-finished samples didn't show significant changes with the applied pressure. Also samples from fresh juices in these conditions kept their shelf life only for one week before they were deemed to be ineligible for the further research.

Vitamin C, total carotene and total phenol content in enteral food samples made from semi-finished juices slightly decreased with the increased pressure.

Initial results also show that after using the HPP at 400 MPa, 500 MPa and 600 MPa it is possible to obtain microbiologically safe products.

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SENSORY PROPERTIES OF HIGH-PRESSURE-TREATED MILK

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Abstract

The acceptance and preference of milk and dairy products by consumers is determined by the products sensory characteristics. High pressure processing (HPP) can be applied as an alternative to thermal treatment to provide that sensory quality remains unaffected. The aim of this work was to evaluate the influence of high-pressure treatment on the acceptance of the sensory profile of milk, compared to untreated and thermally-treated milk. Milk was processed at 400 MPa or pasteurized at 78 °C for 15–20 seconds. Sensory evaluation of milk samples was carried out in two different groups – untrained panellists (at the international food industry fair 'Riga Food 2016') and trained panellists (finished basic course of sensory evaluation). Both groups evaluated the samples using a five point hedonic scale. The hedonic evaluation of liking of untreated, pasteurized and high-pressure treated milk samples' sensory properties showed that there were no significant differences ($p>0.05$) in aroma, but there were significant differences in the liking of milk samples colour, taste and aftertaste ($p<0.05$) depending on treatment type. The overall sensory properties of HP-treated milk were evaluated higher comparing to the traditionally pasteurized milk. The colour (which was measured in CIE L*a*b* colour system) difference was well visible in high-pressure treated milk in comparison to untreated and pasteurized milk.

Keywords: milk, high pressure, sensory properties, colour.

Introduction

Many activities in the food sector are devoted to improving already existing products and developing new products for the purpose of satisfying consumer preferences and needs (Næs et al., 2010). Nowadays consumers demand high quality foods, which are fresh, natural, free from chemical preservatives, nutritionally richer and microbiologically safe, but with extended shelf-life.

Milk is a widely consumed beverage due to its nutritional importance, a pleasant aroma and mouth feel, and a slightly sweet taste (Chugh et al., 2014). Milk and dairy products are treated at temperature range from 70 to 145 °C ensuring the safety and stability of the product during its shelf life. Unfortunately thermal treatments above 100 °C lower the nutritional quality of milk because many nutrients are heat labile. Furthermore, heat treatment is known to cause changes in the flavour of milk. Gandy et al. (2008), Walstra et al. (2006) established that heat treatments (72–145 °C) cause changes in sensory quality of milk with the development of tastes, such as a cooked or caramel flavour, and odours, such as a cooked smell.

To overcome this problem in recent years, several non-thermal technologies (hurdle technology, high hydrostatic pressure, power ultrasonic, pulsed electric field, etc.) have been explored to meet new demands of consumers for fresh products without food additives. Among these technologies, high-pressure processing (HPP) seems to be promising one for food applications (Chopde et al., 2014) and at this moment is the most commercially developed non-thermal technology, with very good acceptance by consumers (Bello et al., 2014). One of the important goals of milk preservation methods by its high-pressure treatment is to reduce microbial loads and inactivate enzymes. Pressure ranges between 100 and 1200 MPa have been considered as effective to inactivate microorganisms including food-borne pathogens (Chawla et al., 2011). At the same time this technology is capable to introduce the least possible undesired changes of physicochemical and sensory properties of product, as well as preservation of its nutritional value (Popov-Raljić et al., 2008). Hogan et al. (2005) reported that high pressure treatment at moderate pressure generally results in minimal changes in the odour, flavour, or other sensory characteristics of foods.

Sensory analysis is the most straight forward way to evaluate the quality and consumer acceptance of food products (Hogan et al., 2005). So far, few studies (Andres et al., 2016; Contador et al., 2015; Fitria et al., 2015; Gervilla et al., 2001; Trujillo et al., 2002) have been conducted to assess the effect of HPP on the volatile profile and sensory quality of milk, and these preliminary results are encouraging. Young and George (2013) established that consumer perception about non-thermal treatments is that they provide more natural or fresher foods than the heat treated samples.

The aim of this work was to evaluate the influence of high-pressure treatment on the acceptance of the sensory profile of milk, compared to untreated and thermally-treated milk.

Materials and Methods

Individual cow milk samples were collected from the morning milking during sampling procedure of milk quality monitoring according to the standard LVS 175:1999 'Sampling of raw milk'. After collection, milk samples were transported to the laboratory of the Faculty of Food Technology, Latvia University of Agriculture.

Processing of milk samples

For obtaining maximally equal samples milk was mixed (2 min) and filled in polyethylene terephthalate (PET) plastic bottles (NF2 – ∅28 mm, 120 ± 10 mL). Milk samples were pressurized in the Iso-Lab High Pressure Pilot Food Processor S-FL-100-250-09-W, (Stansted Fluid Power LTD, UK) with a pressure chamber of 10 cm diameter and 23 cm length. Milk underwent high pressure treatment at 400 MPa for 15 minutes. The pressurization was completed at room temperature.

Product temperature increased during pressurization up to 30 °C and dropped during pressure release to about 17 °C.

High pressure processing was compared to the thermal processing (high-temperature short-time (HTST) pasteurization at 78 °C for 15–20 s).

Sensory analysis

Sensory evaluation of milk samples was organized in two-steps:

1. in the evaluation at the international food industry fair *Riga Food 2016* participated totally 55 untrained panellists (80% women and 20% men), who represented all Latvia regions: Riga (36.4%), Zemgale (22.7%), Latgale (18.2%), Vidzeme (13.6%) and Kurzeme (9.1%). There were three noticeably larger panellists groups: up to 20 years (22.7%), from age 21 to 40 (27.3%) and from age 41 to 60 (36.4%), while the smallest group of panellists (13.6%) was aged 61 and older. More than half of untrained panellists (54.5%) were aware that high-pressure technology can be applied in the manufacture of foods, what may indicate their relation to food technology area;
2. in the evaluation at Latvia University of Agriculture, Faculty of Food Technology participated 25 trained panellists (92% women and 8% men). The sensory evaluation tests were performed in individual cabins with controlled temperature and lighting conditions.

A preference test was used to evaluate the acceptance of the sample sensory properties. Panellists used a 5-point hedonic scale (Meilgaard et al., 1991) ranging from 1 (dislike very much) to 5 (like very much) for scoring the following sensory properties of milk: colour, aroma, taste and aftertaste.

The panellists received approximately 30 mL of each sample at 16±2 °C temperature in cups with a volume of 50 mL, coded with three-digit random numbers. Warm water was provided to the panellist for cleansing palate between sampling.

Instrumental evaluation of colour

Before instrumental evaluation of colour milk was thoroughly mixed and filled in Petri dish.

The colour parameters of milk samples were measured in CIE L*a*b* colour system by direct reading using the colorimeter Colour Tec – PMC (Accuracy Microsensors, USA).

The following colour parameters were evaluated: L* – lightness is ranging from 0 (black) to 100 (perfect white); a* – redness to greenness (positive to negative values, respectively); b* – yellowness to blueness (positive to negative values, respectively). The values provided for each sample were the average of ten replicates.

In order to compare the total colour difference (ΔE) between milk samples, the following equation (1) was utilized:

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \qquad (1)$$

Depending on the value of ΔE the colour difference between the milk samples could be estimated as not noticeable (0–0.5), slightly noticeable (0.5–1.5), noticeable (1.5–3.0), well visible (3.0–6.0) and great (6.0–12.0) (Cserhalmi et al., 2006).

Data analysis

The processing of the obtained data was performed using mathematical and statistical methods with Microsoft Excel software (Microsoft Office Enterprise 2007, License: Shareware *N/A***)**. The results represent the mean ± standard deviations, and were analysed using analysis of variance (ANOVA) and Tukey's test when significant differences among the milk samples were found (Næs et al., 2010). Statistical differences with p-values under 0.05 were considered as significant.

Results and Discussion

Sensory evaluation at the international food industry fair Riga Food 2016 – untrained panellists

Sensory properties of the product are the main parameters, which are used by customers for choosing appropriate food products, therefore the current research aimed to indicate HPP influence on milk sensory properties and compare it with traditional pasteurized milk. In the current sensory evaluation step, untrained panellists evaluated overall acceptance of sensory properties (colour, aroma, taste and aftertaste) of pasteurized (PM) and high-pressure processed (HPM) milk samples.

The evaluation of milk sensory properties by untrained panellists was analysed in both samples, and the results are presented in Table 1.

According to the statistical analysis, there was no significant difference ($p>0.05$) in the liking of pasteurized and pressure-treated milk samples aroma. In the hedonic scale panellists rated aroma of PM and HM sample in the range from 3 (neither like nor dislike) to 5 (like very much).

Table 1

Sensory evaluation of milk samples by respondents at the international food industry fair

Sensory properties	Milk samples	
	PM	HPM
Aroma	4.09±0.3a	4.36±0.9a
Colour	3.91±0.6a	4.63±0.7b
Taste	3.82±0.7a	4.36±0.9b
Aftertaste	4.00±0.7a	4.50±0.7b

PM – pasteurized milk, HPM – high-pressure processing milk
*values marked with the same letters in the rows are not significantly different ($p>0.05$)

Evaluation of acceptance of pasteurized and high-pressure processed milk sensory properties shows that there are significant differences in colour ($p=0.022$), taste ($p=0.019$), and aftertaste ($p=0.038$).

The current evaluation demonstrates that some sensory properties of HP milk, were preferred comparing with PM. Next step of the research was to detect if pressurized milk had similar sensory properties comparing with untreated milk, although in scientific literature (Andres et al., 2016; Fitria et al., 2015;

Trujillo et al., 2002) is mentioned, that HPP doesn't affect it.

Sensory evaluation at LLU, Faculty of Food Technology – trained panellists

Untreated, pasteurized and high pressure-treated milk samples were evaluated based on their sensory attributes: aroma, colour, taste, and aftertaste. Trained panellists evaluated sensory properties of milk samples in the range from 3 (neither like nor dislike) to 5 (like very much).

The diagram given in Figure 1 illustrates the results of the evaluation of sensory properties by trained panellists.

According to ANOVA, there were significant differences in degree of liking among the three milk samples colour ($p=0.000$), taste ($p=0.000$), and aftertaste ($p=0.002$). Results of the hedonic scores show that there are no differences ($p=0.103$) in the preference of three milk samples aroma.

The sample UM had the highest degree of preference compared to samples HPM and PM.

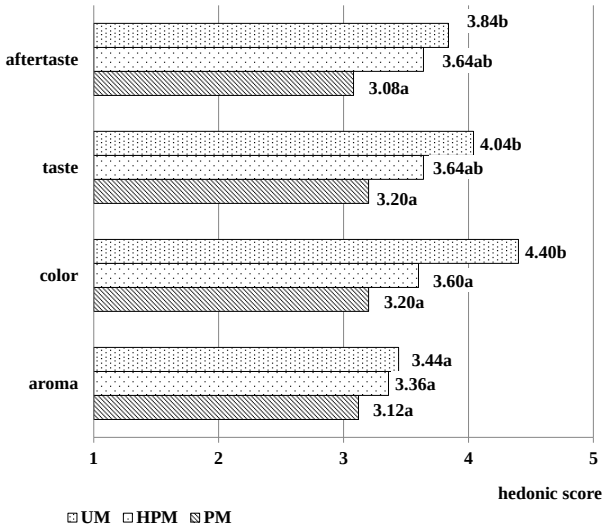

Figure 1. Sensory evaluation of preference of milk samples properties completed by trained panellists

UM – untreated milk, HPM – high-pressure processing milk, PM – pasteurized milk

*values marked with the same subscript letters are not significantly different ($p>0.05$)

The aroma of the sample HPM remained stable after high-pressure processing with score 3.36±0.96, and there was noted no significant difference compared to the sample UM (mean score: 3.44±0.72). This is in agreement with observations by Chawla et al. (2011), who reported that flavour compounds remain unaffected by HP treatment. Also Vazquez-Landaverde et al. (2006) reported that high-pressure processing at low temperature causes minimum change of the volatile composition of milk. Although sample PM had the lowest mean score (3.12±0.44), there was no significant difference ($p>0.05$) from the samples UM and HPM.

The trained panellists found significant differences ($p<0.05$) in taste and aftertaste according to the type of milk treatment. The sample UM had the highest mean score of taste (4.04±0.61) and aftertaste (3.84±0.63) among all milk samples. No significant differences from the sample UM were detected in sample HPM. However, the mean scores of taste and aftertaste of sample PM revealed significant decrease in the degree of liking compared with the control sample (UM).

The obtained results are in agreement with the research results of Popov-Raljić et al. (2008) and Hogan (2005) and can be evaluated positively, HPM sample combines pasteurised and untreated milk properties: is microbiologically safe and with sensory properties, which are close to untreated milk .

Colour of UM had the highest degree of liking therefore for better understanding, which milk colour nuances are preferred by panellists and how milk was changed after different treatment technologies instrumental evaluation of colour was completed.

Instrumental evaluation of colour

White colour of milk is due to scattering of light particles by fat globules and casein micelles (Naik et al., 2013). Hunter Luminance value (L* value) of milk is generally used as a measure of whiteness (Harte et al., 2003).

The results of colour CIE L*a*b* measurements of the milk samples are shown in Table 2.

A moderately intense heat treatment causes colour changes, presumably due to serum protein denaturation and aggregation. As a result pasteurized milk become a whiter (Walstra et al., 2006; Chopde et al. 2014). Also in the present study, pasteurized milk was whiter comparing with sample HPM resulting in higher L* values.

Table 2

Colour values of milk samples in CIE *Lab* system

Parameter	Milk samples		
	UM	PM	HPM
L*	89.08±0.1	90.05±0.18	84.31±0.16
a*	-2.5±0.21	-2.48±0.18	-2.62±0.25
b*	6.67±0.46	6.52±0.69	7.66±0.57

UM – untreated milk, PM - pasteurized milk, HPM – high-pressure processing milk

After high-pressure processing colour parameter L* value of whole milk decreased, indicating significant changes ($p<0.005$) comparing to UM. This is in agreement with observations by Kim et al. (2008), who reported that the L* value of HP treated (at 200 MPa) whole milk was significantly lower than that of the raw milk.

Similar tendency was established by Naik et al. (2013) in skimmed milk after treatment at 250–450 MPa, significant decrease in the L* value was observed, and in ewe`s milk, by Gervilla et al. (2001). Also Harte et al. (2003) reported that milk subjected to HP treatment and thermal treatment followed by HP, loses its white colour and turns yellowish.

Johnston et al. (1992) proposed that the decrease in L* value could have been mainly due to disintegration of casein micelles by pressure into small fragments and that might be a possible explanation for obtained results in our research. Obtained changes of L* value in milk

can be explained also with denaturation of whey proteins during pressurizing (Pandey et al., 2003), but for significant conclusion further studies should be done evaluating milk structure.

Colour parameter a* of pressurized milk was lower comparing with samples UM and PM, but difference was not significant ($p>0.05$). Meanwhile, high-pressure treatment caused a significant ($p<0.005$) increase in the b* value (yellowness) when compared with unprocessed milk. The increase was more pronounced comparing with pasteurized samples.

The total colour difference (ΔE) indicates the magnitude of colour difference between processed and unprocessed fluid foods (Barba et al., 2012). The calculated values for total colour change in milk samples are provided in Table 2.

Table 2

Colour difference (ΔE) of milk samples

Sample	Sample		
	UM	**PM**	**HPM**
UM	0		
PM	0.98	0	
HPM	4.87	5.85	0

UM – untreated milk, PM – pasteurized milk, HPM – high-pressure processing milk

According to the classification of Cserhalmi et al. (2006), colour differences were slightly noticeable ($0.5 < \Delta E < 1.5$) in pasteurized milk, and well visible for sample HPM, comparing with UM. The colour difference between samples HPM and PM was well visible ($3 < \Delta E < 6$) to the human eyes.

Obtained results are unambiguous, according to the data obtained in Table 2, well visible difference was established between PM and HPM milk, but sensory evaluation results (Fig. 1) indicated no significant difference ($p>0.05$) between the degree of liking of the same samples. Combining results of sensory and instrumental evaluation of colour unexpected conclusion of the current results can be drawn – panellists prefer white milk, over the yellowish one.

Conclusions

The hedonic evaluation of untreated, pasteurized and high-pressure treated milk samples showed no significant differences ($p>0.05$) in aroma, but there were significant differences in the liking of milk samples colour, taste and aftertaste ($p<0.05$).

Although high-pressure treatment cannot retain the original sensory properties of raw milk, the overall sensory properties (colour, taste and aftertaste) of HP-treated milk were evaluated higher comparing to the traditional pasteurized milk.

In the present study all the CIE L*a*b* colour parameters were changed by the high-pressure treatment: significant decrease ($p<0.05$) of the L* value and increase of b* value was observed, whereas the value of colour parameter a* increased insignificantly ($p>0.05$). The total colour difference was well visible in high-pressure treated milk in comparison to untreated and pasteurized milk.

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FACILITIES OF BREAD ENRICHMENT WITH CALCIUM BY USING EGGSHELL POWDER

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Abstract

Today's food ration does not often contain enough vitamins, fibre, macro- and micronutrients, especially calcium. Research aim: to study possibilities for the use of the eggshell powder in the production of bread enriched with calcium. For the study, the free-range hen eggshells, main components of which are inorganic salts, were used. The eggshells have been washed, dried and ground. To avoid changes in the texture of bread, the solubility of the eggshell powder was tested by incorporating different quantity of eggshell powder (2.5 g,) into rye sourdough with *Lactobacilli* for 12 hours. After discovering good solubility of the eggshell powder in the fermented leaven samples, sourdough of rye bread was prepared (control, 2.5, 5.0, 7.5, 10.0, 12.5 g addition of eggshell powder) and using them six samples of wheat bread were baked. To estimate an influence of eggshell powder on a product quality and acceptability of bread by consumers, sensory evaluation for bread samples was carried out. Besides porosity, moisture content, acidity, the actual acidity, specific volume of bread were defined, and also nutritional value and amount of calcium were calculated. The study showed that when making the bread enriched with calcium, it is recommended to add an eggshell powder to rye sourdough with lactic acid bacteria. The sensory evaluation shows that the bread with eggshell powder had a better appearance of the crust, colour of the crumb, flavour and overall acceptability compared to control bread, however the taste and flavour remained similar or got worse. By increasing the amount of eggshells, bread porosity and specific volume of bread increased, while acidity and active acidity decreased. Bread containing 5.0 g of eggshell powder was marked as a bread of the best quality.

Keywords: Eggshell powder, calcium carbonate, bread, lactic sourdough.

Introduction

Calcium (Ca) is one of the important macronutrients necessary for normal functioning of the human body. Calcium is not only the major component of bones and teeth, it also participates in the regulation of hormone secretion and activation, muscle contraction, neuronal conduction via ion channels, regulation of inflammatory processes, maintaining the permeability of cell membranes, and many others (Dri et al., 2011). Deficiency of calcium in the diet causes bone loss or osteoporosis. Studies have found that only 10 percent of adults consume adequate calcium dose with food. Older people get with food only 606.02±217.35 mg of calcium per day (Tamulaitienė et al., 2006). This is below the acceptable daily consumption of calcium. It is therefore very important to ensure an adequate amount of calcium in the diet. This can be achieved by the use of calcium supplements, but calcium is better absorbed when consumed in small amounts throughout the day, so nutritional fortification with calcium is a good solution. (Romanchik-Cerpovicz et al., 2007). Results of study (Juma et al., 1999) indicate that enriched bread can serve as a good source of bioavailable calcium. Calcium absorption was significantly higher in the bread in comparison to the milk and control groups. In addition, the use of eggshells can contribute to the reduction of waste.

It was thought that the eggshell is not a usable product, but opinion has changed after extensive research (Daengprok et al., 2003). It was suggested that eggshell calcium promoted strengthening bones and improving their growth.

Several Ca sources are available for food fortification. $CaCO_3$ is the most widely used Ca salt because 40% of the compound is well absorbable Ca. This Ca salt can be formulated from $Ca(OH)_2$ or chalk but can also be derived from fossilized or fresh shells (e.g., chicken eggshell and oyster shell). The egg consists of 9.5% eggshell, 63% egg white (albumen) and 27.5% yolk. Eggshell components are inorganic salts (91.87%), the main ones of which are calcium carbonate (98.4%), magnesium carbonate (0.8%), tricalcium phosphate (0.8%) (Dri et al., 2011). Chemical composition of white and brown eggshells is slightly different. A little more calcium is in a white eggshell powder (34.12±0.10% in white; 33.13±0.10% in brown), but in brown – more magnesium, phosphorus and potassium (EP 2780114 A1). Natural Ca sources are of interest because they contain not only Ca but also other elements (e.g., Sr and F), which may have a positive effect on bone metabolism (Olgun et al., 2015). Concentrations of other elements (Na, K, P) in eggshells are very low compared to the daily recommended allowance (Neunzehn et al., 2015). Studies have proven natural calcium supplement safety – eggshell powder has a smaller amount of V, Cr, Pb, Al, and Cd compared to oyster shells or purified $CaCO_3$ (Schaafsma et al., 2000). Least quantity of hazardous heavy metals - mercury and cadmium is in the organic eggs and in the eggs from free-range hens (Schaafsma et al., 2000).

There is a number of registered patents how to separate the egg inner membrane from the eggshell. One of the best ways – eggshells should be treated by the high air velocity at ambient temperature, thus preserving the original shell and membrane lipids and protein structure (EP 2780114 A1).

There are few papers describing the use of chicken eggshells as Ca supplement in human beings. Schaafsma et al. (2000) showed an increase in lumbar spine, total proximal femur and trochanter bone mineral density in osteoporotic postmenopausal women

who received eggshell powder with vitamin D_3. Chicken eggshell powder (ESP) has been proposed as an attractive source of calcium for human health to increase bone mineral density in an elderly population with osteoporosis (Rovenský et al., 2002). Chicken eggshell contains about 1.0% matrix proteins in addition to a major form of calcium carbonate (95%). Soluble eggshell matrix proteins that remarkably enhance calcium transport in to cells and the potential significance of eggshell calcium as a nutraceutical are discussed (Daengprok. et al., 2003).

Hirasawa et al. (2001) showed that eggshell calcium is one of the most effective sources of Ca – eggshell Ca could have greater effects to $CaCO_3$ on bone metabolism. In contrast with $CaCO_3$, vitamin D_3 supplementation did not significantly increase Ca content. Studies showed that calcium absorption from eggshell powder is slightly higher (45.59%) than from $CaCO_3$ (39.88%) and other calcium supplements (Brun et al., 2013).

It is often assumed that the calcium carbonate is poorly absorbed by the body because of its insolubility. Maybe calcium citrate or calcium lactate soluble in water (in such a form it is present in milk) is better absorbed (Szeleszczuk et al., 2015). Calcium solubility *in vitro* study (Bradauskienė et al., 2016) demonstrated that calcium carbonate from eggshell is readily soluble in gastric juice comparing to solubility of other commercial calcium supplements such as Calcigran®, Calcivit® Coral Calcium®. *In vitro* study showed that in the 0.1 M HCl, pH=1.00 solution the eggshell powder is dissolved at 37 °C temperature within 90 min to 75.0±4.5%.

The eggshell of one egg contains 2.07±0.18 grams of calcium (Dri et al., 2000). This means that half of an eggshell can provide the amount of Ca needed per day in adult human beings. Argentine scientists Brun et al. (2013) provide methods that are easy to use at home. One of them – to dissolve the eggshell powder in lemon or orange juice or vinegar solution. Eggshell powder was added to bread, breaded fried meat, pizza and spaghetti. The sensory evaluation shows no change in the taste, but changes in the texture were identified that may not be acceptable to consumers, so the authors advise to look for ways to avoid them (Brun et al., 2013).

The calcium additives are particularly useful for fortifying leavened baked goods with calcium. Methods for preparing the calcium additives and using the calcium additives to fortify baked goods are also patented (US8221808). Generally, the calcium additives comprise intimate admixtures of calcium carbonate and an acid such as citric acid.

In previous research carried out by Food Technology Department of Klaipeda State University of Applied Sciences, seeking to enrich traditional Lithuanian light bread with calcium, eggshell powder (ESP) (2.5 g) was added to a leavened bread dough. The dough was allowed to rise for 5 hours, but in all cases undesirable texture changes in the baked bread were identified – undissolved eggshell powder was perceptible during bread chewing. The next experiment discovered that the ESP perfectly dissolved in rye leaven and graininess in baked bread was not perceptible, so it was decided to bake bread using rye sourdough with lactic acid and incubating the eggshell powder in it. The aim of this study: to study possibilities for the use of the eggshell powder in the production of bread enriched with calcium.

Materials and Methods

For the study brown eggshells of free range chicken, 7–8 days old, were used, average mass of one egg was 58±2.38 g, eggshell mass was 5.43±0.79 g. Eggshells were prepared properly: thoroughly washed, the inner membrane was removed, dried in oven for 15 minutes at +63±0.3 °C degrees, grinded up in a coffee grinder and crushed in a ceramic mortar and pestle.

Preparation of bread with ESP

Semi-wheat bread samples were prepared at Laboratory for Food Technologies at Klaipeda State University of Applied Sciences in accordance with the leavened bread production technology.

First of all, rye leaven was prepared from flour, water and the mother leaven sourdough with *Lactobacilli* at 1 : 1.25 : 0.25 ratio. It was divided into 6 equal pieces, 250 g each. Six rye leaven samples were prepared with different content of eggshell powder: 1st control, 2nd with 2.5 g of ESP, for the next, the ESP amount was increased by 2.5 g respectively till 12.5 g. All leaven samples with different concentration of ESP were mixed and leavened at 32–34 °C for 12 hours.

Further the bread samples were prepared according to the recipe given in Table 1, which shows ESP content in the sourdough.

Table 1

Recipes of bread samples

Sample	Ingredients, g					
	C	**P2.5**	**P5**	**P7.5**	**P10**	**P12.5**
Sourdough	250	250	250	250	250	250
Eggshell powder	–	2.5	5.0	7.5	10	12.5
Wheat flour 550D	510	507.5	505	502.5	500	497.5
Water	300	300	300	300	300	300
Sugar	30	30	30	30	30	30
Salt	12	12	12	12	12	12
Caraway	10	10	10	10	10	10

Obtained mass of bread samples was mixed in a mixer "Kitchen Aid" (Germany) for 20 min, the dough was leavened in a thermostat at 32–34 °C temperature for 120 minutes.

Then the dough was put up in forms, then leavened once more for 30 minutes at 32–34 °C and baked in an oven "Metos Chef" at 240 °C for 40 minutes.

Baked goods were stored for 60 min, then placed in plastic bags and stored for 24 hours at room temperature (18–20 °C).

Physical chemical investigation

Loaf volume was measured by rapeseed displacement method, cm^3, of the standard ICC 131.

Specific volume was calculated as loaf volume to weight ratio, $cm^3\ g^{-1}$, of the standard ICC 131.
pH was measured by pH-meter ORION 3STAR.
Bread **crumb moisture** content was determined by drying of a crushed sample at 130±2 °C (LST 1492:2013 Bakery goods – Methods for determination of moisture content).
Total titratable acidity expressed as the amount of NaOH (mL) consumed for the neutralization of free acids per 100 g of bread sample (LST 1553:1998 Bakery goods and confectionery. Methods for determination of acidity and alkalinity).
The **bread porosity index** was determined by using the Zuravliov equipment according to the standard LST 1442:1996 Bread, rolls and buns. Determination of porosity.
Sensory evaluation of the breads was carried out using 5-point scale by a group of 6 assessors. Breads were evaluated for colour, taste, aroma, appearance of crust, texture and overall acceptability, with the score 1–5, where 1 represented extremely disliked and 5 – extremely liked.
Nutrition and energy value calculation method – nutritional and energy values were calculated using a specific Excel spreadsheet.
Mathematical statistical analysis of the data
The wheat bread baking experiments were repeated twice along with the investigation of 6 samples. Mathematical statistical data analysis was performed using SPSS 17.0. P-values less than 0.05 were interpreted as statistically significant.

Results and Discussion

All six baked bread samples output turned out to be similar and amounted from 88.87% to 90.89%, the output dependence on the eggshell powder content was not observed. The quality of baked bread depends on the moisture content, volume, crumb grain quality, and the texture of the bread. It was found that upon increasing the eggshell powder the moisture content of the product increased slightly (see Table 2).

Table 2
Characteristics of bread samples

Sample	Moisture content, %	pH	Total titratable acidity, mL 1N NaOH
C	39.78±0.95	4.33±0.12	4.11±0.16
P2.5	40.21±0.75	4.92±0.09	3.76±0.14
P5	40.66±0.68	5.60±0.10	3.42±0.10
P7.5	41.56±0.81	5.87±0.12	2.75±0.12
P10	42.28±0.80	5.95±0.11	2.40±0.14
P12.5	42.82±0.90	5.98±0.09	2.37±0.08

As the difference of the moisture content is low, it can be determined by reduced flour content respectively with the eggshell powder content. Data of other study (Khan et al., 2017) revealed that with the increasing concentration of calcium sources, moisture content of leavened breads and unleavened breads was decreased. The total titratable acidity varied between 2.37 and 4.11 mL 1N NaOH. There was determined a significant ($p<0.05$) increase in the pH and a decrease in titratable acidity, because the calcium contained in the eggshell neutralizes acids formed in bread during fermentation. Calcium carbonate contained in the eggshell reacts with the acids releasing CO_2 gas, which raises the product upon heating, increases the porosity and specific volume of bread (see Table 3).

Table 3
Crumb porosity and specific volume of bread samples

Sample	Crumb porosity, %	Specific volume, $cm^3\ g^{-1}$
C	48±0.49	1.73±0.11
P2.5	52±0.40	1.99±0.06
P5	57±0.54	2.02±0.07
P7.5	64±0.51	2.13±0.06
P10	68±0.45	2.21±0.08
P12.5	67±0.52	2.16±0.09

The quality of the bread crumb can depend on calcium ions which affect the permeability of cell membranes, increase the ability of the yeast fermentation at a concentration from 0.01 to 0.2 mol (Salinas et al., 2016). Calcium ions also are activators and stabilizers of flour α-amylase and amylolytic enzymes, improve the structural and mechanical properties of the bread crumb at the insignificant increase in volume of baked products. However, overdose of this element can inhibit the fermentation process (Young, 1998).
The mean score values of colour, taste, texture and overall acceptability for control and eggshell powder fortified breads are presented in Fig. 1.

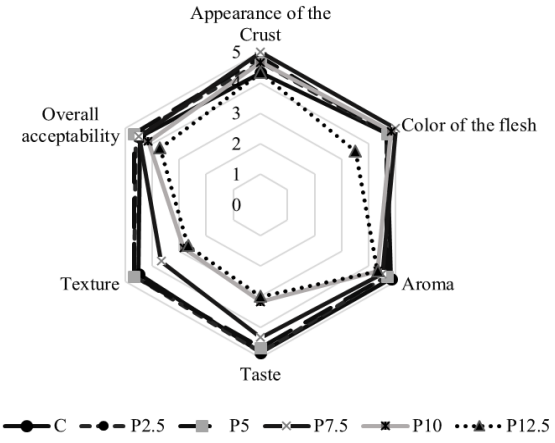

Figure 1. Sensory evaluation of the bread samples
C – control, P2.5 – 2.5 g ESP, P5 – 5 g ESP, P7.5 – 7.5 g ESP, P10 – 10 g ESP, P12.5 – 12.5 g ESP

Bread with ESP had a better appearance of the crust colour of the flesh, flavour and overall acceptability compared to control bread, however the taste and flavour remained similar or got worse.
Highest mean appearance of the crust score (5.0) was observed in P7.5 breads.

Table 4

Nutrition and energy values of 100 g bread samples

Parametrs	Sample					
	C	P2.5	P5	P7.5	P10	P12.5
Calories, kcal	222.00	221.00	220.00	220.00	219.00	218.00
Total fat, g	1.13	1.13	1.12	1.12	1.12	1.11
Saturated Fat, g	0.14	0.14	0.14	0.14	0.14	0.13
Sodium, mg	468.78	468.77	468.76	468.74	468.73	468.72
Total carbohydrates, g	47.62	47.44	47.26	47.08	46.90	46.72
Sugars, g	3.62	3.62	3.62	3.61	3.61	3.61
Protein, g	6.99	6.97	6.96	6.95	6.93	6.92
Calcium, mg	28.77	114.01	199.25	284.49	369.74	454.98

Table 5

Reference content of nutrients in 100 g of bread samples, %

Parameters	Samples					
	Control	P2.5	P5	P7.5	P10	P12.5
Calories	11.00	11.00	11.00	11.00	11.00	11.00
Total fat	1.60	1.60	1.60	1.60	1.60	1.60
Saturated fat	0.70	0.70	0.70	0.70	0.70	0.70
Sodium	19.53	19.53	19.53	19.53	19.53	19.53
Total carbohydrates	13.60	13.60	13.50	13.50	13.40	13.30
Sugars	4.00	4.00	4.00	4.00	4.00	4.00
Protein	14.00	13.90	13.90	13.90	13.90	13.80
Calcium	3.60	14.30	24.90	35.60	46.20	56.90

Whereas, ESP at all concentrations decreased the taste score of bread as compared to control (Figure 1). No significant difference in texture of breads was found in control and eggshell powder fortified samples P2.5-P5 but higher ESP content considerably reduced the texture of breads – graininess was perceptible when chewing. Fortification of bread with eggshell powder in various levels significantly ($p<0.05$) influenced the overall acceptability. Highest overall acceptability value (4.67) was observed in breads P5 (fortified with 5 g addition of eggshell powder), which shows the preference of panellist, bread samples P2.5 and P7.5 have also been estimated well.

In order to assess bread nutritional value and calcium content increase, nutritional and energy values were calculated using the recipe and product composition database (see Table 4). Calculation was based on chemical composition of brown eggshells, determined by Dri et al. (2011): protein (6.4%), lipids (0.03%), water (1.7%), and amount of calcium 33.13±0.10%.

As the results showed, the eggshell supplement significantly increased calcium content in all bread samples. Under 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, adults are recommended to consume 800 mg of calcium per day. Reference calcium content in all bread samples is presented in Table 5.

Calculated reference calcium content in 100 g significantly increased in all bread samples. If a person consumes 100 g per day of recommended bread sample P5, he will receive almost 25% of the daily - recommended amount of calcium.

Conclusions

Eggshell powder has been perfectly dissolved in rye leaven with lactic acid in 12 hours. Bread with eggshell powder had a better appearance of the crust, colour of the flesh and overall acceptability compared to control bread. The baked products have a texture, crumb structure, taste, and "mouth feel" substantially identical to baked products that do not have added eggshell powder. Bread did not have a "grainy" texture. As calcium was the main concern of the current study, calculated calcium content in 100 g significantly increased in all bread samples. By best quality indicators was marked bread with 5 g of eggshell powder.

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INVESTIGATION OF FRUCTANS INCREASING POSSIBILITIES IN RYE BREAD

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Abstract

Nowadays, consumer's great attention is drawn to food with high nutritional value and its functional significance, and as we know the bread is one of the products consumed in the diet. The regular intake of prebiotics such as fructans, improves a few physiological features of human. Fructans concentration in rye flour is not sufficient to have a functional value for bread, but it is possible to add raw materials that contain a high fructans concentration in bread production process. Therefore, the aim of the research was to evaluate and increase the fructans content in rye bread using *Jerusalem artichoke*, chicory powder and sourdough with *Gluconobacter nephelii* acetic acid bacterium. During the research it was found that using *Gluconobacter nephelii* acetic acid bacterium, *Jerusalem artichoke* and chicory powder it is possible to increase the amount of fructans in rye bread. Obtained new rye bread samples have the same energy value as rye bread samples from Latvia market. But for functional products obtaining 12% *Jerusalem artichoke* powder and 9% chicory powder could be used in rye bread prescription, as a result the amount of fructans per 100 g of product could be 5 g. Sourdough is the appropriate substrate to improve the use of bacterium (*Gluconobacter nephelii)* for increasing the content of fructans in the bread. Obtained products were not only with elevated nutritive value, it also has an appealing look, good texture, aroma and taste, customers like it and it is considered as a potentially functional product in the future.

Keywords: rye, fructans, *Gluconobacter nephelii*, *Jerusalem artichoke*, chicory.

Introduction

Nowadays, consumers pay more attention to products purchased on a daily basis and their most attention is focused on food with high nutritional value and its functional properties. The functionality in food mainly can be given by fructans which come from cereals and other products. Fructans are carbohydrates that consist mainly or exclusively of fructose and contain no or one glucose unit (Lewis, 1993). Fructans set an example of functional ingredients: also known as non-conventional sugars due to their prebiotic properties; make up a good opportunity to add value to the product either in terms of functionality or in profitability for the food industry. The regular intake of prebiotics such as fructo-oligosaccharides (FOS) and inulin improve a few physiological features, enhancing resistance against intestinal as well as extra-intestinal pathogens and promoting good immune response development, including the decrease of allergies (Roberfroid et al., 2010). As we know cereals are basic, popular and healthy raw materials, providing excellent opportunities for nutrition, health, diversity and innovation (Collar, 2015). The rye (*Secale cereale* L.) is the second most used grain for bread making, is likely to gain interest and popularity after wheat (Bushuk, 2001). In scientific literature data is available that the fructans concentration in rye is between 3.6 and 6.6% on dry matter basis. The fructans concentration in rye can be influenced by several factors as variety, growing and harvesting conditions, fertilizers and soil composition (Andersson et al., 2009). However, it is not possible to provide fructans concentration in rye bread in same amount as in grains mainly because of fructans thermal volatility. Nowadays there are some opportunities to increase the amount of fructans in rye bread. One of them is fructans production during sourdough making using *Gluconobacter nephelii* acetic acid bacteria. It has been shown that these bacteria in established substrate and in favourable growing conditions are producing fructans (Semjonovs et al., 2015). For a long time sourdoughs have been used for the leavening of the dough's as well as for the acid and flavour formations in wheat and rye dough's (Arendt et al., 2007). They are a mixture of flour and water fermented by lactic acid bacteria (LAB) and yeasts in a complex ecosystem, resulting in several biochemical processes such as acidification, proteolysis, synthesis of enzymes, antifungal compounds (Lavermicocca et al., 2000) as well as exopolysaccharides (EPS) (Tieking et al., 2003). Some other researchers have recently screened and identified other several strains of acetic acid bacteria (AAB) for/as being able to produce high amounts of polysaccharide from sucrose (Kowalsky et al., 2011; Semjonovs et al., 2017). Another option is to add additional raw materials, which composed a high fructans concentration, such as *Jerusalem artichoke* and chicory powder with high FOS content. The *Jerusalem artichoke* is widely known product in the world and the powder is obtained by drying and grinding of *Jerusalem artichoke* tubers. *Jerusalem artichoke* powder contain high amount of dietary fibre as – non-starch dietary fibre – 14%, and inulin – 59%, also minerals, and vitamins, it not contain bitter taste, but has pleasant, slightly sweet taste (Bekers et al., 2007; Gedrovica, 2012). The chicory (*Cichorium intybus)* belongs to the family *Asteraceae* having six species which may categorize on the basis of their geographical location in Europe or Asia. Most of the part of these plants possess a great medicinal importance due to the presence of various types of important compounds such as fructans, where its concentration in root is from 35.7 to 47.6 g 100 g^{-1} (Peshev, van den Ende, 2014; Abbas et al., 2015). Wherewith, developing recipes and

technology, it is possible to obtain rye bread with higher fructans concentrations what we cannot purchase in Latvian markets. Therefore, the aim of the research was to evaluate and increase the fructans content in rye bread using *Jerusalem artichoke*, chicory powder and sourdough with *Gluconobacter nephelii* acetic acid bacterium.

Materials and Methods

Analyses were carried out at the Department of Food Technology in the Latvia University of Agriculture in Year 2016, but bread preparation was performed in the bakery company.

Materials

All used raw materials for the current study were purchased from wholesale trade companies in Europe.

Preparation of the recipe

Recipe was calculated per 100 kg cereal products including whole grain rye flour, wheat flour, non-fermented rye malt, rye flour, sugar, salt, yeast, caraway, malt and water.

Sourdough preparation

Two types of sourdough were prepared – one (as control) with *Lb. fructivorans* and *C. milleri* bacteria, but second for fructans formation by two phases with lactic (*L. reuteris* 20016) and acetic acid (*Gluconobacter nephelii*) bacteria`s.

The control sourdough (used for bread control and bread samples with *Jerusalem artichoke* powder and chicory powder) was prepared by mixing rye flour with water and added bacteria mixture (industrial technology). Fermentation time and temperature for control sourdough were 24 h and 32 °C. But sourdough for fructans production was prepared by two phase's method where rye flour and water was divided in two parts. In the first part half of rye flour, water and sugar were mixed together with bacteria mixture, and placed for fermentation 24 h at 28 °C, after the first fermentation stage second half of flour, water and sugar was added to fermented mass and fermented again for 24 h at 32 °C.

Dough and bread preparation

Eight dough samples were prepared with control sourdough in the present research, three samples with 4, 8 and 12% *Jerusalem artichoke* powder and control sourdough; three samples with 3, 6 and 9% chicory powder and control sourdough, and one sample with sourdough which was made with *Gluconobacter nephelii* acetic acid bacteria.

All ingredients were mixed in a spiral mixer (Wendel Diosna WV 400A) for 8±1 min at low speed (100 rpm). The initial temperature of dough was 32±2 °C, dough moisture 43±1%. Pre-fermentation time was 2 h 30 min. After pre-fermentation dough was divided in 790 g pieces and formed. After placing dough in forms, it was proofed in fermentation chamber for 50±2 min in 36±2 °C with relative air humidity 75±5%. Dough samples were baked in tunnel-type oven in 5 zones: 1st zone baking temperature 260±10 °C, steam 0.05 Bar; 2nd zone – 240±10 °C; 3rd zone – 210±10 °C; 4th and 5th zones – 190±10 °C. Total baking time was 52±2 min. Baked bread was cooled in a spiral cooler for 3 h. The dough fermentation and baking time were the same for all dough samples.

Determination of sourdough and bread quality

The following parameters were analysed in obtained prepared sourdough and bread samples and in bread samples purchased from local markets:

1) *Moisture content* of bread was determined using air-oven method (AACC method 44-15.02).

2) *Acidity* of sourdough and bread was determined using AACC 02-31.01 method.

3) *Fructans content* in rye flour, sourdough and bread was determined using AACC 32.32 method.

Sensory evaluation

The ranking test (ISO 8587:2006) to rank samples according to their degree of liking and line scale method, based on the ISO 4121:2003 Sensory analysis – Guidelines for the use of quantitative response scales was used for this study. In line scale method the samples were evaluated for flavour, aroma, acidity, structure, porosity and colour. Using ranking test the bread samples were evaluated sensory by 55 untrained panellists, but line scale method was done by 16 trained panellists.

Statistical analysis

The results (mean, standard deviation, p value) were processed using mathematical and statistical methods. Data were subjected to one-way analysis of variance (ANOVA) by Microsoft Office Excel 2013 and *XLSTAT* program; significance was defined at $p<0.05$.

Results and Discussion

Determination of optimal Jerusalem artichoke and chicory powder ration in bread

In the first step of experimental study six new products were developed – rye bread with 4%, 8%, 12% *Jerusalem artichoke* powder, 3%, 6%, 9% chicory powder. This experiment was done to understand the optimal amount of *Jerusalem artichoke* and chicory powder in rye bread composition in order to get functional characteristics and not reducing product quality – particularly the appearance and taste. After sensory evaluation of obtained bread samples taste differences, it was determined that, there is no significant difference ($p>0.05$) comparing with control sample. Therefore, the added amount of powders does not significantly affect the taste of rye bread. The highest fructans concentration was detected in rye bread with 9% chicory powder 13.67±0.33 g 100 g^{-1} DW, but in rye bread with 12% *Jerusalem artichoke* powder the fructans content was 12.21±0.36 g 100 g^{-1} DW (Figure 1). In control sample the fructans concentration was 12-fold and 13-fold less, comparing with bread samples with added powders. However, fructans concentration in used rye flour was 3.16±0.12 g 100 g^{-1} DW.

The fructans concentration does not increase in proportion to the quantity of added powders amount,

because it depends on fermentation time, baking conditions and yeast activity (Jasińska-Kuligowska et al., 2013; Verspreet et al., 2013).

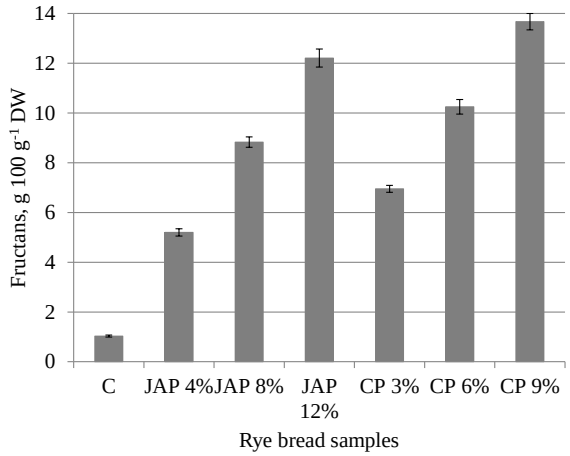

Figure 1. Fructans amount in new developed products, g 100 g^{-1} DW

C – control,
JAP – rye bread with *Jerusalem artichoke* powder,
CP – rye bread with chicory powder

In the scientific literature is mentioned, the minimum amount of fructans at which they have a positive impact on favourable growth for bacteria in humans should be 5–8 g per day (Kolida, Gibson, 2007; Ramnanil et al., 2014). Therefore, for further experiments rye bread samples with 12% *Jerusalem artichoke* and 9% chicory powder were used.

Fructans content evaluation in sourdough

The differences of microflora what can be used for sourdough production give diversified taste, aroma, acidity and consistency (Demarigny, Gerber, 2014) of final product. Control sourdough was used for control rye bread and for bread samples with *Jerusalem artichoke* and chicory powder, but the newly developed sourdough with acetic acid (*Gluconobacter nephelii*) bacteria was used for rye bread to increase fructans content. Sourdough acidity was measured every 4 h (Figure 2) to get the sourdough with acceptable properties which should be suitable for rye bread production.

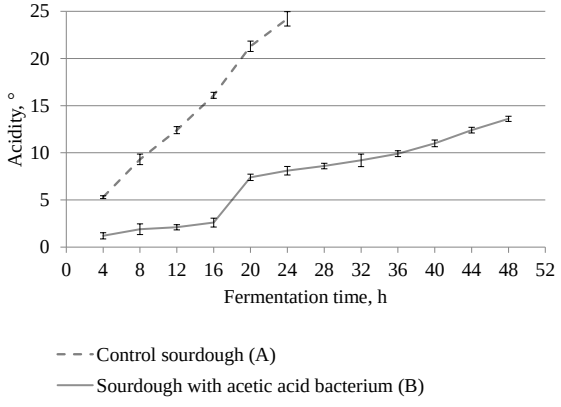

Figure 2. Sourdough acidity (°) changes during fermentation

It can be seen in Figure 2, the sourdough (A) acidification increasing tendency is very rapid and after 24 h of fermentation the sourdough has reached the necessary acidity – 24.2±0.76°.

The acidity increase in sourdough (B) was not so rapid, therefore the fermentation time is two times longer and after 48 h of fermentation the acidity increases (13.6±0.27°), comparing with sourdough (A). The most rapid increase of acidity in sourdough (B) was observed during fermentation from 16 to 20 hours, depending on bacteria adaptation to the environment, optimal temperature and nutrients.

To provide the necessary acidity of dough after 2.5 h of fermentation, the sourdough to the dough was added two times more, not exchanging other raw material amounts. Before and after the fermentation the fructans content in sourdough was determined (Figure 3). According to the results using acetic acid (*Gluconobacter nephelii*) bacteria and lactic acid (*L. Reuter* 20016) bacteria, it is possible to increase the fructans concentration in sourdough.

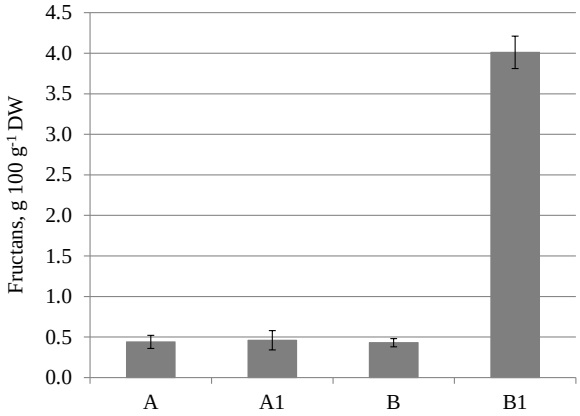

Figure 3. Fructans content in sourdough samples before and after fermentation, g 100 g^{-1} DW

A – control sourdough before fermentation
A1 – control sourdough after fermentation
B – sourdough with acetic acid bacteria before fermentation
B1 – sourdough with acetic acid bacteria after fermentation

Fructans content in sourdough (B) after fermentation reached to 4.01±0.21 g 100 g^{-1} DW. But in sourdough (A) fructans content did not change.

In addition to the nutrients in the flour, sucrose was added to sourdough, because it is required for the synthesis of fructans and the disaccharide concentration is one of the main preconditions for fructans synthesis (Pollock et al., 2003).

Rye bread moisture, acidity, fructans content and sensory evaluation

Rye bread technological process takes many hours, including sourdough, dough preparation, fermentation, and baking. In order to check out fructans formation process in rye bread, for the control sample there was not used raw materials, which are composed of a high (>20 g to 100 g^{-1} DW) fructans content. The analyses and sensory evaluation were done for three new products obtaining in comparison with control sample (Table 1).

Table 1

Physically-chemical parameters of rye bread

Parameters	Control sample	Rye bread samples		
		JAP	CP	SAA
Moisture, %	44.68	46.21	45.83	44.93
Acidity, °	9.23	9.11	8.75	8.45
Fructans, g 100 g^{-1}	0.46	5.63	6.06	2.10

JAP – rye bread with 12% *Jerusalem artichoke* powder
CP – rye bread with 9% chicory powder
SAA – rye bread with added sourdough with acetic acid bacteria

Results of present experiments demonstrate that new developed products have higher moisture content compared to the control sample, mainly because *Jerusalem artichoke* and chicory powders has lower water-binding capacity than flour, due to the fact that these raw materials contain high concentration of fructooligosaccharides. Adding to the recipes raw materials containing fructooligosaccharides, dough water absorption capacity decreases, because fructooligosaccharides create a barrier around the starch molecules, which restrict the water binding capacity (Karolini-Skaradzinska et al., 2009), therefore after baking moisture content of obtained bread samples was higher.

The lowest (8.45±0.09°) acidity was detected in the rye bread sample SAA, but acidity of control sample was the highest – 9.23±0.11°. Traditionally, sourdough quality depends on the incorporated microflora, resulting increase of dough acidity, which was slower compared to dough with control sourdough (A1). It should be noted that the required fermentation time (2.5 h) was sufficient to implement the technological process and provide final product quality. Statistically significant ($p<0.05$) changes were detected for fructans content in obtained bread samples. Higher fructans content was obtained in rye bread with 9% chicory powder and 12% *Jerusalem artichoke* powder (Table 1). In the control sample fructans content was 0.46±0.04 g 100 g^{-1} – by 92% less than in rye bread with 9% chicory powder. It is necessary to indicate, that fructans concentration in bread could be affected not only by pH or acidity, but of increased processing temperature too, as higher than 90 °C (Matusek et al., 2009).

However, essential difference in fructans concentration in commercial rye bread samples purchased in Latvian markets was found (Figure 4). Commercial rye bread has comparably low fructans concentration, which can be mainly based on used flour and other cereal products which are included in the recipes.

Appraising the results, it is evident that the newly developed rye bread samples have higher fructans content (Table 1) in comparison with commercial rye bread samples (Figure 4).

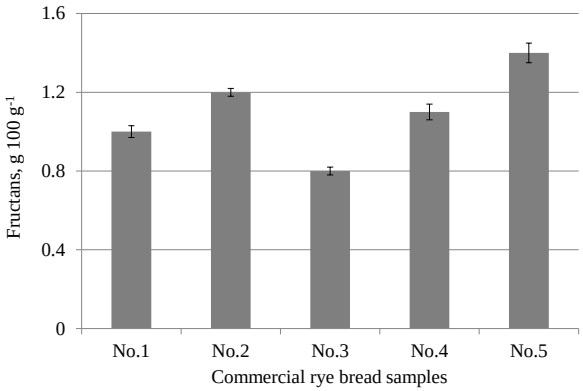

Figure 4. Fructans content in commercial rye bread samples

Sensory properties of new products mainly could provide offering to consumers. Results of sensory evaluation demonstrate that sensory properties of developed rye bread samples were significantly different in comparison with the control sample.

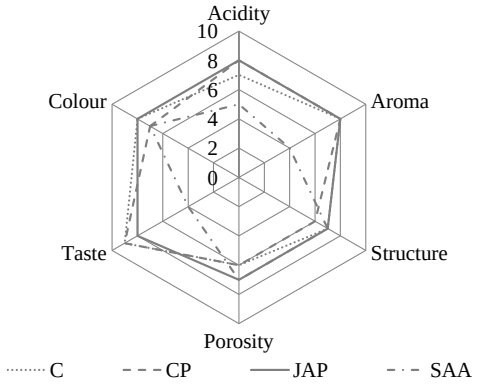

Figure 5. Sensory properties star diagram

C – control sample
JAP – rye bread with 12% *Jerusalem artichoke* powder
CP - rye bread with 9% chicory powder
SAA – rye bread with added sourdough with acetic acid bacteria

Fundamental difference was obtained in taste and aroma, which was described as a specific, unusual for sample SAA and all experts felt bitter aftertaste. Other parameters as acidity, structure, porosity and colour didn't differ significantly ($p<0.05$), comparing to other samples and control. The experts noted, that the samples are qualitative and don't require significant changes.

The degree of liking of rye bread was done by 55 untrained persons (consumers) and in summarized data (Figure 6) can be seen, that they really liked the sample with 9% chicory powder which they described with a intrinsic aroma and sweet aftertaste. Consumers dislike a rye bread with acetic acid bacteria, because it gives lasting bitter aftertaste and some consumers didn't like the flavour. Significant difference of rye bread samples liking were detected. ANOVA results showed that the sample (CP 9%) with $F_{cal} = 45.45$ and the sample (SAA) with $F_{cal} = 10.44$ are higher than $F_{crit} = 3.93$ ($n_1=3$, $n_2=216$, $\alpha=0.05$), therefore for those samples

there are significant difference in degree of liking, comparing with control sample.

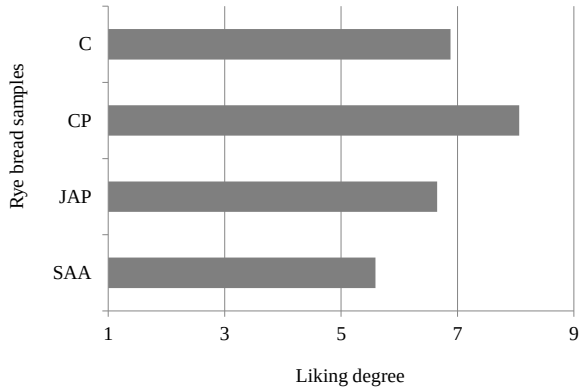

Figure 6. Hedonic evaluations of rye bread samples

C – control sample
JAP – rye bread with 12% *Jerusalem artichoke* powder
CP – rye bread with 9% chicory powder
SAA – rye bread with added sourdough with acetic acid bacteria

But in comparison with control sample and the rye bread with added 12% *Jerusalem artichoke* powder, doesn't have differences in degree of liking, because F_{cal}=3.17. But it should be noted that fructans concentration in products should not exceed 20 g per 100 g^{-1} dry matter. High intake of fructans can contribute to abdominal discomfort, flatulence but higher than 30 g per day can cause diarrhoea and vomiting (Den Hond et al., 2000).

Conclusions

Obtained rye bread with 9% chicory powder fructans content reached to 6.06 g 100 g^{-1}, but in bread with 12% *Jerusalem artichoke* powder – 5.63 g 100 g^{-1}; bread aroma and taste was not changed in comparison with traditional rye bread without additives. Using special microflora, like *Gluconobacter nephelii* acetic acid bacterium, it is possible to increase the fructans content in the final product. Fructans content in rye bread with developed sourdough (contain *Gluconobacter nephelii*) is higher compared to the control sample.

Acknowledgment

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RESEARCH OF HALF-FINISHED FROZEN BERRY PRODUCTS

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Abstract

The great significance of berries in human's diet is determined by the biologically active substances in them. Good quality is one of the main issues in every stage of freezing. It is necessary to research suitability of different berries cultivars to freezing, as well as influence of freezing on quality of the final product. Food quality and safety issues depend on the quality of raw stuffs and half-finished products. Research was carried out at the University of Agriculture, strawberries and blackcurrants grown in Latvia were used in the research. The freezing dynamics of fruits is characterised by temperature measurements in a layer and on its surface. Experience shows that only few products need very quick freezing, such us berries, whose quality is largely influenced by freezing rate. The aim of the research is the quality evaluation of processed products made of fresh and frozen berries. Desserts prepared from berries are products, quality of which depends on the storage conditions of half-finished products. The nutritive value of biologically active substances is retained, while the organoleptic indices worsen only a bit. The objective characterization of physical properties of products allowed to evaluate quality and to incorporate it into technological regimes of the treatment. Also new information was gained on the freezing processes that are mutually interconnected. It provides scientifically practical evidence for some technological developments of qualitative improvement in berry freezing methods and their usage range.

Keywords: human's diet, quality, quick freezing, dessert.

Introduction

Fruits and berries are considered as a commercially important and nutritionally essential food commodity due to providing not only the major dietary source of vitamins, sugars, organic acids, and minerals, but also other phytochemicals including dietary fiber and antioxidants with health-beneficial effects. In addition, fruits and vegetables provide variety in color, shape, taste, aroma, and texture to refine sensory pleasure in human's diet (Kader, 2002).

Practically it is not possible to preserve berries for a long time therefore it is necessary to consider their processing. The new developed product – frozen fruit and berry half-finished products of high readiness – will increase the range of existent products. Recently more and more innovations enter the manufacture of food products and one of them is food hydrocolloids. They are food additives, which are used to carry out certain functions of food products. At present one of the branches of food production, where there are many unused applications of food hydrocolloids, is fruit processing, which by all means occupies a remarkable part of food industry in Latvia. Research shows that freezing is one of the best methods of fruit and berry preservation. In fruit and berry processing frozen fruits and berries as raw materials are increasingly being used. Therefore research into the freezing impact on the quality of further food processing is essential. Up to now relatively few investigations have been carried out on the impact of freezing on the quality of the further processing fruits and berries (Aboltins et al., 2007). Quality of production is of great importance in application of new technologies in catering enterprises. Food quality and safety issues depend on the quality of raw stuffs and half-finished products. Raw and frozen berries are likely to be contaminated with bacteria, which may include food poisoning bacteria. Even when produced under hygienic conditions raw foods must be considered potentially hazardous. The HACCP approach was used for the potential hazard identification and risk assessment in all steps of berries processing. It aims to identify problems before they occur and establish measures for freezing temperature and storage time for their control at stages in production that are critical to ensuring safety of final product. Control is based on results of scientific investigations. The three main aspects influencing safety of frozen berries: quality of raw material; the used processing method; storage conditions, involving storage temperature and time, packaging method (Notermans et al., 2005; Sprenger, 2005). The nutritive value of biologically active substance is retained while the organoleptic indices worsen only a bit. The objective characterization of physical properties of products allowed evaluating quality, to incorporate this into the technological regimes of the treatment. Therefore research of freezing impact on the quality of further food processing is essential. Up to now relatively few investigations have been carried out on freezing impact on the quality of further processing berries. The aim of the research is the quality evaluation of processed products, which are made of fresh and frozen fruits and berries. During frozen storage berries maintain their typical sensory properties, nutritional value and their texture better than after other types of processing (Ancos et al., 2000). However, only small portions of berries are consumed unprocessed after frozen storage, mostly they are further processed in different products and even further processed in catering technologies. Food quality and safety issues is another aspect of the quality in raw stuffs and half – finished products.

Materials and Methods

The study was done in Institute of Horticulture at the Latvia University of Agriculture. Research object: puree from fresh strawberries – 'Polka', 'Honeoye' and blackcurrants 'Selechenskaya' and 'Zagadka' grown in

Latvia. An actual berry puree freezing temperature was -25 °C– -30 °C, the layer thickness was 100 mm. For the determination of the layer density weight-volume method was used. Puree from the same berries was made also immediately after harvesting.

The technology of the puree making was the following: berry sorting, extrusion of puree (mechanically through sieve), adding sugar (30%) and homogenising, putting into containers (500 g), air-freezing at -25 °C, stored 3 and 6 months at -18±2 °C.

Content of vitamin C was used as an index of quality during freezing. The content of ascorbic acid was determined by titration with 0.05 M iodine solution. Alternative hypothesis is accepted with 99% probability ($p<0.0001$) – the mean of the fourth index of samples 1–4 is less than the mean index of samples 5–8. It is indicated by the t-test.

Multivariate ANOVA without replications was performed to test the significance of the influence of freezing. Addition of structurisers – gelatine 6 g per 100 g maturation in water 30 min., dissolves by heating to 60 °C, cooling to 40 °C, adding to puree. Jellies were made from frozen puree after thawing.

The most important attribute of gelatine is its gel strength when determined by the standard method. This is the force in grams required to press a 12.5 mm diameter plunger 4 mm into 112 g of a standard gelatine gel at 10 °C. Several penetrometer type instruments have been adapted to determine Bloom Strength (Cole et al., 2000).

A frequent question is how to substitute gelatine of one Bloom Strength for gelatine of another. As a guide one can say:

$$C \times B^{½} = k$$

$$\text{or } C_1(B_1)^{½} \div (B_2)^{½} = C_2,$$

where: C = concentration; B = Bloom Strength; k = constant, however, there are other considerations besides gel strength which can invalidate such a substitution calculation.

For example, in a gummy formulation, the texture using 250 Bloom gelatine is far shorter than when 180 Bloom gelatine is used.

Data was processed using 2-factor nonlinear regression analysis method. The second row two-argument function $Z = Z(x, y)$ is examined and using the least square method such coefficients α_1 of the function are searched, with which the square difference between the distances of experimental data and appropriate theoretical data would be minimal. Since the impact of the factors is nonlinear, application of the first row – argument function would be incorrect. To evaluate the compliance closeness of the theoretical coherence, determination coefficient η^2, describing the compliance of obtained theoretical coherence with experimental data, is calculated. To process experimental data mathematical programme packages MathCad and Matlab were used.

Results and Discussion

The new products are developed on the bases of plant raw materials. These raw materials are not much researched; cultivars with high content of anthocyanins, flavonoids, carotenoids, and ascorbic acid are not selected. In order to improve continuous supply of Latvian inhabitants with locally produced good quality products of fruits and berries, it is necessary to carry out their complex study (Kampuse et al., 2003).

Therefore for each kind of product appropriate conditions have to be chosen for freezing, as also the state of products before freezing has to be taken into consideration to diminish to the minimum the harmful influences on their quality. An advanced preservation method – freezing is used, which enables obtaining a product with a high content of biologically active substances. It is provided by vitamins, enzymes and other substances included in berries, preservation of which is possible by using positive properties of quick freezing. Biologically active substances very quickly change into less valuable substances if the products are continuously or incorrectly treated (Kampuse et al., 2003).

Good quality is one of the main challenges in every stage of freezing berries. On the basis of worked out functional analysis of freezing, it is possible to optimize regulation possibilities of heat processes for a particular kind of berries. Freezing and storage in frozen condition substantially change content of the most labile vitamin of berries – vitamin C, what proves that maximum loss after freezing of blackcurrants does not exceed 20%. The most important changes after frozen storage are losses of water soluble vitamins (ascorbic acid, panthotenic acid) (Fellows, 1996).

To increase the storage time of production and not cause serious qualitative changes, it is necessary to determine the important question: how the freezing temperature and storage time influences preservation of the product. For the qualitative aspect using losses of vitamin C as the basis of measurement found that changes of vitamin C in prepared berries puree depended on storage time and temperatures. These measurements are shown in a contour plot (Fig. 1). Apparently the least loss of vitamin C is found at storage temperatures between -26 to -21 °C, that can be connected with the transformation of most part of water into ice and lowering the temperature of the frozen product at this temperature limit occurs the radical changes of product quality.

By mathematical processing of experimental data correlation has been obtained between loss of vitamin C in puree depending on the storage time of the puree t (months) and storage temperature T (°C).

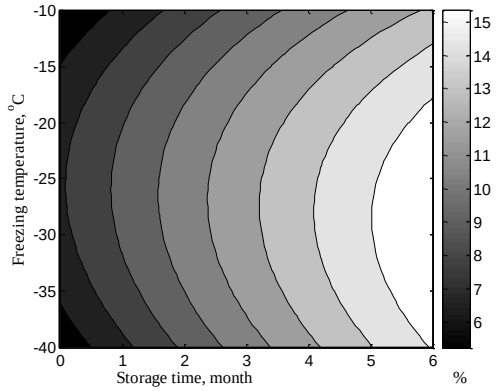

Figure 1. Influence of the freezing temperature and storage time on the preservation of strawberries puree

Thus loss of vitamin C (%) in strawberries puree during storage is characterised by correlation:

$$z = 1.5 \cdot t - 0.05 \cdot t^2 - 0.51 \cdot T - 0.01 \cdot T^2 - 0.012 \cdot t \cdot T + 1.06,$$

where: t - storage time (month); T - storage temperature (°C); z - loss of vitamin C (%).

Analyzing the obtained measurements it is apparent, that storage time plays an important role in the lessening of the content of vitamin C. The interconnection of storage time and the temperature of storage where in berries it is positive (i.e. goes down the level of vitamin C). The contour plot shows the correlation between storage temperature T, storage time t and the loss of vitamin C in berries. The amount of loss depends on the temperature of storage. The duration of storage doesn't considerably influence loss of vitamin C; it does only at lower temperature.

The new developed product – frozen half-finished fruit and berry products of high readiness – will increase the range of existent products. Food hydrocolloids are food additives, which are used to carry out certain functions of food products. Researchers showed that it is possible to store berry purees in frozen condition with no essential quality changes in comparison with fresh purees because essential difference between fresh and frozen puree was not established. Freezing was used in the research both, for continuous storage of berries, and preparation of jellies made of fresh berries, as half-finished products of high readiness. If a gelled jelly is frozen, the product will suffer from syneresis and on thawing the clear jelly will disintegrate with much exuded water. However, if water containing 0.5% gelatine is frozen, the water will freeze as millions of small discrete crystals, instead of forming a single solid block of ice. Gelatine is an amphoteric protein with isoionic point between 5 and 9 depending on raw material and method of manufacture. The only other animal product containing hydroxyproline is elastin and then at a very much lower concentration, so hydroxyproline is used to determine the collagen or gelatine content of foods (Barbosa-Canova et al., 2000).

Range of goods or services will increase related to development of many more appealing new products concerning texture and flavours. At the same time it would provide the consumer with a healthy product, because hydrocolloids are nature polymers with certain functions.

Widely applied gelatine was used in researches as it is more studied. Whereas for the mass with only gelatine added firmness of the product increases by increasing gelatine concentration (Fig. 2). The results indicate that the best solidity of the product is gained with only gelatine added of concentration 45 g per100 g of fruit mass. Researchers showed that mathematical correlation is possible between jelly forming ingredients what is indicated by the high determination coefficient $\eta^2 = 0.93$ and correlation expression:

$$p = 173 - 7.45 \cdot g + 0.12 \cdot g^2 + 6.7 \cdot s - 0.034 \cdot s^2 - 0.17 \cdot s \cdot g,$$

where: p – impact of the substance in jellies, %; s – sugar, g; g – gelatine, g.

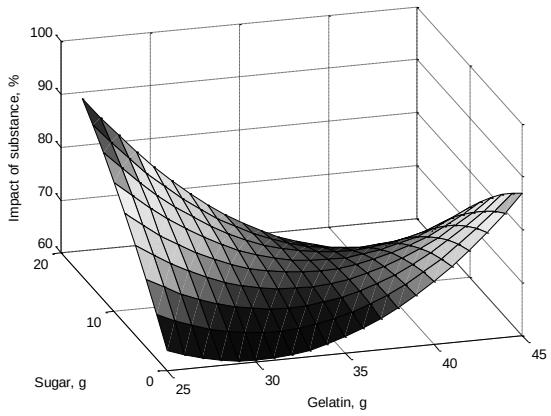

Figure 2. Impact of sugar and gelatine on jellies

The results of the research indicate that concentration of gelatine content influences the texture of the product. It is established that gelatine forms a very stabile disperse phase which provides higher stability during freezing and retains the shape of the product after thawing. Taking into consideration that nature of gelatine containing products rely on Hooke's law, concentration and temperature parameters are considered in the research. Polymer carbohydrates and proteins, included in the content of products, taking part in formation of jellies, if they are hydrated up to a particular level, are considered in the technological process. Researches show that the most efficiency is gained with sugar content 9–16 g at gelatine content 25–27 g per 100 g, when impact of substance in jellies reaches more than 80%.

Fruit jelly is a kind of half-finished product little used in practice, which is connected with the limited proportion of resources and equipments. By adding not only gelatin but also sugar to the fruit mass, solidity of the product decreases. Whereas for the mass with only gelatin added firmness of the product increases by increasing gelatin concentration. The results indicate that the best solidity of the product is gained with only gelatin added of concentration 45 g per 100 g of fruit mass.

Ice formation in food products takes place at relatively low temperature and products stored at the temperature

-18 °C are not yet fully frozen. Products containing higher concentration of small molecular sugar at low temperature contain more unfrozen water because quick frozen carbohydrate solutions as well as most of biological products are characterised by non-equilibrium formation of ice crystals with its specific concentration of solution (Kampuse et al., 2007). This is ascertained taking into consideration such storage temperature which is the temperature practically used also in Latvia. Substantial diversity was established in the blackcurrant jellies made of fresh and frozen berries, what proves that freezing of raw material influences quality of blackcurrant jellies.

Range of goods or services will increase related to development of many more appealing new products concerning texture and flavours. At the same time it would provide the consumer with a healthy product, because hydrocolloids are nature polymers with certain functions. In fruit and berries processing frozen berries as raw materials are increasingly being used. Half-finished products containing biologically active substances, healthy berry products requiring minimal expenditure of time and energy in order to reach adequate consumption quality are researched. On the bases of the research carried out it will be possible to develop technological instructions and regulations for the cultivars approbated in Latvia indicating the volume of natural mass losses, treatment and storage conditions, as well as economic effect for successful establishment of commercial orchards. As a result of the research a data base will be developed for technical and informative indices, which will enable to improve the technological process of storage, ensuring quality maintenance of berries and biological properties by minimal losses.

Conclusions

Development of the new product foresees reduction of these losses to the utmost already at the initial stage of the process, what is connected with correct preparation and storage of raw materials.

The results of the research indicate that concentration of the gelatine content influences the texture of the product. Further researches are necessary for the optimum usage of the product texture in order to enable use of fresh and frozen berries.

Frozen mousse contained significantly less vitamin C than fresh and frozen berries. It means that more ascorbic acid retains when frozen currants are kept as whole berries, not processed in mousse.

The obtained mathematical expression indicates that efficiency of sugar decreases if it is added more to the mass, with gelatine it is contrary – the more it is added, the efficiency increases; although by adding both, the efficiency decreases. It means that the limit has to be found, up to which this addition is with the positive character.

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APPLICATION OF DIFFERENT ANTI-BROWNING AGENTS IN ORDER TO PRESERVE THE QUALITY OF APPLE SLICES

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Abstract

Apple cultivar 'Auksis' was chosen for testing in the research of anti-browning efficiency by slicing and treating them with anti-browning agents: solutions of sea buckthorn, quince and currant juices and NatureSeal® AS1. Therefore, impact of the treatment by sea buckthorn, quince and white currant diluted juice, and solution of 4% and 5% antioxidant NatureSeal® AS1 on browning of fruit slices was evaluated. Strained apple slices were packed into polypropylene boxes and stored in refrigerator for 12 days at 4 °C temperature. During storage samples were analysed every third day by estimating changes in colour, content of vitamin C, total phenolic content, antioxidant activity (FRAP, DPPH). The best results of efficiency for used anti-browning agents on the quality of fresh-cut apple slices were reached by treating them with 5% NatureSeal® AS1 solution. The selected anti-browning agent provided higher content of vitamin C, phenolic compounds, antioxidant activity (FRAP, DPPH) in the product, as well as maintained initial light colour of apple slices in comparison with other anti-browning agents.

Keywords: fresh-cut, anti-browning, colour, vitamin C, phenolics, antioxidant activity.

Introduction

In last few years supply of fresh-cut fruits in market has raised due to society's awareness of fruits potential health benefit. The polyphenolic antioxidants found in apples are responsible for most of the antioxidant activities of the fruit (Liu, 2003).

Fresh fruit browning reaction is one of the main challenges for fruit processing industry. During fruit peeling or cutting fruit cell membranes excrete cell substrate, which contains polyphenol oxidases that at the presence of oxygen dehydrogenate polyphenols to unstable hinons. These compounds are responsible in further reactions for development of dark-coloured pigments (Arias et al., 2009). To prevent browning reactions different additives could be used – ascorbic acid, citric acid, calcium propionate, calcium lactate, calcium ascorbate, carboxylic acids, chelators, thiol-containing compounds, cysteine, glutathione or specific enzyme inhibitors such as 4-hexylresorcinol (Chiabrando, Giacalone ,2013; Rojas-Graü et al., 2006; Oms-Oliu et al., 2010; Gomesa et al., 2010). Different substances and their compositions are described as anti-browning agents for fresh-cut fruits (e.g. Rojas-Graü et al., 2006; Suttirak, Manurakchinakorn, 2010). Combined treatment methods are thought to be more effective (González-Aguilar, 2000).

Nowadays consumers focus not only on appearance and shelf-life of fresh-cut fruits but also are beware on synthetic additives used for colour improvement and consistency retention (Corbo et al., 2009). Therefore, impact of different natural additives instead of artificial ones against fruit browning have been studied: grapefruit seed extract (Park et al., 1999), onion extract (Kim et al., 2005), rice bran extract (Theerakulkait, Boonsiripiphat, 2007) and rosmarine extract (Xiao et al., 2010). Toivonen (2008) stated, that combined browning inhibitors NatureSeal® AS1 and AS5 could be successfully used, whereas Jeon and Zhao (2005) applied honey as anti-browning agent for fresh-cut apples and got good inhibition quality. Krasnova et al. (2011) was used in experiments natural juice of cranberries (*Vaccinium macrocarpon Ait*) to made combinations of several browning inhibitors, which tested to provide the quality of fresh cut pear salad. There are limited data about use of natural juices to prevent this undesirable process in fresh-cut apples – Perera et al. (2010) studied pineapple juice and Son et al. (2000) rhubarb juice, but for fresh cut pears slices treatment Krasnova et al. (2013) was tested Japanese quince (*Chaenomeles japonica)* juices dilution.

The aim of the research was to evaluate sea buckthorn (*Hippophae rhamnoides* L.), quince (*Chaenomeles japonica*), white currant (*Ribes rubrum* L.) juice and antioxidant NatureSeal® AS1 impact on quality of apple slices. Quality measurements were vitamin C, content of total phenols, antioxidant activity and titratable acidity as well as colour measurements.

Materials and Methods

The research has been conducted at the Processing and Biochemistry Department of the Institute of Horticulture, Latvia University of Agriculture (Formerly Latvia State Institute of Fruit-Growing) in Dobele. In this study cultivar of apples (*Malus domestica* L.) 'Auksis' was used. After harvesting apples were stored for one week at temperature +4 °C and relative humidity 90%.

In the present study complex inhibitors NatureSeal® AS1 (AgriCoat Ltd., Great Shefford, UK) for apple slices and NatureSeal® FS for surface of whole apples were taken. Producer AgriCoat NatureSeal® has recommended to prepare approximately 6% concentration of NatureSeal® AS1 water solution for the apple slices anti-browning treatment (AgriCoat NatureSeal® Apple Protocol). Solutions of NatureSeal® AS1 (4% and 5%) were prepared 1 h before experiments. For the apple slices treatment were prepared two solutions of the NatureSeal® AS1, to find the lowest concentration of inhibitors

NatureSeal® AS1. Natureseal® FS for treatment of apple surfaces were dissolved in distilled water till solution pH 2.4. That is recommended amount of FS which is added to water to make a solution with a pH 2.4. This will be generally depended a 1.0–1.5% solution concentration, but will be related on local water pH (AgriCoat NatureSeal® Protocol for Washing).

As anti-browning materials pasteurized natural sea buckthorn, quince and white currant juices were used. Sea buckthorn juice after pasteurization was left to settle down, after 24 hours clear part divided and used for experiments. Samples were treated by diluted juices. The dilution ratio was 2 : 8 (20% juice and 80% water). The watered natural juices (concentration 20%) was made by mixing of 20 mL natural juice and 80 mL water, following by pasteurization at temperature 95 °C and cooling till ambient temperature 20±2 °C. Control sample was treated by distilled water.

Whole apples were washed in running water and whole dipped for 30 minutes in NatureSeal® FS to eliminate microbiological contamination. After that apple was peeled and cut into 1.5–2.0 cm slices and dipped in NatureSeal® AS1 solutions, diluted juices or distilled water (control sample) for 10 minutes, then strained on a sieve for another 10 minutes. Treated apple pieces were packed in the polypropylene boxes in air atmosphere condition, and quickly covered with lids and stored in refrigerator at temperature +4±0.5 °C for 12 days. Samples (in 3 replicates) were analysed after 0, 3, 6, 9, and 12 days.

The packaged samples were labelled as follows: Contr-control; SBJ-diluted sea buckthorn juice; QJ-diluted Japanese quince juice dilution; WCJ-diluted white currants juice dilution; 4NS – 4% NatureSeal® solution; 5NS – 5% NatureSeal® solution. Accordingly, further in the paper below tables and figures will be indicated only abbreviation without a full name of the sample.

Colour characteristics (Hunter L* a* b*) were measured for 20 apple slices using a colorimeter ColorTec-PCM BenchTop Model-45. The colour of apple flesh was expressed as whiteness index (WI), which was calculated using colour indices L*, a* and b* as reported by Albanese et al. (2007). For determination of titratable acidity content (% malic acid 100 g^{-1}) 20 g of sample weighted and blended with 100 mL distilled water. 25 mL of filtrate were used for titration with 0.1 N NaOH till pH 8.1. For the potentiometric titration pH meter (Jenway 3510) with combined electrode was used. The content of vitamin C (ascorbic acid content) was determined by the standard method EN 14130:2003 "Foodstuffs – Determination of vitamin C by HLPC". Total phenolic (TP) content was determined by the photometric method using Folin-Ciocalteau reagent by Singleton et al. (1999). The absorbance of blue colour was measured at wavelength 760 nm. The total phenol content was expressed as gallic acid equivalent per 100 g^{-1} of fresh weight of apple sample (mg GAE 100 g^{-1} FW).

Two methods were used to measure the antioxidant activity (AOA): radical scavenging activity method using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) method. DPPH analysis was conducted according to Brand-Williams et al. (1995) with minor modifications. 100 µL of apple extract in ethanol mixed with 2900 µL of a DPPH ethanol solution. FRAP analysis was done according to Benzie and Strain (1996) with some modifications. Experiment was performed using 2700 µL of FRAP reagent which was mixed with 300 µL of apple extract in ethanol and incubated for 10 min at 20 °C. The antioxidant activity was expressed as mmol of Trolox equivalent per 100 g of fresh weight of apple sample (mmol TE 100 g^{-1} FW).

Data processing was carried out by General Linear Model procedure SPSS 15 software package. Sheffe criterion was used in the clarification of significant differences ($p<0.05$) among studied samples. Closeness of the relationship between the parameters was determined by analysis of Pearson correlation coefficient. A correlation between storage time (days), total phenol, content of vitamin C and antioxidant activity in fresh-cut apple sample has been found using experimental data and program MathCad package for statistical multi parametrical processing.

Results and Discussion

Colour indicators L* value and whiteness index (WI) reflects the colour of fresh-cut apple slices equivalent (Mao et al., 2007). In addition, the L* value reflects the changes associated with the browning of apple surface (Lu et al., 2007). At the beginning of the research samples of the diluted sea buckthorn juice (SBJ) showed better inhibitor properties compared to samples with quince juice (QJ) and white currant (WCJ) juices (Fig. 1 and 2). The colour of these sample after became lighter, L* value and whiteness index were higher in comparison with the control sample, which was dipped in distilled water ($p<0.05$).

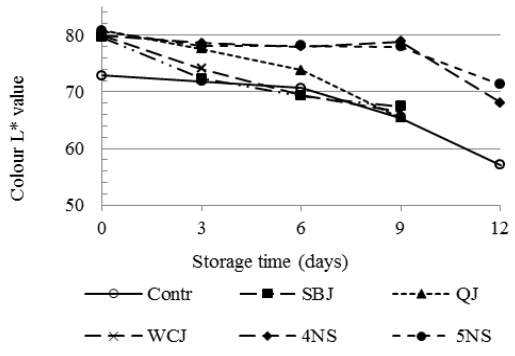

Figure 1. Changes in colour component L* in fresh cut apples slices during storage time

Contr – control; SBJ – diluted sea buckthorn juice; QJ – diluted Japanese quince juice dilution; WCJ – diluted white currants juice dilution; 4NS – 4% NatureSeal® solution; 5NS – 5% NatureSeal® solution.

Relatively good results also showed samples treated by diluted sea-buckthorn and white currant juices. 4% and

5% solutions of NatureSeal® AS1 showed good browning inhibitor properties compared with the control. Fresh-cut apple slices had light colour. As it was mentioned by other researchers the fastest browning of fresh-cut fruit slices proceeded in the first days that is associated with enzyme activity (Perez-Gago et al., 2006). During the further storage colour of samples dipped in diluted natural juices, became distinctly darker. L* value of these samples decreased on average by 17.3% after 9 days of storage.

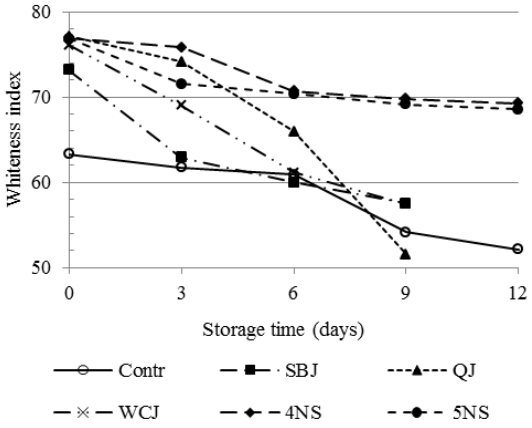

Figure 2. Changes of Whiteness index in fresh cut apples slices during storage time

Contr – control; SBJ-diluted sea buckthorn juice; QJ-diluted Japanese quince juice dilution; WCJ-diluted white currants juice dilution; 4NS – 4% NatureSeal® solution; 5NS – 5% NatureSeal® solution.

It was found that the colour intensity decreased by 10.5% in comparison to the control sample, while for samples 4NS and 5NS treated by NatureSeal® AS1 L* value reduced by only 2.2%. In the last day of the study apple slices dipped in diluted natural juices were deteriorated and were not analysed. Whereas, samples 4NS and 5NS apple slices quality at the end of the storage time treated with the browning inhibitor NatureSeal® AS1 was retained.

At the beginning of the study titratable acidity (TA) in fresh-cut apple slices treated by diluted natural juices were on average of 1.32%, for treated by NatureSeal® AS1 and 0.76% for the control sample 0.97% (Table 1). The largest decrease in TA was found in the first days, which was associated with a respiration rate of apple slices after peeling and cutting. Organic acids in combination with other compounds joined in respiratory reactions that resulted in the decreasing of acid content in fresh-cut fruits, but increasing of pH (Bico et al., 2009). At the ninth day of the study TA for the samples SBJ, QJ, and WCJ decreased in average of two times, but for samples 4NS and 5NS decreased slightly. In addition, the acid content on 9th and 13th day of samples dipped in NatureSeal® AS1 did not differ significantly ($p=0.26$).

Diluted natural juices were used as browning inhibitor for apple slices treated, those little increasing of the vitamin C content in treated samples in comparison with the control (Table 2). Complex inhibitor NatureSeal® AS1, due to its composition ensured the highest content of vitamin C at the beginning of research. The significant changes of the vitamin C content after three days of storage were observed ($p<0.05$). Ascorbic acid is usually degraded by oxidative processes, which are stimulated by the presence of light, oxygen, peroxides and enzymes, such as ascorbate oxidase or peroxidase (Plaza et al., 2006). After 9 days of storage, the vitamin C content in the samples dipped in diluted sea buckthorn juice was higher in comparison with the sample dipped in diluted quince, and white currant juices. Overall at the end of the study content of vitamin C was significantly higher for samples treated by NatureSeal® AS1 in comparison with control.

Content of total phenolic (TP) was significantly ($p<0.05$) higher in the samples treated by inhibitor NatureSeal® AS1 (in average 146 mg 100 g^{-1} FW), but in samples dipped in diluted natural juices in average of 2 times less (in average 87.2 mg 100 g^{-1} FW).

Table 1

Titratable acidity content changes in fresh cut apples slices during storage time

Sample	Storage time, days				
	0	3	6	9	12
Contr	0.97±0.04*	0.77±0.01	0.69±0.10	0.63±0.01	0.67±0.00
SBJ	1.23±0.01	0.77±0.01	0.72±0.00	0.71±0.01	ND
QJ	1.37±0.01	0.90±0.02	0.82±0.01	0.67±0.00	ND
WCJ	1.37±0.01	0.77±0.01	0.62±0.00	0.60±0.01	ND
4NS	0.70±0.02	0.70±0.02	0.66±0.02	0.63±0.01	0.63±0.01
5NS	0.81±0.05	0.77±0.03	0.63±0.03	0.63±0.01	0.60±0.02

* Titratable acidity, %, malic acid 100 g^{-1} FW, ND – not analysed.

Table 2

Vitamin C content changes in fresh cut apples slices during storage time

Sample	Storage time (days)				
	0	3	6	9	12
Contr	11.50±0.03*	10.73±0.37	10.58±0.92	7.35±0.23	7.40±0.31
SBJ	17.41±0.80	12.48±0.28	11.98±0.28	10.27±0.62	ND
QJ	28.46±2.12	13.75±0.30	10.71±0.34	8.24±0.31	ND
WCJ	11.22±0.19	11.47±0.16	11.30±0.46	8.21±0.17	ND
4NS	72.40±0.35	49.48±0.60	30.34±0.75	24.09±0.32	19.71±0.25
5NS	82.35±0.36	27.35±0.41	30.17±1.85	20.39±0.24	20.34±0.17

* Vitamin C content mg 100 g^{-1} FW, ND – not analysed.

Table 3

Total phenolic (TP) content changes in fresh cut apples slices during storage time

Sample	Storage time, days				
	0	3	6	9	12
Contr	47.37±4.71*	69.81±1.63	85.32±2.16	35.12±0.73	34.77±1.61
SBJ	88.20±6.47	72.87±1.57	56.70±2.51	46.77±2.70	ND
QJ	85.42±7.74	79.75±4.42	37.57±2.38	28.51±0.38	ND
WCJ	87.95±6.58	83.28±3.98	37.37±2.01	33.03±1.33	ND
4NS	229.38±6.90	163.57±2.73	140.73±0.59	148.23±1.76	148.15±4.12
5NS	145.28±2.98	144.94±2.57	138.01±0.26	138.33±4.10	138.15±0.65

* TP content, mg gallic acid 100 g^{-1} FW; ND – not analysed.

The lowest TP content (47 mg 100 g^{-1} FW) was for control sample (Table 3).

TP content of the apple slices during storage decrease and after 9 days storage different results was observed. The sample dipped in diluted sea buckthorn juice contained two times, while the samples dipped in diluted quince and white currant juice contained in average three times less of TP. The inhibitor NatureSeal® AS1 showed the best results, and at the end of the study these samples contained a significant amount of TP. Similar results were also observed in other studies, where minimal changes in content of phenolic were observed in fresh-cut apple treated by this browning inhibitor (Rößle et al., 2009). Cocci et al. (2006) observed that there is positive correlation between ascorbic acid and content of TP due to reducing of ascorbic acid activity that prevents a degradation of phenolic compounds. In our case Pearson correlation coefficient between the content of vitamin C and TP r = 0.76 was observed.

Using the free radical scavenging activity (DPPH) and the ferric reducing ability (FRAP) was applied for measurement fresh-cut apple sample antioxidant activity (AOA) (Fig 3. and 4.)

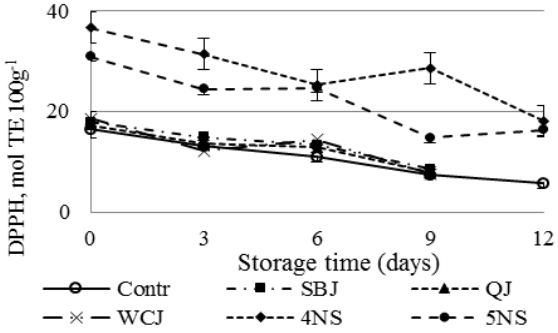

Figure 3. Antioxidant activity DPPH changes in fresh cut apples slices during storage time

Both approaches showed similar results during the study. At the first day AOA activity of all samples was significantly ($p<0.5$) higher than that of the control sample (Fig.4).

This trend continued throughout the whole period of study. It could be explained by TP and vitamin C content in the samples. A strong positive correlation between parameter of antioxidant activity there was found: between TP and AOA (r = 0.83 and r = 0.88) as well between vitamin C and AOA (r = 0.83 and r = 0.76, respectively by DPPH and FRAP).

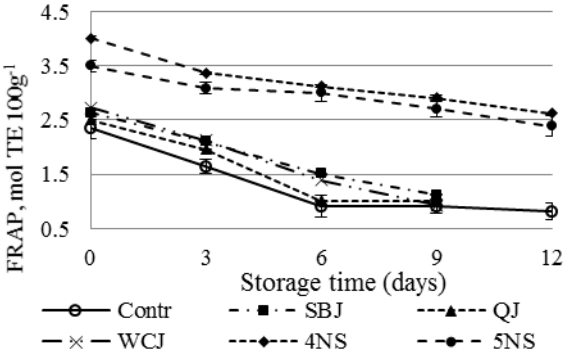

Figure 4. Antioxidant activity FRAP changes in fresh cut apples slices during storage time

As mentioned in study using pineapple, content of vitamin C is strongly correlating with antioxidant capacity determined by both DPPH radical scavenging activity and FRAP reducing power (Kongsuwan et al., 2009).

Antioxidant activity during storage was presumed as main evaluation quality criteria of the fresh cut apple slices after treatment by diluted juices and solution of 4% and 5% antioxidant NatureSeal® AS1 from anti-browning. Changes of the correlation between total phenolic, ascorbic acid (AA) content and antioxidant activity (FRAP) depend mostly on the storage time were found in the study.

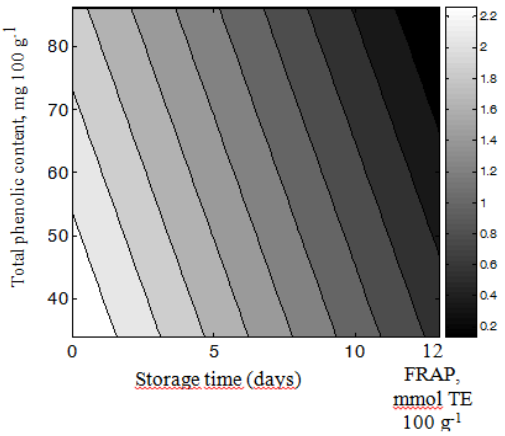

Figure 5. Changes of the correlation $\eta^2 = 0.97$ between total phenolic content and antioxidant activity (FRAP) in storage time of control sample

The graphical interpretations of these analysed parameters in the control sample are shown in Fig. 5 and Fig. 6.

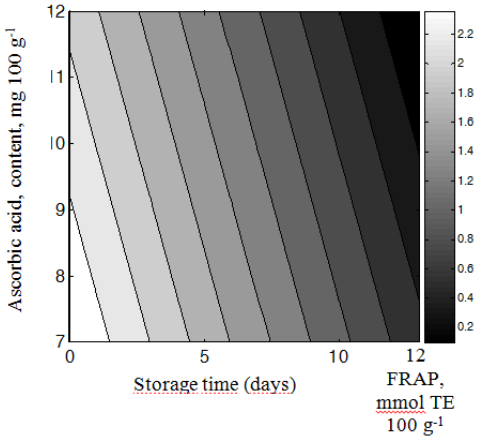

Figure 6. Changes of the correlation $\eta^2 = 0.87$ between ascorbic acid content and antioxidant activity (FRAP) in storage time of control sample

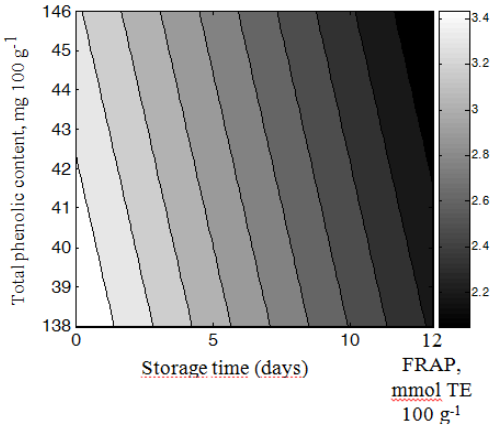

Figure 7. Changes of the correlation $\eta^2 = 0.98$ between total phenolic content and antioxidant activity (FRAP) in storage time of 5% NatureSeal® AS1 solution sample

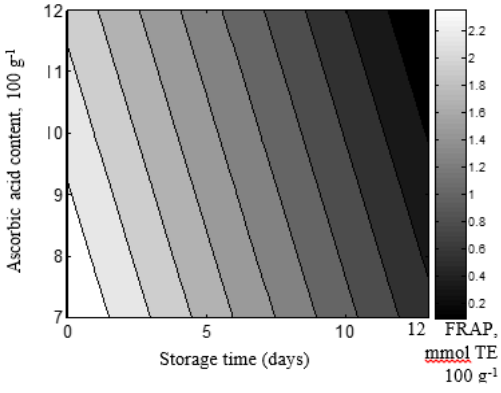

Figure 8. Changes of the correlation $\eta^2 = 0.96$ between ascorbic acid content and antioxidant activity (FRAP) in storage time on 5% NatureSeal® AS1 solution sample

The higher correlation was determined in the control sample during the storage time between total phenolic content and antioxidant activity ($\eta^2 = 0.97$), but between ascorbic acid content and antioxidant activity (FRAP) was smaller ($\eta^2 = 0.87$).

Very good results of the correlation between total phenolic and antioxidant activity (FRAP), ascorbic acid and antioxidant activity (FRAP) during storage time showed sample 5NS (Fig.7 and 8.). Between total phenolic content and antioxidant activity (FRAP) $\eta^2 = 0.98$ and between ascorbic acid content and antioxidant activity (FRAP) $\eta^2 = 0.96$.

Conclusions

The present study verified that the best effect on fresh-cut apple slices has 5% solution of complex inhibitor NatureSeal® AS1. Treated samples showed the highest L* value, content of vitamin C and antioxidant activity during the all storage time.

The highest total phenol content was detected in samples treated by 4% solution of NatureSeal® AS1. Using of diluted natural juices for treatment of apple slices, samples dipped in diluted quince juice had higher L* value and contained more vitamin C in the first three days of storage. Superior antioxidant activity by FRAP and DPPH showed diluted white currant juice on the first day, while diluted sea buckthorn juice by DPPH on the third day of storage. In general, treatment by diluted natural juices ensured good quality of fresh-cut apple slices in the first days of storage. However, such treatments did not provide stability of samples during the all storage due to changes of quality parameters.

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THE EFFECT OF HYDROGEN PEROXIDE, OZONISED WATER AND *NATURESEAL*® AS5 SOLUTION ON THE MICROBIOLOGICAL PARAMETERS OF FRESH-CUT CARROT

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Abstract

Innovative (ozonised water, hydrogen peroxide) treatment solutions is one of the newest treatment agents used to decrease the count of microorganisms in vegetables. *NatureSeal*® AS5 is a commercial solution used for the treatment of vegetables worldwide. The aim of this work was to study the effect of the various treatment solutions – hydrogen peroxide, ozonised water and *NatureSeal*® AS5 (in various concentrations and for different treatment time) with the main purpose to ensure the microbiological safety of fresh–cut carrots. Non–treated carrots were used as control sample. The following treatment regimes were investigated: ozonised water treatment time 60; 120; 180±1 s and concentrations 1.0; 2.0; 3.0 mg L^{-1}; hydrogen peroxide solutions, treatment time 30±1 s, 60±1 s 90±1 s and concentrations 0.5%; 1.0% 1.5%; *NatureSeal*® AS5 water solution 2.5% 5 min ± 1 s were prepared for treatment of shredded carrots. *E.coli* in fresh carrots was not detected. Mathematical processing of the obtained results revealed that various concentrations of hydrogen peroxide ($p=0.019$; $\alpha=0.05$) and treatment times ($p=0.049$; $\alpha=0.05$) significantly influenced the TPC colony forming units in carrots. The obtained results prove the treatment agent properties of hydrogen peroxide. The comparison of chosen treatment regimes detected a significant ($p=0.003$; $\alpha=0.05$) influence of the ozonised water on the decrease in the count of TPC colony forming units in carrots after treatment. During experiments it was proved that *NatureSeal*® AS5 solution could be recommended as treatment agent for the treatment of fresh shredded carrots.

Keywords: hydrogen peroxide, ozone, *NatureSeal*® AS5, microbial contamination, carrot

Introduction

Carrot is one of the important root vegetables rich in bioactive compounds like carotenoids and dietary fibers with appreciable levels of several other functional components having significant health–promoting properties. The consumption of carrot and its products is increasing steadily due to its recognition as an important source of natural antioxidants having anticancer activity (Sharma et al., 2012).

Minimally processed vegetables (MPV) are prepared and handled to maintain their fresh nature while providing convenience to the consumer, as ready-to-eat. Producing MPV involves cleaning, trimming, peeling, coring, slicing, shredding, washing, etc. (Augspole et al., 2014; Ayhan et al., 2008). Fresh–cut carrots can be found in the market place as: whole peeled (baby), sticks, or sliced, shredded, grated and diced. There is a shelf-life limitation for minimally processed carrots from 4 to 5 days due to high respiration rate, development of off–flavour, acidification, and loss of firmness, discolouration, and microbial spoilage (Augspole, Rakcejeva, 2013). The only step for reducing microorganisms during processing is washing. A variety of disinfectants (hydrogen peroxide and ozone) have been used to reduce bacterial populations on fruit and vegetables (EU Scientific Committee on Food, 2002). *Alegree et al*. (2013) reported that these treatment agents have proved incapable of completely removing or inactivating microorganisms on fresh produce.

One of the new approaches is the use of "generally recognized as safe" (GRAS) compounds due to minimal concerns about their environmental impact and low residues in the treated commodity (Loredo et al., 2013). The US Food and Drug Administration (FDA, 2011), under the ruling 21 C.F.R. 173.315, has approved the use of hydrogen peroxide as plant protection agent in the processing of fresh fruits and vegetables (Rodrigues et al., 2012). Hydrogen peroxide is a strong oxidizing agent proposed as an alternative for decontamination of fruits and vegetables due to its low toxicity and safe decomposition products (Alexandre et al., 2012; Loredo et al., 2013). Hydrogen peroxide has been shown to inactivate a wide variety of infective biological agents ranging from the vegetative cells and spores of bacteria and fungi, protozoa and their cysts, viruses and even prions (Malik et al., 2012; Delgado et al., 2012; Loredo et al., 2013).

The US Food and Drug Administration (FDA, 2011), under the ruling 21 C.F.R. 173.315, has approved the use of ozone as plant protection agents in the processing of fresh fruits and vegetables. The fresh produce industry is showing interest in O_3 applications due to growing consumer preference for minimally processed foods, frequent outbreaks of food–related illnesses, identification of new food pathogens, and the passage of legislation governing food quality and safety (Rodrigues et al., 2012; O'Donnell 2012). Within the food industry, ozone has been used routinely for washing and storage of fruits and vegetables. Ozone can be bubbled through water into which it will partially dissolve. This ozonised water then can be used for washing and/or in transfer flumes to reduce the microbial loads of berries and other fruits and vegetables. Controlled studies report that ozonised water may actually provide greater than 90% reduction of total bacterial counts for some vegetables. Such

treatments also have been shown to reduce fungi populations and, subsequently, reduce fungal decay (Farid, 2010). Ozone is a naturally occurring substance found in our atmosphere and it can also be produced synthetically. The characteristic fresh, clean smell of air following a thunderstorm represents freshly generated ozone in nature (Farid, 2010). Therefore, O_3 technology is a good option for the wash–water disinfection for the fresh-cut industry, because it will reduce the need for water replacement and for high sanitizer concentration.

A commercially available anti-browning agent *NatureSeal*™ is a calcium ascorbate powder used extensively in the fresh cut industry. Ascorbic acid functions as reducing agent to deter surface browning and $CaCl_2$ treatment provides tissue firming and has been reported to reduce browning (Saha et al., 2009; Rößle et al., 2009). This is the first commercial antioxidant product of its kind that doesn't have a bad aftertaste or residue (Asrey et al., 2008). Initially developed for apples and pears, the technology has been expanded for use on 19 different produce items including fresh-cut carrots (Agricoat, 2010). Using *NatureSeal*™ preparations carrot processing can adjust the pH of the product − reducing, hindering the development of microflora. *NatureSeal*® is sold commercially to food retailers who use the coating to treat fresh–cut fruits and vegetables. It uses a special blend of vitamin salts and minerals to extend the shelf life of sliced fruits for up to 21 days under refrigeration, without detectable changes in colour, flavour or texture (Asrey et al., 2008).

There are several processing steps in the fresh–cut produce production chain and many points for potential microbial contamination exist in each of these steps. The objective of the current research was to evaluate the effect of the various treatment solutions – hydrogen peroxide, ozonised water and *NatureSeal*® AS5 (in different concentrations and different treatment times) with the main purpose to ensure the microbiological safety of fresh–cut carrots.

Materials and Methods

Materials

Experiments were carried out in Department of Food Technology at the Latvia University of Agriculture. The research was accomplished on 'Nante' type 'Forto' variety carrots (*Daucus carota* L.) grown in Latvia and harvested in Zemgale region in the first part of October, 2015.

Preparation of shredded carrots

Fresh, whole, non–damaged and washed (with drinking water) carrots were used for the research purpose. Carrots were peeled with a Baumann vegetable peeling knife made of non–corrosive steel and covered with a special coating – ceramic layer protecting the product from sticking. Peeled carrots were washed in running water and dried at temperature of +20±2 °C in air ambience for 3±1 min. Carrots were shredded using a kitchen combine (Philips Comfort HR 7605, Austria) with the power capacity 350 W. The volume of a shredded carrot chip was: cross–section 1.5±3.0 mm and length 35–50 mm.

Treatment with H_2O_2

30%, 34.01 g mol^{-1} hydrogen peroxide was used for experiments (Czech Republic). 0.5%; 1.0% and 1.5% hydrogen peroxide solutions in deionised water 0.055 μS cm^{-1} at temperature of 20±2 °C were prepared for treatment of shredded carrots. Solution was prepared one minute before the treatment of shredded carrots to avert the decomposition of hydrogen peroxide. Shredded carrots were immersed in the hydrogen peroxide water for 30±1 s, 60±1 s and 90±1 s. After treatment carrots were placed on a non-corrosive steel sheave (grid diameter 0.3 mm) to draw off the excessive water (3±1 min). The influence of hydrogen peroxide on the carrots quality was analysed immediately after treatment and during storage.

Treatment with ozone

Ozonised water was obtained in high concentration ozone and oxygen generator SOZ–YMS (BNPOZONE Company, China) equipped with the water pump, where the ozone was dissolved in water by means of the ejector. The maximum ozone concentration could be up to 12.0 mg L^{-1} per one circulation time. The ozone concentration in water mg L^{-1} was measured with a portative ozone detector DO3 (Eco Sensors Division of KWJ Engineering Inc., USA). The amount of ozone in the container was measured over the water surface. The measurement was done manually by taking of 10 mL ozonised water in the bottle and measuring it with the portative measurer DO3 (Eco Sensors Division of KWJ Engineering Inc., USA). The electrochemical T–Series sensor (3ET1PO3) with sensibility ±0.05% (0–0.05 mg L^{-1}) was used in the device; the maximum detected concentration was up to 5.0 mg $L^{-1.}$ After determination of ozone concentration (1.0 mg L^{-1}, 2.0 mg L^{-1} and 3.0 mg L^{-1}), shredded carrots were immersed in the prepared solution (2.0 L) at temperature of 20±2 ºC for 60; 120 and 180±1 seconds. After treatment carrots were placed on a non-corrosive steel sheave (grid diameter 0.3 mm) to draw off the excessive water (3±1 min). The influence of ozonised water on the carrots quality was analysed immediately after treatment and during storage.

Treatment with NatureSeal®AS5 solution

NatureSeal® AS5 solution – a blend of dry vitamin and mineral substances patented in the USA, a safe product with the active compounds of ascorbic acid and calcium. 2.5% *NatureSeal*® AS5 water solution was used for the treatment of carrots. 2.5% *NatureSeal*® AS5 water solution was prepared right before the use where shredded carrots were immersed at temperature of 20±2 °C for 5 min±1 s. After treatment carrots were placed on a non–corrosive steel sheave (grid diameter 0.3 mm) to draw off the excessive water (3±1 min). The influence of *NatureSeal*® AS5 on the carrots

quality was analysed immediately after treatment and during storage.

Microbiological analysis

Microbiological evaluation of carrot was performed according to the standard 'Microbiology of food and animal feeding stuffs' LVS EN ISO 7218:2007. All microbiological evaluations were conducted with threefold repetition. Plate counting method was used for microbial detection. Total plate count of mesophilic aerobic and facultative anaerobic microorganisms was investigated on Nutrition agar (dilutions 1:1000; 1:10000) in conformity standard method LVS EN ISO 4833:2003. Yeast plate count was investigated on Malt extract agar (dilutions 1:100; 1:1000) in conformity standard method ISO 21527–1:2008. *E.coli* (LVS ISO 7251). Counting of colonies formed and calculating the number of CFUs was accomplished by automatic colony counter Acolyte.

Results and Discussion

The influence of two alternative treatment methods – hydrogen peroxide (H_2O_2) and ozonised water on the quality parameters of fresh–cut carrots was analysed in the present research. The commercial *NatureSeal*® AS5 solution (control sample) was used to treated carrots for the comparison purposes.

Pre–treatment (washing, peeling, shredding) of carrots damages the carrots tissue and results the growth of microorganisms. Therefore, hydrogen peroxide (H_2O_2) was used as a treatment agent for the treatment of fresh–cut carrots. The use of hydrogen peroxide is recommended for treatment of vegetables thanks to its low toxicity and safe decomposition of O_2 and H_2O.

However, microbial safety is one of the most important factors to be considered for the preservation of minimally processed foods (Bico et al., 2009). *E.coli* in fresh carrots was not detected.

A significant ($p=0.09$; $\alpha=0.05$) difference in the dynamics of TPC between the control carrots sample and carrots treated with H_2O_2 was found after analysing the influence of H_2O_2 on the quality parameters of carrots using different concentrations of H_2O_2 and treatment times (Figure 1).

The TPC of control sample was 2.61 log cfu g^{-1}. Higher TPC (2.25 log cfu g^{-1}) was found in carrots treatment with the 0.5% H_2O_2 for 30±1 s (decrease by 13.97% in comparison with non–treated carrots sample); however, lower TPC (1.65 log cfu g^{-1}) was detected – after carrots treatment with the 1.5% H_2O_2 for 90±1 s (the TPC decrease by 36.78%). After evaluation of H_2O_2 treatment activity, it may be concluded that it is possible to decrease significantly (by 27.20%) TPC of carrots during treatment with the 1.0% H_2O_2 for 30±1 s. Mathematical processing of the obtained results revealed that various concentrations of H_2O_2 ($p=0.019$; $\alpha=0.05$) and treatment times ($p=0.049$; $\alpha=0.05$) significantly influenced the TPC count in carrots (Figure 1).

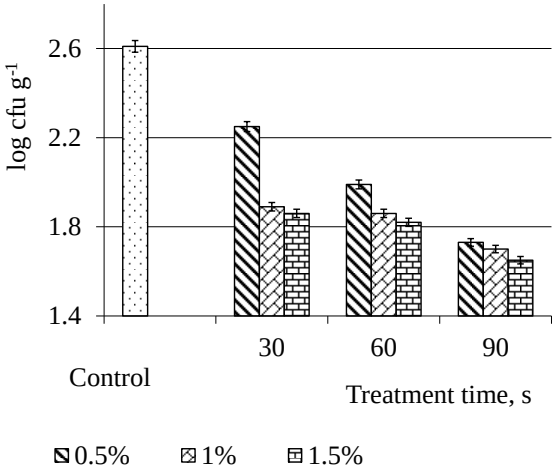

Figure 1. Influence of H_2O_2 treatment regime parameters on the TPC units in carrots

The obtained results prove the treatment agent properties of H_2O_2.

A significant ($p<0.05$) decrease in the yeast count was detected after treatment of fresh-cut carrots with the 1.0% and 1.5% H_2O_2 for 90±1 s – yeasts were not found after treatment (Figure 2).

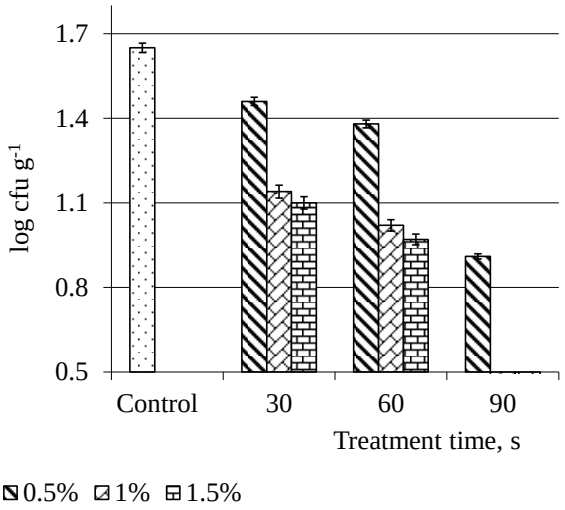

Figure 2. Influence of H_2O_2 treatment regime parameters on the yeast units in carrots

Yeasts (1.47 log cfu g^{-1}) were found in carrots treated with the 0.5% H_2O_2 for 30±1 s. Therefore, a longer (90 s) treatment time and higher concentration of treatment agent (1.0% and 1.5%) is more suitable for the carrots microbiological safety providing.

Ozone (O_3) is one of the newest treatment agents in the world used to decrease the count of microorganisms in vegetables. Ozone is safe and friendly (GRAS – Generally recognized as safe) in the food. Inhibition of the microorganisms' growth is the main advantage of ozone treatment; as a result fresh–cut carrots keep their freshness and quality for longer time.

Different treatment regimes as ozonised water concentration and treatment time were investigated to evaluate their influence on the quality of shredded

carrots. The following treatment regimes were investigated based on the data presented in scientific literature: treatment time – 60; 120; 180±1 s and concentrations 1.0; 2.0; 3.0 mg L^{-1}. The comparison of chosen treatment regimes detected a significant ($p=0.003$; $\alpha=0.05$) influence of the ozonised water on the decrease in the count of TPC in carrots after treatment (Figure 3).

An essential decrease in the TPC count from 2.34 to 1.13 log cfu g^{-1} and 1.10 log cfu g^{-1}, i.e. – by 51.71% and 52.99% respectively – was obtained after carrots treatment with 2 mg L^{-1} and 3 mg L^{-1} of ozonised water for 180±1 s.

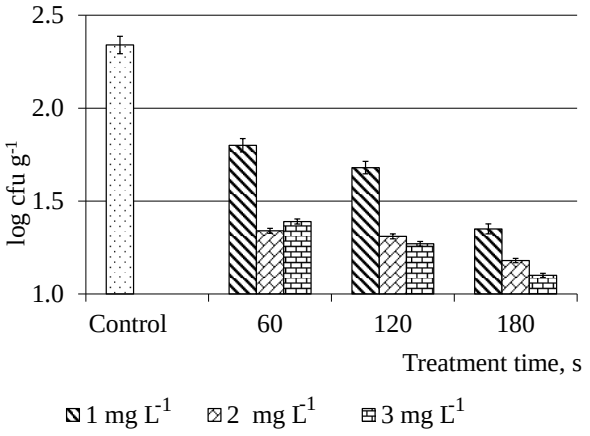

Figure 3. The TPC units in carrots after treatment with the ozonised water

Essential decreases (by 96.36%) in the yeast content (Figure 4) were obtained after treatment of carrots with 2 and 3 mg L^{-1} of ozonised water for 180±1 s, yet not so–pronounced ones (by 50.63%) – after treatment of carrots with 1 mg L^{-1} of ozonised water for 60±1 s.

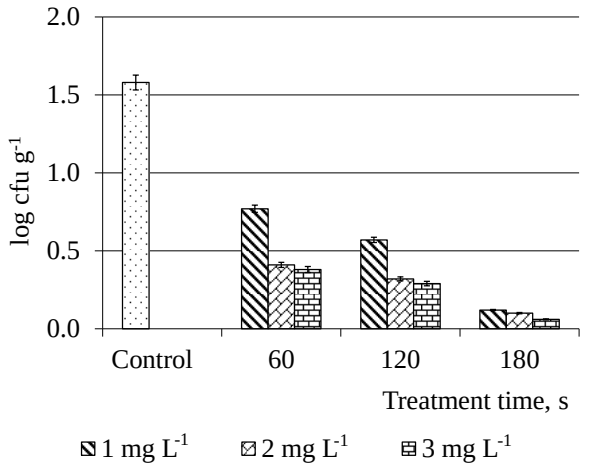

Figure 4. The yeast units in carrots after treatment with the ozonised water

Therefore, a longer (180 s) treatment time and a lower treatment agent concentration (2 mg L^{-1} and 3 mg L^{-1}) are advisable to ensure the microbiological safety of carrots.

NatureSeal® AS5 is a commercial solution used for the treatment of vegetables worldwide. Ascorbic acid and calcium are the active components of *NatureSeal*® AS5. It is the first commercial product, which does not create a non–acceptable aftertaste and does not remain in the product. Therefore, it is possible to control the pH value (decrease) of a product by the treatment of products with the mentioned solution; as a result the growth of microorganisms could be prevented similarly as it is done using other treatment agents (Bhagwat et al., 2004). The recommended treatment time for the use of the commercial *NatureSeal*® AS5 solution (AgriCoat *NatureSeal* Ltd, England) is 5 min±1 s and preparation concentration in the water solution – 2.5%. The present experiments were done to verify the possible influence of *NatureSeal*® AS5 on the microbiological indicators of 'Nante' type 'Forto' variety carrots grown in Latvia and to compare this influence with alternative treatment methods (H_2O_2 and ozonised water) of carrots. The initial count of TPC in non–treated carrots was 2.62 log cfu g^{-1}, which decreased up to 1.28 log cfu g^{-1} after treatment with *NatureSeal*® AS5 (Figure 5).

Comparing the results of the microbiological analyzes of shredded carrots after treatment with *NatureSeal*® AS5 preparations (Figure 5) and 120 ± 1 s to 2 mg L^{-1} ozonated water (Figure 3), it found that treatment with *NatureSeal*® AS5 preparation gives similar TPC count decrease in carrots.

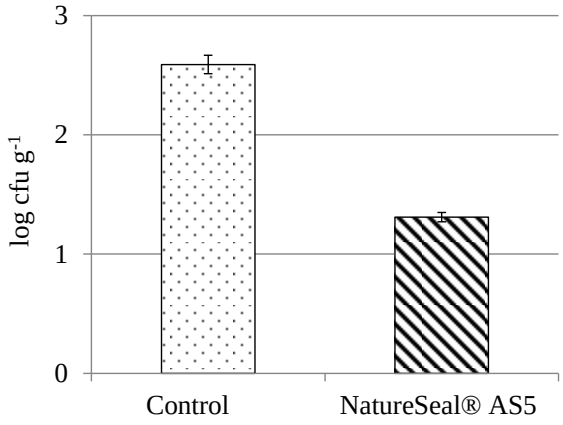

Figure 5. The TPC units in carrots after treatment with the *NatureSeal*® AS5

During experiments it was proved that *NatureSeal*® AS5 solution could be recommended as treatment agent for the treatment of fresh–cut carrots.

Conclusions

The study confirmed that commercial *NatureSeal*® AS5 preparations and ozonised water may be recognized as an effective treatment agents to inactivate undesirable microflora in fresh–cut carrots. Therefore, ozonised water should be alternative for commercial *NatureSeal*® AS5 preparations. However, in the present research obtained results demonstrate hydrogen

peroxide low potential as disinfectant for fresh–cut carrots safety providing.

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EVALUATION OF OAT GRAIN PERTINENCE TO PRODUCTION OF FLAKES, FLOUR AND PORRIDGE PREPARING

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Abstract

Oats for human consumption are primarily used as oat flakes and breakfast mixes. Although cultivar differences in the parameters directing oat breeding and cultivation such as seed size, yield, lodging resistance, and nutritional attributes - protein, lipids, are widely studied, knowledge concerning the suitability of cultivars for production of flakes and other oat products is limited The aim of present investigation was to study chemical composition – protein, fat β-glucans content of new oat genotypes and its pertinence to needs of producers – thousand grain weight, kernel outcome and colour of porridge.

The grain of twelve oat genotypes VP2, VP3, VP4, VP5, VP6, VP8, VP9, VP10, VP11, VP12 and two recognized varieties – 'Laima' and 'Peppi' were the materials used in study. In the studied samples content of protein, lipids, β-glucans same as grain outcome, damaged and dark grain were determined. Oat flakes were made and prepared porridge. Colour difference of oat porridges between genotypes was tested. The results showed that protein content in oat grain ranged from 10.2% to 14.7%, lipids from 5.3% to 7.7% and β-glucans from 1.8 to 3.6% depending on genotype. More perspective breeding lines from nutritional point of view were VP4 and VP3, but from the higher outcome and grain quality perspective, VP8 and VP12. Colour analysis of porridge confirmed that all analysed oat genotypes fulfilled colour requirements and were in agreement with products, which are on the market.

Keywords: hulled oats, nutrition value, production, quality, outcome.

Introduction

Grains, including oats (*Avena sativa* L.), have been recognized as a healthy food containing significant amounts of soluble dietetic fibre, β-glucans, polyunsaturated fatty acids and vitamin E because provide beneficial effect on the health of the consumer and decrease the risk of various diseases (Lásztity, 1998; Sterna et al., 2014; Zielinski et al., 2001). The incorporation of oat grains and oat bran in the food products improves not only the nutrition but also a therapy against various maladies (Butt et al., 2008).

The discussion on oat grain dietetic value and suitability to the production of functional foods is more frequently mentioned in scientific literature by Biel et al. (2009). Food uses for oats include oat flour, oat bran, oat flakes, which mainly used for porridge and breakfast cereals. Oat flour is also used as a thickener in many infant foods (Arendt, Zannini, 2013).

It is possible to produce oats with specific health benefits and genotype traits, therefore it is necessary to compare the chemical composition and quality traits of new breeding lines grains with producers recognized varieties. Although cultivar differences in the parameters directing oat breeding and cultivation such as seed size, yield, lodging resistance, and nutritional attributes such as protein, oil, and β-glucans are widely studied, knowledge concerning the suitability of cultivars for rolled oats production is limited (Lapveteläinen et al., 2001). Critical manufacturing criteria such as degree of dehulling, kernel outcome and amount of dark grain could be desirable determined in the evaluation of grains.

The aim of present investigation was to study chemical composition – protein, fat β-glucans content of new oat genotypes and its pertinence to needs of producers – thousand grain weight, kernel outcome and colour of porridge.

Materials and Methods

The research was conducted at the Institute of Agricultural Resources and Economics, Stende Research Centre from 2015 to 2016.

The characteristic of environmental conditions in 2015 and 2016 years showed in the Table 1.

Table 1

The characteristic of environmental conditions in years 2015 and 2016

Month	Temperature		Precipitation	
	2015	2016	2015	2016
April	5.6	5.8	98.5	37.0
May	9.8	13.3	65.9	61.0
June	13.5	15.6	50.5	92.5
July	15.9	17.2	75.5	91.7
August	17.5	16.0	26.6	110.4
Soil traits			**2015**	**2016**
pH			5.6	5.6
Organic matter, %			2.6	1.7
P_2O_5, mg kg^{-1}			180	174
K_2O, mg kg^{-1}			117	162

Twelve oat genotypes VP2, VP3, VP4, VP5, VP6, VP8, VP9, VP10, VP11, VP12 and two millers recognized varieties – 'Laima' as a widely grown variety in Latvia, and 'Peppi' from Finland as a preferred among imported grains, were the materials used in this study.

Mean samples from all (4) replicates (0.5 kg) were taken for laboratory testing. Hulled grains were dehulled mechanically. In the studied samples (n=96) test weight, 1.000 grain weight (TGW), protein, lipid and β-glucans

content were determined by using automatic grain analyser Infratec Analyser 1241.
Critical manufacturing criteria such as degree of dehulling (grain outcome), kernel outcome and amount of dark grain were determined.
Oat flakes were made from whole oats grain, which were rolled info flat flakes under heavy rollers using laboratory equipment MARGA Mulino (Marcato, Italy). Porridge was prepared by mixing 20 g of oat flakes with 50 mL boiling water and left to swell for 15 min.
The instrumental measurement of porridge colour was performed in CIE (International Commission on Illumination) L*a*b* using a ColorTec-PCM/PSM (Accuracy Microsensors Inc., USA). In colour measurement, CIE L* a* b* coordinates show the degree of brightness (L), the degree of redness (+a), or greenness (–a), and the degree of yellowness (+b), or blueness (–b), respectively (Phimolsiripol et al., 2012). Porridge colour measurements were made by placing the samples directly under the colorimeter. The colour was measured at five different points within the porridge region and mean values were reported for each porridge. The total colour difference (ΔE) was defined by the Minolta equations (1) (Sanz et al., 2009):

$$\Delta L = (L - L_0), \Delta a = (a - a_0), \Delta b = (b - b_0),$$
$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}, \quad (1)$$

where L, a, and b are the measured values of oat genotypes porridge samples, L_0, a_0, and b_0 are the values of oat variety 'Laima' and 'Peppi' porridge (control), which were selected as reference values.

The reference values for calculating ΔE then were the colour difference between control and each porridge sample. The values used to determine whether the total colour difference is appreciable by the human eye were the following:

- ΔE<1 – colour difference is not obvious for the human eye;
- 1<ΔE<3 – colour difference is not appreciative by the human eye;
- ΔE>3 – colour difference is obvious for the human eye (Sanz et al., 2009).

The results were statistically processed using methods of descriptive statistics, analysis of variance, using programme package SPSS 20. Statistical significance was declared at $p<0.05$ or at $p<0.01$.

Results and Discussion

The results of analysed protein, lipid, β-glucans content and thousand grain weight in the hulled oat varieties and breeding lines grain are summarized in Table 2.
The results of investigation showed that the content of protein in samples of oat grains depended on genotype and ranged from 10.7% to 13.9%. The significantly highest protein content – 13.9±1.2% determined in the samples of oat variety 'Peppi' ($p<0.05$). From tested new genotypes highest protein content determined in the samples of VP4 11.8±0.6%. Our results are similar with other research findings, where protein content for husked oat was reported to be 11.5% (Biel et al., 2009) and 15.9% (Asp et al., 1992).

Table 2

Content of protein, fat, β-glucans and thousand grain weight in different genotypes grain

Samples	Protein	Fat	β-glucans	TGW
	%	%	%	g
'Laima'	11.8±0.4	7.7±0.3	3.3±0.3	35.6±1.8
VP2	11.7±0.4	6.4±0.2	2.5±0.2	36.4±2.6
VP3	11.4±0.4	6.8±0.1	2.6±0.1	38.5±4.2
VP4	11.8±0.6	6.6±0.3	2.7±0.2	39.4±3.6
VP5	11.6±0.5	6.6±0.1	2.6±0.1	38.6±4.9
VP6	11.3±0.4	6.5±0.2	2.4±0.2	39.6±2.8
'Peppi'	13.9±1.2	6.7±0.2	3.0±0.1	38.4±1.5
VP8	10.7±0.6	5.7±0.1	2.0±0.1	37.3±3.8
VP9	11.3±0.6	6.3±0.3	2.5±0.1	40.5±2.8
VP10	11.1±0.7	6.4±0.3	2.4±0.1	36.5±4.7
VP11	10.7±0.4	6.7±0.1	2.6±0.1	41.7±0.8
VP12	11.2±0.6	6.5±0.3	2.5±0.1	35.1±3.3

The fat content ranged from 5.7±0.1% in the grains of genotype VP8 to 7.7±0.3% in the grains of variety 'Laima'. From tested new genotypes highest fat content determined in samples of VP3 (6.8±0.1). The fat content of oat grains depends on genetic and environmental factors. The range of 3.1 to 11.6% was found among more than 4 000 entries in the world collection (Zhou et al., 1999).
Content of β-glucans besides health benefits has also high impact on the oat flakes production quality (Lapveteläinen et al., 2001). The content of β-glucans in hulled oat grains varied from 2.0% to 3.3%. The varieties 'Laima', 'Peppi' and genotypes VP4 and VP3 had on average 3.3%, 3.0%, 2.7% and 2.6% β-glucans content, respectively. The content of β-glucans significantly differed among genotypes ($p<0.05$), and it is in agreement with other scientific investigations (Welch, 1995, Lapveteläinen et al., 2001, Arendt, Zannini, 2013).
Data of investigation must be concluded that the better new breeding lines from nutritional point of view were VP4 with highest protein and β-glucans content and VP3 with highest fat and β-glucans content (Table 2). Comparison of oats grain yield and quality criteria such as grain outcome, kernel outcome, amount of dark and damaged grain content are showed in Table 3.
Data of investigation showed (Table 3) that genotypes with higher protein content – 'Peppi', 'Laima' and VP4 had lower grain yield 4.83, 5.73, and 5.74 t ha^{-1} respectively. The high protein content in grains often accompanied by low yield. A tendency toward a negative relationship between protein concentration and yield is generally expected (Burrows, 1986), although various genotypes may respond differently (Givens et al., 2004).
Investigation of grain quality showed that the smallest content of damaged grain was for oat variety 'Laima', the smallest content of dark grain has samples of breeding line VP3, VP4, and VP8 (Table 3).

Table 3

Grain yield, outcome and quality traits in different oat genotypes

Genotypes	Yield, t ha^{-1}	Grain outcome, % above 2 mm sieve	Kernel outcome*, %		
			Damaged grain*	Dark grain	Clean
'Laima	5.73±0.43	99.0±0.1	3.74±0.34	1.85±0.14	68.6±0.2
VP2	6.00±0.42	99.4±0.1	7.41±0.55	2.47±0.18	73.4±0.5
VP3	6.43±0.21	99.3±0.2	7.06±0.54	0.94±0.12	66.7±0.2
VP4	5.74±0.41	99.5±0.2	8.20±0.34	1.62±0.14	78.9±0.5
VP5	6.16±0.45	99.4±0.1	12.65±0.58	2.18±0.14	66.3±0.2
VP6	6.25±0.42	99.3±0.3	7.32±0.54	3.84±0.25	65.3±0.2
'Peppi'	4.83±0.65	99.0±0.4	5.92±0.56	4.19±0.34	75.8±0.5
VP8	6.36±0.50	99.6±0.2	5.40±0.34	1.78±0.14	67.1±0.3
VP9	6.60±0.48	99.0±0.3	8.97±0.64	1.82±0.14	68.0±0.4
VP10	6.53±0.32	99.1±0.1	10.18±0.65	3.20±0.35	66.2±0.3
VP11	6.00±0.58	99.8±0.2	5.00±0.34	2.38±0.14	66.8±0.4
VP12	6.80±0.42	98.0±0.1	8.45±0.64	2.03±0.18	76.6±0.5

* – average values and standard deviations

The highest outcome of clean kernel observed in samples of variety 'Peppi' and breeding lines VP12 and V4. The results of damaged and dark grain content significantly differed by breeding lines ($p<0.05$). A three-year study made by Lapveteläinen et al. (2001) showed that both cultivar and crop year significantly influenced yield, TGW, kernel size value, damaged and dark grain amount, and damaged flake particles. Evaluation of grain yield, grain outcome and most important traits from producer's point of view showed that better quality often combined with lower yield.

Evaluation of new breeding lines technological characteristic were included quality of flakes and colour values of porridge. Oat grains after being rolled info flat flakes, control and analysed sample average flake size are 11–13 mm (Fig. 1). Oat flakes characterise oval and elongated shape, and they are fragile.

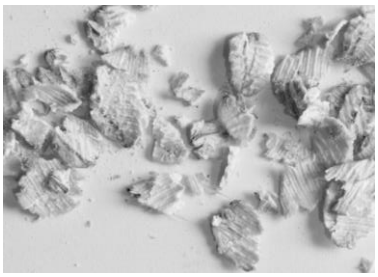

Figure 1. Oat flakes after rolling

After oat flakes are mixed with boiling water, flakes begin to lose shape, divided into smaller pieces; starch granules swell and give porridge homogeneous structure.

Colour is variable measurement, depends on used materials, temperature, storage conditions and time, and spot of the measurement (Mondal, Datta, 2008). Colour values of oat porridge are shown in Table 4.

No significant differences were found in L* and b* values of oat porridge between each type of genotype and variety. Oat porridge had grey, dark grey colour. Significant differences ($p < 0.05$) were found in a* values, it range from -0.23±0.87 genotype VP9 to -2.56±0.52 genotype VP3.

Table 4

Colour values of oat porridge

Samples	Colour		
	L*	a*	b*
'Laima'	89.81±0.76*	-2.39±0.88	21.04±0.71
VP2	87.88±0.87	-1.86±0.55	23.13±0.83
VP3	87.61±0.69	-2.56±0.52	21.48±0.56
VP4	89.90±0.59	-1.53±0.72	21.71±0.49
VP5	88.06±0.13	-1.78±0.41	23.97±0.61
VP6	88.39±0.28	-0.82±0.54	22.94±0.69
'Peppi'	89.94±0.46	-2.02±0.33	22.42±0.49
VP8	87.86±0.40	-0.58±0.11	22.20±0.37
VP9	88.36±0.85	-0.23±0.87	22.01±0.84
VP10	88.44±0.40	-0.46±0.25	23.77±0.75
VP11	88.34±0.42	-1.69±0.60	23.82±0.72
VP12	89.92±0.25	-1.95±0.52	22.78±0.48

* – average values and standard deviations

In order to study if there are colour differences between varieties 'Laima' and 'Peppi', and each oat genotype were detectable by human eye, the parameter ΔE was calculated (Table 5).

Parameter ΔE revealed that the colour difference between porridge of variety 'Laima' and porridge of genotypes VP5, VP10 and VP11 was obvious for human eye ($\Delta E > 3$).

Porridge of genotypes are darker than from variety 'Laima'. Colour difference of oat porridge between variety 'Laima' and other genotypes are not appreciative by the human eye ($1<\Delta E<3$).

Table 5

Colour difference (ΔE) of oat porridge

Genotype	Variety	
	'Laima'	'Peppi'
VP2	2.89	2.18
VP3	2.25	2.57
VP4	1.09	0.86
VP5	3.47	2.45
VP6	2.84	2.03
VP8	2.90	2.54
VP9	2.78	2.42
VP10	3.61	2.55
VP11	3.22	2.15
VP12	1.80	0.37

Colour difference of oat porridge between variety 'Peppi' and genotypes VP4 and VP12 was not obvious for the human eye ($\Delta E<1$), but comparing with other analysed genotypes difference were not appreciative by the human eye ($1<\Delta E<3$).

'Laima' and 'Peppi' are most common oat varieties, which are used in oat flake production in Latvia, colour analysis of porridge reveals that each of analysed oat genotype can be equal in colour with products, which are on the market.

Conclusions

The content of protein, fat, β-glucans, same as a quality of traits significantly differed by genotypes. More perspective breeding lines from nutritional point of view are VP4 and VP3, from the higher outcome and grain quality perspective VP8. Evaluation of grain yield, grain outcome and most important traits from producer's point of view showed that better quality is often combined with lower yield. Colour analysis of porridge reveals that each of analysed oat genotype can be equal in colour with products, which are on the market.

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GRAIN QUALITY OF WINTER RYE CULTIVARS GROWN IN LATVIA

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Abstract

Rye (*Secale cereale* L.) has been cultivated in Europe since ancient times and is second only to wheat among the grains most commonly used in the production of bread. Rye is traditionally consumed as wholemeal products in Baltic countries. In Latvia, rye bread is rich with traditions, and is one of the more favourite types of bread. Our objectives were to determine the grain quality and suitability of the most popular winter rye cultivars in Latvia for wholegrain flour production and bread baking. Three population winter rye cultivars 'Kaupo', 'Amilo', 'Dankowskie Amber' and three hybrid rye cultivars 'Brasetto F1', 'Su Drive F1', 'Su Mephisto F1' were obtained from research field trials (2014 / 2015, 2015 / 2016) at the Priekuli Research Centre, Institute of Agricultural Resources and Economics in Latvia and used for evaluation. Rye grain quality indices were analysed at the Latvia University of Agriculture, in Grain and Seed Research laboratory. Average data in our investigation (two years) show that cultivar, environment and cultivar × environment interaction significantly ($p<0.05$) affected 1000 kernel weight, volume weight, protein content, starch content and Hagberg falling number. The thousand kernel weight in hybrids cultivars grains was statistically significantly higher comparing population cultivar grains. Differences between hybrids cultivars grains volume weight, protein content, falling number comparing with population cultivar grains was not observed. The results of the current research show that the quality of all the studied cultivars meet the requirements for high-grade rye grains for food consumption and are suitable for the wholegrain flour production and bread baking.

Keywords: winter rye, grain quality, varieties.

Introduction

Winter rye (*Secale cereale* L.) is one of the most important bread grains in colder parts of Europe. The chemical composition of rye grain promises health benefits and it contributes to higher intake of dietary fibre. Grain quality adversely affects price and consumer acceptance of finished products (Hansen et al., 2004).

Quality indices of winter rye are not stable between production years because of the inconsistency of the variables, such as initiation of the growing season, distribution of rainfall and heat units available for crop growth during corresponding phases of plant growth and development (Hansen et al., 2004). During ripening rye needs sunny and warm weather and moderate moisture. These conditions secure biological maturity and acceptable technological properties of grain (Kunkulberga et al., 2007; Stepien et al., 2016).

The volume weight is used as an index of rye grain quality, and minimum volume weight requirement for food grain grown in Latvia is 700 g L^{-1}. The 1000 kernel weight depends on grain density and size and the 1000 kernel weight of rye grain in Latvia is about 44 g (Tupits, 2008; Jansone, 2015), while in Lithuania – between 33.5 g and 38.3 g (Vidmantiene, Joudeikiene, 2010). The kernel weight was negative influenced by high temperature and drought during the ripening stage (Shmielwski, Koln 2000).

The main chemical constituents of rye grain are starch and protein. The starch content is limited mainly to the endosperm, and contents between 57% and 66% of dry mater are reported in rye growing in Poland (Hansen et al., 2004), while in investigation grown in Latvia between 53% and 63% (Maļecka, Strazdiņa, 2004; Jansone, 2015). The content of protein reported in rye cultivars, grown in different countries, was between 7.0 and 14.6% (Hansen et al., 2004; Banu, 2006; Ruzgas, Plycevaitiene, 2005; Zdubel et al., 2009; Jansone, 2015).

The baking quality of rye is mainly affected by the activity of amylases measured as Hagberg falling number (hereinafter falling number). It is well known in the milling and baking industry that quality of rye flour can be highly different from one year to another because the amylases activity is significantly affected by the temperature and amount of rain during growing season (Salmenkallio-Marttila, Hovineen, 2005).

Cereal grain is of the highest quality during growth period between wax maturity and full maturity. During this period cereal yield forming is already finished and, in case of unfavourable weather conditions, grains can start sprouting, which would result in reduced falling number. Under very wet harvesting conditions rye reaches the limit when α–amylase activity is considered to be too high. Emergence of grain sprouting may also affect the dormancy period. Grain dormancy period is depending on the weather conditions in grain formation and ripening phase. The falling number value depends on the cultivar genetic characteristics (Ingver, 2002); Maļecka, Strazdiņa, 2004).

If the falling number is 65 s, the volume of bulk bread decreased, while the middle value of falling number (150 s) did not affect the bread volume (Dvorakova et al., 2010). In Latvia the minimum falling number requirement for food rye grain is 130 s (GmbH Dobeles..., 2016). A falling number that is too low results in pasty and unacceptable bread (Hansen et al., 2004).

The objective of the research was to determine the rye grain quality and suitability of the most popular rye cultivars in Latvia for wholegrain flour production.

Materials and Methods

Study fields

Field investigation in years 2014/2015 and 2015/2016 was conducted at the Priekuli Research Centre, Institute of Agricultural Resources and Economics (Latvia), on the soil of sod-podzoloic loam with close to neutral acidity (pH_{KCl} 5.6–6.0), medium high phosphorus and potassium, humus content 1.7–2.5 g kg^{-1}.

There were winter rye cultivars of three populations ('Kaupo' (Latvia), 'Amilo' (Poland), 'Dankowskie Amber' (Poland)) and three cultivars of hybrid winter rye ('Brasetto F1', 'Su Drive F1', 'Su Mephisto F1' (all Germany)) examined during research. These cultivars in Latvian farms nowadays are popular. The field experiment was placed randomly in four replications. Nitrogen, phosphorus and potassium fertilizers (6 : 26 : 30) were applied in autumn. Nitrogen (N) was applied N68 kg ha^{-1} in spring after resumption of vegetative growth and N31 kg ha^{-1} at the tillering stage. Grain was harvested at full ripeness; sampling procedure for grain quality evaluation was performed according to the standard ICC 101/1 for obtaining average sample.

Weather data collection

Winter rye sown in 2014 and 2015 overwintered successfully. The air temperature in investigation years (Table 1) in April was close to long-term average observations. May in 2015 was by –1.5 °C colder, while in 2016 was by 2.7 °C warmer, which promoted plant growth and development. Average daily temperature in June 2015 was lower by 0.6 °C compared to long-term average data, in 2016 air temperature was warmer by 1.5 °C witch contributed to the accumulation of protein. Temperature in the grain filling period (July), which is the most decisive for grain quality formation, was in 2015 by 1.6 °C colder, while in 2016 by 0.4 °C higher than the long-term average mean data.

Table 1

Weather conditions during the field investigation

Month	Average temperature, °C		
	2015	2016	LTM*
April	5.4	6.1	4.6
May	10.2	14.5	11.0
June	14.3	16.4	14.8
July	15.9	17.9	16.9
Monthly average	11.5	13.7	11.8
	Sum of precipitation, mm		
April	76	82	40
May	53	10	56
June	39	145	78
July	92	110	93
Monthly average	65	87	67

LTM* – long term mean

Water availability has effect on rye grain quality. Precipitation in April 2015 and 2016 was respectively by 212% and 232% more than long-term mean data.

May in 2015 and 2016 was dry. Precipitation in June 2015 was close to the long-term mean, while in 2016 by 178% more than the long-term mean data. Precipitation in July 2015 by 127% exceeded the long-term average data, in 2016 close to long-term average observations.

Analysis

The rye grains were analysed at the Latvia University of Agriculture in Grain and Seed Research laboratory. Quality parameters: protein content (%), starch content (%) and volume weight (g L^{-1}) were analysed by grain analyser Infratec 1241 (Sweden), which employs the near-infrared analysis within the wavelength range 570–110 nm. Thousand kernel weight determined by LVS EN ISO 520 „Cereals and pulses. Determination of the mass of 1000 grains", this was done by counting 500 grains duplicate with a counter „Contador". The Hagberg falling number – α-amylase activity – was measured by the Hagberg-Perten method using a Perten Instruments (Sweden) „Falling number 1500" assessed according to LVS EN ISO 3093 using 7 g of flour adjusted for moisture content to 15%.

Statistical analysis

Experimental data evaluation was done using two factor analysis of variance by Fisher's criteria and least significant difference ($LSD_{0.05}$) were applied to estimate the effects of year (meteorological conditions) and cultivars. Component of variance ANOVA for each quality characteristic were expressed as percentage to illustrate the relative impact of each source to the total variance. Differences of the grain quality indices between population and hybrid rye cultivars determined by t-Test: Two Sample Assuming Unequal variance. Correlation analysis between starch content and other grain quality indices, also between protein content and other grain quality indices was carried out.

Results and Discussion

In this study the rye grain yield of the cultivars was between 5.1 to 7.8 t ha^{-1}. Average data in our experiment (2 years) determined by t-Test suggest that grain yield from hybrids cultivar was statistically significantly higher comparing population cultivar ($t_{stat.}3.33 > t_{crit.}$ 2.92).

Grain characteristics

Grain qualities of the different cultivars are differing. *Thousand kernel weight (TKW)* varied significantly ($p>0.05$) depending on the cultivars and meteorological conditions. Average 1000 kernel weight was 42.4±0.6 g, V=5.0% (Table 2).

The 1000 kernel weight ranged from 39.7 g ('Kaupo') to 44.6 g ('Su Drive') on average (Table 2). Similar results were obtained in Lithuania (Alijošius et al., 2016) where in seven rye varieties average of 1000 grains weight were 43.9 g.

Table 2

Winter rye quality indices

Quality indices	Mean	min	max	V%
TKW, g	42.4±0.6	38.9	46.7	5.0
VW, g L^{-1}	754.0±3.8	737.0	779	1.8
PC, %	10.1±0.7	7.7	13.1	22.5
SC, %	61.8±0.7	58.8	64.8	4.1
FN, s	212.0±13.0	133.0	305	21.2

TKW – 1000 kernel weight, VW – volume weight, PC – protein content, SC – starch content, FN – falling number

In our investigation, the 1000 kernel weight was highly influenced by cultivar (69%) and cultivar × year (19%), while influence of harvest year was smaller (10%), however in Hansen and colleagues (2016) experiments 1000 kernel weight dependency on year complete to 65% but the influence of cultivar – 25%, whole cultivar × year – 5% (Tab. 3).

Table 3

Rye 1000 kernel weight, g

Cultivar	2015	2016	Average
Dank. Amber	40.8	43.2	42.0
Kaupo	38.9	40.5	39.7
Amilo	41.0	41.5	41.2
Brasetto	42.1	46.7	44.4
SU Drive	44.9	44.3	44.6
SU Mephisto	42.7	42.1	42.4
Average	41.7	43.0	42.4

Average data in our experiment (2 years) determined by t-Test suggest that thousand kernel weight in hybrids cultivar was statistically significantly higher comparing population cultivar ($t_{fac.}$ 2.91 > $t_{crit.}$ 2.13).

Table 4

Impact factors of rye grain quality indices, %

Source of variation	TKW	VW	PC	SC	FN
Year	10	66	96	95	42
Cultivar	69	15	2	3	44
Year × cultivar	19	16	2	2	14

TKW – 1000 kernel weight, g, VW – volume weight, g L^{-1}, PC – protein content, %, SC – starch content, %, FN – falling number, s.

The *volume weight (VW)* in winter rye grain (Table 5) ranged from 753 g L^{-1} ('Dankowskie Amber') to 764 g L^{-1} ('Su Mephisto').

Table 5

Rye grain volume weight, g L^{-1}

Cultivar	2015	2016	Average
Dank. Amber	760	746	753
Kaupo	754	743	748
Amilo	773	749	761
Brasetto	764	749	756
SU Drive	779	737	758
SU Mephisto	774	753	764
Average	767	746	757

Average data show that the *volume weight* for cultivars mean ± standard error was 754±3.8 the coefficient of variation was 1.8%. The content of volume weight measured in this study is in accordance with findings by other authors (Jansone, 2015; Hansen et al., 2016).

The volume weight was highly influenced by harvest year (66%) and cultivar × year (16%), cultivar influence was small (2%) (Table 4). Influence of the year was most remarkable also in the investigation with 19 winter rye cultivars in the three years 2004–2007 in Denmark (Hansen et al., 2016).

Chemical composition

The content of protein and starch belongs to important criteria for the quality of cereals (Stepien et al., 2016).

In our investigation grain protein content, starch content and falling number significantly ($p<0.05$) varied depending on the cultivars and meteorological conditions. The *protein content (PC)* ranged from 9.7% ('SU Mephisto') to 10.4% ('Dankowskie Amber'). The content of protein in rye grain was differentiated by weather conditions in years. Nowotna et al. (2006) showed that the average content of protein (for five test cultivars of winter rye) is 9.6%, while the investigation of west Lithuania region indicates a broader range of protein content from 9 to 19% (Skuodiene, Nekrošeine, 2009).

Table 6

Rye grain protein content, %

Cultivar	2015	2016	Average
Dank. Amber	8.7	12.2	10.4
Kaupo	7.7	12.3	10.0
Amilo	7.8	13.1	10.5
Brasetto	8.4	12.2	10.3
SU Drive	7.7	12.1	9.9
SU Mephisto	7.7	11.6	9.7
Average	8.0	12.3	10.1

Data in our experiment (2 years) suggest that protein content was significantly ($p<0.05$) influenced by harvest year (96%), while cultivar and cultivar × year influence was small (2%) (Table 4). However in Danish (Hansen et al., 2016) experiments protein content in rye grain dependency on cultivar complete to 67%, the influence of year – 17% but cultivar × year influence was small – 4%.

In 2016 harvest year the content of protein in rye grains was higher by 4.3% as compared with the 2015 year. In the 2016 with a higher mean air temperature in summer favoured a greater concentration of protein. Similar dependences of protein accumulation on weather conditions were confirmed by the study of (Stepien et al., 2016).

The average *starch content (SC)* (Table 7) in two investigation years of the six cultivars was 58.8%; it ranged from 60.9% ('Brasetto') to 62.2% ('Su Mephisto'). The content of starch measured in this study is in accordance with findings by other authors (Hansen et al., 2004; Ruzgas, Plycevaitiene, 2005; Jansone, 2015).

The starch content was significantly ($p<0.05$) influenced by year (94%), while cultivar influence and

cultivar × year influence was small, respective 3% and 2%.

Table 7

Rye grain starch content, %

Cultivar	2015	2016	Average
Dank. Amber	64.4	59.7	62.1
Kaupo	64.8	59.3	62.1
Amilo	64.3	58.8	61.5
Brasetto	63.1	58.8	60.9
SU Drive	64.1	59.6	61.8
SU Mephisto	64.1	60.2	62.2
Average	64.1	59.4	61.8

Falling number (FN) is an indication of degree of soundness of rye in terms of freedom from sprouting (Ingver et al., 2002) which causes the production and activation of α–amylase inside the rye kernel which, in its turn, has a very drastic effect on the dough and bread making process. In our investigation the falling number of grain was significantly ($p<0.05$) different for cultivars. Average data show that the falling number for cultivars was 212±13.0 s, range min – max 130–305 s, the coefficient of variation was 21.2% (Table 2). The falling number of six grain samples ranged from 133 s ('Kaupo') to 254 s ('Amilo') (Table 8). Vidmantiene and Joudeikiene, (2010) also confirmed that the falling number of different cultivars may vary in the same growing conditions.

Weather conditions in investigation years influenced grain α–amylase activity. Higher falling number for all rye cultivars was observed in 2014/2015 (214–305 s). Those were higher comparing to 2015/2016 (133–222 s). The falling number was affected by precipitation during grain maturation. High rainfall in grain maturation period resulted in higher α–amylase activity and lower falling number (Tupits, 2008). In our investigation in July 2016 during rye grain maturation the rainfall exceeded the long-term mean data.

Table 8

Rye grain falling number, s

Cultivar	2015	2016	Average
Dank. Amber	229	222	226
Kaupo	208	133	170
Amilo	305	203	254
Brasetto	266	205	235
SU Drive	221	158	190
SU Mephisto	214	189	202
Average	240	185	213

The falling number was similarly influenced by year 42% and cultivar – 44%, whereas the effect of cultivar × year accounted for 14% (Table 4), however in Hansen et al. (2016) experiments falling number dependency on year completed to 77% but the influence of cultivar 11%, cultivar × year – 8%.

Correlations

A statistically significant positive correlation was found between starch content and falling number r=0.594 and volume weight r=0.791 (n=12, $r_{0.01}$=0.576, $r_{0.05}$=0.708), which is in accordance with results obtained in Lithuania (Ruzgas, Plycevaitiene, 2005). Negative correlation was found between protein content and volume weight r= – 0.839 (Figure 1).

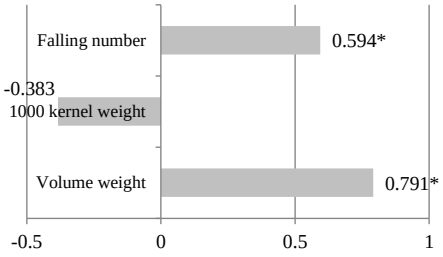

Figure 1. Correlation coefficient (r) between starch content and other quality indices

r* – is significant at 95% level probability

Protein content showed high negative relationship between starch content (r= – 0.981), what in the present experiment was confirmed in Lithuania also (Ruzgas, Plycevaitiene, 2005). Protein content significant negative correlated between volume weight (r=-0.839), these results are in accordance with the ones described by Hansen et al. (2016). A negative correlation between rye grain protein content and falling number identified in our investigation (r=0.616) was also found by Ruzgas and Plycevaitiene (2005) (Figure 2).

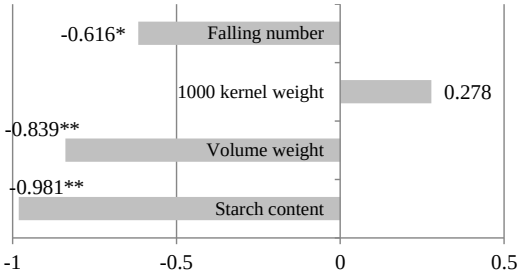

Figure 2. Correlation coefficient (r) between protein content and other quality indices

r**–significant at 99%; r*–significant at 95% level probability

Conclusions

The thousand kernel weight in grains of hybrid cultivars was statistically significantly higher comparing to grains of population cultivar.

Differences between hybrids cultivars grains volume weight, protein content, falling number comparing with population cultivar grains were not observed.

Year meteorological conditions had a much stronger effect on rye grain volume weight, protein content, starch content and falling number than cultivar.

The influence of cultivar was confirmed on higher level for winter rye grain protein content compared with the year meteorological conditions.

The strong negative correlation was found between protein content and falling number, volume weight, and starch content.
The positive correlation was found between starch content and falling number and volume weight.

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INVESTIGATION OF FERTILISATION IMPACT ON FRESH STRAWBERRIES YIELD AND QUALITY PARAMETERS

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Abstract

Strawberries are a choice fruit crop for many home gardeners. The low-growing plants are reliable and quick to produce. June bearers provide a delicious supply of fruit from late spring through early summer, while day-neutral types produce berries throughout the summer. The main purpose of the current research was to evaluate fertilizers impact on strawberries yield and quality parameters. In the experiments freshly picked garden strawberries cv Polka harvested in Koknese region (Latvia) in July 2016 were analysed. For strawberries fertilization Ferticare™ Kombi 1 (NPK 14-11-25+micro) with and without Ca $(NO_3)_2$, YaraLiva™ Calcinit with and without Ca $(NO_3)_2$ were applied at the rate 100 kg N ha^{-1} and 50 kg N ha^{-1}. As a control, strawberries without fertilizer were analysed. The main quality parameters were tested using standard methods: yield (t ha^{-1}); berries firmness (N); pH; nitrate content (mg kg^{-1}); soluble solids (°Brix); extra, proportion of I and II class berries from total yield (%); sensory properties of strawberries (line scale). During the current research significant impact of nitrogen fertilizer on the pH and firmness of strawberries, the content of soluble solids, nitrates content in berries, as well as the yield has been established. As a result, 50 kg of nitrogen fertilizer per hectare should be recommendable during spring strawberries fertilization with NPK 14-11-25+micro when the strawberry yield were 12.7 tons, the berry firmness was 0.94 N, pH was 3.67, nitrates in berries was below 36 mg kg^{-1}, the content of the soluble solids was 11.8 °Brix and the proportion of Extra, I and II categories berries was 83% from total yield.

Keywords: *strawberries, fertilization, quality parameters*.

Introduction

Fruits such as strawberry (*Fragaria* × *ananassa* Duch.) are characterized as being small and fleshy and are consumed in many countries, primarily fresh however as processing products too. Recently, consumers' interest in berries has grown due to their beneficial health effects because of the antioxidant and anticancer properties and for being a good source of fibre, vitamins, folic acid, fatty acids, polyphenols and minerals (Corrêa Pereira et al., 2016). Traditionally strawberry growth requires a considerable amount of water to establish plantings, and depends on frequent applications of pesticides to produce acceptable fruit quality (Pritts, 2002). Cultivation, variety, fertilization, region and weather conditions as well as sampling time and degree of ripeness significantly affect the nutritive value of strawberries and its final quality parameters. Strawberries need moderate fertilization and irrigation, as well as nearly neutral (6–6.5) soil pH (Hakala et al., 2003). Some research data suggest that nitrogenous fertilizers used in agriculture contaminate surface and underground waters, enhance N_2O emissions into the atmosphere resulting in possible nitrate accumulation in plants (Pešaković et al., 2013). In research, based on fruit quality, fruit quantity, water and nitrogen use efficiencies, nitrogen treatment (450 kg N ha^{-1}) would be the most appropriate nitrogen fertilizer application for vegetables by subsurface drip irrigated under the similar plant growth conditions in solar greenhouses (He-xi et al., 2011). Nitrogen is a special mineral nutrient absorbed by the roots of plants. It can be present in the soil either in mineral form, such as NO^-_3 and NH^+_4, or as organic compounds, such as amino acids, resulting from the mineralization of complex organic N forms. In general, strawberry is highly sensitive to mineral N in soil. Many farmers fail to cultivate this crop because it is not possible to apply chemical N fertilizers properly (Wang et al., 2009). However, $CaCl_2$ dips or controlled atmospheres can help maintain firmness and visual quality resulting in a longer shelf-life of the fresh-cut products as strawberries (Aguayo et al., 2006) after harvesting mainly. Calcium is considered as one of the most important nutrient elements in controlling the metabolism of plant cells. Its role in preventing various physiological disorders is well known. In addition, it is also known as retardant of fruit ripening and senescence processes. Though, the mechanism by which calcium prevents physiological disorders is not well understood, however it is known that it principally acts on middle lamella of cell wall and plays its role of cross-linking, where it may influence membrane bound enzymes too (Sharma et al., 2006). The consumer attitude towards food is very complex, as it is influenced by sensory and non-sensory attributes, as well as by the interactions between them. Consumer concerns include production and preservation technologies, contextual influences, social factors, health, ethnic and cultural concepts and price (Filho et al., 2014). Sensory characteristics of food can be considered as one of the key factors in food acceptance. People prefer to eat what is tasty, but the importance of food taste may differ between individuals. The healthiness of berries or berry products can also be an important factor affecting their acceptance and consumption. Possible disclosure of the health-related effects may have a positive impact on consumer acceptance (Jaeger et al. 2009; Laaksonen et al., 2016). The main purpose of the current research was to evaluate fertilizers impact on strawberries yield and quality parameters.

Materials and Methods

Materials and experiment steps

The investigations were carried out at Latvia University of Agriculture and JSP-Latvia in 2016. For strawberries non-root fertilization Ferticare™ Kombi 1 (NPK 14-11-25+micro) *(Kombi)* (content: N_2 14%, P_2O_5 11.6%, K_2O 25.3, MgO 2.4%, S 13.75%, B 0.02%, Cu 0.01%, Fe 0.1%, Mn 0.1%, Mo 0.002% and Zn 0.01%), as well as YaraLiva™ Calcinit *(Calcinit)* (content: N 15.5%, CaO 26.2%) were used. During fertilization extra 50 *(N50)* or 100 *(N100)* kg N ha^{-1}, however $Ca(NO_3)_2$ *(Ca)* were added. The scheme of the experiments was as follows:

1) no fertilizers (control)
2) Kombi + $Ca(NO_3)_2$ + N 100 (K/Ca/N100)
3) Kombi + $Ca(NO_3)_2$ + N 50 (K/Ca/N50)
4) Kombi + N 100 (K/N100)
5) Kombi + N 50 (K/N50)
6) Calcinit + $Ca(NO_3)_2$ + N 100 (Ca/Ca/N100)
7) Calcinit + $Ca(NO_3)_2$ + N 50 (Ca/Ca/N50)
8) Calcinit + N 100 (Ca/N100)
9) Calcinit + N 50 (Ca/N50)

The investigations were carried out in a strawberry plantation located in Koknese region (Latvia). Strawberry cv Polka was used for the experiments. Berries were harvested in July 2016. The experiment was designed in random blocks, number of replications – 3. The soil in the experimental plot was with pH 6.4. For strawberries' providing with calcium six time fertilization with $Ca(NO_3)_2$ was applied by 0.45 kg N ha^{-1} during one fertilization.

Yield calculation

Strawberries were picked three times a week, before they reached fully red colour, but when they had reached 85 to 89 growth stage (according to the scale). Harvested strawberries were divided in three classes regarding requirements that are needed for realization of fresh berries. Extra class strawberries are equal and normal regarding readiness (right shape and colour) as well as size; they are bright and clean, without any soil or any other dirt. Berry size was above 25 mm (measuring by the biggest diameter). Class I, class II strawberries can have slight shape defects, small white spots, they may differ in size. They are practically clean – without soil or any dirt. Size was above 18 mm. To ease work for berry pickers and have berries sorted by standards already at the farmstead there are informative guidelines with strawberry class samples. Class II strawberries have shape defects, white stains that are not more than one fifth of berry surface, slight dry abrasions. Berries may have a little soil, but no other dirt. In both sorted harvestings berries were healthy, clean (without any visible dirt, fresh and unwashed, without visible pest damage, with sepal, without foreign smell or taste).

Firmness

Texture analyser TA.HD plus (United Kingdom) was applied for evaluation of strawberries surface firmness. For the testing 5 mm cylindrical stainless probe was used with speed of 1 mm s^{-1} and trigger force 0.049 N on berries surface. For texture evaluation of 10 separate fruits was completed.

pH

Berries pH was evaluated using pH meter "Lutron PH-212" (Taiwan) according to LVS ISO 1132:2001 by immersing an electrode in blended berry mass.

Nitrate content

Nitrate content in berries was analysed according to GOST 29270-9 ionometric method. Experiments was realised in 3 reiterations.

Soluble solids

Soluble solids content of berries was measured with a Kruss Optronic digital hand refractometer DR 301-95 (Germany) in °BRIX according to LVS ISO 12143:2001.

Berries sensory properties

Berries sensory properties were evaluated using 15 cm line-scale (taste, hardness and colour) according to ISO 4121:2003.

Statistical analysis

Experimental results were analysed by Microsoft Excel 2013. Analysis of variance (ANOVA) and Tukey's tests were used to determine differences among samples. Differences were considered as significant at $p<0.05$.

Results and Discussion

Yield and Nitrates

Nitrogen (N) fertilizer has been used mainly to enhance crop yield, whereas lack of access to fertilizer N in most countries is a major cause of low crop yield and food shortages (Sims et al., 2013). Optimized N fertilizer application rate is one of the predominant aspects achieving food and environment security. Agronomic practices, as well as target yield and soil N supply need to be considered when determining optimal N fertilization rate (Ren et al., 2017).

In the present experiments significant N fertilization, however chosen commercial fertilizers impact on strawberries yield was established (Table 1). Low yield was detected on field without fertilization; it was 2–3 times lower comparing with yield obtained from differently fertilized fields. Higher yield was obtained from fields fertilized with K / N100 and K / Ca / N100 in comparison with control and other analysed samples. What mainly could be explained with used fertilizer composition (N_2, P_2O_5, K_2O, MgO, S, B, Cu, Fe, Mn, Mo and Zn) and its positive effect on strawberries yield. Lower yield was obtained using *YaraLiva™ Calcinit* fertilizer (Table 1).

Significantly higher nitrates content was obtained in strawberries fertilized with commercial fertilizer *Ferticare™ Kombi 1* and with both commercial fertilizers with extra fertilization with 100 kg N ha^{-1} (Table 1). Significantly ($p<0.05$) lower nitrates content was found in control berries sample and in Ca / N50 and Ca / Ca / N50. Taghavi et al. (2004) revealed the significant effect of different NO_3^- : NH_4^+ ratios on

yield, number of fruits, total nitrogen and nitrate content of different parts of the plants and leaf nitrate reductase activity was determined; also, the strawberry plants showed higher nitrogen content in this ratio compared to the treatment lacking ammonium. Yield and number of fruits are higher in nutrient solutions having both nitrate and ammonium.

Table 1

Assessment of strawberries yield and nitrates content

Sample	Yield, t ha^{-1}	Nitrates, mg kg^{-1}	Amount of Extra, I and II class berries % from yield
Control	4.7^{f}	<36^{d}	46^{c}
K / N100	15.0^{a}	76^{b}	85^{a}
K / Ca / N100	15.5^{a}	97^{a}	86^{a}
K / N50	12.7^{b}	<36^{d}	83^{a}
K / Ca / N50	12.6^{b}	40^{d}	80^{a}
Ca / N100	11.3^{c}	64^{c}	79^{a}
Ca / Ca / N100	10.6^{d}	72^{b}	81^{a}
Ca / N50	8.4^{e}	<36^{d}	82^{a}
Ca / Ca / N50	8.4^{e}	<36^{d}	84^{a}

* Results indicated with different letters in the column are significantly ($p \leq 0.05$) different.

Significantly ($p<0.05$) low amount of Extra, I and II class berries in % from total yield was obtained for control sample. It was approximately two fold lower in comparison with fertilized berries samples. As a result significant fertilizers impact on berries quality parameters was proved.

Firmness

Pronounced strawberries firmness was obtained for control berries and for berries extra fertilized with $Ca(NO_3)_2$ during harvesting (Table 2). However, obtained results demonstrate positive $Ca(NO_3)_2$ influence on the berries quality. However, opposite results were obtained in Lanauskas et al. (2006) research, where calcium nitrate applied to the soil decreased berry firmness. However Conway et al. (2002) in their research concluded that calcium in adequate amounts helps to maintain apple fruit firmness and decreases the incidence of physiological disorders such as water core, bitter pit and internal breakdown.

Soluble solids and pH

Cao et al. (2015) in their experiments asserted that soluble solids content is one of the main parameters for evaluating the nutritive value of strawberry (*Fragaria* × *ananassa*), which is affected by a number of factors including genetics, environmental conditions and cultivation practices. However, Keutgen and Pawelzik (2007) indicated close correlation of strawberries soluble solids, titratable acidity and taste.

Non-significant ($p>0.05$) impact of fertilizer on strawberries soluble solids was detected. In the present research obtained results of fertilized berries was very close to control berry sample. Obtained results are very similar with Cao et al. (2015) research, where content of strawberries soluble solids varies from 7.6 to 11.2 °Brix.

Oliveira et al. (2015) summarized that at the pH values typically found in fresh and processed fruits and vegetables will most probably be represented by a mixture of equilibrium forms.

Table 2

Assessment of strawberries quality

Sample	Firmness, N	pH	Soluble solids, °BRIX
Control	1.22±0.07^{e}	3.59±0.09	10.80±0.04
K / N100	0.76±0.02^{c}	3.47±0.05	10.30±0.02
K / Ca / N100	0.83±0.02^{b}	3.57±0.07	10.50±0.03
K / N50	0.94±0.01^{a}	3.67±0.09	11.80±0.03
K / Ca / N50	0.99±0.03^{a}	3.69±0.08	11.60±0.01
Ca / N100	0.93±0.02^{a}	3.61±0.02	11.00±0.07
Ca / Ca / N100	0.97±0.03^{a}	3.65±0.03	11.10±0.02
Ca / N50	1.37±0.04^{e}	3.61±0.04	10.90±0.03
Ca / Ca / N50	1.42±0.01^{e}	3.66±0.07	10.50±0.07

* Results indicated with different letters in the column are significantly ($p \leq 0.05$) different.

The pH modification can influence chemical reactions in phenolic compounds like isomerization or mechanisms of intra- and intermolecular co-pigmentation as well as self-association between compounds such as anthocyanins and chlorogenic acid, caffeic acid and rutin. Similarly, as soluble solids content, non-significant ($p>0.05$) impact of fertilizer on strawberries pH was detected too. In the present research obtained results of fertilized berries were very close to control berry sample.

Sensory properties

To have more detailed evaluation of the harvested berries, a sensory evaluation has been done. Following criteria were used – berry external appearance (shape), colour, taste, berry firmness. Strawberries for sensory evaluation were picked at the third harvest in order to avoid first harvest's berry size effect. In the sample without any additional fertilizer berries size did not reach Extra class criteria and were close to 18 mm, so that was the reason for lower external appearance evaluation (Fig. 1). Firmness results show that berries with fertilizer application rate 100 kg N ha^{-1} are not firm enough. In samples with additional fertilization for cv Polka colour was evaluated better compared to samples of berries with no additional fertilization. Strong flavour was observed in samples with complex fertilization, where strawberries received all necessary nutrients. Also sensory evaluation results show high scores for berries with 50 kg nitrogen provision with complex fertilizers. Experts could not determine any taste differences in berries that were cultivated with 100 or 50 kg nitrogen provision with calcium nitrate, therefore, soil provision with nutrients is not sufficient even after the additional fertilization. In order to improve not only harvest mass, but also taste and firmness, it is important to make complex fertilization. Evaluated strawberry size was corresponding to class I and class II berry sizes. Colour of the berries is essential, because that is the first thing that customer

notices, then follows external appearance, but taste is only rated after purchasing the strawberries. Thereby fertilizers role in harvest formation significantly affects berry sensory attributes.

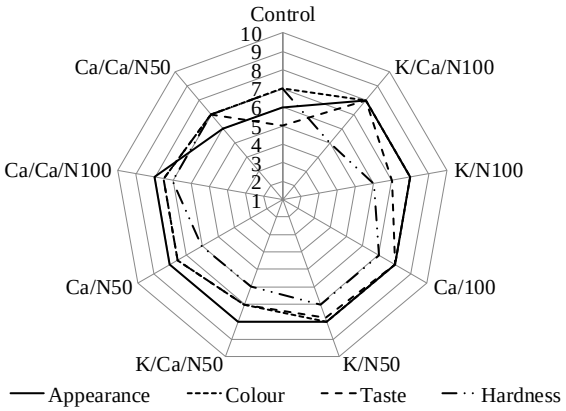

Figure 1. Sensory characteristics of strawberries

The common quality parameters of strawberries for consumer acceptance are appearance, firmness, and flavour. However, the instrumental measures of strawberry quality most reported are colour, firmness, juice, volatile compounds, sugars and organic acids. As well as fruit-to-fruit variation is substantial in fruit firmness and sensory characteristics (Gunness et al., 2009).

Conclusions

Results of present experiments demonstrate that using complex fertilizers at the rate of 50 kg nitrogen per hectare, as well as using 100 kg nitrogen per hectare together with Ca $(NO_3)_2$, allows producing berries with higher quality parameters.

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QUALITY OF TOMATOES DURING STORAGE

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Abstract

Tomatoes (*Solanumly copersicum*) are well known antioxidants, vitamins and other health beneficial compounds containing vegetable. Different qualitative and quantitative changes of chemical composition take place during tomato fruit ripening and storage. Research with the aim to evaluate the chemical composition (soluble solids, titratable acidity, vitamin C, total phenols) of tomatoes stored under ambient conditions was set up during year 2016. The study involved five tomato varieties cultivated and collected from greenhouse at green stage of ripening, then stored at room air temperature from 18.0 °C to 19.0 °C and relative humidity from 40.2% to 50.6% for 36 days. Collected data showed that the highest increase (in average for 12.6%) of total soluble solids content was observed till 24 days of storage. It was found that content of vitamin C during ripening increased till the 24 day of the storage and it significantly depends on tomato variety (from 4.21% in variety Sakura F1 till 33.72% in variety Black Cherry F1). Further the content of vitamin C decreased and after 36 days of storage it was less than 7% compared with the beginning of the experiment. The titratable acidity was significantly ($p<0.05$) different among the tomato varieties and depended on the stage of ripening. It varied between 0.841±0.012 g 100 g^{-1} (Sakura F1) at harvest and 0.302±0.009 g 100 g^{-1} (Golden Nudget F1) at the end of storage. According to results the content of phenols during storage was variable and therefore the correlations were not observed.

Keywords: tomatoes, bioactive compounds, storage.

Introduction

Tomato (*Solanumly copersicum*) is second the most important vegetable crop worldwide (Pantheen, Chen, 2010), providing an important nutritional value to human diet. There is a growing interest in the beneficial health effects of tomato derived antioxidants (Carlsen et al., 2010; Korekar et al., 2011) and many scientific studies have been performed for demonstrating the benefits of tomatoes for human health (Burton-Freeman, et al. 2012; Gómez-Romero et al., 2010; Selli et al., 2014). These health benefits have been associated with nutritional value and bioactive phytochemicals along with carotenoids, vitamin C, and the phenolic content of tomatoes (Mordente et al., 2011; Vallverdú-Queralt et al., 2011).

The chemical composition of tomatoes depends on different factors such as variety, maturity, light, temperature, soil composition, fertilization, irrigation, handling practices, storage, and environmental conditions in which they are grown (Adubofuor et al., 2010).

Different qualitative and quantitative changes of chemical composition take place during tomato fruit ripening. The postharvest ripening stage has been associated with the production of flavour and aromatic compounds (Požrl, et al., 2010), an increase in ascorbic acid content and total soluble solids (Toor, Savage, 2006). Physicochemical profile of tomato fruits changes significantly over time and with the storage temperature (Okolie, Sanni, 2012). The optimal ripening condition for red tomatoes lies between 18 °C and 21 °C. Temperature below 5 °C and 10 °C for longer than 7 and 14 days, respectively, prevents ripening and full colour development (Suslow, Cantwell, 2013).

The main antioxidants in tomatoes are carotenoids such as β-carotene, a precursor of vitamin A, lycopene, vitamins such as ascorbic acid and phenolic compounds such as flavonoids and hydroxycinnamic acid derivatives (Kotkov et al., 2011; Vallverdú-Queralt et al., 2011). These compounds may play an important role through free-radical scavenging, metal chelation, inhibition of cellular proliferation, and modulation of enzymatic activity and signal transduction pathways (Crozier et al., 2009). Phenolics are important compounds for plants, due to acting as phytoalexins, contributors to plant pigmentation and reproduction as well as UV-light protectors. These compounds determined nutritional quality of fruits, vegetables, and other plants; they have been reported to be food preservatives as well as having a primary role in protection against pathological and degenerative disturbances (Ignat et al., 2011).

The aim of study was to evaluate the chemical composition (soluble solids, titratable acidity, vitamin C, total phenols) of tomatoes stored under ambient conditions.

Materials and Methods

Investigations were carried out at the Latvia University of Agriculture, Institute of Soil and Plant Sciences.

Five tomato varieties Sunstream F1, Sakura F1, Black Cherry F1, Golden Nudget F1 and Rhianna F1 produced in the conventional way in greenhouse from 1st of May till 15th of September 2016 were studied. Sample fruits were harvested at green stage of maturity, each variety had a sample of 30 fruits per replication. Seven tomato fruits having similar size and colour of each variety were randomly selected for analysis, weighed, hand-rinsed with pure water, shaken to remove water, blotted with a paper towel, mixed, homogenized, and immediately analysed.

Tomatoes were stored under ambient conditions at room temperature from 18.0 °C to 19.0 °C and relative humidity from 40.2% to 50.6% for 36 days with four replications. On each sampling date (after 24 and 36 days), seven tomato fruits per experimental unit

were randomly selected from each replication for analysis.

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.

Analytical methods

The total soluble solids content was determined using a Refractometer A.KRÜSS Optronic Digital Handheld Refractometer Dr301-95, calibrated at 20 °C with distilled water and expressed as ºBrix.

The content of vitamin C was determined titrimetrically using 2.6- dichlorphenolindophenol. For determination 2±0.001 g of tomato puree was quantitatively transferred in 100 mL tubes, added 50 mL of 1% HCl and 5% HPO_3 mixture (1 : 1 v/v) and mixed thoroughly. After 30 minutes solution was filtered through a filter paper No. 89th. For determination 10 mL (V_a) of filtrate was titrated with 0.0005 molar solution of 2.6 dichlorphenolindophenol (V_{titr}). The content of vitamin C was calculating according to the equation (1):

$$\text{Vitamin C (mg 100 g}^{-1}) = \frac{V_{titr} \cdot 0.044 \cdot V_{total} \cdot 100}{V_a \cdot weight} \quad (1)$$

The titratable acidity was measured by the direct titration method with a strong alkaline solution. For determination 5±0.001 g of tomato puree was quantitatively transferred in 100 mL tubes, added 40 mL of pure water, mixed thoroughly. After 20 minutes solution was filtered through a filter paper No. 89th.

For determination 10 mL of filtrate was titrated with 0.1 M NaOH solution and expressed as g 100 g^{-1} of citric acid.

For total phenols extraction 1.0±0.001 g of finely ground tomato samples was weighed into volumetric flasks, 10 mL of extract, a mixture of methanol, distilled water and hydrochloric acid (79:20:1 v/v/v) was added. The vials were shaked at 20 °C for 60 min in the dark, then centrifuged for 10 min at 5000 rpm. The total phenolic content of the tomato samples was determined using the Folin-Ciocalteu reagent. To 0.5 mL of extract 2.5 mL of of Folin-Ciocalteu reagent (diluted 10 times with water) and, after 3 minutes 2 mL of sodium carbonate Na_2CO_3 (75 g L^{-1}) was added. The sample was mixed. After 1 hour of incubation at room temperature, the absorbance was measured at 765 nm. Total phenols were expressed as gallic acid equivalents (GAE) 100 g^{-1} FW of tomatoes.

Statistical analysis

Analyses were performed in three replicates and each one was measured for three repetitions. Data were expressed as mean of triplicates assay ± standard deviation; for mathematical data processing the value of $p<0.05$ was regarded as statistically significant. One-way analysis of variance (ANOVA) was used to determine the significance of differences.

Results and Discussion

It has been shown that that skin and seeds are important contributors to the major antioxidant compounds of tomatoes (Toor, Savage, 2006), therefore chemical analyses were performed on whole tomatoes.

Total soluble solids

Significant ($p \leq 0.05$) difference was observed in total soluble solids content of tomato varieties during 36 days storage at ambient conditions. In tomato fruit samples the content of soluble solids (Figure 1) was found to be from 5.3 °Brix (Sunstream F1 at harvest) till 8.3 °Brix (Golden Nudget F1 and Rhianna F1 after 24 days of storage). The results indicated that the highest increase of soluble solids in all analysed tomato samples was observed till 24 days of storage, and after that time the values were decreased.

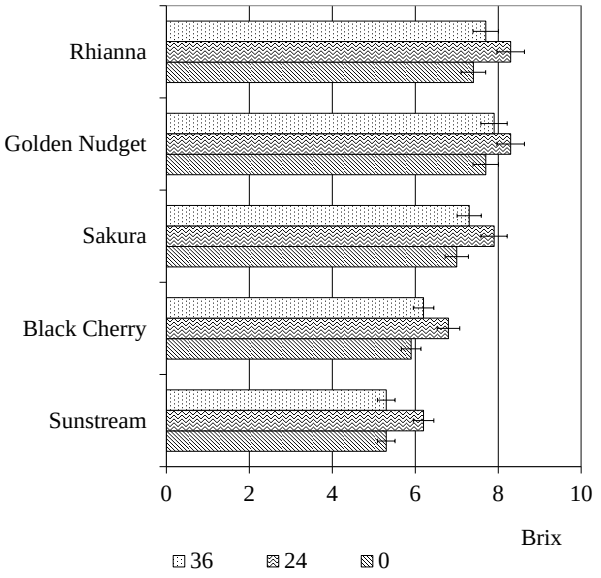

Figure 1. Total soluble solids content of tomato varieties depending on storage time (days)

These results are in agreement with Tigist et al. (2013), Ochoa-Velasco et al. (2016) and Talens et al. (2016).

Content of vitamin C

Vitamin C or ascorbic acid is one of the most important nutritional value parameter in fruits and vegetables (Tigist et al., 2013). The content of ascorbic acid at harvest was from 10.41 mg 100 g^{-1} (Black Cherry F1) till 14.95 mg 100 g^{-1} (Sakura F1). These values are in agreement with the concentration of ascorbic acid reported by Ochoa-Velasco et al. (2016), but less than findings of Vinha et al. (2013) and Kelebek et al. (2017).

In our study it was found that content of vitamin C during ripening increases till the 24 day of the storage (Figure 2). Moreover we could conclude that increase significantly ($p \leq 0.05$) depends on tomato variety – from 4.21% variety Sakura F1 till 33.72% variety Black Cherry F1.

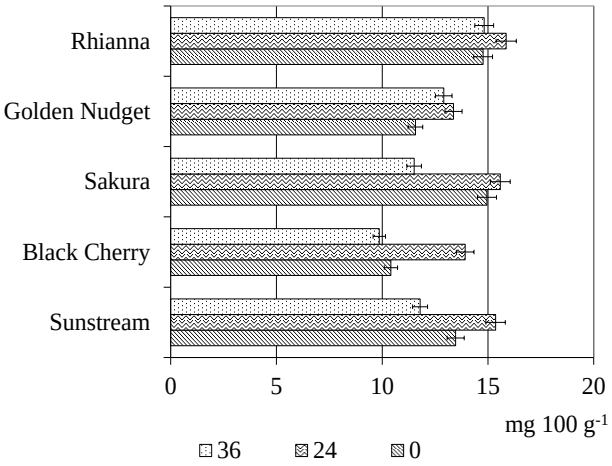

Figure 2 Ascorbic acid content of tomato varieties depending on storage time (days)

Further during storage reduction of values were observed. The content of vitamin C decreases and after 36 days of storage it was less than 7% compared with the beginning of experiment. Similarly Ajayi and Oderinde (2013) observed the decrease of vitamin C.

Titratable acidity

In tomato fruit samples the titratable acidity was significantly ($p \leq 0.05$) different among the tomato varieties and depends on time of storage (Table 1).

It was found that the titratable acidity at harvest was in the range of 0.61 g 100 g^{-1} till 0.84 g 100 g^{-1}. Our findings are higher than results noted by Mladenovic et al. (2014) who determined that the total acidity can be from 0.193 g 100 g^{-1} till 0.49 g 100 g^{-1} in cherry tomato. After 24 days of storage at ambient conditions, the decrease of titratable acidity was observed from 30.5% (Golden Nudget F1) till 34.9% (Sunstream F1), but after 36 days the decrease was in average 24.7%.

Table 1

Titratable acidity* of tomato varieties depending on storage time (days)

Tomato variety	Storage time (days)		
	0	24	36
Sunstream F1	0.662±0.098	0.431±0.025	0.322±0.023
Black Cherry F1	0.816±0.102	0.558±0.124	0.404±0.045
Sakura F1	0.841±0.147	0.569±0.073	0.421±0.058
Golden Nudget F1	0.609±0.092	0.423±0.056	0.322±0.037
Rhianna F1	0.739±0.087	0.508±0.101	0.404±0.067

* expressed as citric acid g 100 g^{-1} FW of tomatoes

The decrease of titratable acidity during the storage could be related to higher respiration rate as ripening advances where organic acids are used as substrate in respiration process (Tigist et al., 2013).

Analysing obtained results we can conclude that tomato variety Rhianna F1 is more suitable for storage taking account the changes of titratable acidity (the decrease during storage 45.3%), but the highest decreases was determined in tomato variety Sunstream F1 (51.4%). It is known that high levels of acidity are responsible for the stability of vitamin C during storage of fruits and vegetables (Vihna et al., 2013).

Total phenols content

According to obtained results (Table 2) the content of total phenols in analysed tomato samples during storage is variable and therefore the correlations were not observed.

It was find out that at green stage of maturity the richest tomato variety with phenolic compounds is Golden Nudget F1 (more than 40 mg GAE 100 g^{-1}), but Black Cherry F1 contains about two times less these compounds.

Table 2

Total phenols content* of tomato varieties depending on storage period (days)

Tomato variety	Storage time (days)		
	0	24	36
Sunstream F1	32.91±2.05	25.31±1.25	33.18±3.21
Black Cherry F1	23.63±1.95	28.08±2.03	35.62±2.69
Sakura F1	35.87±2.98	35.75±2.93	32.61±3.47
Golden Nudget F1	42.70±3.67	31.67±3.07	29.89±2.57
Rhianna F1	37.96±2.36	32.39±2.95	27.65±1.72

* expressed as mg gallic acid equivalents (GAE) 100 g^{-1} FW of tomatoes

Determined total phenols content are in agreement with Vinha et al. (2013), but higher than results reported by Ochoa-Velasco et al. (2016),

The content of total phenols changes after 24 or 36 days of storage. We observed less phenolic content in tomato varieties Sakura F1, Golden Nudget F1 and Rhianna F1. One explanation could be that the decrease in levels of phenolic compounds observed at the end of storage could be due excess of rate of maturation (Vinha et al., 2013). In addition, other reasons could have been the binding of phenols to proteins and changes in chemical structures (Miranda et al., 2010).

Phenolic substances have also been linked to the stability of vitamin C due to its protective effect (Vinha et al., 2013). Therefore, increased levels of total phenols might also be explained with higher contents of other antioxidants.

Conclusions

The major finding of this work was that content of antioxidants in tomatoes changed during storage at ambient conditions and it depended on tomato variety and duration. Content of vitamin C increased till the 24 day of the storage and it significantly depended on tomato variety (from 4.21% variety Sakura F1 till 33.72% variety Black Cherry F1). Further the content of vitamin C decreased and after 36 days of storage it was less than 7% compared with the beginning of the experiment. The titratable acidity was significantly ($p<0.05$) different among the tomato varieties and depended on the stage of ripening. It varied from 0.841±0.012 g 100 g^{-1} (Sakura F1) at harvest till 0.302±0.009 g 100 g^{-1} (Golden Nudget F1) at the end of storage. According to results the content of phenols

during storage was variable and therefore the correlations were not observed.

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EFFECT OF MUSHROOM POWDER IN FRESH PASTA DEVELOPMENT

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Abstract

Fresh pastas with *Shiitake* mushroom flour (MF) were produced. The MF was produced by drying the mushrooms at 40 °C. Proportions of 5%, 10%, and 15% MF were used to prepare the fresh pastas (FP), with two types of wheat flour (regular (RWF) and 30% semolina wheat flour (SWF)). Mushroom pastas were analysed before (FP) and after cooking (CP). FP presented moisture and water activity lower than 35% and 0.95, respectively. The L* and b* colour parameters were similar for pasta produced with RWF and SWF, with the major differences for pastas produce with high content of MF. Thus, with increasing of MF, pastas were redder, darker and less yellow. The CP showed high colour similarities between them. Generally, the introduction of MF changes the texture of pastas. Internal and external firmness, and adhesiveness, decreased with increasing MF and for higher drying temperatures. CP presented similar tendencies, with low firmness, high adhesiveness and high stickiness. The soluble solids were determined in CP cooking water, and range between 5.8 and 9.0%, without a consistent pattern, allowing classifying the pastas as high and medium quality. Sensory analysis revealed that consumers preferred pastas produced with high content of MF with similar profiles for all the parameters tested, and the influence of the type of wheat flour was not important. It could be concluded that mushroom powder could be used to produce pastas with an improved quality and similar characteristics to regular pastas.

Keywords: shiitake, flour, noodle, physicochemical properties, sensory analysis.

Introduction

Pasta is a cereal-based food product and its importance is related with low cost, easy preparation, appreciated sensorial properties, long shelf life, playing an important role in human nutrition and culture (Bergman et al., 1994; Fu, 2008). Pasta is usually made with semolina from durum wheat and water (Marti, Pagani, 2013), but other ingredients can be added, like eggs, whey and herbal products (Nouviaire et al., 2008; Schoenlechner et al., 2010; Krishnan, Prabhasankar, 2012), which are becoming very important in the modern pasta manufacture.

The overall quality of pasta is related to its cooking properties, nutritional value, and textural characteristics (Lu et al., 2006). The high cooking quality is related to low cooking losses and minimal increase in volume during cooking (Lu et al., 2016). The nutritional and textural properties of pasta are important to the acceptability by the consumers (Sobota et al., 2015; Ficco et al., 2016).

Several studies were done in order to improve the nutritional value and functional properties of pasta by substituting the semolina flour by other raw materials, such us fibres, inulin, and other materials (Tudorică et al., 2002). This replacement lead to an increase in availability of gluten-free products, and a decrease in the demand of durum wheat flour, which production is very limited leading to a partial or complete replacement of semolina with common wheat flours or other flours (Mastromatteo et al., 2011; Kim et al., 2016; Biernacka et al., 2017). However, the replacement of semolina is still a challenge, since the addition of other ingredients affects pasta properties (Lorusso et al., 2017).

Shiitake (*Lentinula edobes*) mushrooms are the second most produced mushrooms in the world (about 25%) (Jiang et al., 2015). These mushrooms have a wide range of health benefits, like antiviral, antioxidative, antitumor and hypocholesterolaemic effects (Kim et al., 2014). They also present high nutritional value, having some bioactive components, like dietary fibre, antioxidants, minerals, folates, essential amino acids (such us lysine) as well as vitamins B_1, B_2, B_3 (niacin) and C (Li et al. 2014; Jiang et al., 2015).

Mushrooms are quite perishable and deteriorate very quickly after harvesting due to their high moisture content (87–95%) (Walde et al., 2006). Drying processes are usually used to remove water from mushrooms in order to avoid the growth of spoilage microorganisms, and biochemical reactions, such as enzymatic activity (Krokida et al., 2003). Furthermore, when there is an overproduction of mushrooms or they present commercial defects to be consumed in fresh but with quality to be used in food processing, they could be dried to produce flour for human consumption. Dried Shiitake mushrooms present an intense flavour, similar to meat or cheese, when compared with fresh mushrooms due to the breakdown of proteins into amino acids during the drying process (Qi et al., 2014), and due to lenthionine and guanosine 5´-mono-phosphate (5´- GMP) (Baek et al., 1989). Shiitake mushrooms could be added to food directly fresh or dehydrated, whole or in pieces or as powder, enlarging their consumption and providing beneficial health effects through food products (Lin et al., 2008).

Moreover, the introduction of mushroom powders or flours, and some derivative products from them, in pastas had been studied by several authors (Kaur et al., 2013; Kim et al., 2016; Lu et al. 2016).

In this study, *Shiitake* mushroom flour was added, at different proportions (5, 10 and 15%) to regular wheat flour (RWF) and 30% semolina wheat flour (SWF) with the aim of replacing wheat flour by mushroom flour in pasta production, with acceptable quality, in order to valorise this mushroom, creating economic benefits for producers and avoid unnecessary wastes.

Materials and Methods

Shiitake mushrooms (*Lentinula edodes*) were provided by the company Ementa Saudável (Viseu, Portugal). Mushrooms were cleaned and sliced, before being dried in an FD 115 Binder ventilated drying chamber (with an air flow of 300 $m^3 h^{-1}$) at 40 ºC, during approximately 10 hours and 30 minutes (until constant weight). After drying, shiitake mushroom slices were milled in a SK 100 Cross Beater Retsch (Kiwa International, Germany) knife with a 1 mm sieve to obtain the mushroom flour (MF). The powder or flour was put in a sealed bag and stored at refrigeration temperature until used.

For the **preparation of the pastas** two types of wheat flour were used: regular wheat flour (RWF), type 65, and wheat flour with 30% semolina (SWF) (from durum wheat). It was also tested the proportion of mushroom powder: 5, 10 and 15%. Control pastas with the RWF and SWF (without mushroom flour) were produced. Tap water was added in a percentage of 30%. All the ingredients were hand mixed and passed through a pasta machine Urban Living (Unclepodger enterprise, Belgium) equipped with sheet roller and sheet cutter section, in order to obtain the definitive form of pastas (Figure 1). The final pastas samples look like *tagliatella*, with a thickness of 0.2 cm, width of 1 cm and length of 18 cm. Pastas were used for analysis immediately after preparation.

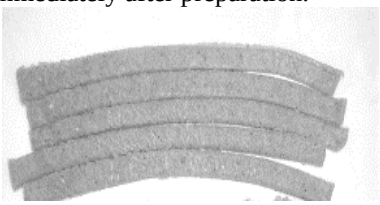

Figure 1. Example of a mushroom pasta produced

The cooking analysis of fresh pastas included cooking time and cooking loss. Optimum **cooking time** (the time necessary to obtain total starch gelatinization, corresponding to the disappearance of the white core) was determined following by the method 16–50 of AACC (2000). Fresh pasta (50 g) was cooked in 500 mL of boiling water and at every 30 s it was scooped out of water and squeezed between 2 pieces of glass.

Therefore, the cooking time of fresh pastas (50 g) cooked in 500 mL boiled tap water was 5 minutes for all cases. Despite this result, some authors found that the supplementation of mushroom powder significantly increase the minimum cooking time of pasta as compare to control (semolina pasta without addition of *Agaricus bisporus* mushroom flour) (Kaur et al., 2013).

Cooking loss (expressed as percentage) was evaluated by determining the amount of solids lost in the cooking water (Curiel, et al., 2014). Cooking water collected from each sample was subjected to evaporation until constant weight at 105ºC in an air oven FD 115 Binder (Binder, Germany) to determine the solids.

Colour was evaluated by a colorimeter CR-400 (Konica Minolta, Japan) in the CIE LAB colour space: L* (whiteness / darkness), a* (redness / greenness), b* (yellowness/ blueness). Ten measurements were performed on each produced pastas.

Texture was evaluated by texture profile analysis (TPA) with a texture analyser TA.XT Plus (Stable Micro Systems, United Kingdom), using a cut test with a Warner-Bratzler probe. The experimental graphs allowed estimating external firmness, inner firmness, adhesiveness and stickiness, as shown in Figure 2. The cut distance was 40 mm, the activation force was 0.2 N, and the test velocity was 2 $mm\ s^{-1}$. Ten measurements for each pasta were performed.

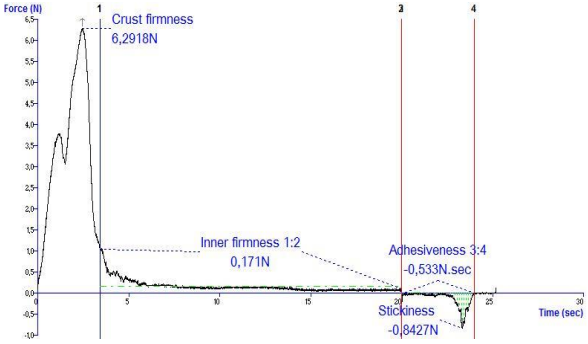

Figure 2. Example of a TPA texture profile performed to a pasta

The **moisture content** of pastas was determined by oven drying (AOAC, 2012). Water activity was determined by a hygrometer (Rotronic) and five determinations were made

For the **sensory evaluation** a sensory panel of 25 non trained assessors were used, for evaluation as a consumer panel. Although the assessors were not trained for this type of product, they were experienced in making sensory analysis. For the assessment of the sensory profile the following attributes were analysed on a 10 point scale varying from 1 (least intense) to 10 (most intense): homogeneity, proportion of smudge, colour, mushroom aroma, mushroom taste, firmness and general appreciation.

All the determinations were performed at least in triplicated for each mushroom pasta. All the results were express in mean ± standard deviation, which was used to quantify the amount of variation or dispersion of data values.

Results and Discussion

Cooking loss

Cooking quality is related with the ability of pasta to maintain the textural properties at the optimum cooking time (Del Nobile et al., 2005). The high cooking quality of pastas is related to low cooking losses (Lu et al., 2016). Aravind et al. (2013) stated that cooking loss correlated with cooking pasta quality and explained the capacity of gluten-starch matrix to maintain the physical integrity of pasta during cooking.

The percentages of soluble solids in the cooking water of pastas are showed in Table 1. The high values of soluble solids are presented by pastas produced with SWF, and the SWF pasta produced with 15% of MF has the highest amount of soluble solids (8.6%). The

RWF pasta with 15% MF shows the lower value of soluble solids (5.8%). The solids loss in cooking water should not exceed 9% (AACC, 2000), thus the values obtained in this work were within that limit. Similar results were found by Kaur et al. (2013) and Lu et al. (2016) for semolina pastas enriched with mushroom powder. The addition of mushroom flour seems to enhance the solids loss in cooking water in more obvious way in SWF, which is not the case for common wheat flour pastas. The results for SWF pastas are in accordance with those found by Kaur et al. (2013).

Table 1

Soluble solids in the water of cooked pasta

% MF	RWF,%	SWF, %
0 (Control)	6.8±0.4	7.1±0.5
5	6.1±0.3	7.8±0.6
10	6.7±0.5	7.8±0.4
15	5.8±0.4	8.6±0.9

The encountered results could be explained by the protein content and quality, and also by the formation of a continuous protein network in entrapping starch (Pagani et al., 1996). Kim et al. (2016) mention that the increasing of cooking loss values could be attributed to a dilution of gluten matrix and protein-starch matrix due to β-glucan substitution. Thus, this disturbs result in a discontinuous network, leading to a leak solid material into the cooking water (Resmini, Pagani, 1983).

Colour

Food colour is an important characteristic considering food quality. Pasta colour results from the properties of flour or semolina but also from other raw materials used (Biernacka et al., 2017).

The results showed that the L* values decrease with the increasing of MF content, and this is observed both for fresh and cooked pastas, probably due to the colour of shiitake flour, which is dark. It was also noticed that pastas became darker with cooking. Moreover, the SWF pastas presented a lighter intensity of colour when compared with RWF pastas, however after cooking there is no noteworthy difference.

The a* coordinate showed quite different values in fresh pastas, with high values for pastas produced with RWF and with high content of mushroom flour, meaning that RWF pastas are reddish brown, and this is intensified by the addition of mushroom powder. It is also possible to notice that SWF pastas presented similar tendencies as RWF pastas. Moreover, cooking causes a change in a* values, leading to a reduction. Generally, the a* values are quite similar for the wheat flours used, and the main differences are in the pastas with MF. The a* value increases with the increasing of MF, mainly when the proportion is equal or higher than 10%.

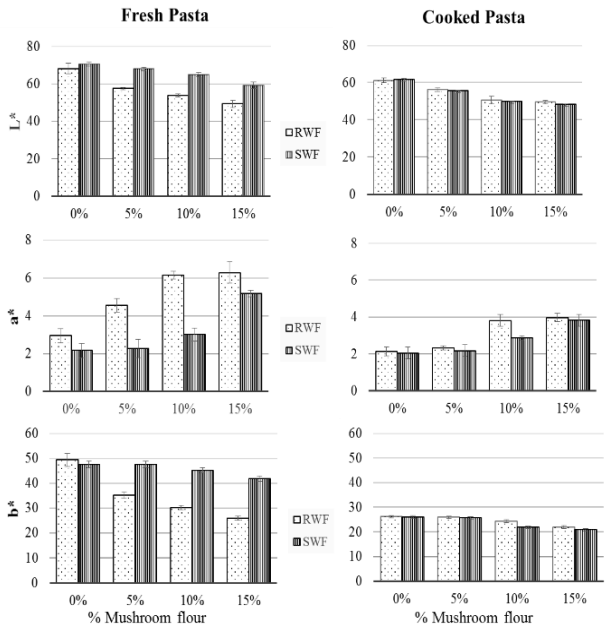

Figure 3. Colour characteristics of mushroom pastas

Kim et al. (2016) also found similar results for common wheat pastas with *Pleurotus eryngii* mushroom β-glucan.

Figure 3 also showed that the b* colour coordinate presented high values, meaning that yellow is the predominant colour. The b* decreased with the increasing of MF, and the SWF pastas presented high values. This means that pastas produced with SWF are more yellow and when the proportion of MF increases they became less yellow. After cooking, the b* values are quite similar for all pastas produced, with a slight decrease when the MF proportion increases.

In general, the CIELAB colour coordinates presented higher values for fresh pastas, which could be due to the lixiviation of colour compounds to the cooking water, what was effectively observed.

Texture

The textural characteristics of pasta are determinant in the final acceptance by consumers, and could be affected by several factors, such as type and rate of fibre and proteins included into the pasta (Tudorică et al., 2002). The textural properties of fresh and cooked pastas are presented in Tables 2 and 3, respectively. According to the results, the inner and external firmness in fresh RWF and SWF are very similar, but with pasta cooking the SWF presented lower values. Moreover, the inner and external firmness of fresh pastas decrease with the increasing of MF proportion for all the pastas, being the lowest values found for the pastas produced with 15% MF. Thus, pastas with MF were less firm, especially in SWF pastas.

Table 2

Textural properties of fresh pasta

Property	% MF	RWF	SWF
Inner firmness, N			
	0 (Control)	2.63±1.41	2.26±0.68
	5	2.33±0.55	1.60±0.14
	10	1.93±1.52	0.72±0.12
	15	1.88±0.78	0.64±0.12
External firmness, N			
	0 (Control)	6.81±1.87	6.01±1.44
	5	4.65±0.55	2.31±0.36
	10	4.15±1.54	1.57±0.26
	15	4.01±0.80	0.86±0.19
Adhesiveness, N.s			
	0 (Control)	-11.12±2.29	-8.15±1.69
	5	-7.13±1.01	-3.76±1.01
	10	-5.30±0.77	-2.62±0.09
	15	-3.73±0.33	-2.48±0.23
Stickiness, N			
	0 (Control)	-10.79±2.39	-6.07±2.44
	5	-3.45±0.45	-1.71±0.19
	10	-2.24±0.44	-1.56±0.15
	15	-1.13±0.25	-1.43±0.26

The pasta adhesiveness and the stickiness decrease with the addition of MF, with lower values for pastas produced with SWF.

Cooked pastas showed higher values for firmness parameters when compared with fresh pastas, and these textural characteristics also decreased with the increasing of MF, but SWF pastas presented lower values of firmness parameters (Table 3). Generally, the adhesiveness and the stickiness showed low values in cooked pastas. The results also allow to say that all the pastas have similar values, considering the same textural property.

Table 3

Textural properties of cooked pasta

Property	% MF	RWF	SWF
Inner firmness, N			
	0 (Control)	10.82±0.27	5.21±1.13
	5	3.87±0.71	1.93±0.36
	10	2.12±0.56	1.67±0.26
	15	1.93±0.57	1.26±0.20
External firmness, N			
	0 (Control)	13.22±2.18	9.06±1.44
	5	8.24±0.91	5.60±0.57
	10	5.80±0.94	5.16±0.12
	15	4.86±1.06	4.65±0.18
Adhesiveness, N.s			
	0 (Control)	-2.88±0.48	-3.23±1.17
	5	-3.01±0.83	-3.01±0.79
	10	-2.58±0.88	-2.37±0.28
	15	-2.37±1.01	-1.77±0.35
Stickiness, N			
	0 (Control)	-1,51±0.88	-1.65±0.18
	5	-1.71±0.26	-1.71±0.30
	10	-1.63±0.31	-1.38±0.13
	15	-1.60±0.27	-1.46±0.19

The encountered results could be explained by the physical competition between protein interaction and coagulation in a continuous network and starch (Lu et al., 2016). Moreover, the firmness is one of the most important evaluation indexes of pasta, which measures the force required for shearing the pasta. The reduction of pasta firmness could be also associated with the role of fibre from mushroom powder in disrupting the protein-starch matrix of pasta (Tudorică et al., 2002).

Moisture and water activity

The moisture and water activity (a_w) are important factors for food storage. Thus, these parameters were also determined for both fresh and cooked pastes to estimate if there were considerable differences between them. The results of these determinations were determined just for RWF pastas (Figure 4). In fact, considering the above results, it is possible to notice that in a general overview, the pastas with better characteristics were the ones produced with RWF.

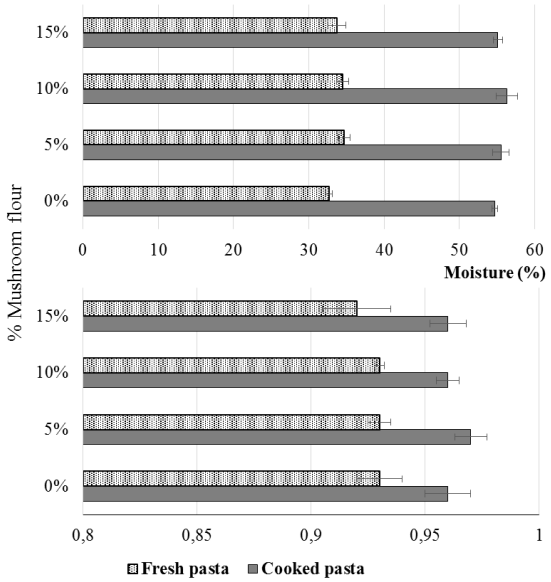

Figure 4. Moisture and water activity (a_w) of RWF mushroom pastas

Generally, the moisture content and a_w of all pastas were similar for pastas produced with the same wheat flour, and the cooked pastas presented higher values, as expected, about 30% more. Krugel at al. (1996) mention the limit of 35% of moisture content for fresh pasta, being this fulfilled for all analysed pastas. Furthermore, pastas presented also similar a_w between them, for fresh pastas varying between 0.92–0.93 and for cooked pastas between 0.96–0.97. Regarding these results, it is possible to state that the water present in all pastas is available to react with other components of the pasta matrix and also the fungi development is a possible concern. According to Neto et al. (2005), most of the microorganisms grow in the range 0.90 to 0.99 (medium and high values of aw), and hence the studied pastas may be susceptible to the growth of microorganisms.

Sensorial evaluation

The sensorial profiles for both wheat cooked pastas, RWF and SWF, and with addition of mushroom powder are shown in Figure 5.

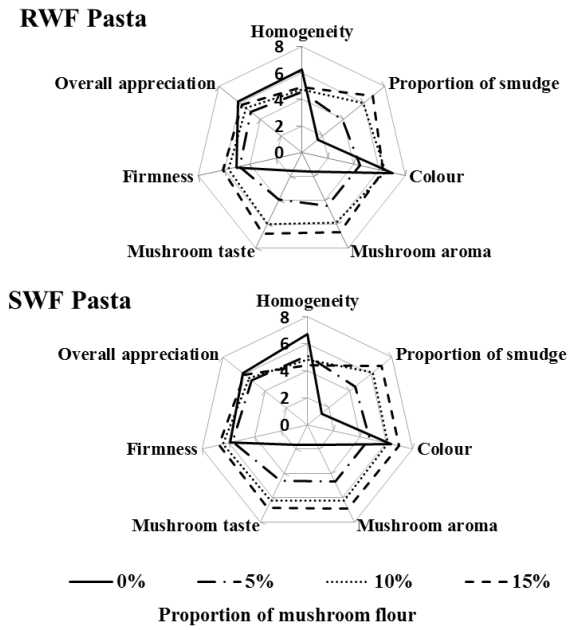

Figure 5. Sensory profiles of RWF and SWF cooked pastas

The profiles of mushroom pastas are quite different from the control pasta, without addition of MF. However, the assessors had a similar sensorial appreciation for pastas with high proportion of MF, and for both wheat flours. The pasta with 5% of mushroom powder presented low scores for proportion of smudge, colour, mushroom aroma and taste when compared with the other two mushroom flours. Furthermore, the firmness and the global appreciation were similar for all the pastas. The 15% mushroom flour pastas were the most appreciate ones, presenting high scores for all the sensorial attributes evaluated.

Conclusions

The results of this study revealed that regular wheat flour type 65 with shiitake powder shows potential as an alternative to flour with 30% of semolina for pasta production. The addition of mushroom flour had a little effect on the cooking time of pastas, but it had a pronounced effect on cooking loss in semolina wheat flour pastas. The mushroom flour also strongly influenced the colour, especially causing a decrease in the lightness and an increase in redness of the obtained products. With cooking procedure the colour and texture of regular wheat flour and semolina pastas were less discrepant, meaning that they did not present marked differences. Moreover, the addition of mushroom flour decreased the firmness, the adhesiveness and stickiness of fresh pastas. With cooking, the pastas became more firm, with less adhesiveness and stickiness when compared with the respective fresh pastas. This means that cooking can improve the textural properties of pastas. The assessors also attributed similar scores to pastas with high content of MF, being the 15% MF pastas the most appreciated.

Therefore, the results showed that addition of 15% mushroom powder was considered as optimum amount to make pasta with regular wheat flour, with good acceptable sensorial scores, and also with health and economic benefits. On this last account, the addition of mushroom flour could possible substitute semolina pasta, which is more expensive, and on the other hand it is possible to avoid waste of shiitake mushrooms and the producer could also leak all the product, enhancing the profits.

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DEVELOPMENT OF NUTRITIVE SNACKS: KIWI BARS

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Abstract

Presently there is an increasing concern about not wasting any type of product with potential commercial utility, like food waste. Thus, this work was undertaken in collaboration with a company in order to find a nutritionally interesting form of valorisation of kiwi waste, namely the pulp from fruits not compliant with commercial standards (like size or shape).

Based on the proposed aims, kiwi based snacks (bars) were developed from kiwi pulp with the addition of gelling agents and some other ingredients. The product development was an intensive task, with multiple experiments until the desired characteristics could be achieved. Because the company intends to commercialize the bars it was also necessary to make a sensory evaluation, to assess the opinion about the developed product(s). Two final products were obtained: a simple kiwi bar and a bar with nuts and oat.

Based on the results obtained it was possible to conclude that, at the nutritional level, the bars revealed low values of energy and carbohydrates, which is positive because nowadays foods with low caloric density and little sugar are valued. Moreover the values of fibre and minerals were high, thus bringing added nutritional advantages.

Through sensory evaluation it was possible to understand that the simple kiwi bar was preferred by the panellists. Colour evaluation showed that the simple kiwi bar had more intense yellow and green colourations as compared to the other bar. Also textural results showed that the simple kiwi bar was softer, more elastic and more cohesive.

Keywords: chemical properties, nutritional properties, physical properties, sensory analysis.

Introduction

The kiwi fruit (*Actinidia deliciosa*) is original from Asia but today is widely consumed all around the world due to its sensory and nutritional properties (Soquetta et al., 2016).

Kiwi was domesticated in New Zealand in the beginning of the nineteenth century, and then reintroduced for commercial production in China (Lu et al., 2016). Widely consumed, kiwi fruit has many cultivars and is nowadays produced in many diverse regions of the globe. Nevertheless, cultivar variability is quite important because different cultivars grown in the same geographic and climatic conditions show significant differences in their nutritive and bioactive properties (Park et al., 2014).

Kiwi fruit is rich in fibre, minerals and bioactive compounds with antioxidant capacity, which are phytochemicals that act as scavengers of free radicals, or prevent their formation. Kiwi is considered a fruit with high nutritional value because of the high vitamin C content and antioxidant capacity (Le, Knulst, 2015; Soquetta et al., 2016).

The fruit marketing and processing industry generates important quantities of residues, which could pose environmental problems as well as economic losses. The utilization of off-size fruits and residues constitutes therefore a necessity to minimize harmful impacts. In this way, by-products of kiwi fruit can contribute to the reduction of agro-industrial waste (Sharma et al., 2016; Soquetta et al., 2016).

The aim of this research was to develop new food products (snacks in the form of bars) made from kiwi pulp as a form of valorisation of the fruits not compliant with commercial standards. Moreover, it was intended to obtain products with a balanced and improved nutritional composition to provide bioactive properties.

Materials and Methods

Fruits of the cultivar Hayward were provided by the company KiwiCoop (Mealhada, Portugal).

For the preparation of the bars the ingredients used were kiwi pulp and juice, pectin (Sigma-76282 from Sigma-Aldrich; Mr 30000-100000), agar-agar (granulated, CAS 9002-18-0 from Merck), sugar, oat, and walnut.

For the development of the new food products many experiments were made until the desired properties would be found. At last, two products were made, according to the formulations shown in Table 1.

Table 1

Kiwi bars formulation

Ingredient	Simple Kiwi Bar (SKB)	Kiwi bar with walnut (KBW)
	g per bar	g per bar
Kiwi pulp	26.3	26.5
Kiwi juice	25.8	30.2
Pectin	2.0	2.0
Agar-agar	1.1	1.3
Sugar	4.2	4.2
Walnut + Oat mix	0.0	5.9

The analyses made to the kiwi bars were moisture content (by drying to constant weight), crude fibre (by digestion acid / basic using Dosi-Fiber), crude protein (by Kjeldhal method, mineralization of protein), ash (by incineration at 550 ºC) and ascorbic acid (by titration with 2,6-dicloroindofenol) following standard procedures (AOAC, 2012). Acidity was determined by titration following the Portuguese Standard NP-1421. Carbohydrates were determined by difference.

Colour was evaluated by a colorimeter CR-400 (Konica Minolta) in the CIELAB colour space: L* = lightness, a* = green / red, b* = blue / yellow (Guiné et al., 2015).

Texture was evaluated by texture profile analysis (TPA) with a texturometer TA.XT Plus (Stable Micro Systems, UK), using a compression test with a flat P/75 probe. The experimental graphs allowed to estimate hardness, resilience, cohesiveness, springiness and chewiness, as defined by Guiné et al. (2015).

For the sensory evaluation a panel of 26 non trained judges were used, for evaluation as a consumer panel. Although the judges were not trained for this type of product, they were experienced in making sensorial analysis. For the assessment of the sensorial profile the following attributes were analysed on a 5 point scale varying from 1 (least intense) to 5 (most intense): consistency, hardness, kiwi odour, kiwi taste, acidity, sweetness and homogeneity. Also a preference test was made to identify the sample preferred by the panellists.

Results and Discussion

Chemical composition

The results of the chemical analyses made are shown in Table 2, being the values and standard deviation calculated from 3 repetitions for each analysis in each sample.

The values obtained for moisture content were very similar in both cases, around 41%, and the same was verified for the ash content, meaning that the amount of minerals in the bars was similar (~1.6%).

Table 2

Results of the chemical analyses

Property	Simple Kiwi Bar (SKB)	Kiwi bar with walnut (KBW)
Moisture content, g 100 g^{-1} sample	41.20±0.45	40.77±1.02
Crude fibre, g 100 g^{-1} sample	5.70±0.14	6.38±0.20
Crude protein, g 100 g^{-1} sample	0.89±0.04	1.16±0.03
Ash, g 100 g^{-1} sample	1.57±0.01	1.67±0.02
Carbohydrates, g 100 g^{-1} sample	22.35±0.04	24.90±0.02
Acidity, mL citric acid 100 g^{-1}	2.47±0.02	2.34±0.02
Vitamin C, mg ascorbic acid 100 g^{-1}	23.60±0.40	20.00±0.30

Regarding fibre, the bar with oat and walnut presented a slightly higher content (6.38%) as compared to the simple kiwi bar (5.70%), which had been expected since both the oat and walnut are foods rich in fibre (USDA, 2016). These results further indicate that both variations of the developed product are very rich in fibre, with much higher contents as compared to raw fruits such as kiwi (3.0%), apple (2.4%) or pear (3.6%) (USDA, 2016).

The protein content was higher in the bar containing oat and walnut, which would be expected because these two products are much richer in protein as compared to kiwi (1.1% in kiwi, 15.2% in walnut and 16.7% in oat) (USDA, 2016).

The carbohydrates were just slightly lower for the simple kiwi bar, but acidity was a little higher for this sample, due to the higher proportion of kiwi, which is naturally acidic. However, the vitamin C content was quite different, being higher in the simple kiwi bar, owing to the higher amount of kiwi pulp, which is much rich in this vitamin. The vitamin C content in raw kiwi is 92.7 mg 100 g^{-1} (USDA, 2016).

Calculation of the caloric value for both bars is presented in Table 3. The results show that the simple kiwi bar is considerably less caloric (120 kcal 100 g^{-1}) when compared with the kiwi bar with walnut (~182 kcal 100 g^{-1}). For this certainly contributes the presence of walnut, which is a nut with a high caloric density (654 kcal 100 g^{-1}), partially due to its elevated fat content (65.2 g 100 g^{-1}) (USDA, 2016).

Table 3

Caloric value

Samples	Energy		
	kcal 100 g sample	kJ 100 g sample	kcal portion
Simple Kiwi Bar (SKB)	120.0	502.3	42.0
Kiwi bar with walnut (KBW)	181.8	761.0	63.6

* 1 portion = 35 g

Colour

The values of the Cartesian colour coordinates are shown in Table 4, being the values and standard deviation calculated from 40 measurements in each sample.

The lightness was equal in both samples, with values around 42, corresponding to slightly dark samples. The value of L* varies between 0 for black and 100 for white (Guiné, Barroca, 2014).

The opposing colour coordinate a* ranges from green (for negative values) to red (positive) (Guiné, Barroca, 2014). Hence, while the simple kiwi bar showed a green coloration (a* = -2.12) the kiwi bar with walnut was just slightly red, with a positive value, but very close to zero (a* = 0.39). This is due to the presence of the oat and walnut that make the colour tend to brown and diminish the predominance of the green colouration of the kiwi.

The other opposing colour coordinate, b*, varies from blue (negative) to yellow (positive) (Guiné, Barroca, 2014). The values for both samples are similar (around 16), meaning that in both cases the intensity of yellow is relevant.

Table 4

Colour coordinates

Colour coordinate	Simple Kiwi Bar (SKB)	Kiwi bar with walnut (KBW)
L*	41.96±2.14	41.81±3.38
a*	-2.12±0.70	0.39±0.86
b*	16.28±2.75	15.55±2.24

Texture

The textural properties are presented in Table 5, being the values and standard deviation calculated from 8 determinations in each sample.

Hardness is the force required to compress a food between the teeth or between the tongue and mouth, and it comprises the force required to cause deformation. Cohesiveness represents the internal forces inside the food that maintain the sample as a whole, i.e., cohesive. Springiness or elasticity measures the ability to recover the shape after compression, and corresponds to the rate with which the product returns to the initial state after removal of the force which caused the deformation. Resilience is the energy used when applying a force to a material without occurring rupture, with or without any residual strain, and corresponds to an instant springiness. Adhesiveness represents the force required to remove the material that adheres to a specific surface, for example lips, palate or teeth. Finally, chewiness measures the energy required to disintegrate a food to such a state that it could be swallowed (Cruz et al., 2015; Guiné et al., 2014; 2015).

According to the results in Table 5, the kiwi bar with walnut was harder as compared with the simple kiwi bar (~54 N and ~59 N, respectively), and also had higher chewiness (~34 N and ~37 N, respectively), which is expected since these two textural properties are intimately related.

Table 5

Textural properties

Property	Simple kiwi bar (SKB)	Kiwi bar with walnut (KBW)
Hardness, N	54.25±3.03	59.15±3.53
Cohesiveness	0.78±0.03	0.73±0.04
Springiness, %	81.93±1.68	77.25±1.95
Resilience, %	36.57±1.57	36.05±2.76
Adhesiveness, N·s	-0.80±0.08	-0.62±0.08
Chewiness, N	33.83±2.13	37.15±1.57

Cohesiveness, on the other hand, was lower for the sample containing walnut and oat (0.73 against 0.78), because the linking of the ingredients was poorer due to the presence of these components. Hence the simple kiwi bar showed a higher capacity of maintaining integrity.

Springiness was higher for the simple kiwi bar (82% against 77%), because the bar containing only kiwi was more elastic as compared with the bar containing oat and walnut. Nevertheless, the results for resilience were practically equal in both variations of the product.

Adhesiveness was higher for the simple kiwi bar (higher absolute values) when compared to the kiwi bar with walnut (-0.80 and -0.62 N·s, respectively).

Sensorial evaluation

The sensorial profiles for both samples are shown in Figure 1, resulting from the mean values for each attribute evaluated by the 26 panellists.

The simple kiwi bar was evaluated by the panellists as more homogeneous, sweeter, softer, with more intense odour and taste to kiwi and slightly more acidic. On the other hand, regarding consistency, both samples were perceived equally.

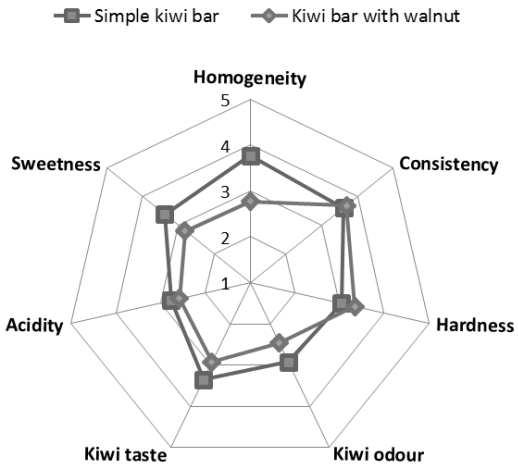

Figure 1. Sensorial profiles of the kiwi bars

The preference test indicated that 19 of the 26 members of the panel preferred the simple kiwi bar, corresponding to 73%. Hence, it was concluded that from the two variations of the product developed the simple kiwi bar has more potential to be accepted by the consumers when compared to the kiwi bar with walnut.

Conclusions

In the formulation of bars made from kiwi, it was possible to see that the gelling agent used, as well as the amounts of all ingredients, greatly interfered with the characteristics of the final product. In view of this, it was necessary to test different formulations and try various types and amounts of gellants until obtaining the desired product.

In the study of physicochemical evaluation, it was possible to perceive that the simple kiwi bar had lower caloric value and was less rich in fibre, protein, ash and carbohydrates as compared to the kiwi bar with walnut. On the contrary, acidity was higher and so was the content of vitamin C.

Regarding colour, the differences between both bars were essentially in the coordinate a*, so that the simple kiwi bar showed a green colouration while the kiwi bar with walnut showed predominance of red. Lightness and yellowness were similar in both bars.

In what concerned the textural properties, the simple kiwi bar was softer, more elastic and more adhesive as compared to the bar with walnut and oat.

Sensory evaluation allowed establishing the sensorial profiles of both samples tested, with the simple kiwi bar showing higher average scores for the majority of the attributes evaluated: homogeneity, sweetness, acidity, kiwi taste, and kiwi odour. Furthermore, the preference test clearly indicated that the simple kiwi bar was the most appreciated of the two varieties of the product developed.

Acknowledgment

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EXPIRATION DATE DETERMINATION OF THERMALLY PROCESSED POTATO MAIN COURSE IN RETORT PACKAGING

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Abstract

Consumer interest in health and wellness prompted the food industry to develop alternative processing technological solutions for preserving low-acid shelf-stable foods. The aim of this study was to assess the expiration date and quality determining factors of potato main course in retort packaging. The study was conducted at the Faculty of Food Technology and the Faculty of Veterinary Medicine, Latvia University of Agriculture. Four different potato main course types – with amaranth (66% potato, 33% amaranth, 1% spices and salt), quinoa (66% potato, 33% quinoa, 1% spices and salt), bulgur (66% potato, 33% bulgur, 1% spices and salt) and chicken fillet (49.5% chicken fillet, potato 49.5%, 1% spices and salt) – were investigated. All main courses were packaged in three different packaging materials, two-layer laminated transparent polyamide/polyethylene film (PA/PE), and four-layer opaque, laminated polyethylene terephthalate / aluminium / polyamide / polypropylene (PET/ALU/PA/PP) packaging. During the 22-month storage, mesophilic aerobic and facultative anaerobic microorganisms (TPC), pH and colour L* a* b* changes in all potato main courses were tested once every month. The results demonstrated that the expiration date of potato main course in PA/PE packaging is 8 months, while the expiration date of potato main course in PET/ALU/PA/PP packaging reaches 22 months.

Keywords: potato main course, expiration date, retort packaging.

Introduction

In the recent years, the growth in the sector of prepared meals has been observed in the food industry (Calderón et al., 2010; Olsen et al, 2010; Kanatt et al., 2013; Regueiro, Wenzl, 2015; Stratakos et al., 2015; Hanssen et al., 2015). Prepared meals can be defined as pre-prepared, chilled or frozen foods, which do not need additional ingredients and require minimum preparation before consumption (Mahon et al., 2006; Regueiro, Wenzl, 2015; Remnant, Adam, 2015). These products have gained considerable popularity because of the lack of time for most consumers (Calderón et al., 2010; Stratakos et al., 2015). The main reason for choosing prepared meals is the convenient use and the low cost compared to home-cooked meals (Remnant, Adam, 2015). Consumers are becoming more demanding for high quality food products with excellent organoleptic and health neutral properties (Moronta et al., 2016).

Canned products in packaging of convenient shape with shelf-life over one year are an important component of the diet for the majority of the population in the developed counties (Patras et al., 2009).

Thermal processing, specifically in retort packaging, has been used as a common preservation technique in food industry for shelf stable low-acid foods (Byun et al., 2010). The United States Food and Drug Administration (FDA) has classified foods in the federal register (21 CFR Part 114) as follows: (i) acid foods, (ii) acidified foods and (iii) low acid foods. Acid foods are those that have a natural pH of 4.6 or below. Thus, a pH of 4.6 represents a demarcating line between low and high acid foods. During thermal processing of low acid foods (pH≥4.6), attention is given to *C. botulinum* – the highly heat resistant, rod-shaped, spore former that thrives comfortably under anaerobic conditions to produce the botulism toxin (Awuah et al., 2007). The aim of sterilization is to ensure that all cold points (worst case processing scenario) in the food product receive thermal treatment capable of reducing the *Clostridium botulinum* load for 12-log cycles. The time required to reach this final microbial load is known as 12*D*, where *D* is the treatment time required to reduce the number of microorganisms to the tenth. These processing conditions allow the ability to ensure shelf stable low-acid products (Patras et al., 2009; Barbosa-Cánovasa et al., 2014). Processors of low-acid canned foods must have an effective and efficient control system over the retort sterilization process to avoid unexpected process deviations that would question the lethality of the resulting process (Simpsona et al., 2007). Commercial retort processing ensures a reduction or inactivation of spore-forming microorganisms sufficient to guarantee commercial sterility (Byun et al., 2010).

Retorting is a method of preserving food by heating it in hermetically sealed containers like cans, glass jars, semi-rigid thermoformed containers and retortable pouches (Bindu et al., 2012; Barbosa-Cánovasa et al., 2014). The retort pouch is a 3-ply multi-layer flexible packaging consisting mainly of polypropylene, aluminium foil and polyester. Pouches can withstand sterilization temperatures up to 130 °C. Retortable pouches allow more rapid heat transfer than cylindrical metals and glass containers of equivalent volume. Commodities that have been packed in thin profile pouches include meat curries, stews, high-quality meat products, frankfurters, ready meals, gourmet sauces, corn, green beans, sliced or diced carrots (Awuah et al., 2007).

The choice of packaging is essential for products intended for long-term storage, as the storage time and temperature significantly affect the appearance, aroma,

flavour and structure of the product (Clark et al., 2002). Packaging plays an important role in maintaining food quality, because each package is an important part of the food that isolates the product from adverse external environment factors (Ahvenainen, 2003; Pardo, Zufía, 2012). Traditionally, canned food products have not been perceived by consumers to have high quality, however, the emphasis is placed on shelf stable retort pouch products of higher quality. The retort pouch minimizes the thermal damage to nutrient, sensory, and other food quality characteristics due to quicker heating based on the thinner package profile when compared to metal cans (Awuah et al., 2007; Byun et al., 2010). The colour of processed foods plays a role by influencing consumer acceptability. Natural occurring pigments in foods are susceptible to changes or degradation from heat. Chlorophylls (in photosynthetic tissues), anthocyanins (the red and blue hues associated with many fruits and vegetables), carotenoids (found in fruits, dairy products, egg, fish and vegetables) and betanins (present in red beet roots) form the major classes of pigments. Anthocyanins are converted to brown pigments by heat. While traditional retorting can affect some of these pigments due to prolonged heat exposure, high-temperature short-time operations can be expected to minimize these changes considerably (Awuah et al., 2007).

Materials and Methods

Experiments were carried out at the laboratories of the Faculty of Food Technology and the Faculty of Veterinary Medicine, Latvia University of Agriculture. Microbiological parameters were determined at the Molecular Biology and Microbiology Research Laboratory, Latvia University of Agriculture and the laboratories of Institute of Food and Environmental Hygiene, Faculty of Veterinary Medicine. Prepared meal samples were made and subjected to physical analysis (pH, colour components L* a* b*) at the Packaging Material Properties Research Laboratory, Department of Food Technology. Four different types of potato main course samples and control sample (potatoes) were prepared for this study: potatoes with amaranth *(Amaranthus* L.) (66% potato, 33% amaranth, 1% spices and salt), potatoes with quinoa (*Chenopodium quinoa* Wild.) (66% potato, 33% quinoa, 1% spices and salt), potatoes with bulgur (*Triticum durum* Desf.) (66% potato, 33% bulgur, 1% spices and salt) and potatoes with chicken fillet (49.5% chicken fillet, 49.5% potato, 1% spices and salt) (Fig. 1).

Peeled potatoes were cut by Robot Coupe vegetable preparation machine CL50 in equal-sized cubes (10 × 10 mm). Cut potatoes were mixed with chicken fillet, which was cut into medium-sized pieces, or amaranth, quinoa, or bulgur, then 1% spices and salt was added to each sample. After mixing, products (300±10 g) were filled in 200×250 mm sized laminated pouches. Three different packaging materials suitable for thermal treatment were used: two-layer PA / PE (polyamide / polyethylene) laminated packaging material with 80 µm thickness, PET / ALU / PA / PP (polyethylene terephthalate / aluminium / polyamide / polypropylene) packaging material with aluminium layer, 110 µm thickness and three-layer PA / EVOH / PE (polyamide / ethylene vinyl alcohol / polyethylene) laminated packaging material with UV barrier properties and 80 µm thickness.

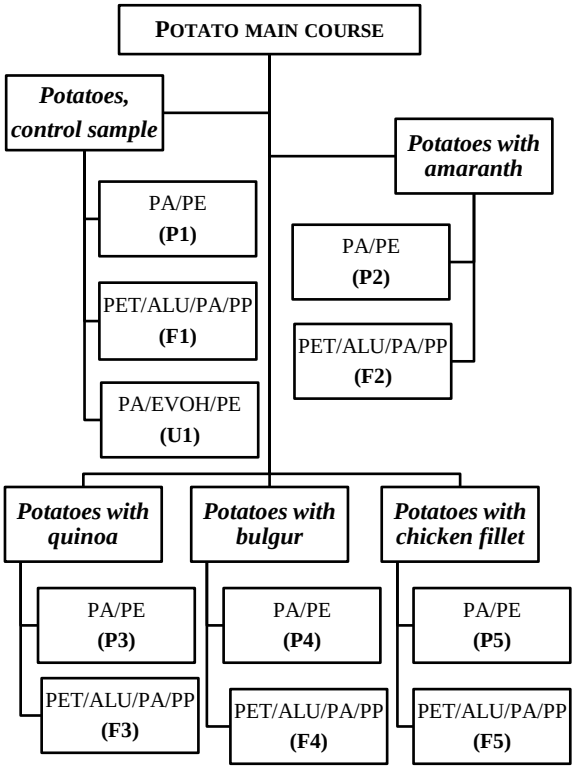

Figure 1. Potato main courses samples

After filling, pouches of potato main course were hermetically sealed using chamber type vacuum packaging machine Multivac C350; hermetic sealing mode – vacuum, 20 mbar, sealing time for PA / PE and PA / EVOH / PE packaging – 3.8 seconds, sealing time for PET / ALU / PA / PP packaging – 5 seconds. Vacuum sealed pouches were then thermally treated in a pilot autoclave HST 50/100, ZIRBUS Technology GmbH (Germany). Sterilization was carried out at 120±2 °C for 10 min, the cooling temperature was 20±2 °C in product. After thermal treatment, sterilized products were stored at 20±2 °C for 22 months. Physical analysis and microbiological parameters were determined on the production day and once every month during 22-month storage.

Microbiological parameters

Aerobic and facultative anaerobic, mesophilic bacteria (hereafter referred to as TPC, total plate count) were determined according to the standard EN ISO 4833: 2003 "Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of microorganisms – Colony-count technique at 30 degrees C" on Plate Count Agar (PCA) medium.

Determination of pH

pH of potato products was determined using pH-meter JENWAY 3510 with electrodes JENWAY 3 mol / KCl (standard method ISO 1842:1991).

Colour analysis

Colour of potato product of samples was determined using *Colour Tec PCM / PSM* with CIE L*a*b* colour system. Measurements were completed in tenfold repetition for each sample for more precise calculations of the mean value and standard deviation. Measurements were recorded using data program Colour Soft QCW. Total colour difference (ΔE*) of potato products between the initial value and after storage was calculated using the following equation 1:

$$\Delta E^* = \sqrt{\left(L^* - L_0^*\right)^2 + \left(a^* - a_0^*\right)^2 + \left(b^* - b_0^*\right)^2} \qquad (1)$$

where

ΔE* – total colour difference of the product during storage,

L* – colour intensity value at the final product storage day,

L_0* – colour intensity value at day 0,

a* – value of colour component green – red at the final product storage day,

a_0* – value of colour component green – red at day 0,

b* – value of colour component blue – yellow at the final product storage day,

b_0* – value of colour component blue – yellow at day 0.

Data analysis

The obtained data were processed using SPSS software package 16.0; differences among results were considered significant if p-value<0.05. One way analysis of variance (ANOVA) and Tukey's test were used to interpret the results.

Results and Discussion

Foodborne illnesses are still at a very high level in Europe. Plant based raw materials are mainly contaminated with the undesirable microflora during growth, harvesting, primary processing, manufacturing, as well as during distribution and transport (Birmpa et al., 2013). Food producers and processors must control products during all of the processing stages in order to prevent or reduce the contamination of food to acceptable levels, therefore, the knowledge of food safety and hygiene are necessary to protect consumers from food poisoning caused by food infections (Smigic et al., 2016; Gomess et al., 2014). Potato main courses in PA / PE packaging (samples P1, P2, P3, P4, P5), PET / ALU / PA / PP packaging (samples F1, F2, F3, F4, F5) and PA / EVOH / PE packaging (sample U1) were tested for the presence of aerobic and facultative anaerobic microorganisms (TPC) immediately after the heat treatment and during 22-month storage at 20±2 °C.

The results showed that TPC in potato main course packed in PA / PE and PA / EVOH / PE immediately after heat treatment and during 8-month storage did not exceed <10 CFU g^{-1}. Microbiological testing of these samples in PA / PE (P1, P2, P3, P4, P5) and PA / EVOH / PE (U1) packaging was not carried out after 8-month storage, because the sensory characteristics (taste, colour, structure) did not meet the desired product quality requirements. It was concluded that the shelf-life of potato main course in the above-mentioned packaging materials is 8 months. By contrast, potato main course in PET / ALU / PA / PP (F1, F2, F3, F4, F5) packaging demonstrated microbial contamination below 10 CFU g^{-1} immediately after heat treatment and during 22-month storage, without significant changes in organoleptic characteristics.

Based on the results of microbiological testing, it can be concluded that the potato main course in PA / PE and PA / EVOH / PE packaging is safe for human consumption till 8-month storage at 20±2 °C room temperature, whereas potato main course in PET / ALU / PA / PE packaging is safe for human consumption after 22-month storage at 20±2 °C room temperature.

pH value is the measurement of acid and alkali ratio. Environment pH is one of the key factors in determining which microorganisms can grow in the product. Microorganisms which cause food infections typically have an optimum around neutral pH – 6 to 7. The characteristic pH for vegetables is 4.2 to 6.5. pH of fresh potatoes ranges from 5.4 to 5.8 (Suryawanshi, 2008). It should be taken into account that the bacterial resistance to treatment will be different with various pH of products (Garcia-Segovia et al., 2007).

pH value of potato main courses in PA / PE (P1, P2, P3, P4, P5) and PA / EVOH / PE (U1) packaging during

8-month storage is shown in Fig. 2, while pH of potato meals in PET / ALU / PA /PP (F1; F2; F3; F4; F5) packaging during 22-month storage at 20±2 °C is shown in Fig. 3.

pH dynamics of potato main course in PA/PE and PA/EVOH/PE packaging showed that none of the samples had significant changes of pH value (p>0.05) during 8-month storage. Control sample in different packaging materials – P1 (packed in PA/PE) and U1 (packed in PA/EVOH/PE) – had insignificant changes in pH dynamics during storage. The initial pH of sample P1 was 5.77±0.01, while the initial pH of sample U1 was 5.75±0.01. After an 8-month period, pH of samples P1 and U1 was 5.71±0.01 and 5.7±0.01, respectively. For the other samples with different potato main course types, the highest initial pH value was observed for potatoes with chicken (P5) – 6.07±0.01, however, during 8-month storage it decreased to pH 5.96±0.01 which was not considered significant (p>0.05). Similar pH changes were observed in sample P4 – potatoes with bulgur. Throughout the storage period the least changes of pH were observed in sample U1 (potatoes, control sample) packed in PA / EVOH / PE, a total of 0.05 units.

The results of pH dynamics of potato main courses in PET / ALU / PA / PP packaging during 22-month storage at 20±2 °C showed insignificant changes (p>0.05).

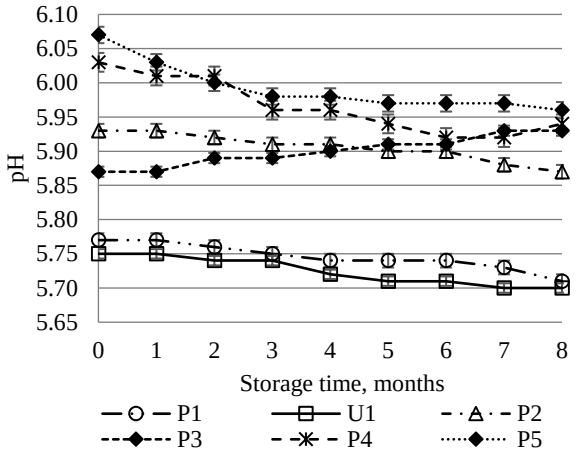

Figure 2. Changes in pH of potato main course in PA / PE (P1, P2, P3, P4, P5) and PA / EVOH / PE (U1) packaging during 8-month storage

P1 – control sample in PE / PA packaging, U1 – control sample in PA / EVOH / PE packaging, P2 – potatoes with amaranth in PE / PA packaging, P3 – potatoes with quinoa in PE / PA packaging, P4 – potatoes with bulgur in PE / PA packaging, P5 – potatoes with chicken fillet in PE / PA packaging

The comparison of potato main courses in PA / PE packaging (8-month storage at 20±2 °C) with samples in PET / ALU / PA / PP packaging (22-month storage at 20±2 °C) indicates that pH value of samples did not depend on the type of packaging.

The initial pH of potato main course control sample P1 was 5.77±0.01, and after 8-month storage it decreased to 5.71±0.01 (Fig. 2), whereas the initial pH of potato main course control sample F1 was 5.79±0.01 and after 22-month storage it decreased to 5.69±0.01 (Fig. 3). Such comparison of potato main course with quinoa in PA / PE packaging (P3) and PET / ALU / PA / PP packaging (F3) showed that initial pH values were 5.87±0.01 and 5.90±0.01, respectively, but after 8-month storage – 5.93±0.01 (P3) and 22-month storage – 5.98±0.01 (F3). Throughout the storage period the least changes of pH were observed in sample F3 (potatoes with quinoa) packed in PET / ALU / PA / PP, a total of 0.08 units, whereas sample F2 (potatoes with amaranth) in PET / ALU / PA / PP packaging showed the greatest pH changes – a total of 0.15 units which was considered significant ($p<0.05$).

Colour changes can be observed in foods during storage due to the exposure to fluorescent light. Light promotes the discoloration of products, the formation of unwanted odours, and it reduces the shelf life in spite of the compliance to the temperature regimes (Murcia et al., 2003). Consequently, appropriate food packaging materials and processing technology can significantly extend the shelf life of products.

The total colour changes of potato main course during storage were characterised by the total colour difference ΔE*, which was calculated from L*, a* and b* values. The total colour difference of all potato main course types in PA / PE, PA / EVOH / PE and PET / ALU / PA / PP packaging are summarised in

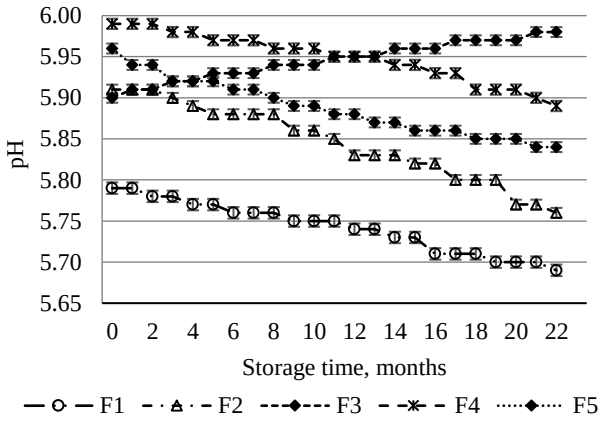

Figure 3. Changes in pH of potato main course in PET / ALU / PA / PP (F1, F2, F3, F4, F5) packaging during 22-month storage

F1 – control sample in PET / ALU / PA / PP packaging, F2 – potatoes with amaranth in PET / ALU / PA / PP packaging, F3 – potatoes with quinoa in PET / ALU / PA / PP packaging, F4 – potatoes with bulgur in PET / ALU / PA / PP packaging, F5 – potatoes with chicken fillet in PET / ALU / PA / PP packaging

Figure 4. The results demonstrate that potato main courses packed in PA / PE (P1, P2, P3, P4, P5) had significant colour changes ($p<0.05$) after 8-month storage, which considerably influenced the visual appearance of the product and cannot be considered appropriate for further research, as colour is one of the main factors which the consumer perceives as the quality indicator of the product (Suryawanshi, 2008).

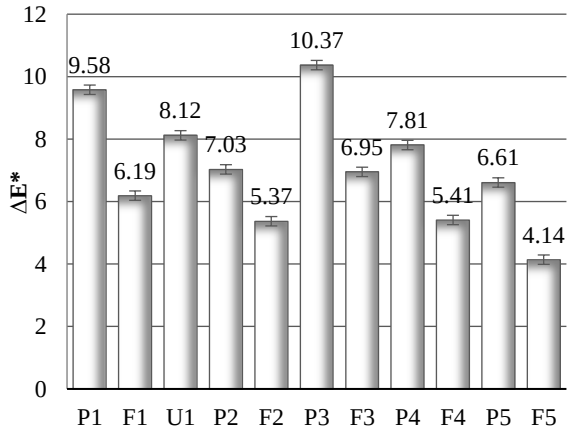

Figure 4. The total colour difference ΔE* of potato main course after 8-month (P1, U1, P2, P3, P4, P5) and after 22-month (F1, F2, F3, F4, F5) storage

P1 – control sample in PE / PA packaging, F1 – control sample in PET / ALU / PA / PP packaging, U1 – control sample in PA / EVOH / PE packaging, P2 – potatoes with amaranth in PE / PA packaging, F2 – potatoes with amaranth in PET / ALU / PA / PP packaging, P3 – potatoes with quinoa in PE / PA packaging, F3 – potatoes with quinoa in PET / ALU / PA / PP packaging, P4 – potatoes with bulgur in PE / PA packaging, F4 – potatoes with bulgur in PET / ALU / PA / PP packaging, P5 – potatoes with chicken fillet in PE / PA packaging, F5 – potatoes with chicken fillet in PET / ALU / PA / PP packaging

Evaluating the packaging material PE / EVOH / PE with UV protective layer, it can be concluded that the overall colour difference ΔE* for sample U1 was not significantly different ($p>0.05$) compared to potato main course in PA / PE packaging (sample P1). Thus, the total colour difference was studied further only for potato courses in PET / ALU / PA / PP packaging (samples F1, F2, F3, F4, F5) during the storage period of 8 to 22 months.

Figure 4 shows the total colour difference ΔE* for all types of potato main courses and all three packaging materials to illustrate the variation of colour difference and demonstrate the importance of packaging materials in maintaining product quality. Potato main course sample P1 (potatoes – control sample) had the total colour difference of ΔE* 9.58 units after 8-month storage, which is about 1.5 times higher than for sample F1 (potatoes – control sample) that was stored for a 14-month longer period. The highest total colour difference was observed for potato main course P3 – potatoes with quinoa in PA/PE packaging, compiling up to ΔE* 10.37 units, which was 1.49 times higher than for potatoes with quinoa in PET / ALU / PA / PP packaging (F3) after 22-month storage.

These results can be explained by the difference in light transmission and barrier properties of various packaging materials, which have a significant role in maintaining the product quality during prolonged storage.

Conclusions

The most suitable packaging material for prepared ready-to-eat potato main course was PET / ALU / PA / PP, as it was able to ensure consistent product quality during 22-month storage at 20±2 °C temperature. Potato main course packed in PA/PE and PA / EVOH / PE maintained the quality up to 8 months at 20±2 °C temperature. An essential quality determinative factor, which influenced the shelf life of potato main course in PA / PE and PA / EEVOH / PE packaging, was the increase in the total colour difference ΔE*. It was affected by the significantly lower packaging light transmittance compared PET / ALU / PA / PP packaging material, thereby substantially reducing product quality.

Acknowledgment

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STABILITY AND ISOMERISATION OF LYCOPENE IN OIL-BASED MODEL SYSTEM DURING ACCELERATED SHELF-LIFE TESTING

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Abstract

Shelf-life prediction of a product could be assessed by measuring quality attributes through accelerated shelf-life testing (ASLT) under extreme conditions. The ASLT could be beneficial to specify the effects of different storage temperatures on quality properties of food products in some cases where the environmental conditions exceed the limits. The objective of this study was to evaluate the effect of temperature and light on lycopene stability in oil-based food model system during 100 days of storage. Extract of lycopene in oil-based model system was prepared from 'Tolstoi H' tomatoes and virgin rapeseed oil and poured into transparent vials. Samples were tested at 1±1 °C temperature in absence of light, ambient temperature at 20±1 °C in natural light, ambient temperature at 20±1 °C in absence of light, at 40±1 °C temperature in absence of light and at 40±1 °C temperature in UV irradiation. Lycopene and its *cis*-isomers in oil-based model system and control were determined by high performance liquid chromatography (HPLC/DAD). The colour measurements of the samples were made using MiniScan XE Plus spectrophotometer. The addition of tomato extracts to vegetable oil might increase the level of lycopene in a human diet and enhance its bioavailability. The optimum storage conditions for lycopene-enriched oil were at 20 °C in the dark. The temperature and light irradiation has a combined influence on the lycopene stability and isomerisation (from 17 to 100 % changes from *trans*- to *cis*-lycopene isomers). ASLT is useful and practical tool for the stability monitoring of lycopene.

Keywords: lycopene, stability, isomerisation, accelerated shelf-life testing.

Introduction

Consumers increasingly demand products with high quality (taste, appearance, texture, flavour) whilst keeping their nutritional value. For this purpose, determining properties of food products during their shelf life is very important for the research and the food industry. The definition of self-life provides information regarding the time during which the product appropriately retains its quality (Ganje et al., 2016). This prediction could be performed by measuring quality attributes through accelerated shelf life testing (ASLT) under extreme conditions (Shao et al., 2015). However, the ASLT could be beneficial to specify the effects of different storage temperatures on quality properties of food products in some cases where the environmental conditions exceed the limits. The data in the literature generally agree that carotenoids is quite stable in fresh tomato matrices, but during thermal treatments, UV exposure or when carotenoids is dissolved in organic solvent, degradation and isomerisation can occur rapidly (Maiani et al., 2009). The kinetics of pigments degradation is complex in food products. Kinetics studies are capable of determines parameters such as reaction order and constant rates are required, being equally important to establish the impact on the food acceptability (Van Boekel, 2008).

Pedro and Ferreira (2006) used ASLT as an approach for determining the shelf-life of commercial concentrated tomato products and they reported zero and first order kinetic reactions for the quality factors of the product.

The effects of thermal- and light-irradiation processing on lycopene stability in an oil-based food model system have not yet been completely investigated. The stability of lycopene during heating and illumination has been studied, but the results are controversial. Only a few studies was described the kinetics of *cis*-lycopene formation (Ax et al., 2003; Shi et al., 2000, Colle et al., 2010b).

The objective of this study was to evaluate the effect of temperature and light irradiation on lycopene stability in oil-based food model system during 100 days of storage.

Materials and Methods

Materials

The experiments were performed in the Laboratory of Biochemistry and Technology of the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry. Fresh tomato (*Lycopersicon esculentum* L.) of the hybrid 'Tolstoi H' (grown in the greenhouses of the Institute of Horticulture) and rapeseeds oil (Lithuania) were used.

The HPLC-grade solvents, including tetrahydrofuran, methanol, methyl-tert-butyl ether and ethyl acetate, were obtained from Sigma-Aldrich (Germany).

Sample preparation.

The lycopene rich oil-based food model systems were prepared using virgin rapeseed oil according to Urbonaviciene et al. (2015) with slight modification. The extract with lycopene (containing 25 $mg \cdot mL^{-1}$ lycopene) was poured into 20 unit 2 mL transparent vials, and the extract was divided into five groups. Stability of lycopene in oil-based food model system was investigated during 100 days of storage period. Storage conditions were as follows:

1) at 1±1 °C temperature in dark (1 °C, dark),
2) ambient temperature at 20±1 °C in natural light (day and night illumination was differ (300±10 Lux)) (20 °C, light),
3) ambient temperature at 20±1 °C in absence of light (20 °C, dark),
4) thermostatically controlled temperature at 40±1 °C in UV irradiation (2500±100 Lux)) (40 °C, UV),
5) thermostatically controlled temperature at 40±1 °C in absence of light (40 °C, dark).

The samples were stored in hermetically sealed containers. The control sample in our study was lycopene in oil-based food system on day zero (0). The control sample and all lycopene oil-based food model samples were prepared for HPLC analysis after storage.

Lycopene extraction for HPLC

The samples (1 mL) was extracted repeatedly with tetrahydrofuran and diluted until 25 mL and the total lycopene content and *cis*-lycopene isomers was analysed using HPLC.

HPLC analysis of lycopene and its isomers

The content of total lycopene and lycopene isomers was analysed by HPLC with diode array detection according modified version of the different methods and systems described by Heymann et al. (2013), Melendes-Martines et al. (2013), Urbonaviciene et al. (2015). To quantify lycopene in the extract samples, a calibration curve was generated using an authentic *all-trans*-lycopene standard. Levels of *cis*-lycopene isomers are given in *all-trans*-lycopene equivalents.

Colour measurements

The colour was measured by a spectrophotometer MiniScan XE Plus (Hunter Associates Laboratory Inc., USA). Colour measurements were carried out using the standard CIE L*, a*, b* coordinates (McGuire, 1992).

Kinetic data. The degradation rate constant of the total concentration of lycopene (all-*trans*- and *cis*-isomers forms) were calculated on lycopene stability in oil-based food model system using the following formula:

$$k = -Ln(C_A/C_{A0})/t$$

where C_A is the total amount of lycopene after storage; C_{A0} is the initial amount of lycopene; t is storage time.

Statistical analysis

The analysis were triplicated for each sample and mean values are presented. Differences at $p<0.05$ were considered to be significant. All statistical analysis was performed using Statistica 8.0 (StatSoft, Czech Republic) and Excel 2007 (Microsoft Corporation).

Results and Discussion

The effect of storage conditions (temperature from +1 to +40 °C) and different light irradiation (300–2500 Lux) on lycopene stability and possible isomerisation in oil-based food model system was investigated. The total lycopene and *cis*-lycopene isomers concentration were observed.

Figure 1 shows a change of the total lycopene (μg mL^{-1}) during 100 days of storage. The storage conditions affect stability of lycopene in oil-based food model system. The lycopene content decreased significantly ($p<0.05$) at 40 °C in UV storage conditions, to compare with other treatments. The data of our study shows that the slowest lycopene degradation was in samples stored at 1 and 20 °C temperatures in absence of light. Also the lycopene degradation rate was not significantly different to compare samples stored at 20 °C in light (average value of 300 Lux) and at 40 °C in dark (Figure 1) storage conditions. It could be explained that temperature and light irradiation have a combined impact to biologically active substances. A significant interaction between temperature and light irradiation was detected for lycopene degradation in accelerated shelf life storage conditions.

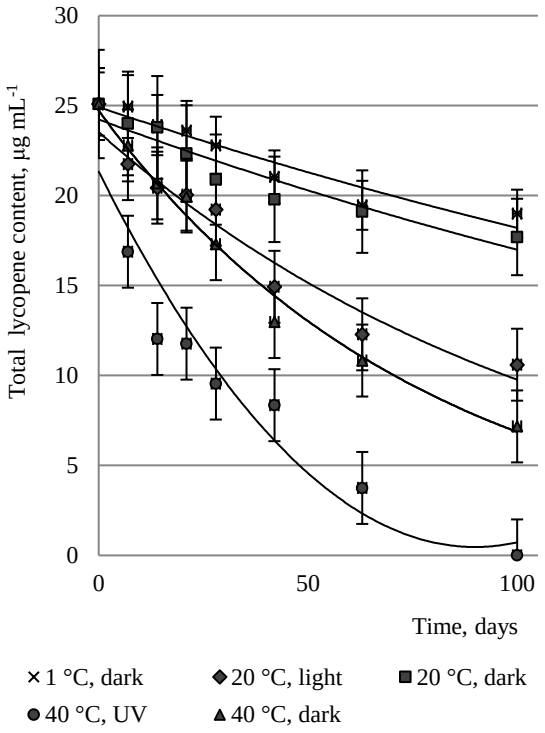

Figure 1. Degradation of total lycopene during the storage at various conditions

The degradation rate constants (k) of the total concentration of lycopene were calculated. The lowest degradation of total lycopene was found at 1 and 20 °C in dark storage conditions (k = -0.002 and k = -0.004, respectively). The degradation rate constants values were similar to compare samples stored at 20 °C in light (-0.010) and at 40 °C in dark (-0.008). The degradation of lycopene in 40 °C UV sample was the most significant (k=-0.024). The decreasing amount of total lycopene may have been due to isomerisation, resulting from additional energy (temperature and / or light) input, which led to unstable, energy-rich situations (Shi, Le Maguer, 2000).

In nature, carotenoids, also lycopene, mostly exist in all-*trans*-isomers form. Thus, red tomatoes typically contain 94–96% all-*trans*-lycopene. The double bonds of the carotenoids molecules can undergo isomerization from *trans*- to mono or poly-*cis*-isomers under the influence of heat, light, oxygen or certain chemical reactions in extracts and in food products from tomato.

The changes of the total *cis*-isomers concentration during 100 days of storage are shown in Table 1. The concentration of *cis*-lycopene increased in all treatments (1 °C at dark; 20 °C at dark; 20 °C at light and 40 °C at dark) in except for 40 °C at UV irradiation after 100 days of storage. The concentration of *cis*-lycopene isomers increased until 45.8% after 42 day and decreased until 0% after 100 days in samples stored at 40 °C in UV irradiation.

Table 1

The changes of *cis*-lycopene isomers in samples stored at different conditions

Sample name	Time (days)	*Cis*-lycopene isomers concentration, %							
		0	7	14	21	28	42	63	100
1 °C, dark		0.00	3.3±0.08	3.64±0.11	4.46±0.12	4.87±0.09	6.50±0.16	10.25±0.11	10.77±0.22
20 °C, dark		0.00	6.1±0.20	11.45±0.23	15.31±0.09	18.57±0.33	23.61±0.22	26.35±0.31	28.69±0.14
20 °C, light		0.00	8.8±0.21	12.44±0.17	15.90±0.17	16.81±0.15	22.20±0.41	29.50±0.36	35.34±0.32
40 °C, UV		0.00	20.6±0.41	31.73±0.24	39.25±0.32	41.54±0.71	45.81±0.38	34.31±0.30	0.00
40 °C, dark		0.00	25.54±0.30	30.38±0.19	32.86±0.24	33.18±0.46	34.32±0.27	36.80±0.42	37.09±0.44

The *cis*-isomers concentration increase was greater about 18% to compare the samples stored at 1 °C in dark and at 20 °C in dark about 25% to compare the samples stored at 1 °C in dark and at 20 °C in light after 100 days of storage. The data in the literature summarized that isomerisation from *trans*- to *cis*-carotenoids isomers could be a result of the overlapping of the methyl group of a carbon atom adjacent to a double bond and the hydrogen (Mercadante, 2007).

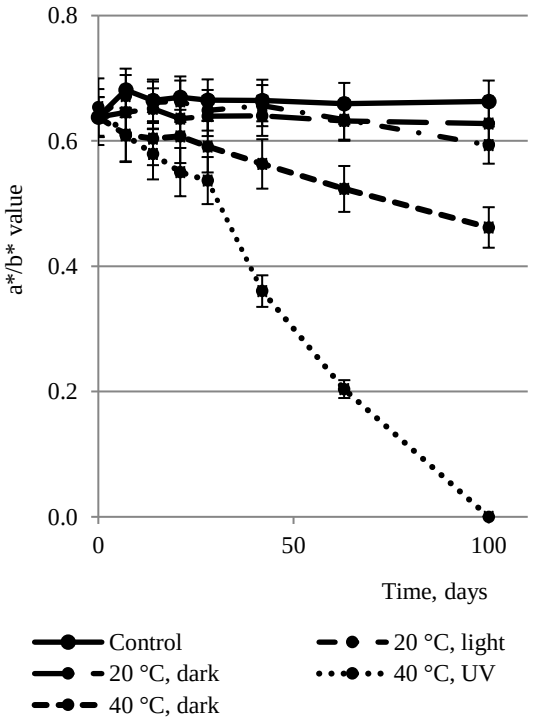

Figure 2. Change of samples colour expressed as a*/b*during the storage

Effect of colour change of lycopene oil-based food model system was investigated to compare the ratio a* and b* change. The ratio of a* and b*, indicate the change of redness, are shown in Figure 2. The a* / b* value of the control was 0.64 and was not significant differences in samples stored at 1 °C in dark, at 20 °C in dark and at 20 °C in light (0.63, 0.62 and 0.59 ($p \geq 0.05$), respectively after 100 days of storage. The a* / b* value of the samples stored at 40 °C in dark and at 40 °C in dark were significantly different to compare with other storage conditions. The a* / b* value was decreased to 0.46 of the samples stored at 40 °C in dark. The a* / b* value of the sample stored at 40 °C in UV decreased until 0.2 after 63 days and 0.0 after 100 days of storage. The results of a low a* / b* value represented the colour change from yellow-red to brown colour due to the breakdown of lycopene (Shi, Le Maguer, 2000; Krebbers et al., 2003). The a* / b* values of samples stored at low temperatures in light were significantly greater to compare samples stored at higher temperatures and especially samples irradiated with UV (Figure 2).

Conclusions

The optimum storage conditions for lycopene in oil based food model system were at 1 °C temperature in the dark. The temperature and light irradiation has a combined influence on the lycopene stability and isomerisation (from 17 to 100% changes from *trans*- to *cis*-lycopene isomers). The ratio of a* and b* colour coordinates indicates the changed as a function of storage illumination. ASLT is useful and practical tool for the stability monitoring of lycopene.

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USED FOOD OILS: PHYSICAL-CHEMICAL INDICATORS OF QUALITY DEGRADATION

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Abstract

Used food oil (UFO), designated as frying oil, is a residue. Degradation by reuse or during storage, may occur by contacting, chemical, enzymatic and microbiological pathways, but oxidation is a major concern of the industry, as it affects sensory and nutritional quality of edible oils, with potentially toxic compounds formation. In Portugal, UFO's main destination still is the sewerage system, an environmental problem and waste of raw material, which can be re-qualified for non-food uses. However, quality control applied to UFO's, often results into expensive analysis inappropriate for small laboratories and catering industry. This project, developed with the Musketeers Group Portugal co-promotion (2012–2016), aimed to identify low-cost physicochemical parameters for further implementation as UFO´s Quality Degradation Indicators (QDI) indicating defects quickly and accurately. UFO´s analysis was tested on the use, for industrial frying, and by degradation induced in the laboratory (frying and heat stability tests) by applying following parameters: moisture, water activity (a_w), total acidity, peroxide index, iodine index, colour (CIE, CIE Lab), UV absorbency, total polar compounds and microbiological indicators. Internal procedures (ESAS) were validated, redefining working ranges and test conditions, as standards procedures did not provide reliable results for the entire life cycle of oils, whose profile changes with time and reuse. Results demonstrate significant differences with quick response parameters as Total Acidity, Peroxide Index and CIE Lab colour, outlined as QDI's. Moisture, a_w and CIE Lab colour proved to be inadequate for this purpose. Iodine Index and UV Absorbency are more complex and time-consuming and were profiled as reference methods.

Keywords: used edible oil, quality degradation, physical-chemical indicators.

Introduction

Food grade oils designate commercial mixtures of two or more edible oils of unlabelled variety, refined separately or together (DL 106 / 2005). For a healthy diet World Health Organization (WHO) has been established that 30% of energy consumption should originate from vegetable oils and fats (Pitts et al., 2007). Yet industrialization and changes in lifestyle leaded to an increase in its use since frying is a fast and economical culinary process of great acceptance, but is also associated with negative aspects, as higher prevalence of obesity and cardiovascular diseases (Saguy, Dana, 2003). Physicochemical changes during processing, storage and use as means of heat transfer, causes changes in its chemical profile, a spontaneous irreversible process that affects its economical and nutritional value and food safety (Gupta et al., 2004). Lipid degradation induced by frying process and reuse or during long term or inadequate storage leading to oil rancidity, may occur by contacting (with prooxidants or rancid materials), chemical, enzymatic and microbiological pathways. Oxidation is probably the best known and studied process of degradation (Silva et al., 1998); it is a major economic concern of the industry, as it affects sensory (*off flavours*, browning, viscosity changes, foam) and nutritional quality of edible oils, with potentially toxic compounds formation (Aladedunye, Przybylski, 2011), like acrolein, ubiquitously present in cooked foods and environment (Moghe et al., 2015). In frying process, oil interacts with air, water and other food components, undergoing a complex chemical process of degradation by chain reactions, which affects the triacylglycerol molecules by hydrolysis and oxidation (Takeoka et al., 1997), the latter through a radical mechanism involving oxygen singlet (^1O), resulting into peroxidation (primary oxidation) and subsequently, fission, polymerization, condensation, interesterification, cyclization, etc. (terminal reactions or secondary oxidation) (Sahin, Sumnu, 2009), with the increase of free fatty acids, carbonyl groups, conjugated dienes and polymeric compounds (Gupta et al., 2004). Temperature, light, pressure, moisture, enzymes, metal ions, influence the radical mechanism and the final products profile (White, 2006). In continuous industrial frying, food is completely submerged and the process is not interrupted, but in homemade frying and restoration a discontinuous process is used; the oil is heated at high temperatures (150–180 ºC) and subjected to temperature cycles and different types of food, being successively cooled and reheated, accelerating its degradation (Choe, Min, 2007). *Used Food Oil* (UFO) is a residue (DL 267 / 2009). In Portugal, although collection is mandatory – resulting into untraceable oil mixtures – UFO's main destination still is the sewerage system, an environmental problem and waste of valuable raw material in production of biodiesel, soap composting, etc. Estimated annual production is of 43 000 t to 65 000 t, generated by domestic sector (62%), hotel and catering sector (37%) and a residual fraction from food industry (IGAOT, 2005). Edible oil's analysis highlights quality ensuring its authenticity and safety; yet, when applied to UFO's intended for non-food recovery, often resorts into inappropriate expensive analysis, pointing to the convenience of further research focused on those degraded oils.

The present research, whose experimental period spanned two biennia, 2012–2013 and 2014–2015, aimed to identify low-cost physicochemical parameters enabling to detect incipient and severe degraded oils, for

further implementation as UFO´s. *Quality Degradation Indicators* (QDI) indicating defects, inexpensively, accurately and quickly. *Experimental Design*. Oils were subjected to real or induced degradation, by industrial frying or laboratory scale equipment (e.g. frying and heat stability tests) performed under *use and abuse* conditions. Oil´s analysis was tested on the use by applying following parameters: moisture content (%H), a_w, total acidity (AT), peroxide index (IP), iodine index (II), colour (CIE and CIE Lab) and UV absorbency (AbsUV), all common and feasible in small laboratories. Monitoring was done by microbiological control and later on (2014–15), also by total polar compounds' (TPC) rapid method. TPC value corresponds approximately to the total change of compounds formed during frying. TPC´s colorimetric quick tests are inexpensive kits alternative to chromatographic standard method (not suitable for a small industry or vendor to use on site), allowing reliable response in minutes without specialized personnel (Chen at al., 2012). Used in restoration and by official entities, they remain unchanged allowing use as legal evidence. Portuguese regulations (Ordinance 1135/95) states maximum permissible limit for TPC in foods prepared in oils as 25% (w/w). Heat stability and fry tests (in 2014–2015), aimed to provide complementary data to the untraced real-use UFO´s (analysed in 2012–2013), using equivalent techniques, but by being performed in a laboratory environment may be controlled and verified, also providing oil´s samples usable as internal patterns.

Materials and Methods

Samples

All came from the Musketeers Group Portugal facilities, in Alcanena. Sampling intended to cover an extended food oil´s use. New food grade oils were delivered in sealed plastic bottles (1 dm^3) of the trade mark partner-supplied (*Bouton d'Or*): 3 units (2012), 6 (2013) and 12 (2014-2015), having the same lot code in each delivery phase. UFO´s were provided in plastic containers (5 dm^3). First trial of indicators (2012), were performed with just 3 samples: new (OAN), twice used (OAU_{2x}), and rejected oil from Alcanena´s base (OAUr). In 2nd phase (2013), 14 samples of untraced UFO (encoded A to N) were provided, having different degrees of sensory perceptible degradation. The first heat stability test was also conducted (2013) using new oil (OS); samples were taken at i = 0, 1, 2, 4, 6 and 8th weeks in a drying chamber (collecting triplicates of 3 independent units – j, samples totalling 18 (OSij). To microbiological first tests (2012–2013), 3 other samples were specifically provided: new oil (O1) and two UFO´s (O2, O3) collected in sterile package both degraded (turbid and brownish). In the last phase of experiments (2014–15), frying tests were performed by immersion of potato and nuggets in heated oils (OFB, OFN, respectively); new oil samples were successively reused and collected at i=0, 2, 5, 10, 15, 20 and 25th fry. Four experiments were done, generating 28 different samples (OFBi, OFNi). A 2nd heat stability test was also carried out with new oil (OE), extending time up to 14 weeks in the oven, collecting combined samples every two weeks, totalling 8 (OEi). All 71 samples obtained during the project were submitted to physical-chemical analysis, and most degraded also to microbiological tests. In analytical assays, number of replicates was standardized under repeatability conditions: five for microbiological and CIE Lab colour (2012–15), ten (2012, for validation purposes) and later on three (2013–15) for all other physicochemical parameters.

Heat stability and fry tests

Heat stability tests (ST) were designed as a forced (accelerated) shelf life test. A convective drying electrical chamber (Memmert, Model 40050 IP20), operated at constant temperature of 39 °C, was used. Food oils have a shelf life of 18 months, but 1 week in an electric chamber at 39 °C in closed bottle is equivalent to 2 months' oil´s exposure under natural storage conditions (Industry personal communications). The test was performed twice (2013, 2015), held in triplicate new oil samples placed closed in the oven in their original bottles. In frying tests (FT), a domestic electric fryer of 1.5 dm^3 max capacity, from Moulinex, was used. Frozen commercial samples of potatoes into cubes or chicken nuggets were weighed (150 g±0.01 g) and fried in 1.5 dm^3 of oil at 180 °C for 4 to 6 minutes. The oil was subjected to reuse as a frying medium for a single food (potato or nuggets), each cycle performed with or without fresh oil replacement to the 10th operation. In each assay, total heating time was 99 min (average) and total frying time about 109 min, being 207 min (3 h 27 min) the actual mean time of use of the oil in those operations. In both ST and FT, sampling over time (120 cm^3 each oil collection) was that described.

Physical-chemical analysis

%H was determined by ISO Standard 3727-1 (2001), using a precision balance (Sartorius BI210s) and an electric oven with ventilation (Memmert 40050 IP20) at 103±2ºC. $\mathbf{a_w}$ was measured by direct method (Rotronic, Model Hygroskop DT; Rotronic cells model DMS 100H) at 25 °C, using water bath (Selecta, Model Unitronic 6320100). AT was determined by minor modification of the titration method described in the Portuguese Standard NP-903 (1987), results expressed in $g_{oleic\ acid}$ 100 g^{-1}. IP and II were determined by classical iodimetry, the first according to NP-904 (1987), results in meq-O_2 kg^{-1}; the last, by adapting NP-941 (1985) and results expressed in g-I_2 100 g^{-1}. Colour in the CIE L*a*b* space (CIE Lab) were determined using a reflectance colorimeter (Chroma Meter CR-400 by Konica Minolta, controlled by the SpectraMagic NX software), measuring the cartesian coordinates L*, a*, b*, where L* is brightness (0=black to 100=white), a* is the green / red chromatic coordinate (negative or positive values, respectively), and b* is blue / yellow coordinate (negative / positive); *chroma* (C*) and hue (Hº), were calculated: $C^* = (a^{*2}+b^{*2})^{1/2}$ and $H^o = \tan^{-1}(b^* / a^*)$. The instrument was standardized each time

with a black and a white tile (Y=89.5; x=0.3176; y=0.3347) using D65 illuminate and a 2° observer. CIE colour was determined by absorbance at 445, 495, 560 and 625 nm against carbon tetrachloride referred to the 1 cm thickness, using a UV-Vis spectrophotometer (Hitachi, Model U-2001); internal procedure was adapted from NP 937 (1987). Apparent and real colour was determined in the original and centrifuged (3000 rpm, 30 min) samples, respectively; stimuli X, Y, Z, was calculated to determine CIE attributes: dominant wavelength (λ), purity (σ), transparency (Y) and Tgα value. AbsUV allows detection of conjugated dienes, absorbing at 230–235 nm, as also conjugated trienes and oxidation by-products at 260, 270 and 280 nm (Wan, 2000). Absorbance was measured against isooctane at 232 nm and 264–272 nm, expressed as absorbance coefficients K_{232}, K_{268} and Δ_{268}, referred to 1 g_{oil} 100 cm^{-3} and 1 cm thickness, according to NP 970 (1986). Actual measures were performed in diluted oil solutions (1:5, 1:10, 1:25) after the originals (1g 100 cm^{-3}), to allow readings in the 0.2–2.0 absorbance range. UV-Vis spectrophotometer (Hitachi U-2001) was used. Internal procedures were validated to repeatability (2012), redefining test conditions and working ranges. Relacre (2000) recommendations were used as validation criteria. In previous trials, oil samples (OAN, OAU_{2x}, OAUr) were used to calculate the coefficients of variation of repeatability (CVr), comparing mean values to the respective standard deviations using its percentage ratio; results leaded to acceptable values (CVr <5–10%) for all physical-chemical indicators except for %H, a_w and CIE apparent colour, due to its highly dispersive results (Laranjeira et al., unpublished data). ST and FT were monitored by TPC using a colorimetric kit (FRITEST®). Numeric scale associates state of the oil to colour response: 1 – good, 2 – still good, 3 – replace, 4 – bad.

Microbiological analysis

Degradation of oils by microbial means is rare, as they are practically water free. However, lipid flora may be responsible for OAU's rancidity since they incorporate water through food. Enumeration of lipolytic microorganisms was performed according to Bourgeois, Leveau (1991), using *Tributyrine Agar*. Lipolytic fungi enumeration was similar, but to *Tributyrine Agar* medium was added chloramphenicol; xerophilic fungi enumeration was done according to the NFV Standard 08036 (2003) using *Dicloran-Glycerol Chloramphenicol Agar* medium.

Statistical analysis

Physicochemical data was analysed with a one-way ANOVA and mean values were compared using *Post Hoc LSD Tuchey´s Unequal* test, except for CIE colour (2013) and for all mean values of FT parameters, as they were compared using *Post Hoc LSD Ficher* test, considering two independent variables "sample" and "treatment". Also for ST assays both tests were used, since it was intended to isolate the "bottle / position" effect (2013), but in the 2nd series (2015), composite samples were prepared. For treatment of multivariate data, Principal Component Analysis (PCA) was used. In all tests a level of significance of 95% ($p<0.05$) was considered (Wilks test) and the software used was Statistica version 7.0 (Stat Soft Inc.) for Windows.

Results and Discussion

It is intended to report most significant results due to screening also highlighting PI and colour data, providing brief information on other parameters in order to allow the understanding of results obtained by TPC analysis. In previous tests (2012), *moisture* (%H) revealed a poor performance as QDI. It was observed (Laranjeira et al., 2014) a clear distinction in drying needs: the more degraded the oil, the longer the time required. In final assays both new (OAN) and twice used (UFO_{2x}) oils were submitted to initial 60 min drying at 103±2 ºC, followed by periods of 15 min extending up to 105 min drying. Oils reached constant weight at 60 min (OAN) and 75 min (UFO_{2x}) and despite high dispersion, recorded minimum (w.b) moisture of 0.044±0.050% and 0.068±0.021%, respectively. From this point, samples registered mass increase. Degraded OAUr were submitted to initial 240 min (4 h) drying, followed by periods of 30 min, total time drying being 360 min (6 h); consistent mass loss were recorded during the first 5 hours (300 min) minimum corresponding to 0.036±0.050% (w.b.) moisture. This lowest result, suggests a later prevalence of water loss by evaporation and hydrolysis (AT is also higher) in relation to its incorporation from food in a large frying reuse. Inversion in oil´s drying curves with mass increased is associated to oxidation (Harpern, 1997). Drying may require a long time run, drying time is not predictable in advance for all UFO and results are low precision, which renders the method uninteresting. a_w was also eliminated in late 2013 as no significant differences between UFO´s samples (OAN, OAU_{2x}, OAUr and oil´s A to N) where observed: measures between 0.5403±0.0006 minimum (E) and 0.73±0.14 maximum (I), led to unsatisfactory highly dispersed results, though data were probably compromised by the inadequacy of equipment: slow and low sensitivity instrumental response for lipid matrices besides non-air-conditioned laboratory (Laranjeira et al., 2014 and unpublished data). CIE and CIE Lab colour were determined in UFO´s samples. CIE is the standard and allows determination of apparent and real colour, unlike CIE Lab. CIE system was appealing only because in frying practise, oils are not centrifuged, allowing a closer observation of the real state of the on-the-use oils. Table 1 shows the CIE colour results (means and standard deviations) obtained in 2012 exploratory tests, presenting λ (dominant wavelength), Y and σ parameters, where (O) refer the original and (C) the centrifuged oil´s samples. Oil not only dim, but the reflected spectral radiation becomes more yellow (λ) and monochromatic (σ) with degradation. Y is significantly lower in OAUr yet higher in centrifuged samples, as expected.

Table 1

Real and apparent CIE colour parameters

Oil samples	λ, nm	σ, %	Y, %
OAN_C	571.52^{c}±0.17	5.96^{c}±0.04	94.34^{b}±0.09
OAN_O	572.87^{e}±0.33	4.62^{a}±0.06	89.72^{d}±0.18
$OAU_{2x\,C}$	571.97^{b}±0.15	5.52^{b}±0.10	94.44^{b}±2.41
$OAU_{2x\,O}$	570.88^{d}±0.55	4.52^{a}±0.38	84.34^{a}±0.11
$OAUr_C$	574.17^{a}±0.03	44.33^{d}±0.21	83.81^{a}±0.85
$OAUr_O$	574.03^{a}±0.13	44.90^{e}±0.15	76.45^{c}±0.27

However, CIE colour is a costly, laborious, reagent dependent technique, uninteresting as QDI and CIE Lab system is a simpler and eco-friendly method. Experiments were repeated with UFO´s A to N, aiming to evaluate the replacement of CIE by CIE Lab system, in used oils. Those 14 untraceable oils led to more scattered results (Laranjeira et al., unpublished data), but data were compared by TPC (Fig 1).

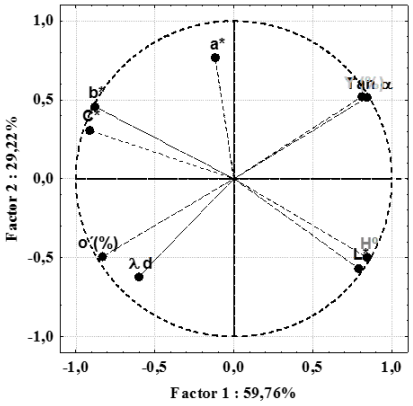

Figure 1. TPC´s variable projection in the plan for used food oils A to N - CIE and CIE Lab colour

Strongest correlations are between internal parameters of CIE or CIE Lab, but factors 1 and 2 evidence correlations between the systems. To this end, it was decided to discontinue the CIE colour assays. Figure 2 presents the results for b* with UFO´s A-N series. Data is presented as a curve to evidence randomness, but these are discrete points of distinctive oils, internally ordered by increasing degradation based on results of classical parameters, AT, IP and II and sensory evaluation. Frying oil´s colour depends mostly on its composition, refining conditions and use. An increase in b* value (yellow) as oil degrades has been reported, due to photo and thermal oxidation. Isomerization of double bonds leads to greater absorbance of blue light, with an increase of colour in the orange and brown hues (Choe et al., 2007); polymerization and absorption of dark pigments from food to oil, due to *Maillard* reactions, also contribute (Gunstone, 2008).

Yet, instead of presenting progress with re-frying, curve (Fig. 2) suggest the influence of individual matrices of different untraced oils on the dispersion of b* values – the same behaviour was observed with other colour parameters and in AbsUV (Laranjeira et al., unpublished data) – as, likely different colour (or UV absorbency) in original (*blend*) oil´s lots, shelf life, unknown conditions of storage and use, and others, as those related to techniques, e.g. instrumental error.

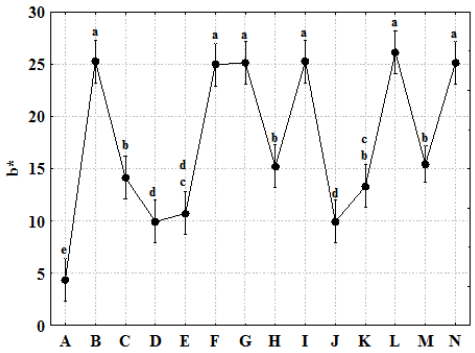

Figure 2. CIE Lab coordinate b* for used food oils

Despite randomness, b* value tends to increase from new (A) to degraded (N) oil; this trend was confirmed by the results of FT and ST, where significant differences were observed along re-frying or heat exposure by ANOVA and TPC analysis. Mean values with different superscript letters are significantly different ($p<0.05$). In ST (2015), b* value increased regularly along permanence in the electric chamber, being 4.59^{b}±1.33 (min) in the original oil (OE) and 22.65^{e}±2.62 (max) on the 14th week; in 2013, values were 4.53^{a}±0.17 (min) in the original (OS) up to 15.62^{h}±0.68 (max) at the 8th week. In potato frying assays, without oil replacement (S), b* also increased from 5.44^{a}±2.38 in new oil (OFB) to 54.92^{f}±1.80 after the 25th fry; in fry with oil replacement to the 10th reuse (assay C) – where maximum was observed (29.14^{d}±2.11) – b* decreased by the 15th reuse to 5.99^{a}±0.01 returning to maximum at the 25h fry (29.18^{d}±3.08), suggesting degradation by contacting fresh and used oils. In experiments with nuggets, variation of the yellow colour was higher and the effect of replenishment was not observed; b* values always increased with reuse: from 5.88^{a}±1.09 in new oil (OFN) to 79.32^{i}±1.76 (assay S) and up to 71.89^{h}±3.64 (assay C), respectively, in 25th fry. In all studies, progress of *chroma* (C*) were very similar to the corresponding coordinate b*, but lightness (L*), a* (green, negative) and hue (H°) data, was characterized by some dispersion, higher in the UFO´s series. FT and ST results indicated differences of relatively small magnitude in progress (although often statistically significant) and the evolution of the curves, were close to a *steady model*, with oscillations around mean value.

Table 2

CIE Lab´s principal data in oils assays

Variable	Mean (combined)	Max	Min
L*	60.8±9.1	$94.9±0.3^{1}$	$46.8±1.0^{2}$
a*	-6.9±7.3	$-0.6±0.4^{3}$	$-24.6±0.3^{4}$
b*	20.7±19.3	$79.3±1.8^{4}$	$1.7±1.6^{5}$
C*	22.3±20.3	$83.1±1.6^{4}$	$2.8±0.9^{3}$
H°	108.7±11.3	$168.2±10.7^{5}$	$94.46±0.3^{6}$

Oil samples: 1 – OAN, 2 – OFB2, 3 – OE2, 4 – OFN25-S, 5 – OE10, 6 – I

Table 2 presents a short compilation of data obtained with the 71 oil samples universe.

Classical parameters AT, IP and to a lesser extent, II, gave the most regular and predictable data in the set of all tests, the shape of the curve with oil´s degradation being very similar, in its respective parameters, in all experiments (Laranjeira et al., unpublished data). *Peroxide index* (IP), the most common parameter used to characterize oils and fats presented a Gaussian evolution, decreasing when the secondary oxidation products appear as noticed by Silva et al. (1998) and Chen at al. (2012). As an example, Figure 3 shows IP curves based on the results of nuggets frying assays and its monitoring by TPC.

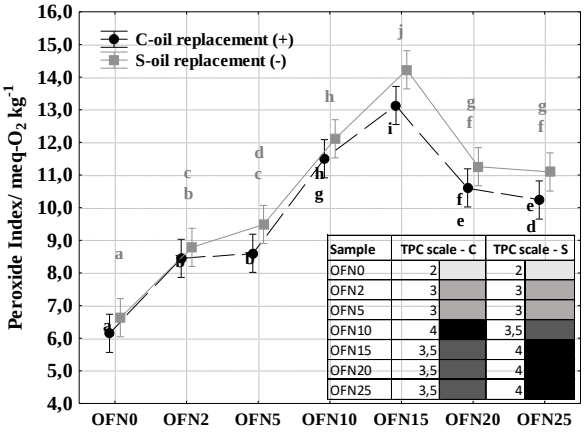

Figure 3. IP and TPC values in nuggets frying assay
TPC´s oil scale: 1-good, 2-still good, 3-replace, 4-bad.

In frying oil´s group, recommended maximum limit of 15 meq-O_2 kg^{-1} (Moigradean et al., 2012) was not exceeded (with one exception), being the absolute maximum of 13.03±0.26 in potato´s frying (OFB10, 10th fry, TPC value: 4 – *bad*), and 10.30±0.28 (G) and 37.55±0.37 (OAU_{2x}) in UFO´s series. It was also overpass in first ST: 34.86±0.28 (OS8, 8th week), but not in the 2nd, 12.78±0.25 (OE6, 6th week) where TPC colour test remained unchanged (1-*good*) during assay. The lowest IP values registered with the most degraded oils were similar (no significant differences) to those of new oil, being 1.99±0.42 (N), 3.71±0.26 (OAUr), 5.30±0.28 (OE14, 14th week) and 10.23±0.29 (OFB25-S, 25th fry). The highest and lowest IP values in unused oils were 20.30±0.45 (OAN) and 2.31±0.03 (OFB). Highest TPC' recorded value for unused oil was 2-*still good* (OFN) (Fig. 3). In *Total acidity* (AT), related to hydrolysis, samples quickly acidified with the first two fried, and later, in most degraded oils. Lowest (initial) and highest (final) values obtained were 0.0067±0.0010 (OFB) and 0.317±0.012 (OAUr) in $g_{oleic\ acid}$ 100 g^{-1}, respectively. In low-medium degraded oils (present in UFO´ series and induced lab tests) - where TPC´ values were 1 – *good* to 3 – *replace* none or minor significant differences were registered. *Iodine index* (II) not being Gaussian, actually presented an initial increase in values, due to non-specific iodization reactions (Ricardo, Teixeira, 1988) giving a more accurate response regarding (secondary) oxidation with the most degraded frying oils, where II declines with increasing degradation (Laranjeira et al., unpublished data). The highest and lowest II values, in g-I_2 100 g^{-1}, on unused oils were 38.12±0.40 (OAN) and 34.727±0.060 (A), being of 37.080±0.076 (OE14) and 19.18±0.15 (OAUr) for degraded oils, respectively. In ST assays minor changes were observed. UV´s absorbance coefficient K_{232} that undergone a Gaussian evolution, closely related to IP; highest, lowest and mean values were 37.86±0.52 (OAU_{2x}), 0.00±0.26 (OFB) and 10.7±5.2 respectively. K_{268}, associated to secondary oxidation, had a less predictable, random progress; highest, lowest and mean values were 9.21^{i}±0.26 (OFN5), 0.77^{a}±0.05 (OFN) and 3.1±2.5. Δ_{268} registered minor or no significant differences in all assays, its combined mean being 0.29±0.41. Microbiological tests held positive results in oils O2, O3, C, D, G, I, L, N, OFBi and OFNi (i=15, 20, 25); highest enumerations were: 1.8×10^3 c.f.u. dm^{-3} for lipolytic microorganisms (OFB20-C), 2 (OFB25-S) and 3 (I) c.f.u. dm^{-3} for xerophilic and for lipolytic fungi. All physicochemical parameters outlined as indicators were treated by TPC. Table 3 and Figure 4 show variables and case´s projection in the plan, considering all 71 samples.

Table 3

TPC – Principal components for used oils

Variables	Factor 1	Factor 2	Factor 3
	41.07360%	**19.64420%**	**16.90663%**
IP	-0.069032	**-0.814257**	0.242260
II	0.216178	-0.131070	**0.848616**
AT	**-0.661266**	-0.165999	-0.458827
b*	**-0.893131**	0.302022	0.177146
C*	**-0.898031**	0.263761	0.210018
K_{232}	-0.473889	**-0.710412**	-0.191190
K_{268}	**-0.746767**	-0.043268	0.286461

The distribution of variables by principal components is consistent with the phenomena they indicate and, despite a large overlap in the projection of cases, sample grouping reflect clockwise trend with the increasing oil´s degradation. Groups 1 to 3 join usable oils (new and ST samples). UFO and FT samples are in groups 4–5, the latter linking visually deteriorated and almost all oils used in frying nuggets.

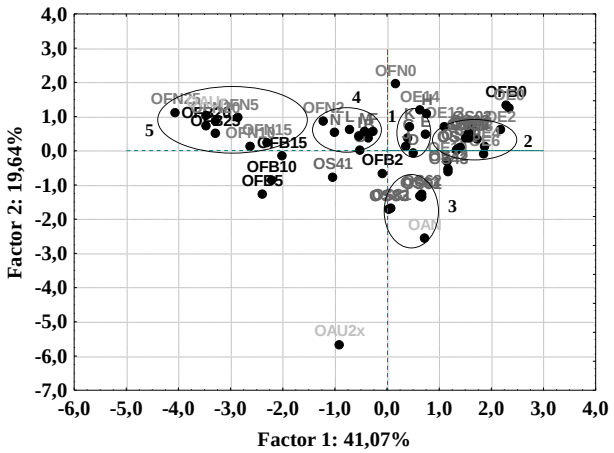

Figure 5. TPC´s sample projection in the plan

Peroxidation indexes (IP, K_{232}) are strongly correlated, like CIE Lab coordinates b* and C* (coordinate a* is mostly stationary), AT and K_{268}. These parameters have a major influence in groups 1 and 2 as in ungrouped samples (3rd quadrant). II, being the least specific and most isolated, still contributes to the positioning of samples of the 2nd quadrant and also to most degraded ones (4th quadrant); in this last case, II progress is negative (specific for oxidation) and b* and C* are major important variables. OAU_{2x} atypical and isolated, with low use in frying (2x) but a moderate depreciation and strongly associated with IP, suggests degradation upstream of the use, probably due to poor storage or senescence by long shelf life of the original oil before frying use.

Conclusions

Frying oils incorporate products of its degradation, externals and water affecting related parameters %H, a_w and AT and indirectly, oxidation indicators (Chen et al., 2012). TPC, IP, II, CIE and CIE Lab colour and AbsUV are associated with oxidative effects. Each method provides only partial information on oil´s degradation (Silva et al, 1998), but CIE system, %H and a_w was, to this end, inadequate. TPC analysis showed correlations in the remaining parameters, suggesting different possible choices in distinct phases of the oil life cycle. TPC signals earlier degradation (in the FT, not in ST assays), recommending oil´s replacement before other parameters show clearly significant changes. Yet, considering the requirements, results suggests that AT, IP and CIE Lab colour (b*, C*) are suitable *as Quality Degradation Indicators* for UFO intended for non-food recovery. AbsUV, and to a lesser extent, II, both more complex and time-consuming, are outlined as complementary, reference methods.

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MIGRATION OF IRON, ALUMINIUM, CALCIUM, MAGNESIUM AND SILICON FROM CERAMIC MATERIALS INTO FOOD SIMULANT

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Abstract

Ceramics are widely used as kitchen ware. Pottery in contact with food can be a source of various compounds from clay and glazing. The potential migration of toxic lead and cadmium from ceramic is well-known and is evaluated by the specific maximum migration levels (acceptable limits being set by the Food Contact Materials Regulation (EC) 1935/2004). Besides lead and cadmium other elements have been detected in glazed ceramic ware that could migrate during food processing. Migration experiments were performed on 10 commercially available glazed and unglazed stewing potteries (produced in Latvia and China). The migration of iron, aluminium, calcium, magnesium and silicon from the ceramics was carried out in 4% (v/v) acetic acid water solution (24, 48 and 72 h at 20 °C; 30, 60 and 90 min at 180 °C). The concentrations of elements which had migrated into the test solutions were measured by absorption spectrometry (Fe, Al and Si) and titration (summary Ca and Mg). The migration of iron, aluminium, calcium, magnesium and silicon was observed in all tested ceramics samples. Overall, the migration of studied elements was higher in unglazed stewing potteries and increased with temperature. Migration was decreased in repeatedly used ware.

Keywords: glazed and unglazed ceramics, migration, food contact.

Introduction

Ceramics stewing potteries are widely used as kitchen ware. As ceramics are made from natural material clay, they contain various elements which have a potential to migrate into food contained within. The potential migration of toxic lead and cadmium from ceramic glazing is well-known.

The EU Framework Regulation EC 1935/2004 determines the specific maximum migration levels (SML) into food only for cadmium and lead (Commission Directive 2005/31/EC). The migration of other elements from ceramic could be expected and was detected in many kinds of glazed ceramic ware.

Previously the migration experiments of toxic metals from ceramic food packaging materials into acid food simulants were carried out (Dong et al., 2014; Lin et al., 2014; Dong et al., 2015; Szynal et al., 2016). The effects of pH, nature of acid and temperature on trace element migration have been described in ceramic ware treated with 18 commercially available glazes. Besides of the well-studied lead and cadmium, the migration of other toxic and non-toxic elements such as aluminium, boron, barium, cobalt, chrome, copper, iron, lithium, magnesium, manganese, nickel, antimony, tin, strontium, titanium, vanadium, zinc and zirconium was investigated in order to evaluate their potential health hazards (Bolle et al., 2012; Demont et al., 2012).

The aim of the study was to investigate the migration of iron, aluminium, calcium, magnesium and silicon into food simulants from ceramic – stewing potteries, available on the Latvian market. Potential consumer exposure can thereby be estimated from the release of these elements into food. Permissible migration limits for these elements have not yet been defined by EU laws.

Materials and Methods

Ceramics stewing potteries were obtained on the Latvian market: four glazed ware (No. 1, No. 2, No. 3, No. 4; produced in Latvia), four unglazed pottery (No. 5, No. 6, No. 7, No. 8; produced in Latvia), two glazed stewing potteries (No. 9, No. 10; produced in China). Ceramics potteries used in this study correspond to category 3 (cooking ware according to Commission Directive 2005/31/EC). Extraction levels of aluminium, iron and silicon were determined in a similar way as the extraction levels of lead and cadmium according to Commission Directive 2005/31/EC) and calculated as mg of element per surface area (Table 1).

Table 1

Surface area of different potteries

Glazed pottery		Unglazed pottery	
No.	**Surface area, dm^2**	**No.**	**Surface area, dm^2**
1	2.63	5	5.65
2	2.55	6	2.32
3	1.45	7	1.67
4	2.92	8	1.86
9	1.37		
10	2.77		

Preparation of the sample

The samples were cleaned from grease or other matter likely to affect the test. The samples were washed in a solution containing a household liquid detergent at a temperature of approximately 40 °C, rinsing first in tap-water and then in distilled water, drained and dried so as to avoid any stain (Commission Directive 2005/31/EC).

Reagents

All the reagents were of analytical quality.

4% (v / v) acetic acid in aqueous solution was prepared as follows: 40 mL of glacial acetic acid were added to water, and then water was added until the final volume of 1000 mL.

Experiments were carried out

The migration of iron, aluminium, calcium, magnesium and silicon from the ceramics was carried out in 4% (v/v) acetic acid water solution (24, 48 and 72 h at

20±2 °C; 30, 60 and 90 min at 180±5 °C). 3 samples were taken from each ware and used for analysis.

Determination of content of iron, aluminium and silicon
Concentration of iron, aluminium and silicon were determined by microprocessor photometer MPM 3000 from WTW and commercially available reagent kits from MERCK according to manufacturer`s protocol. The concentration of iron was determined according to MERCK method No. 14761/2. The concentration of aluminium was determined according to MERCK method No. 1.14825.0001. The concentration of silicon was determined according to MERCK method No. 1.14794.0001.

Determination of content of calcium and magnesium
The calcium and magnesium concentration was determined using titration with a standardized solution of ethylenediaminetetra acetic acid (EDTA) disodium salt (Pastare et al., 2007).

Statistical analysis
All presented data are the averages of triplicate measurements. The results are presented as the mean ± standard deviation (SD). Data analysis was performed using in-built analysis of Microsoft Excel 2010.

Results and Discussion

Main components of clay used to make potteries are $Na_2O{\cdot}K_2O{\cdot}MgO{\cdot}FeO{\cdot}CaO{\cdot}Al_2O_3{\cdot}SiO_2$ and their migration into food is expected. The migration of different elements has been detected in various kinds of glazed ceramic ware. Meanwhile, very scarce information is available on migration of iron, aluminium, calcium, magnesium and silicon from clay ceramics. Current study was designed to study migration of elements present in clay like iron, aluminium, silicon, calcium and magnesium from glazed and unglazed stewing potteries into the acid food stimulant (4% (v / v) acetic acid water solution) at temperatures 20±2 and 180±5 °C.

Migration of iron from glazed ceramic
Figure 1 shows the migration of iron at 20±2 °C. Iron was identified in all samples tested and the amount of iron increased over time. The highest migration of iron was observed for sample No. 3. Such a difference could indicate lower quality of glazing used for sample No. 3. Increase in temperature to 180±5 °C markedly facilitated migration of iron in all the samples (Figure 2) as migration just after 60 minutes was higher than migration at 20±2 °C for 24–72 h (Figure 1). Overall, similar migration tendency was observed at both temperatures tested and the highest migration at both temperatures was observed for sample No. 3, followed by samples No. 9 and No. 1.
Migration of iron from glazed new pottery and repeatedly used pottery was compared and migration was markedly decreased after repeated use of cooking ware (Figure 3). Apparent explanation is that more readily soluble compounds are washed out over two cycles of use, and the remaining clay components are more stable against acidic food simulant.

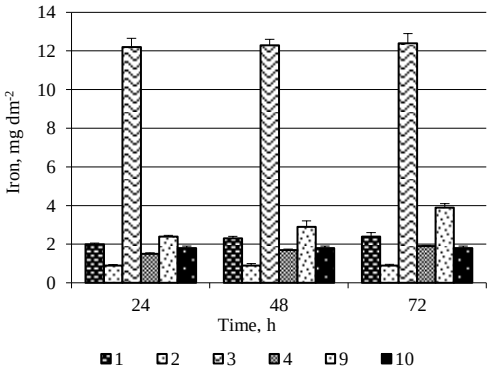

Figure 1. Migration of iron from glazed ceramic in 4% acetic acid at 20±2 °C

Glazed stewing pottery (No. 1, No. 2, No. 3, No. 4 produced in Latvia; No. 9, No. 10 produced in China).
*The results are presented as the mean ± SD (n=3).

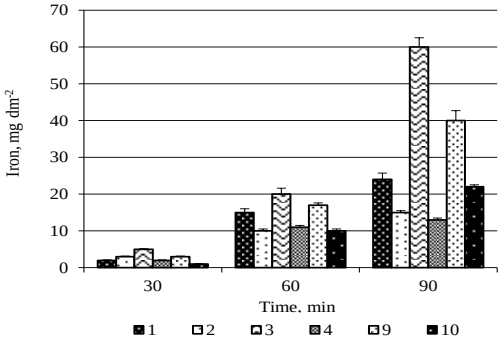

Figure 2. Migration of iron from glazed ceramic in 4% acetic acid at 180±5 °C

*Glazed stewing pottery (No. 1, No. 2, No. 3, No. 4; produced in Latvia; No. 9, No. 10; produced in China).
**The results are presented as the mean ± SD (n=3).

Migration of iron from unglazed ceramic
Figure 4 shows the migration of iron at 20±2 °C from unglazed ceramic. Sample No. 5 had significantly lower migration of iron compared to other samples tested. In general, migration rate of iron from unglazed potteries was higher than that from glazed ware indicating that glazing could offer more protection against migration of clay components into food.

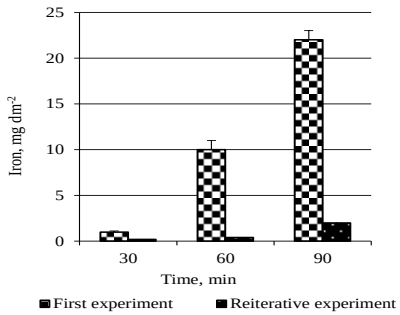

Figure 3. Migration of iron from glazed stewing potteries No. 10 in first treatment and reiterative experiment at 180±5 °C.

*The results are presented as the mean ± SD (n=3).

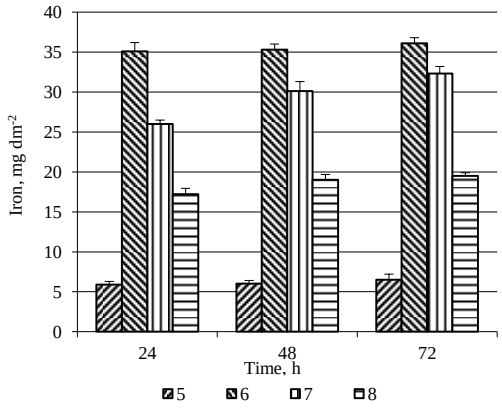

Figure 4. Migration of iron from unglazed ceramic in 4% acetic acid at 20±2 °C

*Unglazed stewing pottery (No. 5, No. 6, No. 7, No. 8 produced in Latvia).

**The results are presented as the mean ± SD (n=3).

Similar to migration observations with glazed ceramic, migration of iron at 180±5 °C from unglazed clay was markedly facilitated in all the samples (Figure 5) as migration just after 60 minutes was higher than migration at 20±2 °C for 24–72 h (Figure 4).

Migration of aluminium from glazed and unglazed ceramic

Figure 6 shows the migration of aluminium at 20±2 °C. Migration of aluminium was similar in all glazed ceramic samples and increased over time. In unglazed ceramic migration of aluminium was slightly higher than in glazed ceramic, but in general, this difference was not as marked as in case of migration of iron. Increase in temperature to 180±5 °C markedly facilitated migration of aluminium in all the samples (Figure 7) and overall tendency was similar to migration at 20±2 °C.

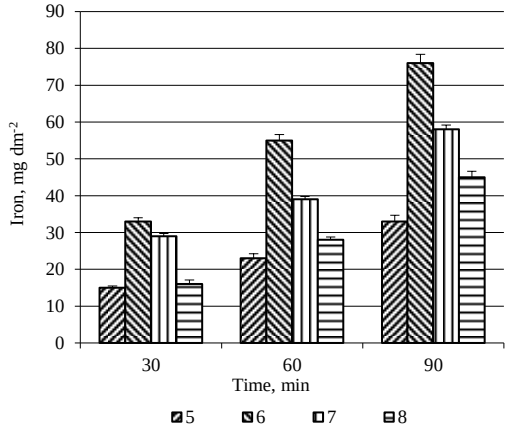

Figure 5. Migration of iron from unglazed ceramic in 4% acetic acid at 180±2 °C

*Unglazed stewing pottery (No. 5, No. 6, No. 7, No. 8 produced in Latvia).

**The results are presented as the mean ± SD (n=3).

It is also clear that the migration of aluminium from the potteries was relatively high in either case. Clay compounds iron, silicon, magnesium and calcium are non-toxic elements. Migrations limits for aluminium have not yet been set in EU legislation.

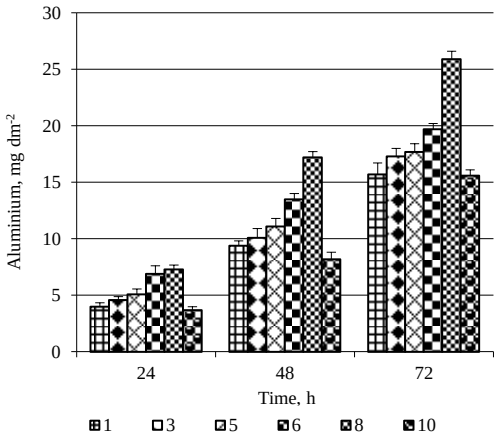

Figure 6. Migration of aluminium from glazed and unglazed ceramic in 4% acetic acid at 20±2 °C

*Stewing pottery (glazed: No. 1, No. 3 produced in Latvia; No. 10 produced in China; unglazed: No. 5, No. 6, No. 8; produced in Latvia).

**The results are presented as the mean ± SD (n=3).

Biologically active aluminium is present in the human body and sometimes can be acutely toxic. Although not clearly proven yet, there are indications that chronic aluminium intoxication may be related to Alzheimer's disease, breast cancer and autism (Exley, 2016). Comparing maximal allowed concentration of aluminium in drinking water (0.2 mg L^{-1}) (Regulation of the Cabinet of Ministers No. 235., 2003) and highest concentration of 37.8 mg L^{-1} of aluminium found in food simulant in this study (sample No. 8, after 90 min at 180±5 °C), it would be advisable to find means to limit migration of aluminium from clay potteries or limit the use of clay ceramics, particularly, unglazed ware at high temperatures. Pre-heating clay potteries filled with acidified water 1–2 times before actual use could be a relatively easy option to decrease aluminium migration in food.

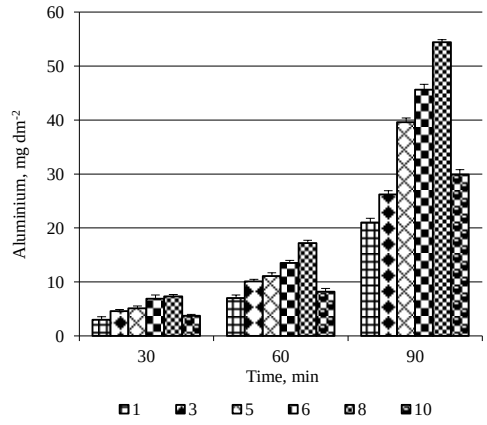

Figure 7. Migration of aluminium from glazed and unglazed ceramic in 4% acetic acid at 180±5 °C

*Stewing pottery (glazed: No. 1, No. 3 produced in Latvia; No. 10 produced in China; unglazed: No. 5, No. 6, No. 8 produced in Latvia).

**The results are presented as the mean ± SD (n=3).

Migration of silicon from glazed and unglazed ceramic

One of main components of clay is SiO_2 (Sedmanis et al., 2002) and it also can migrate. Although silicon was identified in all samples, the variations in migration among different potteries were very high. The content of clay varies a lot and this could explain high variability in migration of silicon (Figure 8).

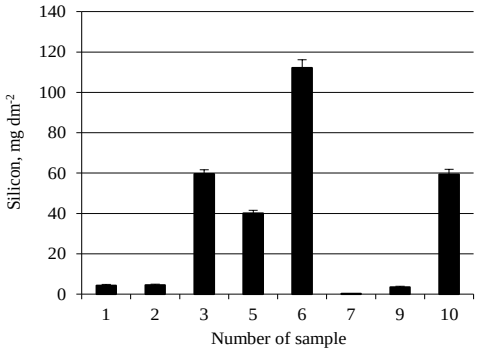

Figure 8. Migration of silicon from glazed and unglazed ceramic in 4% acetic acid at 20±2 °C

*Stewing pottery (glazed: No. 1, No 2, No. 3 produced in Latvia; No. 9, No. 10 produced in China; unglazed: No. 5, No. 6, No. 7, No. 8 produced in Latvia).

**The results are presented as the mean ± SD (n=3).

Migration of calcium and magnesium (sum) from glazed and unglazed ceramic

Figure 9 shows migration of calcium and magnesium (sum after 90 min at 180±5 °C). The migration rate of calcium and magnesium from potteries was highly variable, but in general migration was higher from unglazed ware.

Overall, clay potteries are not inert and various elements, particularly iron, aluminium, calcium and magnesium do migrate into acidic food simulants.

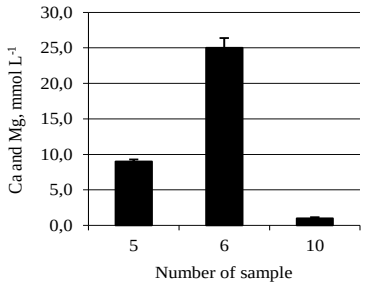

Figure 9. Migration of calcium and magnesium from glazed and unglazed ceramic in 4% acetic acid at 180±5 °C after 90 min

*Stewing pottery (glazed: No. 10 produced in China; unglazed: No. 5, No. 6 produced in Latvia).

**The results are presented as the mean ± SD (n=3).

Migration is markedly accelerated by increasing temperature, while glazing partially delays migration of elements. Of particular interest due to possible health related adverse effects might be migration of aluminium and it could be reasonable to test aluminium migration from clay potteries available on market.

Conclusions

The present study indicates that potteries are not inert ware and can readily interact with food-mimicking acetic acid solution in water, thus, it can be concluded that in a similar manner potteries could interact with real food. Migration rate of studied elements into the food simulant increased with the temperature and initially over time, but was significantly lower in repeatedly used ceramics. The migration rates of iron, aluminium, silicon, magnesium and calcium varied among different ceramics, but in general rates were higher in unglazed ware.

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IDENTIFICATION OF MICROFLORA OF FRESHWATER FISH CAUGHT IN THE DRIKSNA RIVER AND POND IN LATVIA

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Abstract

Identification of freshwater fish microflora is an important tool for evaluation of quality and safety of fish intended for human consumption. The aim of the present study was to detect the microflora of freshwater fish caught by fishermen. Altogether, 23 samples of freshwater fish were collected from fishermen from two different water sources – Driksna river and pond in Dobele. For detection of microbiological contamination, fish samples were tested for the total bacterial count (TBC), coliforms, *Enterobacteriaceae* and zoonotic pathogenic microorganisms - *Salmonella* spp., *Listeria* spp., and *Yersinia* spp. Identification of bacterial species was carried out by the MALDI-TOF MS Biotyper. TBC ranged from 2.7 to 4.78 $\log_{10}$ CFU g^{-1} and 3.68 to 4.11 $\log_{10}$ CFU g^{-1}, coliforms from 2.55 to 4.10 $\log_{10}$ CFU g^{-1} and 1.38 to 2.73 $\log_{10}$ CFU g^{-1}, *Enterobacteriaceae* from 1.95 to 4.05 $\log_{10}$ CFU g^{-1} and 1.72 to 2.69 $\log_{10}$ CFU g^{-1} in pond and river fish samples, respectively. Between two fishing locations, fish caught in pond carried the significantly higher number of TBC, coliforms and *Enterobacteriaceae* than fish from the river ($P>0.05$). Freshwater fish microflora consisted of *Pseudomonas* spp. (55%), *Serratia* spp. (7%), *Candida* spp. (6%), *Rahnella* spp. (7%), *Pantoea* spp. (9%), *Aeromonas* spp. (5%), *Buttiauxella* spp. (8%), *Stenotrophonomas* spp. (2%) and *Enterobacter* spp. (1%). *Salmonella* spp., *Listeria* spp. and *Yersinia* spp. were not identified. Despite the human pathogenic microorganisms were not identified in the present study, the composition of microflora and especially the abundance of *Pseudomonas* spp. indicates that the fish are prone to spoilage process development that potentially may alter the quality of freshwater fish meat.

Keywords: freshwater fish, bacteria, MALDI-TOF MS Biotyper

Introduction

Fish is an important source of protein for human consumption; however, the fish and fish products may contain spoilage, pathogenic and opportunistic pathogenic microorganisms, including foodborne pathogens (FAO, 2000). Foodborne pathogens may cause disease outbreaks, causing a health risk to consumers (Davies et al., 2001; Huss, 1997; Rohde et al., 2014). The presence of foodborne pathogens in fish is related to environmental conditions and microbiological quality of the water at the fishing site, because the contamination of the water and fish from animal, human and agricultural sources may occur (Feldhusen, 2000; Davies et al., 2001; Hosseini et al., 2004). Fishing method and storage conditions also may affect the microbiological quality of the fish. Fish microflora characteristics are important to determine the character of the fish microbiological contamination and consumption validity. Fish may undergo rapid spoilage process associated with intensive microbial growth that leads to rapid deterioration of the quality of the fish meat with the meat become unfit for human consumption (Austin, 2006). Consequently, to make a decision on safety of fish meat, it is important to identify and characterize the fish microflora. Fishing for consumption is very common in Latvia, but the microbiological assessment of freshwater fish caught in rivers and ponds have been not conducted yet. Therefore, the aim of the present study was to detect the microflora of freshwater fish caught by fishermen.

Materials and Methods

Selection of samples

Altogether, 23 freshwater fish were obtained from two different water sources - Driksna river and pond during February to June in 2015. An amount of 18 fish, including seven samples of roach (*Rutilus rutilus* L.), four samples of bream (*Abramis brama* L.), five samples of perch (*Perca fluviatilis* L.), one sample of chub (*Leuciscus cephalus* L.) and one sample of ruff (*Gymnocephalus cernua* L.) were caught in Driksna river located in the central part of Latvia – Zemgale district. A total of five samples of crucian carp (*Carassius carassius* L.) were caught in the pond located in the Dobele district in the central part of Latvia. Fish were immediately placed in a sterile container and transported to the laboratory on ice. Testing was started within 2 to 4 h after sampling.

Sampling

Skin, gill and gut samples of fish aseptically were taken for quantitative microbiological assessment for detection of the total bacterial count (TBC), coliforms and *Enterobacteriaceae* from each fish. *Enterobacteriaceae* were detected as the general indicator of environmental hygiene but the coliforms may signify the possible presence of pathogens able to ferment lactose. Skin samples were taken with a scalpel and a cotton swab from a 10 cm^2 (depending on fish size) fish skin area near to the lateral line of the body. For gill samples, the operculum was opened first and then the gills were dissected with sterile instruments. For gut samples, the belly was cut along the midline from the anal fin and the gut content was removed.

Pooled samples of skin, muscles and gut were used for detection of pathogens - *Salmonella* spp., *Listeria* spp. and *Yersinia* spp.

Bacteriological analyses

For detection of TBC, coliforms and *Enterobacteriaceae* counts, a quantity of 9 mL of 0.1% peptone water (CM0509, OXOID, UK) was added to each gill, skin and gut sample to obtain the decimal dilutions. Then, an amount of 1 mL of decimal dilution was plated out on Plate Count Agar (PCA, 5121452, Biolife, Italy) and incubated at 30 °C for 72 h. After incubation bacterial colonies were counted (ISO 4833:2003). For detection of coliforms, the decimal dilutions were plated out onto Violet Red Bile Agar (VRBA, 5121852, Biolife, Italy) and incubated at 37 °C for 24 h. After incubation, the typical – purple, round bacterial colonies were enumerated (ISO 4832:2006). For detection of *Enterobacteriaceae*, the decimal dilutions were plated out onto Violet Red Bile Glucose Agar (VRBGA, 4021881, Biolife, Italy) and incubated at 37 °C for 24 h. After incubation, the typical purple colonies were enumerated (ISO 21528-2:2004).

For detection of *L. monocytogenes*, a suspension of the sample in Half-Fraser broth (BO0793, OXOID, UK) with ratio 1:10 was incubated at 30 °C for 24 h (ISO 11290-1:2005). After incubation, a quantity of 0.1 mL of suspension was transferred into Fraser broth (CM0895, OXOID, UK) and incubated at 37 °C for 48 h. An amount of 0.1 mL of Half-Fraser and Fraser broth was plated out on Palcam (401604, Biolife, Italy) and Agar Listeria Ottaviani & Agosti (ALOA, 4016052, Biolife, Italy) and incubated for 24–48 h at 37 °C. After incubation, the agar plates were checked for the presence of typical blue with opaque halo colonies on ALOA and grey colonies with a black halo on Palcam agars. Typical colonies were Gram stained and were tested for catalase activity, haemolysis and the biochemical identification was performed.

For detection of *Salmonella* spp., a suspension of the sample in 0.1% buffered peptone water with ratio 1:10 was incubated at 37 °C for 24 h (ISO 6579:2006). After incubation, a 0.1 mL of the suspension was transferred into 9 mL of Rappaport-Vassiliadis (5119802, Biolife, Italy) and Muller Kauffmann Tetrathionate broths (4017452, Biolife, Italy) for selective enrichment at 37 °C for 24 h. After incubation, a 0.1 mL of the enriched broth was plated on Xylose Lysine Deoxycholate Agar (XLD, 4022082, Biolife, Italy) and incubated at 37 °C for 24 h. Then, the plates were checked for the presence of typical pink to red colonies with black centers, which were transferred in Triple Sugar Iron Agar containing tubes (TSI, CM0277, OXOID, UK) and incubated at 37 °C for 24 h. After incubation, the agar tubes were checked for glucose, lactose and sucrose fermentation.

Suspension of the sample in Peptone Mannitol Bile Salt Broth (1 : 10) (PMB, 10.0 g bacteriological peptone, LP0037, OXOID, UK; 10.0 g mannitol for microbiology; 1.2 g bile salts No.3; 7.5 g d-sodium hydrogen phosphate, Scharlau Chemie S.A., Barcelona, Spain) was plated out onto Cefsulodin Irgasan Novobiocin Agar (CIN, 401302, Biolife, Italy) at 30 °C for 24 h for detection of *Yersinia* spp. (ISO 10273:2003). In the case of negative results, the suspension was incubated at 4 °C for 14 days with subsequent treatment with 0.5% KOH before the culturing. Inoculated plates were incubated at 30 °C for 24–48 h and then checked for the presence of typical colonies with red centre and translucent outer zones. Typical colonies were checked for urea hydrolysis (UREA agar, CM0053, OXOID, UK).

Identification of microbial microflora with MALDI-TOF Biotyper MS

MALDI-TOF Mass Spectrometry was applied for the identification of bacteria. Bacterial colonies from PCA, VRBA and VRBGA agars were plated onto Tryptone Soya Agar (TSA, CM0131, OXOID, UK) and inoculated at 37 °C for 18–24 h.

The colonies from the TSA were transferred in 300 µL of sterile distilled water and 900 µL of absolute ethanol (99%, Sigma-Aldrich, USA) was added. The mixture was centrifuged at 13 000 × g for 2 min. After removal of the supernatant, residual ethanol was pipetted and the pellet dried at a room temperature. Then, a 10 µL of formic acid (70%) and 10 µL of acetonitrile (100%) was added to the pellet and mixed. The solution was centrifuged repeatedly and the supernatant was transferred on a polished MALDI plate (Bruker Daltonics, Germany). Then, a 1 µL of the matrix solution (HCCA: α-cyano-4-hydroxycinnamic acid, 50% acetonitrile with 0.025% trifluoroacetic acid. Samples were processed in the MALDI-TOF MS (Microflex LT/SH, Bruker Daltonics) and results were obtained with Realtime Classification software (RTC) (Bruker Daltonics).

Statistical analyses

All microbial counts data were transformed to decimal logarithms.

T-tests for calculating differences among the TBC, coliforms and *Enterobacteriaceae* in different samples and fish species were applied.

Results and Discussion

The highest TBC was found on skin, gills and gut of roach with 3.95, 4.63 and 4.39 $\log_{10}$ CFU g^{-1} (Table 1). The highest number of coliforms and *Enterobacteriaceae* counts were in ruff skin, gill and gut samples, comprising 3.00, 3.66 and 4.10 $\log_{10}$ CFU g^{-1} and 4.71, 3.67 and 2.66 $\log_{10}$ CFU g^{-1} for coliforms and *Enterobacteriaceae*, respectively. TBC ranged from 2.72 to 4.78 $\log_{10}$ CFU g^{-1} in crucian carp skin and gill samples, respectively. Coliforms were detected in all samples and the highest coliform count was 4.10 $\log_{10}$ CFU g^{-1} in gill but the lowest of 2.55 $\log_{10}$ CFU g^{-1} in skin. The highest *Enterobacteriaceae* counts of 4.05 $\log_{10}$ CFU g^{-1} were in gills while the lowest of 1.95 $\log_{10}$ CFU g^{-1} in skin samples.

Table 1

TBC, coliforms and *Enterobacteriaceae* (log_{10} CFU g^{-1}) in freshwater fish caught in Driksna river and pond

Fish species	Sampling site	TBC Mean±SD	Coliforms Mean±SD	*Enterobacteriaceae* Mean±SD
Chub (*Leuciscus cephalus*)	Skin	3.46	0	2.26
	Gills	3.04	2.26	0
	Gut	4.42	3.07	3.32
Roach (*Rutilus rutilus*)	Skin	3.95±1.08[a]	1.29±1.22[b]	0.60±1.03[c]
	Gills	4.63±0.42	2.82±1.29	2.79±1.38
	Gut	4.39±0.57	2.96±0.46	1.97±1.41
Bream (*Abramis bram*)	Skin	3.26±0.52[a]	0.49±0.98[b]	1.55±1.04
	Gills	3.49±0.75	2.54±1.79	2.30±1.54
	Gut	3.86±0.62	2.05±1.37	1.06±1.22[c]
Perch (*Perca fluviatilis*)	Skin	3.98±0.71[a]	2.17±1.43	2.73±0.93
	Gills	4.12±1.34	2.66±1.56	3.19±0.63
	Gut	4.15±0.53	2.39±1.48[b]	2.74±0.61[c]
Ruff (*Gymno-cephalus cernua*)	Skin	2.26	3.00	4.71
	Gills	3.49	3.66	3.67
	Gut	2.66	4.10	2.66
Crucian carp (*Carassius carassius*)	Skin	2.72±0.46[d]	2.55±0.36[d]	1.95±1.15[d]
	Gills	4.78±0.36	4.10±0.34	4.05±0.38
	Gut	4.54±0.62	3.64±0.71	3.25±0.72

[a] no significant differences between TBC in roach, bream and perch skin, gill and gut samples were observed ($p<0.05$)
[b] coliforms in roach and bream skin samples was significantly less than in gills and gut samples ($p>0.05$)
[c] significant differences between *Enterobacteriaceae* in roach and bream skin, gill and gut samples were observed ($p>0.05$)
[d] TBC, coliforms and *Enterobacteriacae* in crucian carp skin samples was significantly less than in gills and gut samples ($p>0.05$)

The higher TBC in comparison with coliforms and *Enterobacteriaceae* in chub, roach, bream, perch and crucian carp is attributable to the widespread occurrence of microorganisms in the aquatic environment. Coliforms and *Enterobacteriaceae* are hygiene indicators and their counts generally are lower, because they indicate a contamination and the possible presence of pathogenic bacteria in fish and aquatic environment.

In the present study, the TBC, *Enterobacteiacea* counts were in line with Austin (2006) reported for Atlantic salmon and rainbow trout with skin contamination rates of 10^2 to 10^3 CFU g^{-1} and from 10 to 10^7 CFU g^{-1} in skin samples, respectively. However, the present results were less than reported for perch and bream sampled from Usmas lake in Latvia with skin contamination of 5.48 and 7.07 log_{10} CFU g^{-1}, respectively (Terentjeva et al., 2015). As the water microbiological quality, angling method and hygiene are crucial in providing of the microbiological quality of fish, the bacterial counts can vary in great extent that could be an explanation for differences in the reports (González et al., 1999). In our mind, the microbiological quality of caught fish was good and indicated that the fish were caught from a clean environment.

The lowest bacterial contamination with TBC, *Enterobacteriaceae* and coliforms was observed in skin, while the highest - in the gill and gut. Since the contamination of skin fish from surrounding environment, including fisherman hands, is possible, the results show that the skin contamination could be avoided. Our results are in agreement with Austin (2006), who reported that fish skin contains the relatively less amount of bacteria than other tissues. In contrast, TBC from 10^{12} to 10^{13} CFU g^{-1} were reported on skin of tilapia in Nigeria (*Oreochromis niloticus*) that emphasize the importance of hygienic handling of fish and storage conditions (Emikpe et al., 2011).

In our study, the high bacterial contamination in gills was observed. The gills usually share a large number of bacteria - up to 10^6 CFU g^{-1} (Austin, 2006). The reason for the relatively high amount of bacteria is the precipitation of water and other organic substances, including bacteria during the water filtration (Austin, 2006).

Our study revealed that the highest bacterial contamination was found in the fish gut. Austin (2006) stated that the highest bacterial population was observed in the fish digestive tract and TBC varied from 10^5 to 10^8 CFU g^{-1}. Composition and counts of microflora in fish gut depend on many factors, but the most important are water quality, fish species, age and the type of feed. Herbivore fish receives additional bacteria from aquatic plants, but predator fish from other living organisms, so in that fish more microorganisms can be found in the gut than in other tissues (Bacanu, Oprea, 2013).

Pathogenic bacteria - *Salmonella* spp., *Listeria* spp. and *Yersinia* spp. were not identified in the present study, however, the *Listeria monocytogenes* and *Yersinia* spp. were isolated from freshly caught fish in Latvia previously (Terentjeva et al., 2015). Our results indicate that the freshly caught fish were safe for consumption.

Bacterial contamination of the fish caught in the pond was higher in comparison with the fish caught in Driksna river (Figure 1). Despite the TBC was

higher in the skin of fish caught in Driksna river (3.68 $\log_{10}$ CFU g^{-1}), the TBC in gill and gut were higher in fish caught in the pond, 4.54 and 4.78 $\log_{10}$ CFU g^{-1}, respectively. Also, the highest coliform counts were detected in fish from the pond – 2.55, 4.10 and 3.64 $\log_{10}$ CFU g^{-1} in skin, gills and gut, respectively. The highest *Enterobacteriaceae* counts were obtained from fish from the pond as well with 1.95, 4.05 and 3.25 $\log_{10}$ CFU g^{-1} in skin, gills and gut, accordingly.

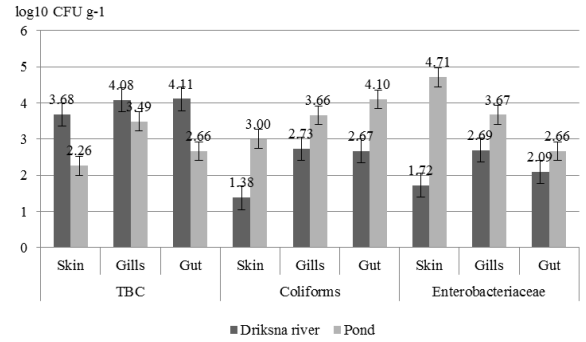

Figure 1. Bacterial contamination of freshwater fish caught in Driksna river and pond

Prevalence and survival conditions for microorganisms in rivers significantly differ from those in lakes and ponds. In rivers, as compared to ponds, the longer coastline, the higher speed of water flow and quantity of the substance exchange protect the water from exceeding contamination with microorganisms. In contrast, in the ponds, water exchange is not possible, thus in such water bodies, the increased concentration of different substances and microorganisms were observed (Bronmark, Lars-Anders, 2005; Kļaviņš, Cimdiņš, 2004).

Among all microbial genera were isolated from freshwater fish, the majority belonged to bacteria with *Pseudomonas* spp. (55%), *Pantoea* spp. (9%), *Serratia* spp. (7%) and *Rahnella* spp. (7%) were predominant. *Candida* spp. (6%) was only the microscopic yeasts isolated (Figure 2).

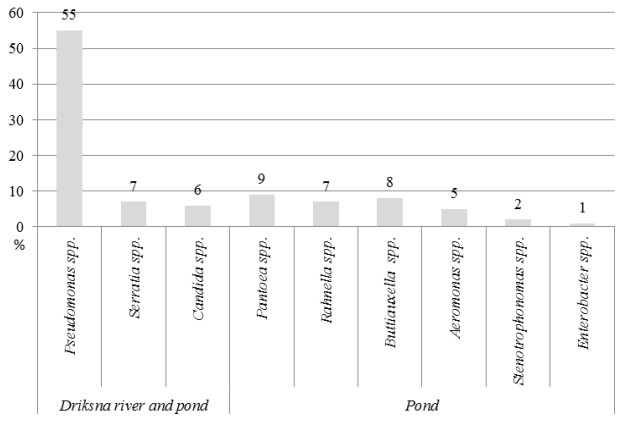

Figure 2. Microflora of freshly caught freshwater fish

Pseudomona spp., *Serratia* spp. and *Candida* spp were isolated from freshwater fish caught in Driksna river. *Pseudomona* spp., *Serratia* spp., *Candida* spp., *Pantoea* spp, *Rahnella* spp., *Buttiauxella* spp., *Aeromonas* spp., *Stenotrophonomas* spp. and *Enterobacter* spp. were identified in freshwater fish caught in the pond. Microflora of freshly caught freshwater fish from the pond was more diverse than in fish from Driksna river. *Pseudomonas* spp. were isolated from both freshwater fish sampling sites - from the river and the pond.

Pseudomonas are well-known fish specific spoilage microorganisms and their abundance in fish may led to rapid fish spoilage processes that causes changes in fish meat quality and makes fish unfit for human consumption (Gillespie, 1981; Gram, Dalgaard, 2002). *Serratia* spp., *Pseudomonas* spp., *Rahnella* spp. and *Pantoea* spp. were isolated from rainbow trout (*Oncorhynchus mykiss*) and bacterial microflora composition corresponds to our study (Austin, 2006). *Serratia* spp. *Rahnella* spp. and *Pantoea* spp. are present in the environment, plant surfaces, soils and water (Blackburn, 2006). *Serratia* spp. have been isolated from fish and other foods. The bacteria are able to grow in an environment that is unsuitable for other microbial growth and *Serratia* spp. also contribute to spoilage of foods. *Candida* spp. can be found in both contaminated and clean waters - rivers, lakes and ponds. Consequently, this microscopic yeast was previously isolated from fish and aquatic components (Batt, 2014). *Aeromonas* spp. is an opportunistic pathogen found in freshwater habitats around the world, in soil, water and food. This bacteria can cause foodborne and nosocomial infections (Cabral, 2010; Gauthier, 2015; Janda, Sharon, 2010). The presence of this microorganisms potentially may cause consumer health concerns.

Conclusions

All the freshly caught fish obtained from fishermen exhibit satisfactory microbiological quality with crucian carp (*Carassius carassius*) was the most contaminated fish among all the tested species.

The *Pseudomonas* spp. was predominant in fish microflora in all cases. *Pseudomonas* spp. cause rapid deterioration of fish and fish products and the dominance of this bacterial group indicates that the fish are prone to spoilage process development.

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LACTOSE CONTENT OF BREAST MILK AMONG LACTATING WOMEN IN LATVIA

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Abstract

Human milk is the best nutrient source providing everything for the infant's development. Lactose, after the fat, is the second most energy-dense macronutrient found in the breast milk but its concentration can be affected by factors like mother's current body mass index (BMI), birth weight of an infant, duration of lactation, etc. The aim of this study was to determine lactose content in mature breast milk among lactating women in Latvia and factors affecting it. The preliminary study was carried out from November 2016 to February 2017. In total, 28 mature milk samples pooled within 24 h were collected from mothers whose off springs had reached the age of at least two months. Participants (28 mothers with singleton deliveries of 13 male and 15 female infants) were 26 to 39 years old with an average BMI 21.85±2.69. Personal information including birth weight and age of an infant, breastfeeding method (exclusive, mixed) and milk expression manner (by hand or using breast pump) was recorded. Lactose content was determined by ISO 22662:2007. Mean lactose concentration was 6.53±0.34% which is comparable to data obtained from other studies. Although breast milk composition is variable and changes according to the needs of an offspring, no significant difference in lactose content was found for age (2 to 21 months), gender or birth weight (3.53±0.63 kg) of an infant ($p>0.05$). None of the recorded maternal characteristics influenced lactose's content in milk. Also milk expression or breastfeeding method did not affect it. Preliminary results show that lactose concentration is kept relatively constant in human milk, however more samples need to be analysed for further conclusions.

Keywords: human milk, lactose, composition

Introduction

Human milk is the ideal food source for infants providing all the nutrients for growing and many other health benefits both for mother and the young one (Fewtrell, 2004; Motee, Jeewon, 2014; Torres, Park, 2013). The composition of human milk varies corresponding to many factors, including infant's birth weight, length of lactation, age of mother and her anthropometrics (Andreas et al., 2015; Soliman et al., 2014; Torres, Park, 2013). Lactose is the most abundant carbohydrate in human milk (90–95%). It is broken down into glucose and galactose prior to intestinal absorption. Comparing to other species, human milk has the highest lactose concentration (~7 g 100 mL^{-1}), serving to the high-energy demands of the brain (Coppa et al., 1993, cited by Andreas et al., 2015; Picciano, 2009; WHO, 2009). Galactose is important for synthesis of galactolipids which are essential for development of central nervous system (Chang et al., 2015). The secretion of lactose initiates concomitant excretion of a large amount of water what is needed to compensate for sweating, respiratory water loss and also for urine formation (Picciano, 2009). A small amount of disaccharide is not absorbed and promotes softer stools, regulates microbiota and may protect neonatal gut against pathogens (Cederlund et al., 2013; Dahl, 2015). Most of the lactose (~80%) in human milk is derived from plasma glucose. Maternal glucose utilization increases by ~30% during lactation (Picciano, 2009; Sunehag et al., 2002). However, mammary glands can *de novo* synthesize both lactose moieties from other substrates (remaining ~20% of lactose) (Sunehag et al., 2002, Sunehag et al., 2003).

Breast milk composition among women in Latvia is not sufficiently studied (Aumeistere, Zavadska, 2016; Broka et al., 2016). The aim of this research was to determine lactose content in mature human milk among lactating women in Latvia and factors affecting it.

Materials and Methods

Study design

The study was carried out from November 2016 to February 2017. In total, 28 mature milk samples pooled within 24 h were collected from mothers whose young ones had reached the age of at least two months. Participant group included 28 mothers (singleton deliveries of 13 male and 15 female infants) from age 26 to 39 years. Personal information of each participant was recorded, including mother's age, weight and height parameters, sex and age of an infant, breastfeeding pattern (exclusive or mixed), milk expression method (by hand or using breast pump) used during the study. Mothers were also asked to complete food frequency questionnaire – a modified inquiry form was taken from World Health Organisation coordinated Survey (WHO, 2007). Mentioned questionnaire was transformed – more food categories (cereals, vegetables, fruits, berries, nuts, canned food, etc.) were included to obtain comprehensive information about woman's dietary habits during breastfeeding.

Milk collection and analysis

Approximately 100 ml of milk pooled within 24 h was obtained by hand expression or using breast pump. Samples were kept frozen at -20 °C until analysis. Collected samples were analysed in the Laboratory of Food and Environmental Investigations of Institute of Food Safety, Animal Health and Environment BIOR. Lactose content was determined according to

ISO 22662:2007 using high-performance liquid chromatography (HPLC).

Limitations of the study

Mothers were allowed to use the most convenient milk expression method (hand expression or breast pump). Maternal Body Mass Index (BMI) was calculated based on given information about height and weight of respondents. Anthropometric measurements were not made during this study.

Statistical analysis

Analysis were done in duplicate. Data were recorded, compiled in Microsoft Excel 2013 and reported as the mean ± standard deviation. Data statistical analysis was performed using software R version 3.3.2. Categorical variables were compared by chi-square test and continuous variables were compared by Kruskal Wallis test. Spearman's rank correlation coefficients were obtained to evaluate possible associations affecting lactose content in human milk (α=0.05).

Ethical considerations

The Study protocol was approved by Riga Stradiņš University Ethic Committee (No. 4/28.7.2016.). Written informed consent was obtained from all participants.

Results and Discussion

We investigated the relation between lactose concentration in mature milk and basic information about the mothers and babies. Table 1 represents obtained information, as well as lactose results.

Table 1

Descriptive characteristics

	Mean ± SD	Range
Lactose (%)	6.53±0.34	5.94–7.14
Maternal characteristics		
Age (years)	31±3	26–39
BMI (kg m^{-2})	21.71±2.70	17.85–28.55
Characteristics of the offspring		
Birth weight (kg)	3.53±0.60	1.60–4.70
Age (months)	5±4	2–21
Sex	54% female, 46% male	

Lactose concentration

Mean human milk lactose concentration was 6.53±0.34%. Our results was almost equal (6.50%) to data obtained from another recent research (Broka et al., 2016) done in Latvia. *Broka et al*. (2016) also used HPLC to determine lactose content but milk samples were collected from day 11^{th} to 28^{th} of lactation, including transitional milk (7 to 21 days *post partum*) (Broka et al., 2016). Our research only included samples of mature human milk (at least 2 moths *post partum*). Our results were also similar to data obtained from other countries (from 6.14 to 7.75%) (Figure 1) (p>0.05). The coefficient of variation for our results was 5.19%, which is higher than observed by Thakkar et al. (2013), Yamawaki et al. (2005), Saarela et al. (2005) and Soliman et al. (2014) (1.56%, 1.70%, 1.84% and 3.87%, respectively) but lower that observed by Chang et al. (2015), Mitoulas et al. (2002) and Shi et al. (2011) (5.63%, 9.77% and 17.50%, respectively). Deviations in results between studies could be related to distinctions in sampling. Also different methods of analysis among researches were used to determine lactose concentration

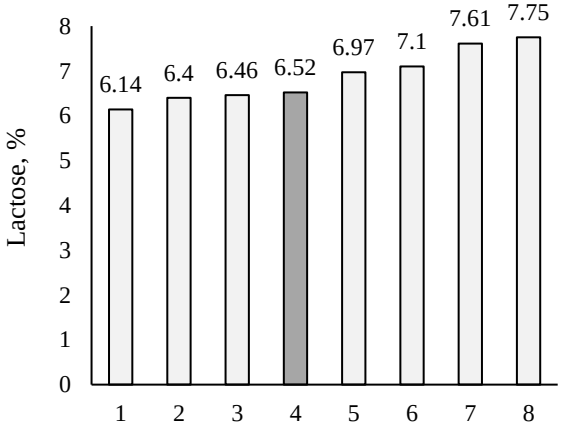

Figure 1. Lactose concentration in mature breast milk among women from different countries

1 – Mitoulas et al., 2002, 2 – Thakkar et al., 2013, 3 – Yamawaki et al., 2005, 4 – current study, 5 – Shi et al., 2011, 6 – Chang et al., 2015, 7 – Saarela et al., 2005, 8 – Soliman et al., 2014

Maternal characteristics altering lactose content

Participants in our study were 26 to 39 years old. Current maternal BMI was calculated based on given information about height and weight. It ranged from 17.85 to 28.55. Our study agrees with Kader and co-authors (1992, cited by Andreas et al., 2015) findings that lactose content in human milk is mother's age independent (Spearman's *r*=-0.17, p=0.39). Michaelsen and co-authors (1990) observed a positive correlation between carbohydrate content in human milk and mother's BMI. In contrary, Quinn together with co-authors (2012) spotted a negative correlation between milk sugar content and mother's BMI. No significant association between lactose content in human milk and mother's BMI was found in this study (*r*=-0.17, p=0.39) but it can be due to small sample size.

Offspring's characteristics altering lactose content

Our study included mothers with singleton deliveries of 13 male and 15 female babies with an average age 5 birth weight of 3.53±0.60 kg. There is an evidence that gender can alter milk composition and mother having a male offspring produce more milk which is also more energy-dense. This association is referred to difference in muscle mass (Altufaily, 2009; Fujita et al., 2012; Powe et al., 2010). However, in our study it seems that gender does not influence lactose concentration in human milk (p>0.05). Broka et al. (2016) observed that concentration of lactose was higher in samples from

mothers who had neonates with low birth weight (<2.5 kg), which disagrees with our study. Broka and co-authors (2016) represented data from mothers with hospitalized infants, therefore the results cannot be used as a reference for the general population. Our study only included participants who in consent form had stated that they and their children were currently in good health Birth weight for babies in our study ranged from 1.60 to 4.70 kg. We concluded that birth weight does not influence lactose content in human milk (r=0.05, p=0.81). Our preliminary study included 27 infants (2 to 11 months old) and one toddler (21 months old). Soliman et al., (2014) observed that lactose content increases with an infant's age, ranging from 7.38±0.05 g dL^{-1} in the first month to 8.08±0.43 g dL^{-1} in the fourth month of child's life. Kader et al. (1972, cited by Andreas et al., 2015) also estimated that lactose production is time associated, reaching the highest concentration from fourth to seventh month of lactation. Quinn and co-authors (2012) observed moderate inverse association between human milk sugar content and infant's age. However, some studies are indicating that lactose concentration remains constant throughout lactation period (Chang et al., 2015; Mitoulas et al., 2002; Saarela et al., 2005; Thakkar et al., 2013). In addition, our research also marked a tendency that lactose concentration in human milk is not related to child's age (r=0.24, p=0.22).

Nevertheless, more samples are needed to verify the above mentioned findings.

Breastfeeding pattern and lactose content

The World Health Organization recommends that all children should be exclusively breastfed for the first 6 months after birth and nursing should be continued up to 2 years of age and beyond (WHO, 2009). Most mothers were still exclusively breastfeeding during the study (n=17). Only two mothers practised partial breastfeeding (breastfeeding + formula feeding) but nine mothers had started weaning. There is no consensus whether breastfeeding pattern alters lactose concentration in human milk (Dewey et al., 1984; Neville et al., 1986, both cited by Michaelsen et al., 1990; Prosser et al., 1984). Prosser et al. (1984) observed gradual decrease in lactose content commencing weaning. However, our preliminary study did not reveal connection between lactose concentration in human milk and breastfeeding manner (p>0.05).

Milk expression method and lactose content

Mothers were allowed to use the most convenient method for milk expression – breast pump (61%), by hand (21%) or combination of both methods (18%). The use of a breast pump can alter milk composition by evaporating water content (Miller et al., 2013; Morton et al., 2012). However, lactose content remains the same regardless of milk expression method (Morton et al., 2012). Also our study did not observe a change in lactose concentration depending on the milk expression method (p>0.05).

The impact of mother's diet on lactose concentration

Lactose concentration in human milk seems to be fairly insensitive to mother's nutrition (Andreas et al., 2015; Emmett, Rogers, 1997; Quinn et al., 2012). However, Prentice et al. (1983, cited by Emmett, Rogers, 1997) discovered that lactation capacity can be modified by dietary interventions. Lactose concentration decreased but fat content increased after Gambian mothers' diet was replenished with nutritionally balanced supplements. Correspondingly, total energy content of human milk remained the same (Prentice et al., 1983, cited by Emmett Rogers, 1997). Authors (Prentice et al., 1983, cited by Emmett, Rogers, 1997) pointed out that this is probably because lactose is metabolically more parsimonious for mammary glands to produce. Dagnelie et al. (1992) observed that lactose content in milk were lower for omnivorous mothers but higher for mothers consuming macrobiotic diet. The macrobiotic diet consists mainly of organically grown cereals, vegetables and pulses. Small amount of fruits, fermented foods, seeds and nuts, fish are consumed. Meat, dairy products and eggs are avoided (Dagnelie et al., 1992). Although difference in lactose content between groups was significant, energetically it was quite little (~1 kcal per 100 g) (Dagnelie et al., 1992).

Fasting also has no significant effect on the macronutrient composition of the human milk (Rakicioglu et al., 2006) suggesting that milk composition may be buffered against variation of food intake (Quinn et al., 2012).

During our study mothers were asked to complete a food frequency questionnaire. Results revealed that lactose content correlates with fresh (r=0.72) and canned legume (r=0.41), as well as salty snack (chips, roasted nuts) (r=0.45) and soda drinks (r=0.53) consumption (p<0.05). Possible explanations for results should be deeply analysed when more data will be obtained.

Although there is still no clarity how much mother's eating habits impact milk composition (Andreas et al., 2015), there is evidence that diet affects sensory quality of milk and can extend suckling duration (Mennella, Beauchamp, 1990), and also can enhance infant's satisfaction to different flavours starting the weaning (Mennella et al., 2001). Therefore, mothers should be encouraged to consume healthy but variable nutrition during breastfeeding.

Conclusions

Although some researches indicate that lactose content differ with lactation period, breastfeeding pattern or it is influenced by mother's or offspring's characteristics, our preliminary results revealed that lactose concentration in mature milk is stable and insensitive to changes. Nevertheless, more samples need to be analysed to make significant conclusions.

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QUALITY AND FOOD SAFETY MANAGEMENT SYSTEMS APPLIED TO THE LITHUANIAN FOOD SECTOR

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Abstract

Food safety assurance is one of the most important and significant issues in the activity of the food sector. Product certification process according to various standards means the highest quality not only for the company but for the consumer as well. Company's executives are free to choose what a management system should be introduced in the organization. It must be effective and viable. International standards and modern quality management systems have been developed and currently are widely used to accomplish this task. In order to ensure the highest quality requirements in all food supply chain, companies often implement and integrate quality, environmental and food safety management. However, the company's executives faces many difficulties to select which of the management systems is superior and the most appropriate for certain company.

This paper analyses the requirements of the international standards towards the food safety and quality for the establishment of food quality and food safety management systems; similarities and differences between them, to evaluate the application of these systems, the main advantages and disadvantages. Implementation of the quality and safety management systems in Lithuanian food companies was analysed. At least one management system is certified at the 87 Lithuanian food companies. Of these, the management system according to the ISO 9001:2015 standard is certified at 48 companies, according to the ISO 22000:2005 – at 53 companies, and according to the requirements of the British Retail Consortium standard – at 31 companies. Even 45% of the companies have implemented and are certified by two or more mutually integrated management systems.

Keywords: food safety assurance, certification, quality management systems.

Introduction

Development in the international food trade introduced a new range of food safety and quality management standards. The company's decision to apply a certain management system according to various standard requirements affects the chance for the company to remain in the competitive market. The applied system has to be effective and viable. Its application in the company should provide top quality results. One way to achieve such results is system integration, when the company introduces a number of management systems (quality, food safety or environmental protection). When properly integrating separate management systems into the process, costs can be reduced by 30–40 percent when compared with the individual management systems. However, company management often has difficulties in assessing, which management system is superior and in what ways management systems are similar to each other and how they differ (Rezaei et al., 2011).

Many of the new safety concepts and quality parameters of the utmost importance are applied: Hazard Analysis and Critical Control Point (HACCP), total quality management, corporate certification to the international ISO (International Organization for Standardization) standards (Venskutonis, 2006).

As food safety is related to the appearance of potential food safety risk factors, risk management is necessary in every step of food processing. Food safety is assured by joint efforts of every party, participating in food processing. To avoid any risks, food businesses must be subject to good hygienic practices or the HACCP – Hazard Analysis and Critical Control Points system. HACCP is applied in food companies according to the following criteria:

- hazard analysis;
- Critical Control Points (CCP) implementation to manage potential risks;
- setting critical limits for set Critical control points;
- establishing monitoring system for CCP's;
- establishing corrective actions;
- establishing food management system and inspection procedures;
- 'essential program' establishment to ensure the hygiene controls.

LST EN ISO 22000:2005 / AC: 2006 standard describes the requirements for HACCP criteria and additional requirements to ensure food safety (clear tracking system, management of potentially unsafe products and others) (Food safety…, 2006). According to LST EN ISO 22000:2005/AC:2006, BRC (British Retail Consortium) and IFS (International Food Standard) standards, food companies must carry out food safety risk analysis and establish management methods for the elimination of risk factors.

Food retail suppliers are subject to the IFS and BRC standards. The IFS is designed for the German, French and Italian retail trade associations (IFS, 2008) and the global standard for food safety BRC – British Retail Consortium (BRC, 2015). IFS and BRC standards are designed to ensure supplier compliance and secure retailers' ability to guarantee the safety and quality of marketed products. Today it is used worldwide as a business (trade or processing) system, enabling the production of safe food and the selection of reliable suppliers.

World practice shows that nowadays the quality management systems (Quality management…, 2015a; Quality management…, 2015b) are very popular in

organizations, and most often they are introduced and developed, based on the total quality management concept and / or the ISO 9000 series of standards (Markevičiūtė, 2007). Practice of total quality management is rarely applied in Lithuanian companies. Total quality management and efficiency of the companies is widely analysed by Kaunas University of technology professor P. Vanagas (2004). Ruževičius (2006a) described the total quality management and integration of information management model. According to Markevičiūtė (2007), some researchers argue that the ISO 9000 series of standards, requirements and total quality management are incompatible, and the other authors believe that these systems complement each other and upon integration of these systems in a single organization, synergy effect can be achieved.

Organizations whose leadership is more interested in health and safety of their employees implement health and safety systems according to the requirements of international standard BS OHSAS 18001:2007 (Occupational health and safety assessment series is an internationally applied British standard for occupational health and safety management systems). This system applies to professional risks associated with normal operations and management of abnormal situations (Occupational health…, 2009).

Lithuanian and foreign author's scientific works (Melece, Romanova, 2007; Ruževičius et al., 2004; Savov, Kouzmanov, 2009) tend to describe and analyse quality (ISO 9001), environment (according to the ISO 14001 standard) and food safety (in accordance with ISO standard 22000) management systems, applied in food companies without conducting extensive research on these systems. Ruževičius (2006b) summarized an integrated quality and food safety management systems (according to ISO 9001 and ISO 22000 standards) implemented in one food company, stated that the complete analysis of this issue has not yet been done not only in Lithuanian, but also in the world's scientific literature. Therefore, it can be said that research in support of the use of integrated management systems, their synergy and positive changes in food sector has not been conducted yet.

The aim of the paper is to examine the development of management system implementation in Lithuanian food companies.

Materials and Methods

An analysis of differences and similarities between the most commonly implemented management systems was conducted using the data of years 2011, 2014 and 2015 published by the Lithuanian Standardisation Department concerning certified management systems as well as international management systems applicable standards (ISO 9001, ISO 22000, ISO 14001, IFS, BRC and OHSAS). In this article, the data cover all Lithuanian registered food companies, which have at least one management system. Processing of the results applied to the comparative analysis method (Steponavičienė et al., 2011). In addition, management system integration opportunities in Lithuanian food sector were presented.

Results and Discussion

Responsibility for food safety rests with the company, which supplies a product to the market. The importance of successful food safety management increases depending to the company size, production amounts, food product, or it's part risks. Widespread method to achieve this goal is an independent certification according to the quality and food safety standards (ISO 9001, ISO 22000, BRC, IFS, etc.), when a certificate means that food safety assurance level of the company matches the standard.

Comparing 2015 to 2014 and 2011, the data showed that the number of certified food companies is growing every year (Fig. 1). Lithuania, under international management systems standards, in 2011, certified 70 food companies, in 2014 – 77 food companies, in 2015 – 87 food companies.

Out of all certified Lithuanian food companies, most companies (44 – in 2011 and 55 – in 2014) were certified according to ISO 9001:2008 standard and 48 – in 2015 were certified according to ISO 9001:2015 standard (Fig. 1). 26 companies (in 2011), 34 companies (in 2014), and 53 companies (in 2015) had a management system under ISO 22000:2005 standard requirements. Food safety according to the IFS standard was certified in 3 food companies (in 2011), 10 food establishments (in 2014) and 13 food establishments (in 2015), and according to the BRC standard requirements – 1 food establishments (in 2011), 28 food companies (in 2014), 31 food company (in 2015). Occupational health and safety standard (OHSAS) requirements in 2011 were applied in 5 and 2014 and 2015 – 9 Lithuanian food companies. The evaluation of the results over the years 2011–2015 show a significant trend of more and more management systems set according to the specific food sector created standards such as ISO 22000:2005, BRC and IFS.

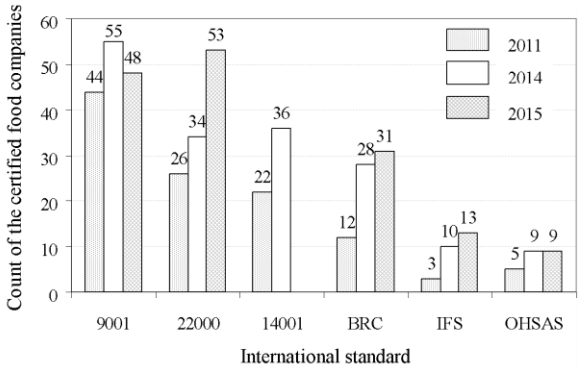

Figure 1. Implemented management systems at the Lithuanian food companies according to the international standards

Implementation and certification of each individual management system in a food company means additional expenses, therefore there is an opportunity to

integrate separate management systems into each other. For example: food companies commonly imply quality and food safety management system integration in accordance to ISO 9001:2015 and ISO 22000:2005/AC:2006 standard requirements.

Implementation and certification of each individual management system (quality, food safety) in company requires additional resources, so it is possible for separate systems to be integrated into each other, e.g., food companies often used in quality and food safety management system integration according to international standards (ISO 9001:2015 and ISO 22000:2005/AC:2006) requirements.

Differences and similarities between management systems (in accordance with international standards ISO 9001:2015, ISO 22000:2005, BRC and IFS requirements) used in quality and food safety assurance are shown in Table 1.

Table 1

Differences and similarities between quality and food safety management systems

Management system	General requirements	Specific requirements
Quality management system (according to the ISO 9001:2015)	Policy	Planning of product sale
	Resource control	Projection
	Documentation control	Risk management
	Nonconformity control	Planning of safe product and its realization
Food safety management system (according to the ISO 22000:2005), BRC, IFS	Planning	HACCP establishment for possible risk control
	Resource management	Establishment of CCP monitoring and management systems
	Internal audits;	
	Continuous improvement	

Out of all certified Lithuanian food companies, 39 companies (in 2011), 48 companies (in 2015) had implemented at least one management system in accordance with international standards, 23 companies (in 2011), 25 companies (in 2015) had implemented two integrated management systems in accordance with international standards (Fig. 2 and 3). A smaller number of companies had implemented three integrated management systems in accordance with international standards – 5 (in 2011), 10 companies (in 2015), while four integrated management systems in accordance with international standards were applied in 3 (in 2011) and in 4 companies (in 2015).

In most cases companies implement integrated management systems certified according to ISO 9001:2015 and the ISO 22000:2005, – 16 companies (in 2015) a little less companies – according to the BRC and the ISO 22000:2005 – 3 companies or by BRC and IFS – 4 companies. 10 companies had integrated 3 management systems. Most often integrated systems are: ISO 22000:2005, ISO 9001:2015, OHSAS and BRC. In conclusion, it can be said that in order to save resources, companies more and more often integrate different management systems taking their similarities into consideration.

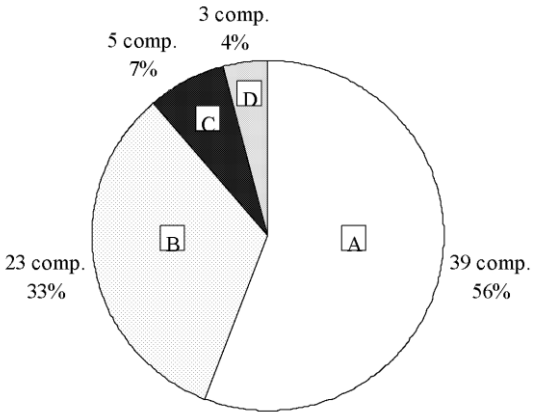

Figure 2. Integration of management systems at the Lithuanian companies in 2011

A – companies with one certified management system; B – with two certified management systems; C – with three certified management systems; D – with four certified management systems

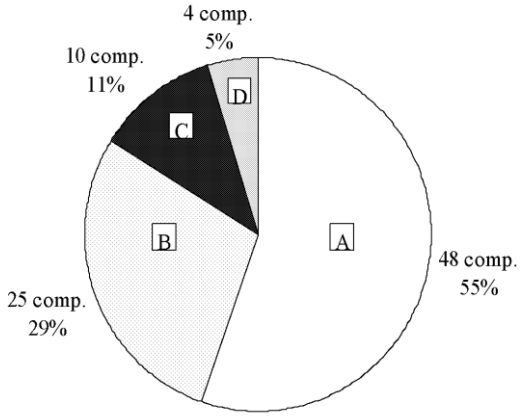

Figure 3. Integration of management systems at the Lithuanian companies in 2015

A – companies with one certified management system; B – with two certified management systems; C – with three certified management systems; D – with four and more certified management systems

Organizations that have a management system implemented in accordance to the standard requirements are considered as reputable and trustworthy partners, who do not aim for short-term goals, but seek long-term goals and quality. After implementing management system, employees clearly understand company policies, goals, their duties and responsibilities. Management systems make the clients put more trust in the company (Vanagas, 2004). Implementation of every separate management system costs resources; therefore it is recommended to integrate different management systems (Ruževičius, 2006b). As opposed to quality

management, environment protection, work safety and healthcare companies, food safety management systems (BRC; IFS; ISO 22000) are implemented only in food companies. Lithuanian food companies successfully apply not only food safety management systems, but environment protection, work safety and healthcare systems.

Conclusions

The company's decision to apply a certain management system according to various standard requirements affects the chance for the company to remain in the competitive market. To achieve the best results, while reducing costs and non-compliances, a constant development of management systems is required.

Comparison of data from 2011, 2014 and 2015, it was concluded that the number of certified food companies is growing. Most Lithuanian food companies are certified in accordance to ISO 22000:2005, second place – 48 in accordance to ISO 9001:2015 quality management standard, third place – 31 in accordance to global standard for food safety (BRC).

In 2015 out of 87 food companies in Lithuania 48 companies were certified according to at least one management system, 25 companies were certified with at least two integrated management systems, 14 companies were certified with three or more integrated management systems.

Most commonly integrated management systems are certified in accordance to ISO 9001:2015, ISO 22000:2005, occupational health and safety standard (OHSAS) or in accordance to BRC.

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BREAD CHOICE AND CONSUMPTION TRENDS

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Abstract

The consumption of bread per capita has been declining in Latvia for several decades. What could change the consumption behaviour of residents to increase the consumption of bread? The research aim is to identify trends in the choice and consumption of bread by consumers in Latvia. To achieve the aim, two specific research tasks were set: to identify the trends in the consumption of bread and to ascertain the opinion of Latvia's residents on determining factors in their choice of bread. An Internet survey of Latvia's residents on bread quality and consumer choice was conducted in November 2016. The survey involved 919 respondents aged 15-74. The main results showed that the consumption of bread declined, yet a stabilisation trend emerged. The reasons for the consumer choice, which were related to the quality and price of bread as well as the confidence and behaviour of consumers, were diverse for different kinds of bread. In choosing wheat bread, the determinant factor was price, while the choice of rye bread was determined by previous experience, i.e. the producer of the bread consumed. Consumers believed that an increase in bread consumption could be achieved by producing tastier breads.

Keywords: bread choice, consumption, Latvia.

Introduction

Bread production is not constant – it is variable and persistently adapts to consumer wishes, which are not always understandable and – what is even more important – are difficult to predict (Bread …, 2013; World …, 2009). The consumption of bread and bakery products steadily increases in the world (Market …, 2008; AIBI, 2015). This is mainly associated with the growing populations. At the same time, a decrease in bread consumption is observed in developed countries. In the last 20 years, the consumption of bakery products persistently decreased in Latvia as well (Eglite, Kunkulberga, 2015; Partikas …, 2016). In the world, forecasts of bread consumption are based on the average consumption in an average developed country, which totals approximately 70 kg of bread per capita per year, and on population increase forecasts. The number of countries where the production, distribution and prices of bakery products are regulated by the government and most of the quantity of bread is produced by order of the central and local governments, often for distribution among the poor, increases (Competition …, 2009; Bread …, 2013; Eglite, Kantike, 2012; German …, 2009; Innovation …, 2009).

The per capita consumption of bread is very diverse across European countries. After AIBI (The International Association of Plant Bakers) information the highest consumption of bread per capital is reported in Turkey (104 kg) and Bulgaria (95 kg), while the lowest one is in Great Britain (approximately 32 kg). European residents consume on average 59 kg of bread per year and this level was stable in recent years (AIBI Bread Market..., 2015) (Table 1). Although the bread consumption in general is stable, it is important to analyse consumer preferences for bread in order to increase bread consumption by launching successful targeted bread promotions initiatives at national level.

Table 1

Bread consumption per capita per year in European countries (2013)

Country	Consumption, kg	Trend with the previous year
Belgium	55.0	stable
Bulgaria	95.0	stable
Denmark	45.0	stable
Finland	42.0	stable
France	57.0	stable
Germany	56.0	stable
Greece	68.0	stable
Italy	52.0	stable
Netherlands	62.0	-1.0%
Russia	55.0	+2.0%
Slovenia	42.0	stable
Spain	37.0	+4.2%
Turkey	104.0	-10.0%
Ukraine	89.0	-7.5%
Great Britain	32.0	-1.0%
Average	**59.4**	

Source: construction based on AIBI Bread Market ..., 2015

The research aim is to identify trends in the choice and consumption of bread by consumers in Latvia. To achieve the aim, two specific research tasks were set: to identify the trends in the consumption of bread and to ascertain the opinions of Latvia's residents on determining factors in their choice of bread.

Materials and Methods

The present research was performed by applying the quantitative approach to statistical data analyses and the survey of residents. The sociological survey was done owing to the responsiveness of residents, who were not reluctant to fill in a questionnaire on their choice of bread and consumption habits, in the Internet environment. The survey involved 919 respondents aged 15–74. Most of the respondents represented

multi-person households, and only 7% of them represented single-person households. The respondents were selected employing the method of multistage stratified random sampling. This sampling method ensures a representative sample from the general population. The maximum statistical error for a sample of 919 respondents was ±5%. The survey was conducted in November 2016; the respondents were aged 15–74. The survey data were processed by statistical analysis methods.

Results and Discussion

In the last two decades in Latvia, the consumption of bread decreased not only owing to the declining population but also, and mainly, because of a decrease in the per capital bread consumption (Eglite, Kantike, 2012; Partikas …, 2016). The per capital consumption of rye bread decreased the most over the last 20 years (Figure 1). A positive fact is that the decrease cannot be precisely described by a linear function, as some stabilisation is observed; the function is as follows:

$$y = 0.0779x^2 - 2.9262x + 42.932 \quad (1)$$

$$R^2 = 0.9938, \text{ where}$$

x – year number;

y – rye bread consumption, kg/capita per year;

R^2 – determination coefficient, which indicates the proportion of the variance in the dependent variable that is predictable from the independent variable.

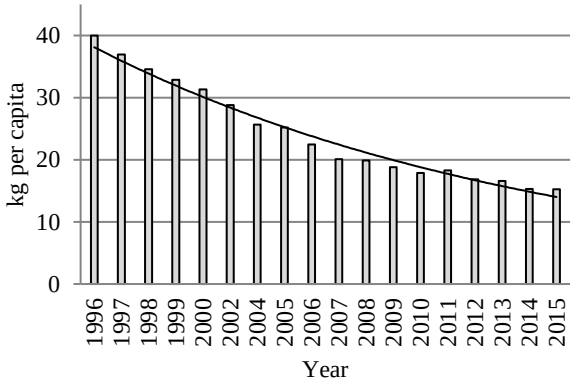

Figure 1. Consumption of rye bread in Latvia in 1996-2015, kg per capita

In the last two decades, the decrease in the per capita consumption of rye bread – from 39.96 kg in 1996 to 15.25 kg in 2015 – was the largest, i.e. at 62%.

A decrease in the consumption of wheat bread was almost linear in Latvia in the last 20 years; however, the decrease could be more precisely described by a polynomial function.

The per capita consumption of wheat bread decreased from 32.76 kg in 1996 to 15.71 kg in 2015, i.e. by 52% over the last 20 years (Figure 2).

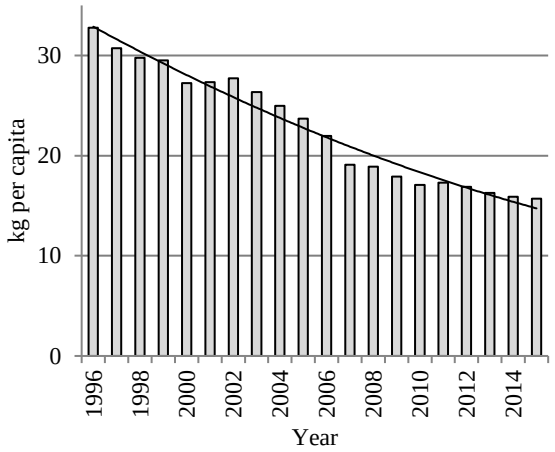

Figure 2. Consumption of wheat bread in Latvia in 1996–2015, kg per capita

The decrease in the consumption of bread could be explained by a number of factors that are described in the scientific literature by a lot of research studies conducted in developed countries (Langhauser, 2009; World …, 2009; Market Trends…, 2008). The pressure of other alternatives available for breakfast and food consumption outside the home are considered to be global trends that result in the decrease in bread consumption. A global demographic change is an increase in the number of single-person households, which are also ageing. The number of mobile and flexible employees whose modern lifestyle promotes the understanding of foreign cultures and culinary diversity increases as well (AIBI, 2015; Bread and Bakery …, 2015). It is believed that a cyclical lack of dynamic growth in developed countries make household incomes stagnate and lead to an inert demand for bread and bakery products. In the last decade, it was associated with changes in shopping behaviour that took the forms of a growing demand for bakery products with value added (snacks, convenience food) and food consumed outside the home as well as one-stop shopping at a supermarket due to the lack of leisure time; a greater diversity and making a particular decision began playing greater roles when shopping, and the demand for organic food and internationally recognisable food products, at the expense of traditional ones, increased as well (Innovation and Market …, 2009; Culinard, 2009).

A sociological survey of Latvia's residents on the choice and consumption of bread was conducted to identify the global trends that influenced Latvia too. In our survey of the respondents, 60% earned a monthly income ranging from EUR 200 to 600 per household member. During the last year, incomes did not change for 58%, decreased for 17% and increased for 25% of the respondents. Of them, 81% were aged 25–54, 6% were under 24 and 13% were over 55 years of age. Of the respondents, 83% were women and 17% were men. On the one hand, this may be regarded as a research imperfection, while on the other hand it allows more objectively assessing shopping habits, as traditionally women take care about food at home and they usually

determine the menu, trends in food consumption and conviction about the right foods for their family.

To better understand prejudices and stereotypes that were present in Latvia's society in respect to the nutritional value of bread, the survey included a number of assertions, like "I choose a bread that I (my family members) like', "Rye bread is better suited for those doing physical work" and others.

The survey revealed that 97% of the respondents preferred the bread they or their family members liked. Of them, 72% believed that bread was an essential source of carbohydrates in their nutrition. However, for lunch, 16% would replace potatoes and sauce with bread, and 60% believed that hot lunch could not be replaced with bread and butter (Figure 3).

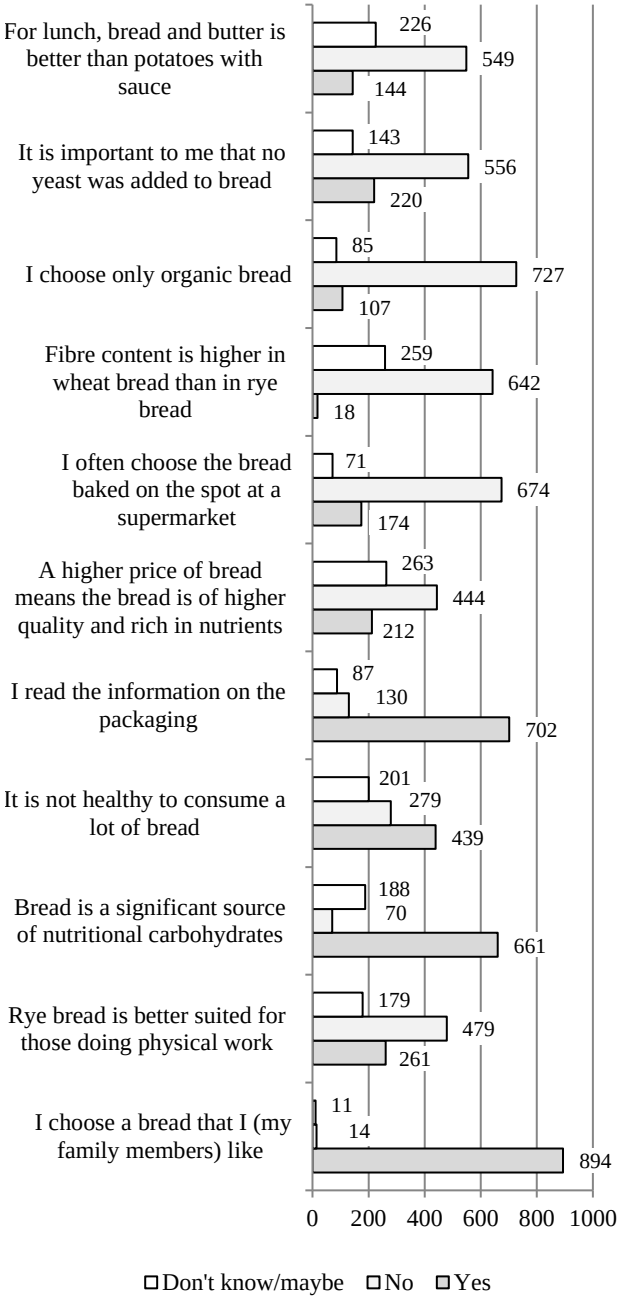

Figure 3. Distribution of replies to the question: „Do you agree with the assertion that: ...?"

Almost half of the respondents (48%) were convinced that it was not healthy to consume a lot of bread. However, compared with a 2009 study, an opinion that rye bread is better suited for that doing physical work has changed, as most of the respondents (52%) believed that rye bread had no association with physical activities. The residents' knowledge about bread improved and two thirds knew bread components (Eglite, Kantike, 2012). One more positive trend was observed with regard to this fact. Compared with earlier surveys at 2009 (Eglite, 2012), 76% of the respondents were interested in information available on the packaging of bread, only 14% were not interested in it, while the others were indifferent to it. It was important to almost a fourth (24%) that no yeast was added to their bread. Of the respondents, 23% thought that a higher bread price meant the bread was of higher quality and richer in nutrients.

A question was asked regarding any change in the quality of bread in comparison with the previous year. Most of the respondents believed that there was no change. Most complaints about a decrease in the quality of wheat bread by the respondents related to wheat bread, while the quality of rye bread, and mainly whole grain bread, increased, according to them. It is worth mentioning that the same results were acquired by another survey conducted two years ago (Eglite, Kunkulberga, 2015). One can note that the consumption of rye and wheat breads still declines, while that of whole grain and sweet-and-sour breads is not captured by statistics.

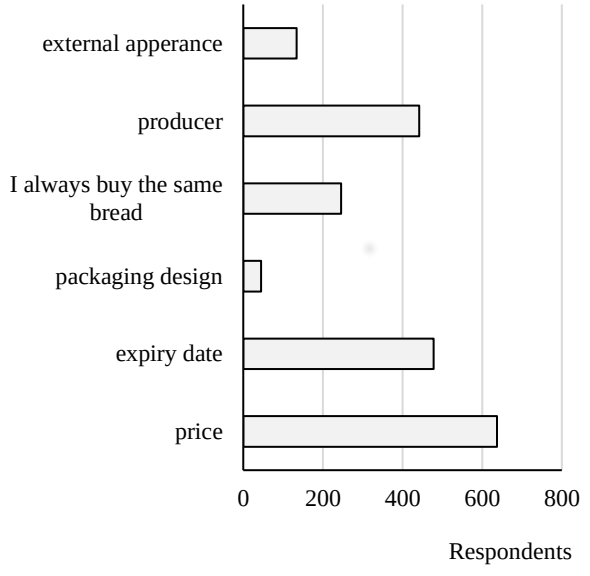

Figure 4. Factors for the choice of wheat bread

The respondents were asked to rate three most important factors, based on which they chose every kind of bread. In choosing wheat bread, price was the most important factor, followed by the bread expiry date and the producer brand. Packaging design and external appearance were the least important factors (Figure 4). In Latvia, wheat bread is bread supplied in the broadest assortment and has relatively similar taste

characteristics. In choosing rye bread, the producer was the most important factor for the consumers (Figure 5). For 46% of the respondents, the producer of rye bread was the most important factor, followed by price and the expiry date. About a fifth of the consumers bought the same kind of bread. Only 7% of them paid attention to the external appearance of bread.

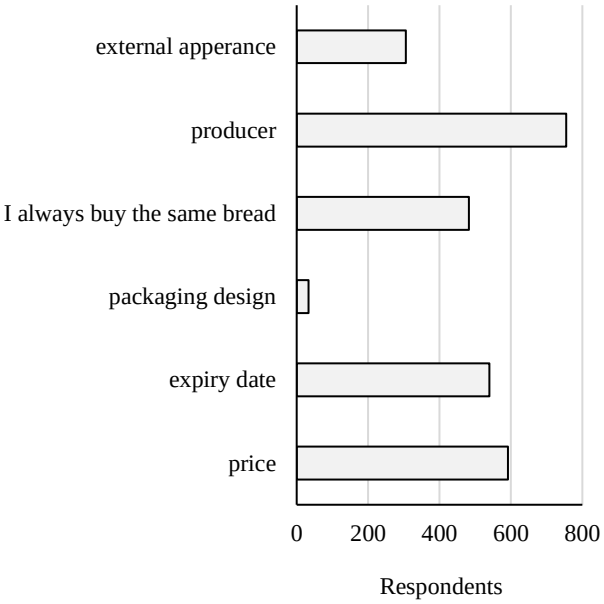

Figure 5. Factors for the choice of rye bread

The respondents were requested to choose the prerequisites under which they would consume more bread. The assertions were like follows: "The price of bread is lower", "The bread is tastier", "I am informed that this bread is healthy" and similar.

With regard to the kinds of bread, the respondents expressed explicit opinions on whole grain bread and rye bread, while their opinions on sweet-and-sour bread and wheat bread were uncertain and implicit.

The most important prerequisite for consuming more bread for all the kinds of bread (rye, whole grain and sweet-and-sour), except wheat bread, was a tastier bread. They would consume more wheat bread if being informed it is healthy.

Information about whether wheat bread is healthy and a bread packet of an adequate size would be important prerequisites for increasing the consumption of the wheat bread. Sweet-and-sour bread would be consumed more if consumers were informed about its healthiness, its price was lower and bread packets of an adequate size were available. To increase the consumption of whole grain bread, the following prerequisites were important: information about its healthiness, a lower price, a broader assortment and longer expiry dates. For rye bread, its taste was the most important factor, and a packet of an adequate size was ranked second, which were followed by a lower price, information about the bread's healthiness and the consumer's physical activity.

Overall, one can conclude that the most important factors for increasing the consumption of bread were the taste of bread, followed by personal factors difficult to explain, while the least important factors were the external appearance of the bread and its expiry dates. There is a paradox – consumers wish longer expiry dates, but when choosing particular criteria, this factor is ranked in last but one position.

Studies on the market of bakery products in Europe (Innovation and Market..., 2009) explicitly show that in view of an increase in the number of older individuals (aged over 65) in Europe, such services as delivery, custom-made orders and consumer personalisation along with the choice of bakery products would be crucial for this consumer group; besides, not only the quality of the product but also its association with the particular site, bakery and local brand would be important.

Since the population's health problems get worse and there is an increased need for dietary bakery products made of rough flour, a broad market niche is available in the market. We concluded that the purchasing power of consumers rises after an economic crisis, and bread is going to be one of the products the consumers are ready to pay more, especially if the bread is made according to an original or ancient recipe or technology.

New niches for bread and bakery products are sought for and found in Europe to meet the wishes of consumers. One can clearly notice a "boom" in sales of frozen bakery products, which has undergone consumer testing. The following trends have emerged in bread production: more pleasure from eating bread as well as healthy, fresh and ready to eat bread. Market researchers predict a lot of changes for bread and confectionery products in the third millennium (Huber, 2000). European consumers increasingly wish to enjoy traditional bakery products of various countries. "Exotic" kinds of bread from foreign countries are more and more often seen on consumer tables along with regional kinds of bread. It is seen not only at international exhibitions and conferences but also in all European cities that an "ethnic food wave" spreads across the entire Europe.

Conclusions

In recent years, bread consumption in Latvia has stabilized and has not been reduced.

Study showed that in choosing wheat bread, the determinant factor was price, while the choice of rye bread was determined by previous experience, i.e. the producer of the bread consumed.

Consumers believed that an increase in bread consumption could be achieved by producing tastier breads.

It was concluded that bread producers, faced by the decreasing market demand for bread, will have to work on maintaining and enhancing the quality of the bread as well as developing diverse alternative products, thereby increasing competition.

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LATVIAN CONSUMERS EATING MOTIVATIONS

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Abstract

The society's dietary habits make an important impact on overall health status, the food that is consumed daily can be one of factors that prevent or, on the contrary, increase health risks. Eating habits, food choices, quantity and frequency of mealtimes are dependent on many variables from physiological to emotional needs. The aim of this research was to find out the factors that influence food choice of Latvian consumers.

The questionnaire was used as a basic tool for studies of Latvian consumers eating motivation, which was completed by 245 respondents (75.1% female and 24.9% male) from different Latvia regions. The survey included ten parts – demographical information, anthropometric data and behavioural and health related elements, sources of information about healthy eating, factors related to food choices according to motivations (health aspect, emotional, economic, availability, social, cultural, environmental, political, marketing and commercials). The results of questionnaire show, that the 48% of respondents have motivation to eat healthy; Latvian consumers haven't united viewpoint regarding emotional motivations for eating – 33% agrees with this motivation form. Frequently physical exercises positively correlate with knowledge about healthy food, healthy motivations and also emotional motivations. More than half of respondents completely agree that food makes them feel good.

Keywords: eating motivation, survey, food choice

Introduction

It is well established that there are close interactions between eating habits and person's overall health condition. There have been studied numerous factors that predict unhealthy eating habits, however equally important is to understand aspects that promote healthy eating habits (Swan et al., 2015). To find out what impacts person's eating habits, there are proposed several scales and factors, e.g., four category model of motivations to eat – coping, social, compliance and pleasure (Jackson et al., 2003). In the different eating motivation survey results indicate 15 main factors that explains eating motivations – liking, habits, need and hunger, health, convenience, pleasure, traditional eating, natural concerns, sociability, price, visual appeal, weight control, affect regulation, social norms and social images (Renner et al., 2012), health and nutritional value, sensory appeal, familiarity and convenience, feeling good and safety, and natural content (Ares, Gambaro, 2007), set of individual level factors, social-environmental and physical-environmental factors (Swan et al., 2015), food liking, emotional coping, self-control with food, costs and health issues may influence food choice (Dressler, Smith, 2013), taste, economy, convenience, health and variety are also widely described factors that determines food choices (Finkelstein et al., 2004), consumer individual attitudes, perceptions, usage of available resources should be also considered (Urala, Lähteenmäki, 2006). Through analysing all these factors, it is possible to find out causes of healthy and unhealthy eating, which is important to promote healthy eating of public, reduce obesity rates, encourage to consume healthier foods (Finkelstein et al., 2004), decrease rates of diseases like high blood pressure, diabetes, heart diseases (Miller, Cassady, 2012).

Eating is human physiological requirement to provide nutrients for adequate functioning of the human body and its growth. Beyond physiological demands for nutrition, psychological motivations also play important role for both healthy and disordered eating (Jackson et al., 2003). Due to increasing number of cases with eating disorders and obesity, there has been studied the role of emotions for development of these problems (Canetti et al., 2002). Emotions, state of mind and food choices can obviously interact with each other, where mood can affect food choice through physiological reactions that change desire for food or, on the contrary, food choice could make a change of mood (Gibson, 2006, Köster, Mojet, 2015). So, it works both ways – mood and emotions can alter food choice and food choice can change state of mind (Gibson, 2006). One of typical emotional states which makes a great impact on altering eating patterns is stress and stressful situations. Many studies are performed regarding stress induced eating changes but the results are contrary. Several studies indicate that restrained eaters under stress increase their amount of food intake more than non-restrained eaters (Zellner et al., 2006, Canetti et al., 2002), and that there are definitely changes in eating under stress, for emotional eaters it is overconsumption but for non-emotional eaters – both under and overconsumption in similar number of cases (Wallis, Hetherington, 2008).

As stated above, there are many psychological factors that make an important impact on consumers' food choice, these factors are possible to combine together and also analyse them separately. Some of aspects can promote eating disorders, weight gain and obesity that usually lead to different diseases and health problems, but some of them can boost healthy eating habits. As every person is individual, researching this topic is complex and must consist of different factors that interact with each other.

As previously declared, not only the emotions make the impact on food choices, social, cultural (Pliner, Mann, 2004), economic, environmental (Popkin et al., 2005), political, marketing and

commercials issues also have essential meaning in defining person's eating habits. It is equally important to evaluate both healthy and unhealthy eating habits, in order to identify causes that promotes one and reduces the other (Williams et al., 2012). After summarising this kind of information it is possible to create recommendations for popularizing healthy nutrition. Also person's daily routine and eating routine should be considered in evaluation of eating habits (Jastran et al., 2009), although it is more helpful for individual improvement of healthy eating practises.

In order to change society's eating habits to healthier tendencies, it is critical to understand what factors influence food choices. The aim of this research was to find out the factors that influence food choice of Latvian consumers.

Materials and Methods

Design of questionnaire

Basic tool of study was questionnaire, which aimed to collect information about Latvian consumers eating motivations. The questionnaire was structured into different sections in order to gather information about respondents' lifestyle, knowledge about healthy eating, healthy and eating motivations.

The demographic information like age, gender, level of education, living environment and behavioural and health related elements like information about physical exercises, dietary regimes were addressed in the beginning of the questionnaire.

The questionnaire consisted from 29 questions (Table 1), which aimed to evaluate knowledge on three distinct areas: 10 questions for knowledge about healthy food, 9 questions for healthy motivation and 10 questions about emotional motivations.

Table 1

Design of questionnaire

Item	Complete question
S1	**Knowledge about healthy food**
S1.1.	A healthy diet is based on calorie count
S1.2.	We should never consume sugary products
S1.3.	Fruit and vegetables are key to being healthy
S1.4.	A healthy diet should be balanced, varied and complete
S1.5.	We can eat everything, as long as it is in small quantities
S1.6.	I believe that a healthy diet is not cheap
S1.7.	In my opinion it is strange that some people have cravings for sweets
S1.8.	I believe that food tastes better when we have company
S1.9.	I believe that tradition is very important to a healthy diet
S1.10.	In my opinion, is very important to eat food that contributes for the preservation of the environment
S2	**Healthy motivations**
S2.1.	I am very particular about the healthiness of food I eat
S2.2.	It is important for me that my diet is low in fat
S2.3.	I always follow a healthy and balanced diet
S2.4.	It is important for me that my daily diet contains a lot of vitamins and minerals
S2.5.	I do not avoid foods, even if they may raise my cholesterol
S2.6.	I try to eat foods that do not contain additives
S2.7.	I do not eat processed foods, because I do not know what they contain
S2.8.	It is important for me to eat food that keeps me healthy
S2.9.	I do not avoid foods, even if they may raise my blood glycaemia
S3	**Emotional motivations**
S3.1.	Food helps me cope with stress
S3.2.	I usually eat food that helps me control my weight
S3.3.	I prefer food that keeps me awake and alert
S3.4.	It is important for me to eat food that helps me relax
S3.5.	Food can make me feel good
S3.6.	When I feel lonely, I console myself by eating
S3.7.	Food helps me to cope with life
S3.8.	I eat more when I have nothing to do
S3.9.	It is important to eat less than usual when I gain weight
S3.10.	I often have cravings for sweets when I am depressed

They are all presented in ordinal Likert scale, where respondents could measure each statement with their opinion – 1 – strongly disagree; 2 – disagree; 3 – neither agree nor disagree; 4 – agree and 5 – strongly agree, and additional option "no opinion".

Selection of respondents

The study was conducted with 245 respondents. The participation in the questionnaire was voluntary and the questionnaire was distributed using internet network (www.visidati.lv). The respondents were selected by convenience, although attempting to reach different parts of society, in terms of age, gender, education, living environment, civil state, professional activities.

Statistical analysis

The software SPSS, from IBM Inc. (version 24), was used for all data analysis. To validate questionnaire's results there was used Cronbach's alpha, the closer Cronbach's alpha coefficient is to 1.0 the greater the internal consistency of the items in the scale, general view for interpreting Cronbach's alpha is if $\alpha \geq 0.9$, then result is excellent, if $0.9 > \alpha \geq 0.8$, then – good, if $0.8 > \alpha \geq 0.7$, then – acceptable, of $0.7 > \alpha \geq 0.6$, then – questionable, if $0.6 > \alpha \geq 0.5$, then – poor, and if $0.5 > \alpha$, then – unacceptable. All obtained data were statistically processed using several descriptive statistics tools, there was determined significance of the test (p-value), where level of significance was 0.05.

There were calculated mean, median for each subscale of questionnaire and, as subscales had normal

distribution, there were also calculated Pearson correlation coefficients between different subscales.

Results and Discussion

The total number of respondents was 245, from which 75.1% were female and 24.9% – male (Table 2), aged between 18 and 77 years, with an average age of 39.4 years.

Table 2

Characterisation of respondents

Variable	Number of respondents	Percentage, %
Gender		
female	183	75.1
male	61	24.9
Living environment		
rural	50	20.4
urban	195	79.6
Education		
primary school	2	0.8
secondary school	33	13.5
university	210	85.7
Practice any specific voluntary dietary regime		
raw foodism	2	0.8
fruitarianism	–	–
vegetarianism	7	2.8
veganism	1	0.4
flexitarianism	7	2.8
caloric restriction / weight control	25	10.2
religious restrictions	5	2.0
general food regime	198	80.9
Physical exercise		
never	20	8.15
sporadically (less than 1 time a week)	64	26.1
occasionally (1 time a week)	84	34.3
moderately (2–3 times a week)	57	23.3
intensively (more than 3 times a week)	20	8.15

More than half of all respondents (80%) are in active working age (between 18 to 50 years), 18.0% – from 51 to 65 years, and 2.0% over 65 years. Most of respondents lived in an urban environment (79.6%), while 20.4% lived in rural areas (Table 2). More than 80.0% of respondents have general food regime, but 19.1% practice some specific dietary regime (caloric restriction / weight control, vegetarianism, flexitarianism, religious restrictions and raw foodism). 65.7% of respondents at least once a week deals with the physical exercises (of which 8.1% more than three times a week), whereas for a balanced diet daily takes care 8.5% of respondents and 23.2% for a balanced diet think never, or very rarely.

There were calculated Cronbach's alpha for each subscale in order to check reliability of questionnaire. For subscale knowledge about healthy food it was 0.692, for subscale healthy motivations – 0.770 and for subscale emotional motivations – 0.817, but global Cronbach's alpha for these 3 subscales with 29 questions was 0.863. It means that questionnaire results are very good in terms of reliability, as 0.8–0.9 suggests high internal consistency.

The data shows that large part of respondents isn't concerned with balanced diet, but regularly makes physical exercises. The studies show that physical exercises alone aren't so effective, as combined with healthy diet for weight loss outcomes (Stephens et al., 2014). Also balanced diet and regular physical activities provides lasting, long-term success (Keller, Hartmann, 2016). Analysing total data for all three subscales (Figure 1), 44% of respondents strongly agree and agree with statements regarding knowledge about healthy foods, 48% agrees with healthy motivations statements and 34% of respondents accepts emotional motivations statements.

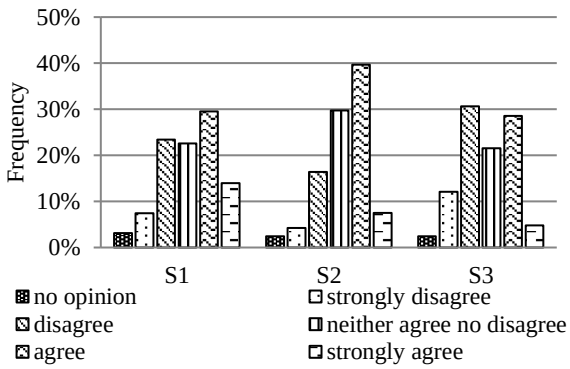

Figure 1. Level of knowledge about healthy diet, healthy and emotional motivations

The lowest agreement level is for the emotional subscale, other two scales positively reach almost half of all respondents.

If compare subscales between each other, it can be concluded that all have significant positive correlations (Table 3).

Table 3

Pearson correlation for the subscales

Item	S1	S2	S3
S1	1.000	0.486*	0.475*
S2	0.486*	1.000	0.365*
S3	0.475*	0.365*	1.000

* Correlation is significant at the 0.01 level (2-tailed)

Moderate, in this case more remarkable, positive correlations (0.40>r>0.59) are between knowledge about healthy food and healthy motivations and emotional motivations. It means that if increases level of knowledge, then proportionally should increase levels of healthy and emotional motivations.

There was calculated correlation between subscales and other variables like gender and physical exercises of respondent.

There is significant, although relatively small, positive correlations between gender and healthy motivations. That is completely acceptable as females tend to be more demanding to healthiness of food. Physical exercises

positively correlate with knowledge about healthy food, healthy motivations and also emotional motivations.
Questionnaire shows that 44% of respondents are well informed about what is healthy diet and what it should contain (Figure 2). And accordingly to that 48% of respondents have marked that they agree to healthy motivations.

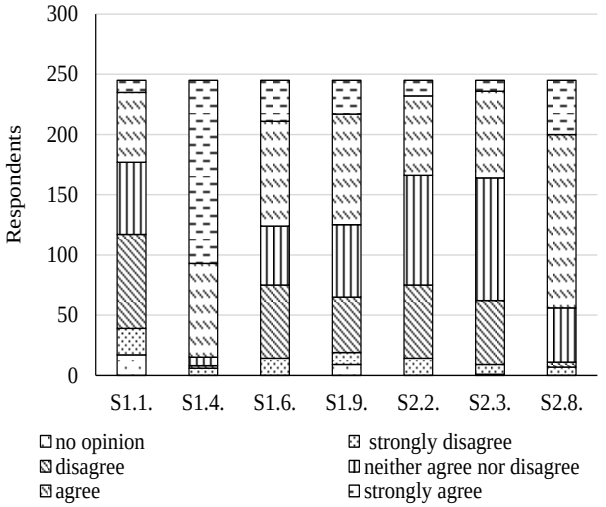

Figure 2. Level of Latvian knowledge and healthy eating motivations

Respondents who agree with the statement that calorie count is important, also agree that it is relevant that their diet is low in fat, questions about balanced diet (S1.4. and S2.3.) doesn't have similar proportion, almost all respondents have knowledge that healthy diet should be balanced, varied and complete, but only less than a half of them agrees that they follow healthy diet.

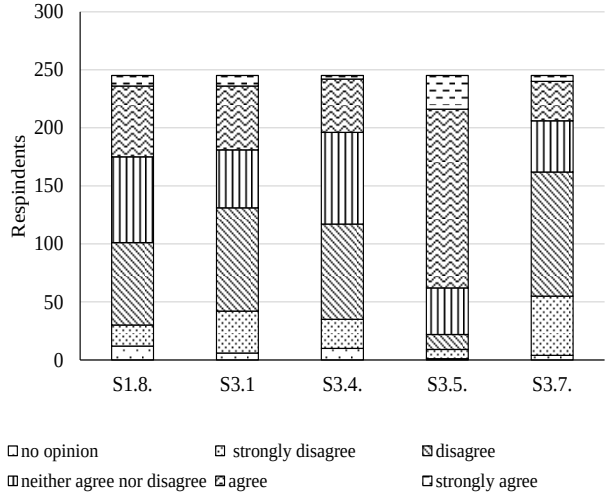

Figure 3. Level of knowledge and emotional motivations

Studies regarding this subject are contrary to questionnaire's results – it is establishes that knowledge about nutrition combined with socio-economic status improves diet quality (Beydoun, Wang, 2007) and it even significantly correlates with higher adherence to some diets (Bonaccio et al., 2013).

More than half of respondents completely agree that food makes them feel good (S.3.5.), smaller proportion, about 25% of respondents, is for similar statements that food helps cope with stress, helps relax and cope with life (Figure 3).
In the research obtained results corroborate with opinions of Torres and Nowson (2007), that in acute stress situations, there may be expected reduced food intake in short term, but in chronic stress situations more likely food intake will increase due to cortisol raise, and it can tempt person to consume hedonic, energy-dense foods.
Largest part of respondents disagrees that they should never consume sugary products and they also disagree opinion that is strange to have cravings for sweets. Similar opinion is also about question that there often are cravings for sweets when depressed, although here agrees only around half of respondents (Figure 4).

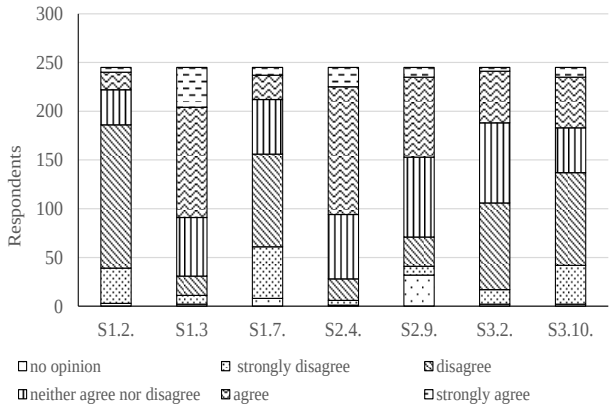

Figure 4. Level of knowledge, healthy and emotional motivations

Fruits and vegetables are highly recommended to consume in order to keep up healthy weight and decrease chronic disease risks, and social marketing campaigns, mass media and policy changes could help to improve community's consumption (Chapman et al., 2016). More than half of respondents have stated that fruits and vegetables are key to be healthy and they also agree with the statement that it is important that daily diet contains vitamins and minerals. The results of Díaz-Garcés et al. (2016) research show that persons with higher education level tend to increase amount of fruits and vegetables.
Obtained results (Table 5; S1.5.) indicate that 61.3% Latvian respondents agree, that eat everything, as long as it is in small quantities, but 22.8% – neither agree nor disagree with this statement. For 46.3% respondents it is very important, that food what they eat is healthy. Regarding question S2.6., 58.1% respondents (agree and strongly agree) try to eat food that does not contains additives.
Shim et al. (2011) present that consumers are concerned about artificial sweeteners, colorants and preservatives, and usually they lack information or it is insufficient about food additives, but the results of this questionnaire show that Latvian consumers have strong opinions regarding food additives and try to avoid them.

Table 5

Statistical regarding the relationship between knowledge about healthy eating, healthy and emotional motivations

Item	Respondents answers, %						Mean	Std. Deviation
	no opinion (0)	strongly disagree (1)	disagree (2)	neither agree nor disagree (3)	agree (4)	strongly agree (5)		
S1.5.	1.6	2.0	12.2	22.8	46.8	14.6	3.55	1.053
S2.1.	1.6	4.9	13.0	34.1	39.5	6.9	3.25	1.041
S2.6.	0.7	4.9	13.0	23.2	44.4	13.8	3.46	1.084
S2.7.	2.4	7.7	39.0	28.9	18.3	3.7	2.63	1.058
S3.3.	4.9	9.3	28.9	26.8	28.1	2.0	2.69	1.174
S3.9.	2.0	3.7	17.5	15.0	47.2	14.6	3.46	1.168

Fifth part of respondents, when purchasing food, does not pay attention if there are added food additives in the product production process. Considering the responses "neither agree nor disagree" (Table 5; S3.3.), the 26.8% respondents are not sure, that the reason for product choice is to keep them awake and provide alert.

Conclusions

44% of respondents are well informed about what healthy diet is and what it should contain, and 48% – have marked that they agree to healthy motivations.

More than half of respondents completely agree that food makes them feel good, but overall agreement of emotional motivations reach only 34% of respondents.

Physical exercises positively correlate with knowledge about healthy food, healthy motivations and also emotional motivations.

Gender has significant, but weak, correlation with healthy motivations.

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THE SIGNIFICANCE OF LOCAL FOOD IN THE CONSUMPTION OF MODERN CONSUMER

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Abstract

With time changing, consumer diet changes as well. Food industry trends show that people increasingly choose the local food. This study aims to explore the local food choices and use by modern consumers. The researchers have conducted the survey to find out the peoples' understanding of different types of food, such as functional foods, novel foods, ecological, etc. The aim of the survey was to explore how often the modern consumers choose the local food and what is their attitude to it. The survey was organized in the beginning of 2017, and more than five hundred respondents were surveyed. The survey data indicate that the consumers when buying food pay attention to food quality, expiry date, and price. The respondents regularly buy food from the local domestic food producers. Consumer behaviour is affected by the economic development and education level in the country. If education level increases, demand for the quality food – in this case the local food – will increase as well.

Keywords: local food, local food producer, consumer behaviour, food logistic.

Introduction

Institutions and economy affect the market development. Food sector is one of the largest industrial sectors in Latvia (food industry comprises about 20% of the total processing industry's Gross Domestic Products (GDP) (Ziņojums par...., 2016). The economic processes determine how the food delivery systems are designed, what decisions are passed at the political level to promote development of the market favourable for the local products. An important aspect is the market and business regulative framework, for instance, restrictions regarding distribution of genetically modified organisms, use of agricultural chemicals, etc., as well as restrictions regarding planning of the land use, such as use of agricultural land, and allotment areas in the city. Food policy and legislative base to the large extent depends on the global policy, particularly the European Union's policies in areas of agriculture, industry, and consumer rights.

In the Maslow's pyramid of needs, food is located at the level of basic needs, nevertheless, although the food is a basic human need, its choice nowadays is determined by various factors or combination of factors, which is not always rational, but often can be irrational. Therefore, an individual choosing food and satisfying personal needs takes into account both food's impact on the health, its quality, balance, safety, price, availability, etc.

According to the Institute of Food Research, there is a growing number of buyers in the European Union countries considering healthiness as a main criterion for choice and purchase of foodstuff (Vaarst, Hovi, 2004). Along with stabilisation of economic situation in Latvia and increase of the household income, this trend gradually will become relevant in Latvia as well (Jemeļjanovs, 2013).

Currently, increasing role of the local foods is enhanced by the communities' willingness to develop the local economic activities and to maintain the local identity; however, they are hold back by the lack of regulatory framework, which does not even define a term "local food".

The research aims to explore the choices of local food and its consumption by the modern consumers.

Modern (nowadays) consumer characterize: around-the-clock-shopping, consumers are in control, omnichannel shopping, content consumers, global experience, collaborators and social sharers (Bolen, 2016).

Materials and Methods

Scientific (article) and practical (law) information was used in the research.

A set of general scientific methods (monographic, logical constructive, graphic) and sociological research method (survey) were used to carry out the research.

Within the research, a questionnaire was developed to assess the extent the population of Latgale planning region (Latvia) consume local foods. The survey was conducted in January – February, 2017 by internet. Altogether 504 valid questionnaires were collected. According to the Central Statistical Bureau data, the number of permanent residents of the Latgale region was 276 358 in 2016 (Centrala Statistikas…., 2016). Applying a simple random sampling method, it was calculated that the required number of respondents, in order to confirm the data obtained is reliable (with probability of 95%) and represents the general sample, is 384. Since, in fact, larger number of respondents (504) was surveyed, it can be stated that the data obtained with a probability of 95% demonstrates the extent to which the local population consumes the local foods (Raosoft, 2004).

The aim of questionnaire was to examine consumers about their behaviour for choice food and knowledge about their consumption of local food. Questionnaire had 23 questions about consumer behaviour and their attitude to the local food and also about quality and consumer knowledge's about new food, functional food etc. Results of these questions (quality, consumer knowledge's etc.) authors do not use in this article because these results authors will use in other research.

Authors use SPSS for data processing and use descriptive statistics, correlations analyse, frequencies. Respondents can give more than one answer for some questions and according it the results were calculated for each answer as for separate question.

Characteristics of the survey's participants: 65.7% of the respondents are women and 34.3% – men; 35.8% of the respondents are under the age of 24, 16.3% –25–35, 20.1% – 36–45, 19.1% – 46–60, 8.7% – 61 and more. 47.0% of respondents are employees, 31.7% – students, pensioners (6.3%), employers (6.0%), housewives / men (5.8%), and unemployed (2.8%). 43.8% of the respondents have graduate (higher) education, 29.4% – secondary education (since 31.7% of the respondents are students), 20.0% – vocational education, and 6.8% – primary education. Most of the respondents (64.1%) reside in cities, 26.3% - in villages, and 9.6% – in farmsteads. It is essential that 67.5% of the respondents have their own backyard farm; hence, it is evidence in favour of the self-produced foodstuff, that is, the local food with a known origin.

Results and Discussion

The concept "local food" is being used more often and more broadly, though, there is no a single and official definition of it (Litavniece, Silicka 2016).

Many researchers have carried out scientific and practical studies on the local foods (i.e., Abate, 2008; Anderson, 2009; Aurier et al., 2005; Bahram, 2003; Best et al., 2009; Brown et al., 2008; Carpio et al., 2009; Futamura, 2007; Hughes, 2007, etc.).

This study particularly is focused on the concept of "local food". This concept hasn't one official definition what is acceptable in the different countries. According it, definition of "local food" usually is explained as combination of two essential aspects:

- Geographical (Hughes, 2007; Best et al., 2009; Bahram, 2003; Aurier et al., 2005; Abate, 2008; Anderson, 2007). In this concept, a distance between the producer and consumer dominates (in line with the above mentioned interpretation of the "food of local identity").
- Characteristics of social and supply chains (Futamura, 2007; Carpio et al., 2009; Brown et al., 2008; Aurier et al., 2005; Abate, 2008; Anderson, 2007). Advantage of short supply chains is related with the relationship between the consumer and product seller. Short supply chains are characterized by a spatial proximity, in this case the food is produced and marketed specifically for this region, and consumers are aware about the local characteristics of the product (Litavniece, Silicka 2016).

In Latvia, the local food distribution channels can be divided using V. Praude's typology of local grocery market participants (Praude, 2011) as follows.

- Business to Customer (B2C) – the company's products are sold to the final consumers. For example, the domestic food markets, direct sales communities.
- Business to Business (B2B) – company sells the product to another company, for example, to the retail stores (*Rimi*, *Uga*, *Satys*), restaurants, cafes, and other catering companies, guest houses.
- Business to Government (B2G) – company sells products to the state and municipal enterprises, for example, *green procurement* program, programs *"Skolas piens" ('School Milk')* and *"Skolas auglis" ('School Fruit')*. Healthy nutrition aspect characterizes these programs.
- Every year, the European Union Member States spend an average 19% of GDP for public procurement purposes. In Latvia, public procurement accounts 17% of GDP. Such an effect on the goods and services market is significant; therefore, by including the environmental requirements in the public procurement (when implementing *green public procurement*) it is possible not only to promote increase of the share of environment-friendly goods and services in the market, but also to achieve financial and social improvements (EK, 2011). Thus, *green public procurement* can be used as a "critical mass" for the environment friendly products' market development (Testa et. al 2012).

The current consumption trends and the local foodstuff characterize a quality of contemporary life. Quality of life, in turn, is no longer just a simple set of social indicators, as Dutch scientist F. Oort (2005) states, rather it is a complex concept including "objective" and "subjective" indicators mutually interacting in different areas of life. The authors of the study consider that it has not changed even now. Alike the society, the concept of life quality changes and evolves.

The World Health Organization defines life quality as "an individual's perception of personal life position in the context of culture and value system in which the individual lives and is linked to his/her goals, expectations, standards, and concerns. This is a broad concept, which is affected in a complex way by individual's physical health, psychological state, personal beliefs, social relations, and the most important environmental factors". The Ministry of Health has developed the Public Health Guidelines for 2014–2020, including three prerequisites of healthy nutrition identified in the Public Health Guidelines of the previous periods (Sabiedribas veselibas ..., 2014):

- food quality and safety;
- balanced nutrition;
- availability of the food based on the local agriculture for every citizen.

To be able to analyse and evaluate role of the local products in nutrition of the consumers, it is necessary to understand the overall tendencies in food consumption.

Structure of the consumption expenditure is one of the indicators characterizing the material welfare of the society. The main priorities of the consumption expenditure for the last two years are – food, housing, and transport (Majsaimniecibu..., 2016).

In the households, 1.7% more on food and non-alcoholic beverages was spent in 2015 compared to 2014, which could partly be explained by wider use of public catering services, as well as by decline of food prices by 1.3%. Consequently, at reference prices, the expenditure on food and non-alcoholic beverages increased by 3.0%. The households spend three-quarters (76%) of the total food expenditure buying food in the retail stores, 8.7% buying *food in kind*, and 14.8% is spent for public catering. Along with improvement of economic situation, the households' expenditure for catering services continues to increase. Namely, these expenditures have increased in average by 11% in 2015, compared to 2014. In 2015, the households spent more on such foodstuff as meat and meat products. (Majsaimniecibu..., 2016)

Though, the structure of consumption is changing. Consumption of non-alcoholic beverages and liquors, fruits, poultry and meat products increases. Thus, the authors conclude that the changes in division of work and leisure, as well as an increasing intensity of life leave the impact on the food choices. These trends contribute to the consumption of semi-processed foodstuff. However, not only the eating habits, but as well the grocery shopping and eating places change. The consumers increasingly use to have a meal outside the home.

An individual's characteristics determining his / her food consumption choices are, for example, level of incomes per household member, and knowledge and awareness about the composition of foodstuff, its impact on health, recognition of markings. These are the considerations related to the individual's value system, role of the status, for instance, when buying expensive liquor, exclusive foodstuff, as well as ethical considerations regarding consumption of the animal food products.

Food seasonality still has a significant role in the food consumption. Nevertheless, the product price, and views of a healthy lifestyle, including a healthy diet has to be considered as the key factors in the foodstuff choices (Eglite, 2010).

Changes in the consumer behaviour involve not only financial aspect, but also phases of the life-cycle, and personal development.

There are still strong traditions of cultivating food for personal use, and preserving it for the winter. In rural areas, 18% of consumed foodstuff is cultivated and produced by the consumers themselves. In Latvia, there still are households using in their daily nutrition agricultural products – vegetables, dairy products, meat, fish caught, or wild animals hunted, that is, products *in kind* – either produced by themselves, or received from relatives, friends for free. (Majsaimniecibu..., 2016) These consumer traditions of cultivating food give us information of local food characteristic and importance criteria what mean local food.

In the empirical research, significance of the local foodstuff in consumption of the modern consumers was explored. The results encourage new ideas requiring in-depth research to approve the identified probabilities. The respondents have provided their replies on frequency of the purchase of the local foodstuff (Fig. 1).

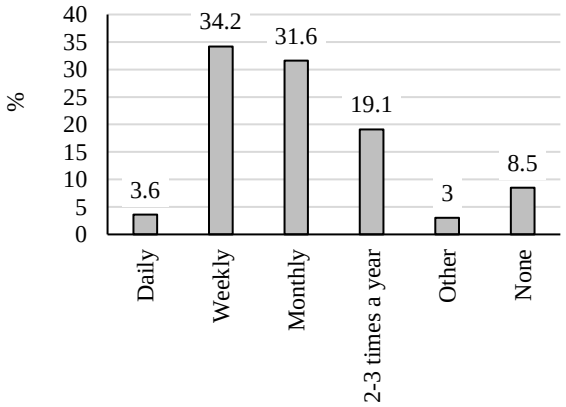

Figure 1. Frequency of buying the food from the local domestic producers

34.2% of the respondents purchase the domestic food on a weekly basis, 31.6% - monthly. It suggests that the domestic food is in demand. Since the vast majority of the respondents purchase the local foodstuff in the domestic producers' markets, it provides an explanation of shopping frequency (Fig. 2).

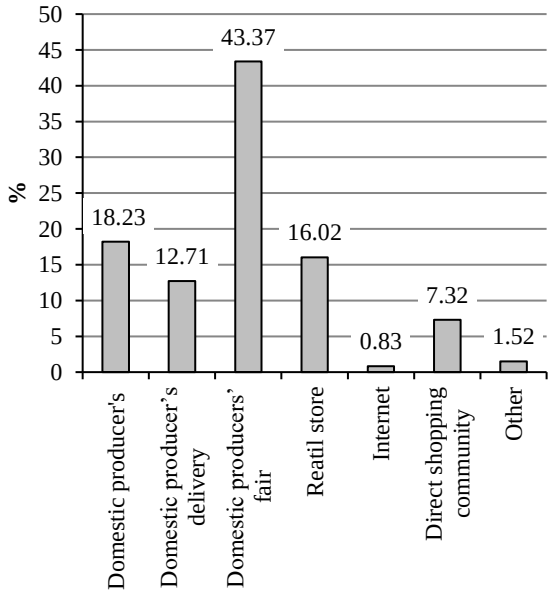

Figure 2. Places of buying the local domestic food

The Figure 2 shows that the most popular local domestic food acquisition place is the domestic producers' fair. Fairs usually take place on weekends or before holidays. People have more free time or a desire to buy something different to serve on a festive table. Also, many local municipalities of the Latgale region, considering the local economic development, take initiative and organize the domestic producers' fairs on the certain days of month, informing the local community (e.g. the association "LEARN" at the Rezekne municipality). Fairs are becoming increasingly popular, as evidenced by engagement of the supermarket chains "Rimi" and

"Maxima" as the domestic producers' are allowed organising their fairs in the parking lots of the mentioned supermarkets.

57.68% of the respondents during a visit to the domestic producers' fair spend in average 6-15 EUR, 20.18% – 16–30 EUR, 17.54%–up to 5 EUR, and 4.61% – above 31 EUR. Level of spending is determined by the level of income and the average price of foodstuff (Table 1).

Table 1

Spending on purchases in relation to income level, %

Incomes	Spending on purchases			
	Up to 5 EUR	6–15 EUR	16–30 EUR	31 EUR and more
Up to 400 EUR	28.26	58.70	10.87	2.17
401 -600 EUR	14.88	59.50	19.01	6.61
601 - 800 EUR	11.11	61.62	24.24	3.03
801 EUR and more	17.16	52.24	24.63	5.97

The Table 1 shows that the respondents with lower incomes spend relatively less money in the domestic producers' fair. Between spending on purchase and income level have close positive correlation (0.87) with significant level 95%. If income levels decrease also decrease spending on purchase. There are a number of probabilities that could justify the above mentioned:

- the domestic food is relatively more expensive than the foodstuff in supermarkets (Fig. 4), and the people with lower income can channel a relatively smaller share of their income for the food;
- more than 31 EUR is spent by a relatively small number of the respondents because period of use of the local domestic food is short.

Every consumer has its own motivation and reasons to buy the local food products (Fig. 3.).

The respondents' most important motivation is awareness that the local product is being acquired (53%), and it also is an opportunity to support the local producers (34.5%). These responses confirm the importance of the local products in the consumption of the modern consumers. 44.8% of the respondents believe that the local food quality and choice is much higher. 40.7% of the respondents consider the significance of eco-friendly products. Often, the local foods are associated with eco-friendly products. The respondents' replies encourage ideas for the further in-depth research that should be focused on evaluation of the consumer behaviour in a particular region.

One of the key aspects affecting the buying motivation is level of education (Table 2).

The level of education contributes to the global way of thinking, for instance, by supporting the local economy, not only receiving direct personal benefits. The respondents with a higher level of education have a wider range of vision and knowledge that may affect the awareness of the product's nutritional value – healthiness, place of origin, usefulness.

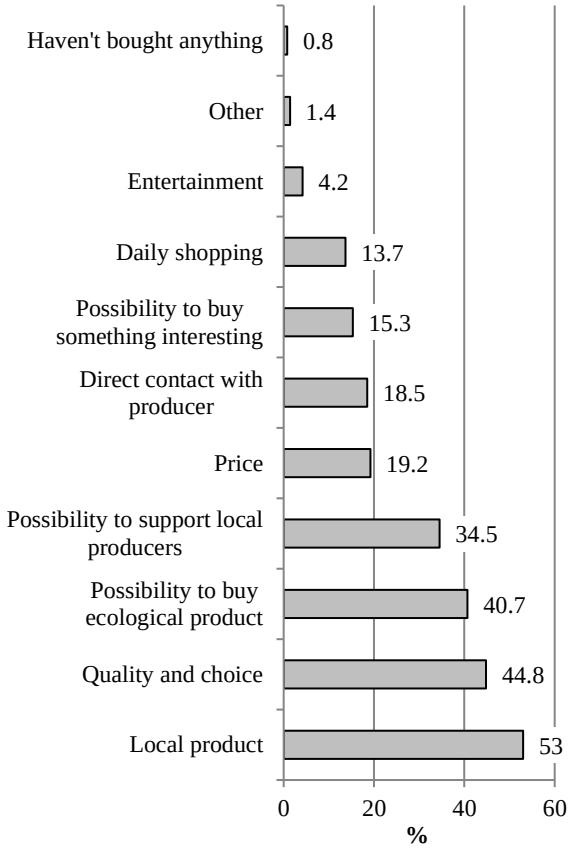

Figure 3. Motivation to buy local domestic producers' foodstuff

Table 2

Respondents' motivation to buy the local domestic food depending on the level of education

Motivation	Level of education			
	Basic	Secondary	Vocational	Graduate (higher)
Daily shopping	4.11	6.39	9.27	3.98
Possibility to buy eco-friendly products*	17.81	14.89	16.10	16.54
Local product	17.81	21.88	24.39	20.06
Quality and choice	13.70	21.58	18.54	16.07
Price	10.96	10.94	8.29	5.21
Direct contact with producer	12.33	5.47	7.81	7.50
Possibility to support local producers	10.96	11.85	9.27	16.39
Entertainment	1.37	1.82	2.44	1.23
Possibility to buy something interesting	9.59	4.86	3.42	7.20
Haven't bought anything	1.37	-	-	5.05
Other	–	0.30	0.49	0.77

*Questionnaire contained explanation what mean local products and eco-friendly products and according it we can accept that respondents understand these questions and also give correct answers.

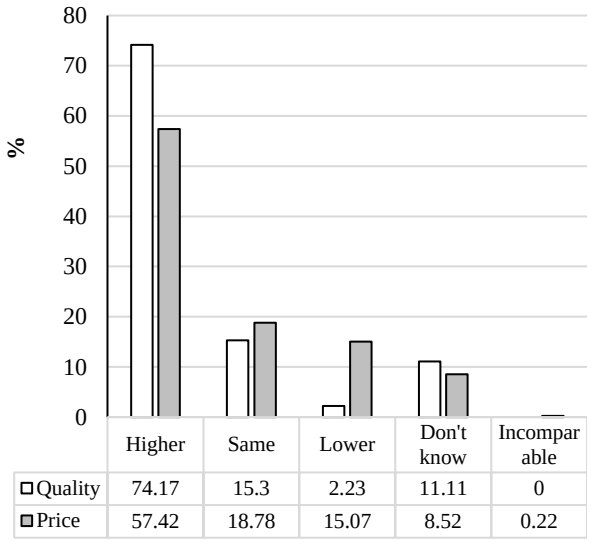

Figure 4. Comparison of the local domestic producers' product quality and prices with the supermarkets'

Product quality consumers understand as the characteristics of a given product or quality score what make features of a set of food (appearance, colour, odour, nutritional value, origin of the product, ease of preparation, safety), what provides the consumer needs and it practical validity (Prasibas partikas kvalitates…., 2014).

Majority (74.17%) of the respondents believe that quality of the domestic food is higher than quality of the products sold in supermarkets (Fig. 4). 93.65% of the respondents are satisfied with the quality of the local domestic food, evidencing that the local domestic food is of high quality and, in compliance with this criterion, it is competitive.

In recent years, short food supply chains (direct selling: farmer —> buyer), the farmers' markets and fairs, and the *slow food* movement gradually develop. As well the organic food becomes more available, though it is still relatively more expensive, therefor its consumption is limited (Silicka, Litavniece, 2016). This is evidenced by the survey data as well, since 57.42% of the respondents believe that prices of the domestic food are higher than of the products sold in supermarkets.

Table 3

Evaluation of the local domestic food prices compared to the supermarket prices, depending on the income level of the respondents

Criteria	Higher	Same	Lower	Don't know
Up to 400 EUR	18.15	24.71	18.18	32.43
401–600 EUR	25.48	30.59	31.82	21.62
601–800 EUR	20.85	20.0	30.30	18.92
801 EUR and more	35.52	24.71	19.79	27.03

However, comparing the estimates by the respondents according to their total family income, it is observable (See Table 3), that the respondents having higher income believe that price of the local domestic food is higher.

The survey results foster debate and encourage the need for in-depth study. There is a likeliness that the respondents with lower income primarily choose discount products, but people with higher incomes are not ready to pay a higher price, unless the quality criteria of the product do not comply with.

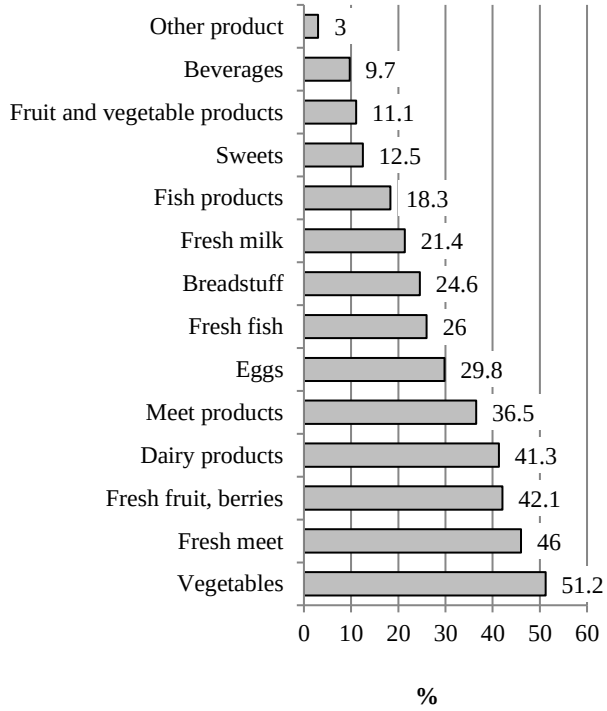

Figure 5. Types of the domestic food

One of the most common types of products purchased at the local domestic producers' (Fig. 5) are vegetables (51.25%).

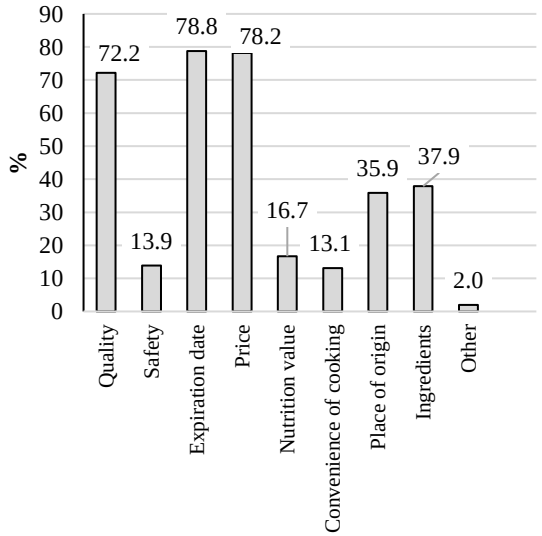

Figure 6. Characteristics the respondents pay attention to when purchasing the local food products

More than 70% of the respondents believe that the quality, expiration date, and price are the most important

characteristics of the product to be paid attention to when purchasing the product.

Conclusions

Modern consumers pay attention to origin of the product. Consumer behaviour impacts education and income level. Higher income and education level provide consumers thinking from the global to the local. According it people more think what they buy and what they eat and for these people local food is significant in their consumption.

Often, in perception of the consumers, the local food is one of the aspects of healthy food (it can be concluded from the survey conducted).

In the result of the empirical research, the authors have concluded that, to evaluate the data obtained, it is necessary to carry out the in-depth research on the choices of the local foodstuff and evaluation criteria in the view of consumers.

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INFLUENCE OF EATING HABITS ON PEOPLE'S LIFE QUALITY

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Abstract

Life quality requirement and indicators is person`s health, which is affected by food quality and eating habits. Healthy food consumption is related not only with food quality standards, but it is influenced by social, economic and cultural factors, in specific cases by physiological, inherited or gained eating habit features corresponding to persons age. Complying with these features, for example, new-born nourishment, different product or substance intolerance, or illnesses etc. determine the intake of necessary nourishment. Eating habits are analysed together with other healthy life style components, as well as physical, social and educational environment influence factors on physical and psychological well-being. In a life quality context researchers have studied eating habits and factors that influence them already for a while, creating a study basis to ascertain the dynamics of behaviour change, as well as carrying out quantitative and qualitative data comparative analysis. Empiric data basis include different age groups, starting from study on factors affecting new-born life quality from 2007, which continued at preschool and elementary school age group from 2008 and at secondary school level from 2011. The aim of this article is to present the outcome of longitudinal study on the eating habits and their impact on quality of life. Conclusions and recommendations for unhealthy habit change can be found in complex approach, focusing on education improvement aspect interrelations with institutional solutions, providing healthy food accessibility and limiting the unfavourable factors.

Keywords: life quality, healthy lifestyle, food quality, eating habits, education.

Introduction

The quality of life in the context of stratification and agedness of today`s society is emphasized both in European and in Latvian strategic planning documents. According to „economic spurt" as set leading motive and growth of national economy, theories supporting human securitability and growth as implementation of set priorities, limited labour force, financial and natural resources turning into competitive products and services, in order to achieve the main goal – growth of life quality (National development Plan of Latvia for 2014–2020, 2012), it is essential to carry out comparative studies regularly to identify the dynamics of populations life quality.

Notion „life quality" has no single definition. Therefore there is no single understanding of how to characterize the life quality. Although until the middle of 20th century economic indicators were uppermost in the development, more and more often opinion was expressed that economic growth is not a goal in itself, if it doesn`t promote well-being and satisfaction with life, because not always the growth of material prosperity creates society of happy people (Ķīlis, 2007). During the second half of 20th century strengthened the opinion that well-being is not defined only by income, but it also includes such areas as security, climate, health, education (Hasan, 2007). Huge impact on life quality has surrounding environment (Biagi et al., 2006). During the second half of 20th century such social conditions that impact life quality subjective elements as participation, entertainment, capacity to act (Lanteigne, 2005) and opportunity (Seed, Lloyd, 1997) were highlighted. Opportunity in this context is not coincidence or luck, but rather a choice, access to resources, services and goods that provide sufficient standard of living. As a result quality of life forms from many components, which objectively affect people`s lives and subjectively manifests as level of life satisfaction, and, incorporating in itself the economic indicators, centre around opportunities and capacity to act of individual (Biagi et al., 2006; Meiselman, 2016).

Life quality is complex social, economic, political notion that contain wide range of living conditions of state residents. It is characterized by the level of consumption and by range and quality of social services available to person, as well as by the possibility to obtain the education, to live long and healthy life, to participate in state`s political life, and by the eradication of discrimination based on sex, ethnic, religion, disability, sexual orientation and age. Therefore person is able to embrace its potential for the creation of well-being for the whole society. Life quality is defined by state`s nature, economic, social and political environment, which can be characterized with different indicators (National Development Plan of Latvia for 2014–2020).

Static and dynamic understanding characteristic to life quality (Tisenkopfs, 2006):

- Static understanding: life quality is achievable or achieved goal or result. In this aspect life quality will always be insufficient and there is big possibility that it will obtain patterns of consumerisms.
- Dynamic understanding: life quality is process of improvement of life itself – set of intentions and activities that are directed towards raising the well-being, using the opportunities, perfection of abilities. It depends on capacity to act and it provides satisfaction.

Ferrell (1995) has defined life quality as feeling of comfort that is established by four areas: life quality consists of physical, mental, social and spiritual feeling of comfort. Food is one of the factors that influence subjective well-being (Grunert et al., 2007; Schnettler et al., 2015), differentiating food- and pleasure-related well-being and food- and health-related well-being (Guillemin et al., 2016).

Siekierski and Ponchio (2014) emphasize that according to studies where demand for uptake of nutrients is simulated (Behrman, Deolalikar, 1990; Barrett, 2002)

and according the studies about link between food choices and health and labour market (Pitt et al., 1990), as well as body weight (Cawley, 2004; Cutler et al, 2003), food in economy is both commodity and investment, because with taste, texture and feeling of satiety food gives instant satisfaction, but it is also related with health and well-being, costs and benefits.

Since food is essential part of quality of life and unhealthy nourishment can leave a negative effect on health (Jackson et al., 2005), along with growing public concerns about eating habits more and more discussions occur on necessity to increase the proportion of health education in educational programs in Latvia, as well as on requirements for food quality in educational institutions (Government regulations No.172), hospitals, providing balanced diet, limiting food colorants, preservatives, sweeteners, flavour enhancers, including amount of mononatrium glutamate (E621), preservatives, sugar and salt. Ministry of Health of Republic of Latvia has made differentiated recommendations for different age groups – infants, children aged 2 to 18 years, adults, persons who are older than 60 years, as well as it has indicated the recommended energy and nutrient intakes for citizens of Latvia.

Food choices and eating habits are influenced by different factors, but most significant are life style and culture (Siekierski, Ponchio, 2014; Ares et al., 2016).

In the context of this study understanding of life style is associated with how person lives (Sheth et al., 1999), spends his time and money (Kotlers, Keller, 2006; Blackwell et al., 2001), reflects on what person thinks of life, values, how he acts and behaves (Dias, 2003), creates self-image, which is defined by previous experience, strains and current situation, social status, subculture, motives, emotions and that is influenced by economic, demographic, social, nutritional, educational, historical and climatic factors, putting forward such indicators as action (work, hobby, social events, vacation, entertainment, shopping, sports), interests (family, home, work, relaxation, food, media, achievements), opportunities (social questions, politics, economics, education, products, culture) and demographic (age, education, income, occupation, size of family, home, geography) (Hawkins et al., 2007; Wells, Tigert, 1971).

Social stratification is based on class division: upper class, upper middle class, where success strategy is the dominant one, and lower middle class, lower class, where survival strategy is dominant (Meņšikovs, 2006). Whereas culture, that has both roots in religion and secular trend, influences fashion and life style choices, and at some point in life it can influence persons behaviour, becoming a habit and at certain conditions to create a tradition. Fashion displays person's reaction on modern life pressure (Rožkalne, 2006).

The aim of this research is to determine changes in people`s eating habits and their influence on life quality in the period from 2007 to 2017.

Materials and Methods

Liepaja University Sociological research centre`s longitudinal study was started on 2007 in Liepaja and in Kurzeme region (Latvia). 1804 respondents from particular city and its surrounding territory were involved. They were both students and experts, who represent all age groups, defining the frame for study that is related to children and youth life quality perspective – healthy life style, nourishment quality and eating habits. Study consists of four stages:

I stage New-born life quality perspective – study in Kurzeme and Liepaja carried out in 2007. Surveyed unit distribution per month was calculated according to proportion of birth each month. Respectively each year data from medical records were collected, it was transferred to anonymous forms according to personal data protection requirements. As a result N 1141 units were selected randomly, they sorted by the years according to expectations, and were supplemented by five independent medical personnel expert interviews;

II stage Study on population reproduction qualitative aspect in Kurzeme and Liepaja carried out in 2008. In order to study preschool and elementary school children life quality aspects qualitative research method was used – 6 focus group interviews and expert interviews, 34 focus group interview participants and 6 experts participated. At preschool level there were two independent focus groups consisting of preschool teachers, psychologists, medics, social workers, parents. Schools were chosen randomly – 3 in Liepaja and one in Kurzeme region – focus group participant content was similar, sometimes school administration representatives participated.

III stage Youngsters` future plans and their implementation possibilities in Kurzeme and Liepaja. Population reproduction study in Kurzeme and Liepaja carried out in 2011. Survey was carried out between elementary school graduates (9^{th} class) and secondary school graduates (12^{th} grade) in the same schools where previous 2008 study was carried out using the survey method – students filled the questionnaires in their classes (N 338).

IV stage Youngster`s life quality and healthy lifestyle aspects in Kurzeme and Liepaja carried out in 2017. To find the answers on set questions student questionnaire was carried out according to methodology used during 3^{rd} stage of study (N 266). Experts and 2 focus group consisted from 14 respondents: general education school pedagogues, school principals, deputy directors on study and education work, medical workers (family doctors, dietician, nurses, and parents – members of parent councils, university pedagogy doctors – experts).

During the collection of data mixed methods approach was implemented, therefore securing the triangulation.

In the basis of developed coding system that consisted of profile codes and content (conceptual) codes, there are categories / criteria and subcategories / indicators identified as a result of theoretical literature analysis: **life quality philosophic understanding** (static and dynamic); **lifestyle** (relationship / interaction /

communication, behaviour / action, interests, stress); **culture** (tradition / rituals, table culture, values and **life quality strategies** (survival (ensuring the basic needs, adaptation to conditions success (capacity to act, possibilities of self-realization)). Respondent profile is formed by **status** (new-born (1141), pupils (656), pedagogue (24), parent (9), expert (6); **gender** (female (869), male (935), residence (Liepaja (1153), Kurzeme region (651)) and **social layer** (upper (55), upper middle (684) lower middle (976), lower (89)).

For the self-assessment of respondent life quality, wellbeing, health and stress Likert scale was used.

Data processing methods:

- Processing of qualitative data in the AQUAD (Huber, Gürtler, 2003) software environment: frequencies, linkages, and implicants.
- Processing of quantitative data in the SPSS 21.0 (Statistical Package for Social Sciences) software environment: Kolmogorov-Smirnov test for definition of result groups, cross tabulation and Chi-Square test to define relationships, Kruskal-Walis H-test to define differences among respondent groups, Kendall's tau-b correlation analysis to define correlations among categories.

Results and Discussion

In a study „***Newborn quality of life perspective***" (Markausa et al., 2007) done by Liepaja Pedagogy academy Social science department Sociological research centre researchers focused on factors influencing and characterizing the new-born life quality perspective. They admitted that „*Fundaments of child`s life expectancy and quality are laid during the pregnancy, they are influenced by mothers overall health condition, nutrition, emotional well-being etc.*" (Markausa et al., 2007, 4). Neonatal pathology, disease causing factor analysis results correlated with mother`s life style, including nourishment quality, bad habits (smoking, alcohol abuse, reproductive behaviour) etc. ($p=0.000$; $r=0.79$), while drawing attention also to a way infant is nurtured starting from the first minutes of his life. The study found that infant feeding not always is mothers choice, it can be influenced both by child`s and by mother's health condition.

In 2017 study in interview with neonatologist with 35 years long experience it was confirmed and found that there is link between life quality dynamic understanding and values:

"*Clinical studies have demonstrated the importance of breast-feeding from the first hours of child's life, which is difficult to compensate with other nutrition. Lately it can be noticed that desire to breast-feed is growing, which to some extent can be called a good fashion tendency. It can be explained by increasing number of publication on social media about new mother`s life style. It lacks deeper knowledge, but it`s alright…*"

However family doctor, paediatrician with 30 years' work experience has noticed not only the positive tendency in new-born feeding, but also pointed at problems that new mother`s face. It allowed to establish a correlation in qualitative data processing AQUAD software environment between categories *life quality philosophy*, *culture* and *strategy* or, more precisely, subcategories – *life quality dynamic understanding*, *health as value* and *survival strategy: "There is no indication that demand for infant specialized food from the parents would have grown. Mothers are trying to breast-feed. Other issue is that while child grows he might need an additional feeding. I assume that restrictions on infant nourishment commercials have partially promoted the positive change. There are also families where mother`s life style and economic situation do not contribute to healthy nourishment choices. Poor people can be not only poorly educated, they can also live in partial families."*

Infant feeding choice is influenced by recommendations from medical experts, "pressure from society" as fashion tendency or etiquette, but not always by thorough knowledge about the influence that healthy nourishment has on new-born life quality, well-being and health.

At all life stages of parents health education is necessary. It would reduce the negative influence of commercialization related to necessary health promoting life style, including food choices and regime.

"***Study on population reproduction qualitative aspect in Kurzeme and Liepaja***" (Markausa et al., 2008) is logical continuation of the first stage. During the study it was found that qualitative balanced nourishment provided in educational institutions at the preschool and elementary school level for children contrasts with eating habits in families.

Results of the surveys from 2008 and 2017 show that healthy nourishment and life style is easier to provide in families with high or average living standard, but there are potential risks also for families with high and average income level per family member. Self-identification with or objective belonging to upper or middle social layer is not the decisive factor for healthy diet. Besides catering at educational institutions, especially at children parties, unhealthy food dominates as a main snack: pizza, pastries, crisps, sweetened soda drinks, although kids at kindergarten level already know what is healthy and what`s not a healthy food. It was confirmed by a preschool pedagogue with 35 years' experience during the focus group interview:

"If there are sandwiches with salami, salad and carrots on the table then sandwiches will disappear first. In the group only one child eats salad and carrots. Sweet drinks are on demand. It is the mirror of family. If parents offer to go to Hesburger for a lunch, then it is obvious, that they are doing so because it is easier. Today it is no rarity that kids who are going home have pack of crisps or some colourful candy in their hands."

Very often parents take their children to sports trainings after work, or to different artistic creativity hobby groups. On the way from educational institution to the place of extracurricular activity very often children eat fast food, sweets, drink sweetened drinks. Also after the

activity, which sometimes ends late in the evening, children have heavy meals before sleep:
"Usually those are late dinners. It depends on son`s football trainings. After the kindergarten 3 times a week we walk to the sports hall and then back. It is a big load, but there are no complaints. Therefore last meal usually is late. It can be at 9 o`clock in the evening. He eats different soups, second course with meat, fish. Without objections he eats salad, tomatoes, and cucumbers. He does not like sweets very much." (Father of preschooler, 34 years old)
Younger students at the beginning of elementary school gladly eat meals provided at school, however later, when they have a pocket money it is obvious that children prefer to eat outside the school. During the teen years girls particularly are concerned about the looks. They show the tendency to lose weight by skipping the meals:
"At the early classes there were free meals, everything happened centralized, but after the second grade I was paying. Also now school offers to eat during particular break and centralized, but my children do not choose that. Daughter keeps an eye on her weight. Because these are teen years, they pay attention to weights also because of peer pressure. There were times when she skipped meals to obtain the desired look, but it had opposite effect, until she realized that you have to eat regularly and cooked meals." (Mother of 2 children, 38 years old)
Preschool educational institutions and schools provide healthy and balanced diet, but parent life style, ambitions and fragmented knowledge about healthy nourishment do not promote good health for their children. Children are well informed about diet and unhealthy habits, but indiscriminate following of fashion tendencies and imitation of friend`s behaviour causes risks.
In 2011 study "***Youngsters' future plans and their implementation possibilities in Kurzeme and Liepaja. Population reproduction study in Kurzeme and Liepaja***" revealed significant connections between implementation of future plans and healthy eating habits:

- Teachers believe that there are several related health, behaviour and school results problem causes: parent busyness and therefore minimal contact with their children; one of parents lack or absence; not sufficient, excessive, misbalanced diet; relationship with peers; family conditions (level of wealth, living conditions, relationship between children and parents); sedentary life.
- 9–12th grade questionnaires, where those who are facing career choices whether to continue studies or to start working were questioned, revealed the possible life style related risks, including health problems caused by diet and bad habits, which threatens the future profession choices.

Student life style is related with physical space and social environment around. Usually the physical space – home, school and leisure – are united, but often students, who live in rural areas, use educational and leisure possibilities in nearest town. Distribution of time between time spent with parents at home, at school and at leisure activities is not constant. If student don`t miss school, then distribution of time for school in one age group is similar. Two variables are time spent at home and at leisure activities. When time spent on leisure activities grow, student needs money. Pocket money is usually spent on entertainment, sweets, cigarettes, while giving up on meals at school canteen.
2011 study revealed that approximately one third of students spend their money on sweets, two times more in ninth grade than in twelve grade (42.4% and 21.4%). During secondary school spending pocket money on cigarettes and alcohol becomes more frequent. However 2017 study ***Youngster`s life quality and healthy lifestyle aspects in Kurzeme and Liepaja*** shows that there are more smokers in eighth grade (78%), smoking frequency varies – 20% admits that they are regular smokers (10-20) cigarettes per day. In comparison with 2008, where 52.6% boys and 51.4% girls had encountered alcohol abuse, but at twelve grade – 76.7% boys and 73.7% girls, 2017 study revealed that at least 89% ninth graders and 73% secondary school students had consumed alcohol "because others in the group were drinking", which can be explained by the fact that usually motivated, success-oriented students continue studies in secondary school, while part of the ninth graders continue their education at vocational schools or find job.
Mother of two youngsters reveal that her children are supporters of active and healthy life style. She admits that youngsters are familiar with alcohol abuse, smoking and even drug abuse:
"*Son quit secondary school because he wanted to study logistics at vocational school. The rumours say that 90% of boys are smoking. There are no indications that he has started, too. He acts disapprovingly. My daughter, when she was at sixth grade, told me that once during a field trip one girl fainted because she was smoking in the morning and had no breakfast before that. My children choose sports, not parties. Their friends have similar life style – they choose trainings, but, I guess, its half to half: others like parties, but not all of them. Secondary school students sometimes have alcohol at the parties, and not just that, other substances are being used, too. I don`t want to believe that its only family`s fault, its more influence from friends. Unfortunately also between those who do sports… Desire to try…*"
Stress, sleepiness appears in 2017 study: 26% ninth graders together with their peers or anonymously long hours during the night play interactive computer games:
"*Less in secondary school, but at 8th and 9th grade they "live" in their smart phones – in social networks. And those, who have trendy clothes and latest smart phone, they don`t have the best grades. Their parents don`t attend school meetings or attend very rarely. They don`t have time or they don`t live with their children. Maybe they just "pay-off" their children to earn the big money undisturbed.*" (Teacher, 28 years old)

2017 study reveals that persons in youngster`s close circle have similar life style, and that at secondary school 48% boys do sports.

Cultural aspect related to previous eating habits and traditional culture change under the pressure of open market economy and globalization. It is positive that fresh fruits, vegetables are available at any time of year, but as symbols of rush and globalization more and more rapidly fast food, exotic synthetic tastes enter one`s life style, which change the eating habits of a person. Popular culture, too, imposes its model, advertising weight loss diets, which sometimes include dietary supplements. They take focus away from the obesity reasons and problems caused by obesity. If somebody relates food intake only with calorie intake, then table culture – being together, discussing experiences, being in cosy atmosphere with aesthetic table setting – loses its significance. Unfortunately also school cafeterias and canteens usually use disposable dishes.

"*More sustainable are family traditions related with holidays, however because of daily habits holiday dinners and rituals become more and more casual: people by convenience foods, ready-made dishes like pastries and cakes, therefore they prevent holiday rituals, but, instead, they focus on result – full table, and the result is achieved without big effort."* (Expert, cultural anthropologist, 48 years old)

In 2017 study 12th graders value health higher, comparing to 9th graders (p=0.047).

Significance of health, which depends on their own choices and is related with dynamic paradigm, compared with 2011 study has increased among 12th graders. For example, consumption of fruits and vegetables has raised from 46.5% in 2008 to 49.6% in 2017. However number of smokers increases in younger classes, but at 12th grade it stays the same. In 9th grade there were 49.1% non-smokers in 2008, and only 42.9% in 2017.

Similar tendencies show non-use of alcohol, but way of socialization that was related to alcohol abuse is being compensated with excessive playing of computer games and spending time on social networks.

Health can be affected by eating regime. In 2011 study it was found that students try to eat during the school time – 43.5% students have lunch at school on regular basis, 18.6% go home, others use different possibilities: go out in town, sometimes home, buy something in the shop or make choices according to possibilities. Nearly one third (32.0%) eat only at home – they don`t eat almost all day long. Students avoid bringing food from home. 2017 study show tendency to takeaways from shops during the breaks, cafes, markets or at canteens, although school provides catering. It is related with wider range of meals offered at those places, students like to enjoy cafeteria atmosphere or they find food outside school tastier.

Kendall correlation analysis show that there is a correlation between life quality and criteria "life quality philosophical understanding" (p=0.000; r=0.53), "lifestyle" (p=0.000; r=0.85), "culture" (p=0.000; r=0.62), and "success strategy" (p=0.000; r=0.81), as well as between indicators "life quality dynamic understanding" (p=0.000; r=0.57), "eating habits" (p=0.000; r=0.66), "healthy balanced diet" (p=0.000; r=0.73), "health as value" (p=0.000; r=0.55), "capacity to act" (p=0.000; r=0.71), and "possibilities of self-realization" (p=0.000; r=0.69). Quantitative secondary data is confirmed by qualitative data - processing of qualitative data in the AQUAD software environment regularity condition was identified – life quality is affected by its dynamic understanding, eating regime, interests, healthy balanced diet, health as value, capacity to act and possibilities for self-realization.

Determination of Chi-Square test result relationship indicates that statistically very significant link exists between respondent`s profile – social class and indicators "healthy balanced diet" ($\chi^2(2)=38.177$; p=0.002), "life quality dynamic understanding" ($\chi^2(2)=29.139$; p=0.009), "success strategy" ($\chi^2(2)=34.121$; p=0.005). Kruskal-Walis H-test for identification of result differences between respondent groups indicate that statistically maximally important differences (p=0.000) between social classes and eating habits exist – healthy balanced diet is consumed by upper class more often, comparing to lower class, as well as capacity to act and possibilities for self-realization, and life quality dynamic understanding are valued more at upper middle class, compared to lower and lower middle class.

Conclusions

Life quality of new-borns, which is influenced by parent`s reproductive health and lifestyle, which in healthy lifestyle context is viewed as balanced nutrition and eradication of bad habits, can be achieved with early preventive educational work, because special risk group are young, unmarried, smoking, little-educated mothers.

Society development potential improves at schools, but value and life style fundaments are founded in family.

To promote healthy diet social educational campaigns should be improved, as well as institutional solutions for derogation of bad habits.

To promote healthy lifestyle and healthy eating habits complex cooperation between parents, educational workers and other social institution representatives should be introduced, in order to provide sustainable life quality.

For the interested cooperation partners life-long learning should be directed towards success strategy, therefore diminishing the survival effect.

As a priority to promote children and youngster health education as an alternative to prohibitions and limitations, which shall rise the capacity to act.

Social stratification creates conditions for poverty trap, which is the main threat for life quality; in order to diminish social stratification it is necessary to carry out responsible policy, creating economic system and cultural environment that motivates learning.

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SHORT COMUNICATION

INFLUENCE OF LYOPHILIZATION AND CONVECTIVE TYPE DRYING ON ANTIOXIDANT PROPERTIES, TOTAL PHENOLS AND FLAVONOIDS IN POLLENS

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Abstract

Pollen is one of the most popular beekeeping products surpassed by honey and wax. In nature there is no food analogue, which can compare to pollen in terms of those biologically active substances, which are necessary for normal development and functioning of human body. The aim of the research was to determine and compare the total antioxidants, total flavonoids, total phenolic compounds and antiradical activity content in fresh, lyophilized and dried pollen samples. Content of total antioxidants (DPPH quercetin equivalent), total flavonoids, total phenols and antiradical activity (with DPPH absorption) was determined by spectrophotometric method. Three different pollen samples were analysed – fresh pollen, dried pollen at +42 °C and lyophilized pollen. Results of the analysis showed that the highest total antioxidant content is in fresh pollen – 29.75 mg QC 100 g^{-1}, but the lowest in both dried samples. Total phenol content in dried pollen was 56.89 mg GAE 100 g^{-1}, in lyophilized pollen – 54.11 mg GAE 100 g^{-1}. Total phenol content in fresh pollen was 61.64 mg GAE 100 g^{-1}. Both thermal treatment and lyophilisation decreased flavonoid content in pollen. Drying pollen at +42 °C affected by lower losses of total antioxidants, total flavonoids, total phenols and antiradical activity than in lyophilisation process.

Keywords: drying, lyophilisation, pollens, total phenols.

Introduction

The chemical composition of pollen is diverse and complex. Nowadays it is used not only as a dietary ingredient, but also as an alternative medical remedy (Kroyer, 2001). Pollen contains carbohydrates, fats, proteins (composed of all the essential amino acids), minerals (containing 28 chemical elements, especially significant amounts of K, Cu, Fe and Co) about 50 enzymes and hormones (Siņakovs, 2009). Natural antioxidants are also found in pollen (de Arruda et al. 2013; LeBlank et al., 2009). Vitamins A, E, C, B-vitamins, niacin, rutin, polyphenols, and selenium compounds are widely represented in pollen (Bonvehi, 2001). Pollen contains 20 times more vitamin A than, for example, carrots (Šteiselis, 2013).

There are various parameters characterizing antioxidants. Flavonoids are a significant group of plant secondary metabolites having a different biochemical and antioxidant effects. Flavonoids possess antiradical activity, thus radicals, as atomic oxygen, hydrogen peroxide, superoxide anion radical, resulting in the plant from UV radiation and natural plant metabolism, are neutralized and eliminated from the plant (Galeotti et al., 2008). Because of this essential factor people are interested in flavonoid rich foods, because human metabolic intermediates are also radicals that cause damage to cells, disrupting the phospholipid membranes. Flavonids have anti-virus, anti-allergic, anti-platelet, anti-inflammatory and antioxidant effects on the human body (Purviņš, Purviņa, 2011).

Antioxidants stops the oxidation in its early stage, preventing ongoing chain reactions, but antiradical activity begins to work later. Also, it neutralizes free radicals. Anti-radical activity is caused by a variety of compounds, including polyphenols that are capable of neutralizing the radicals (Feas et al., 2012).

Polyphenols are sum of phenol compounds in pollen. They are biologically active substances that are found in nature. Polyphenols main feature is the presence of many phenolic structures. This structure is the basis of large number and diversity of this group's unique physical, chemical and biological properties (Quidau et al., 2011).

Pollen composition depends on the plant type and pollen harvesting conditions (Almaraz-Abaraca, et al., 2004).

Variable according to relative humidity of air is the water content - in freshly harvested it is 20–30%. Fresh pollen must be used within a short period of time because of the increased moisture content, they begins to grow mould and microorganisms, which produce toxins are developed. For long-term storage of pollen they need to be treated – water content must not exceed 12.5%, the optimal water content is 8–10% (Ritmanis, 2004). Beneficial nutritional properties of biologically active substances in various honey products, including pollen, are widely studied but rarely changes in composition and properties based on type of storage and treatment are discussed.

Most conventional way to treat pollen is to dry it in convective type dryers. Pollen is laid in a thin layer and dried at 42 °C, the existing water is continuously discharged by forced ventilation (Bogdanov, 2011). Thermal drying is not the only way to achieve the desired result.

In lyophilisation process pollen is not dried by heating but by freezing it under reduced pressure. This causes sublimation - water in pollen immediately goes into the gaseous phase, skipping the liquid phase (Giordano et al., 2011). This technique is indispensable if it is required to dry heat-sensitive substances, which during drying in the oven (42 °C) is significantly affected by temperature and lose its high-value properties (de Melo, de Almeida-Muradian, 2010).

Pollen high-value properties, characterized by biological activity indicators such as total phenol, total flavonoids, total antioxidant content and antiradical activity, is reduced if product is dried or lyophilized (Giordano et al., 2011).

The aim of the research was to determine and compare the total antioxidants, total flavonoids, total phenolic compounds and antiradical activity content in fresh, lyophilized and dried pollen samples.

Materials and Methods

Research was carried out at the Department of Chemistry, Faculty of Food Technology at the Latvia University of Agriculture. The object of the research was bee pollen. Pollen were harvested in April of 2016 from Saldus district Ltd. „ULMUS-MEDUS".

Characterization of drying process

Bee pollen were convective dried with air circulation at 42 ºC using Memmert UFE-400 and lyophilized using Christ Freeze Dryer Alpha 1-2 LD plus at - 60 °C for 24 hours at 0.046 mbar.

Extraction of pollen samples

1.500 g of fresh, dried and lyophilized pollen was extracted with 50 mL methanol. Extraction was carried out for 1 hour. Extract were centrifuged at 13 000 rpm in a centrifuge for 5 min, the supernant was used for further analysis.

Determination of total phenolic content (TPC)

Total phenol compound content was determined by spectrophotometry (Kaškoniene, 2009). Method is based on phenol compound reaction with the Folin-Ciocalteu reagent. Coloured solution is formed, which is measured in the light absorption using a 760 nm wavelength. Total phenolic content levels are expressed as gallic acid equivivalents mgQE 100 g^{-1} dry weight (Singleton, 1999).

Determination of total flavonoids content

The total flavonoid content of pollen was determined by spectrophotometry. Method was based on the pollen flavonoids reaction with $AlCl_3$. Coloured solution was formed, its light absorption was measured using 415 nm wavelength light. Total flavonoid content are expressed as quercetin equivalent mgQE 100 g^{-1} dry weight (Singh, 2012).

Determination of total antioxidants content

Pollen antioxidant properties was characterized by spectrophotometry (Bertoncelj, 2007). The method is based on reagent DPPH (2,2-diphenyl-1-picrylhydrazyl) reaction with antioxidants by light absorption maximum of 517 nm wavelength. Total antioxidants content is expressed as quercetin equivalent mg QE 100 g^{-1} dry weight.

Determination of antiradical scavenging activity

Antiradical scavenging activity was determined by spectrophotometric method with DPPH (2.2-diphenyl-1-picrylhydrazyl) absorption of 517 nm wavelength.

Statistical analysis

The results were processed by mathematical and statistical methods (mean, standard deviation) using Microsoft Office Excel 2016.

Results and Discussion

The highest total flavonoid content was found in fresh pollen: 196 mg QE 100 g^{-1}, while the lowest was in lyophilized pollen: 154 mg QE 100 g^{-1} (Fig.1).

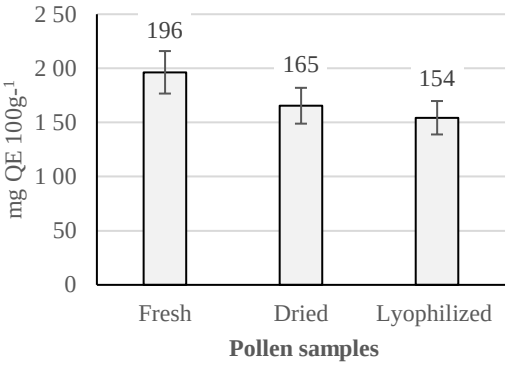

Figure 1. Content of total flavonoids

Flavonoid content in pollen is decreased by both thermal treatment and lyophilisation of pollen. Heat treatment destroyed 15.7%, while the lyophilisation 21.3% of flavonoids, compared to flavonoid content in fresh pollen. When comparing the flavonoid content of the thermally treated and lyophilized pollen, differences are not significant.

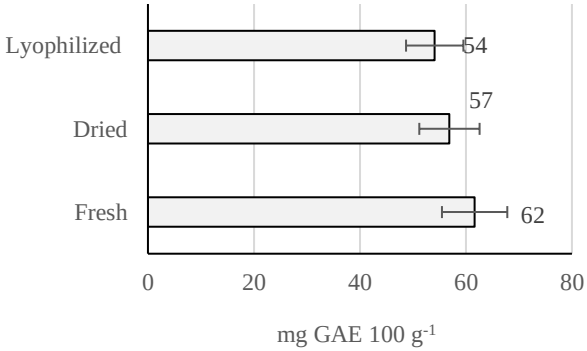

Figure 2. Content of total phenols

In dried pollen total phenol content in the dry matter is 57 mg GAE 100 g^{-1}, in lyophilized 54 mg GAE 100 g^{-1}, in fresh 62 mg GAE 100 g^{-1} (Fig. 2). The differences between the total phenolic content of dried and lyophilized pollen is similar – differing only by 2.1%, which is within the method errors limits.

The highest antiradical activity was detected in dried pollen – 85%, slightly lower in the freeze-dried pollen – 82%, while the lowest was in fresh pollen – 72% (Fig. 3).

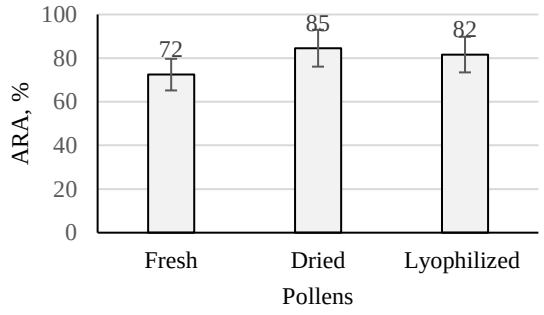

Figure 3. Antiradical scavenging activity

However, the differences between data was within error limit of the method i.e. ±10%. If highest and lowest error detection margin is taken in account regarding the data, then the differences are negligible.

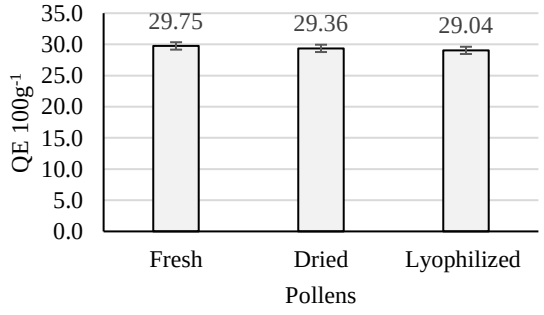

Figure 4. Content of total antioxidants

The total content of antioxidants (Fig.4) in dried and lyophilized pollen dry matter is similar - differing only by 2%, which is within the margin of method error.

Conclusions

The highest total flavonoid - 196 mg QE 100 g^{-1} and phenol content – 62 mg GAE 100 g^{-1} was found in fresh pollen, antiradical activity in analysed samples was insignificantly higher in dried and lyophilized samples, but the total antioxidants content of all samples was similar and in all the pollen ranged from 29.04 to 29.75 QE 100 g^{-1}.

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SHORT COMMUNICATION

ANTI-AGING COMPOUNDS IN LATVIAN WILD GROWING PLANT OF *FALLOPIA JAPONICA*

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Abstract

Fallopia japonica (Japanese knotweed) native to Japan is an aggressively invasive plant in the world, but also it contains a biological active natural compounds. Most important compounds of anti-aging by which can activate responsible gene sirtuin in nature is stilbenes *trans*-resveratrol (*trans*-3,4′,5-trihydroxystilbene), *trans*-piceid (resveratrol-3-β-mono-D-glucoside), flavonoid – butein (1-(2,4-dihydroxyphenyl)-3-(3,4-dihydroxy- phenyl)-2-propen-1-one), flavones – fisetin (3,3′,4′,7-tetrahydroxyflavone, 5-deoxyquercetin) and quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one). The aim of this study was to determine the anti-aging compounds in Latvian wild growing plant roots of *Fallopia japonica*. Before extraction roots of *Fallopia japonica* were convective dried at 60 °C. For the extraction of the anti-aging compounds from *Fallopia japonica* roots the ethanol / water solution with the concentration 70% (v/v) was used. High performance liquid chromatography (HPLC) method was used for analysis of anti-aging compounds: resveratrol, piceid, butein, fisetin, and quercetin. The obtained results showed, that the main anti-aging compounds detected by HPLC method in *Fallopia japonica* were stilbenes: resveratrol and piceid. Flavone – fisetin was detected in significantly lower concentrations than resveratrol and piceid. The remaining compounds, quercetin and butein, were detected in trace amounts.

Keywords: anti-aging, *Fallopia japonica*, trans-resveratrol, trans-piceid, HPLC.

Introduction

Along with the development of advanced technology, we are also developing a detection methods for variety of chemical compounds, as well as the identified compounds in the medical field.

The obtaining of eternal life elixir has been of great interest through human history, which has so far failed. This kind of magical substances or finding solution to this problem is extremely difficult, because the human body is a very complex life structure in which the variety of complicated biochemical transformations and processes take place and which is not completely researched. Aging is an inevitable process and it is linked to lifestyle, environmental and genetic structure (Si, Liu, 2014) and it is one of the most investigated research objects in years. One of the models, for longevity of human organisms are phytochemicals derived from plants. Phytochemicals may be from different classes: phenolic compounds, terpenes, polysulfides, quinones and polyamines (Leonov et al., 2015).

One of the phytochemicals bioactive compounds that influence the human aging, is stilbene phytoalexin *trans*-resveratrol (*trans*-3,4′,5-trihydroxystilbene) (Fig. 1), when they are infected by pathogens (bacteria, fungi) (Cademas, Packer, 2001; Stefani et al., 2007; Likhtenshtein, 2009).

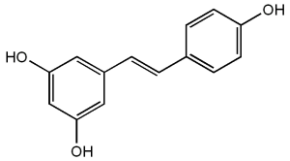

Figure 1. Chemical formula of *trans*-resveratrol

Plants are a great sources of *trans*-resveratrol and can be find in *Fallopia japonica*, red grapes, cranberry, blueberry and peanuts (Likhtenshtein, 2009).

Besides *trans*-resveratrol there is another phytochemical that influence human aging process – *trans*-piceid (polydatin, *trans*-resveratrol-3-β-mono-D-glucoside) (Fig. 2).

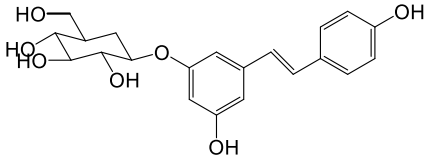

Figure 2. Chemical formula of *trans*-piceid

Trans-piceid is the glucoside of resveratrol (Fraga, 2010) that found in *Fallopia japonica*.

Third important phytochemical is butein (1-(2,4-dihydroxyphenyl)-3-(3,4-dihydroxy- phenyl)-2-propen-1-one) from flavonoid class (Fig. 3).

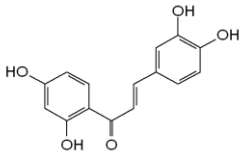

Figure 3. Chemical formula of butein

Fourth and fifth phytochemicals are two flavones: fisetin (3,3′,4′,7-tetrahydroxyflavone, 5-deoxy-quercetin) (Fig. 4) quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one) (Fig. 5)

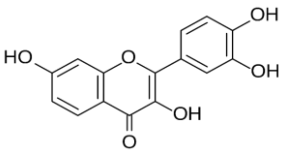

Figure 4. Chemical formula of fisetin

All five phytochemicals can increase the longevity processes of humans (see Figure 6).

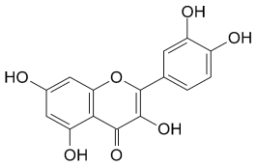

Figure 5. Chemical formula of quercetin

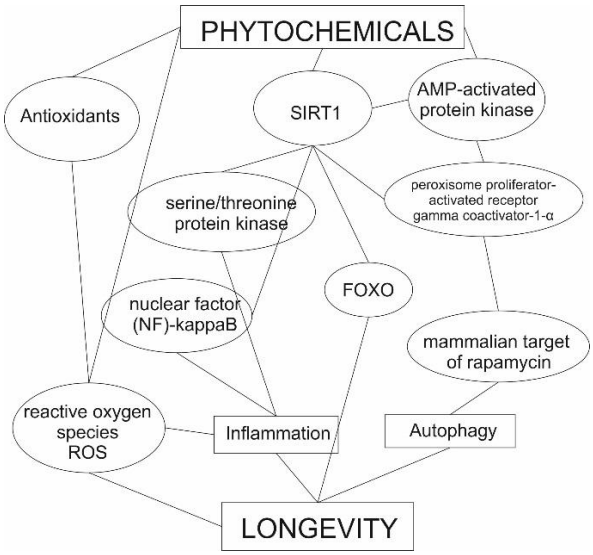

Figure 6. Increase of longevity with phytochemicals (Si, Liu, 2014)

Phytochemicals increase longevity directly or per antioxidants scavenge reactive oxygen species (ROS). Phytochemicals without ROS can activate AMP-activated protein kinase (AMPK) and SIRT1 (Sirtuin1) pathways, through inflammation and autophagy to longevity (Si, Liu, 2014). The aim of this study was to determine the anti-aging compounds in Latvian wild growing plant roots of *Fallopia japonica*.

Materials and Methods

Research was carried out at the Department of Chemistry, Faculty of Food Technology at the Latvia University of Agriculture. The object of the research was roots of *Fallopia japonica*.

Reagents

For research HPLC grade chemical reagents were used after filtration through a 0.45-µm pore size membrane filter. Ultrapure water was used in all work. *Trans*-resveratrol, *trans*-piceid, butein, fisetin, and quercetin was obtained from Sigma-Aldrich.

Preparation and extraction of samples

Roots of *Fallopia japonica* were harvested in the end of December of 2017 from Ogre district. Roots were convective dried for 24 hours at temperature +60 °C. After drying sample was ground n a laboratory mill and fitted through a 0.2 mm sieve. Powder of roots of *Fallopia japonica* 2.5 g was extracted on the magnetic stirrer with 11.0 mL of ethanol / water solution with the concentration 70% (v / v) for 60 min. Extract was centrifuged at 13 000 rpm in a centrifuge for 5 min, the supernant was then filtered through 0.45 µm pore size membrane filter and were kept at temperature of -18 °C until HPLC analysis.

Detection of trans-resveratrol, trans-piceid, butein, fisetin and quercetin with liquid chromatography

Content of *trans*-resveratrol, *trans*-piceid, butein, fisetin and quercetin was determined by high performance liquid chromatography (HPLC) (Schimadzu LC-20 Prominence, Shimadzu USA Manufacturing Inc, Canby, USA), detector DAD SPD-M20A, Solvent Delivery Unit LC-20AD, Column Oven CTO-20A, Autosampler SIL-20A, System Controller CBM-20A and data system LCsolution software.

Preparation of calibration solution

Weight in 50 mL volumetric flask with narrow neck 5.8±0.1 mg *trans*-piceid, 4.9±0.1 mg *trans*-resveratrol, 2.0±0.1 mg butein, 6.0±0.1 mg fisetin, 5.6±0.1 mg quercetin and fill with ethanol till mark and mix. Calibration chromatograms of calibration solution are given in Figures 7, 8 and 9.

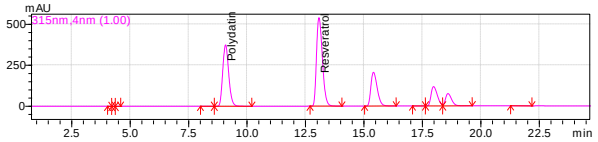

Figure 7. Calibration chromatograms of *trans*-piceid and *trans*-resveratrol

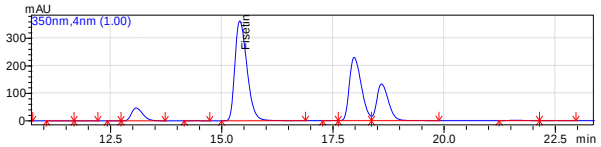

Figure 8. Calibration chromatograms of fisetin

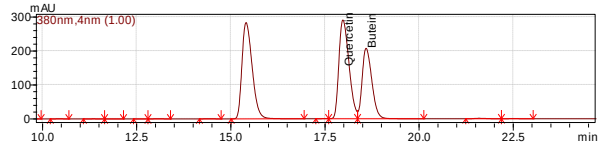

Figure 9. Calibration chromatograms of quercetin and butein

Parameters of chromatography: the analytical column YMC C18, 4.6 mm x 250 mm, 5 µm and temperature of column +30 °C were used for separation. Wavelength 315 nm for *trans*-piceid, *trans*-resveratrol, 350 nm for fisetin and 380 nm for butein, quercetin. Injection volume of sample 1 µL for *trans*-piceid, *trans*-resveratrol, 10 µL for fisetin, butein, and quercetin. Mobile phase: A (*deionized water)*, *B (*HPLC grade *CHROMASOLV®* methanol) and C (Acetic acid solution for HPLC) in the gradient conditions was used. Flow rate was 0.8 mL min^{-1}. Gradient conditions: start B (35 mL), C (2 mL); 15 min. B (55 mL), C (8 mL); 20 min. (55 mL), C (8 mL); 21 min. B (35 mL), C (2 mL); 30 min – stop.

Statistical analysis

The results were processed by mathematical and statistical methods (mean, standard deviation) using Microsoft Office Excel 2016.

Results and Discussion

The obtained results of research showed, that the main anti-aging compounds detected by HPLC method in *Fallopia japonica* were stilbenes: *trans*-resveratrol and *trans*-piceid (Fig. 10).

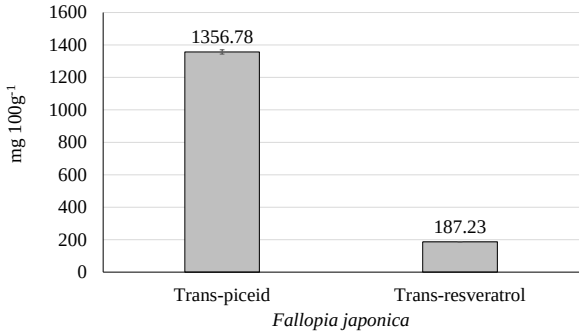

Figure 10. Content of *trans*-piceid and *trans*-resveratrol

In roots powder *trans*-piceid content was very high 1356.78 mg 100 g^{-1} comparing to other anti-aging compounds in the sample. *Trans*-resveratrol content 187.23 mg 100 g^{-1} was approximately seven times lower than *trans*-piceid. Another results showed that content of both substances can be in range from 670 to 1220 mg 100 g^{-1} for *trans*-piceid and 104 to 390 mg 100 g^{-1} for *trans*-resveratrol (Lin et al., 2016; Zhang et al., 2015; Jin et al., 2013). *Trans*-piceid is a glucoside and it is natural precursor of *trans*-resveratrol (De Maria et al., 2013). *Trans*-piceid is metabolized in the small intestine of human body to form of *trans*-resveratrol (Fig. 11) (Wang et al., 2013).

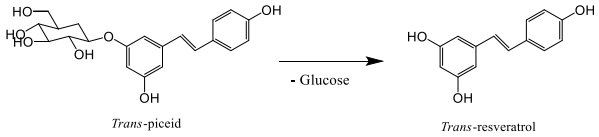

Figure 11. Biotransformation of *trans*-piceid to *trans*-resveratrol (Wang et al., 2013)

Besides *trans*-piceid and *trans*-resveratrol in plant root fisetin was detected in significantly lower concentrations. The remaining compounds, quercetin and butein, were detected in trace amounts (Fig. 12).

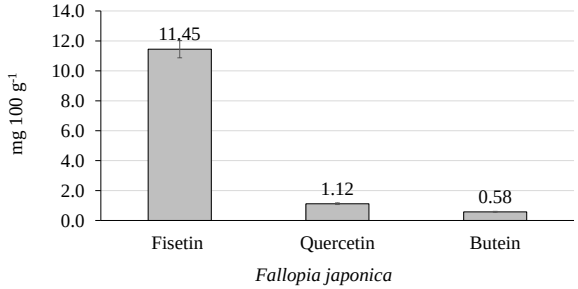

Figure 12. Content of fisetin, quercetin and butein

Fisetin content was 11.45 mg 100 g^{-1}, quercetin 1.12 mg 100 g^{-1} and butein 0.58 mg 100 g^{-1}, respectively

Conclusions

In research it was detected that the main anti-aging compound phytochemicals in extract of *Fallopia japonica* roots were *trans*-piceid and *trans*-resveratrol and those are good source for activating Sirtuin gene that increase longevity.

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SHORT COMMUNICATION

WATER SOLUBLE VITAMINS B_1, B_2 AND B_3 IN TRITICALE AND HULL-LESS BARLEY GRAINS

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Abstract

Whole cereal grains contain nutritionally significant quantities of vitamins that are important for health. The aim of this study was to assess the amount of vitamins B group in mature and immature triticale and hull-less barley. All grains were obtained from the experimental farm of Latvia University of Agriculture in immature (use the scale of Zadoks, GS75) and mature conditions (GS93) in 2016. The vitamins were determined by AOAC official methods. The highest content of vitamin B_1 (thiamine) was found in mature triticale (0.52±0.01 mg 100 g^{-1}). The amount of vitamin B_2 (riboflavin) was higher in immature triticale (0.40±0.01 mg 100 g^{-1}) compared with other grains mature or immature conditions. Vitamin B_3 (niacin) amount was higher in immature hull-less barley (7.11±0.01 mg 100 g^{-1}) than other grains mature or immature conditions. According to the results of the study can be seen that the immature grains can be used to enrich foods with water soluble B group vitamins. Immature triticale grains are great source of vitamins B_2 and B_3. The mature triticale and hull-less barley and immature hull-less barley, accordingly has potential for the use in production of functional foods and increasing nutritive value of products.

Keywords: immature and mature grains, thiamine, riboflavin, niacin.

Introduction

Human health is closely related with food used in their diet. It is well known that lack of vitamins causes serious violations, the nervous system, skin diseases, type two diabetes (Liu et al., 2000), also some cancers type and more.

For the harmonious function of the human body, it must be provided with vitamins daily. Consumption of wholegrain cereals in their diet can reduce the risk of disease and contribute to the preservation of health.

Therefore, the interest in cereals as a source of bioactive and functional ingredients has increased (Awika, 2011). Recent evidence suggests that the complex mixture of bioactive components gained of whole grain food may be more healthful than individual isolated components (Lui, 2004). Good potential for research is the whole grain triticale and hull-less barley.

Triticale (× *Triticosecale*) is a type of small grain created by genetically combining wheat (*Triticum*) and rye (*Secale*), which grows well in Latvia. Triticale can be used not only for the production of alcohol and animal feed, but also for the enrichment of food products with vitamins. Triticale grains, flour, and products made from this type of grain for consumers are available through both health food and commercial trading places, but, unfortunately, on a limited choice of range of products (Kalnina et al., 2014).

Hull-less or "naked" barley (*Hordeum vulgare* L. var. *nudum* Hook. f.) is a form of domesticated barley with an easier-to-remove hull. Barley has always been present in the human diet, and recent studies have shown that during the ripening of barley part of vitamins disappear in the mature kernels. In the last years appearing more publications about the possibility of using advantage of immature barley. For example, immature and mature hull-less barley is possible to use in the preparation of yogurt to increase their nutritional value, including B group's vitamins (Zagorska et al., 2015). Increased knowledge of the health-protective potential of the outer grain layers urges to re-think the way of using and processing grains for food, therefore hull-less barley role in the human food chain is growing more and more (Poutanen, 2012).

The aim of this study was to assess the amount of B group vitamins in mature and immature triticale and hull-less barley, growing and prevalent in Latvia.

Materials and Methods

The analyses were performed at the scientific laboratories of the Institute of Biology, University of Latvia, Salaspils.

Materials

Triticale (*Triticosecale)* and hull-less barley (*Hordeum vulgare* L. *var. nudum* Hook. f.) grains from the experimental farm "Peterlauki" (56° 30.658´ North latitude and 23° 41.580´ East longitude) of Latvia University of Agriculture was harvested at immature conditions (milk stage, use the scale of Zadoks, GS75) and mature conditions(GS93) in 2016. In experiments used grain varieties: triticale 'Ruja' and hull-less barley 'Irbe'. All grain samples with initial moisture content of 65% were dried in a microwave-vacuum dryer at 45–50 °C temperatures till moisture content 11.1%. For determination of B group vitamins, approximately 100 g of sample was ground in a laboratory mill Cyclotec 1093 (AB Foss Analytical, Sweden) and immediately used for analysis.

Methods

The vitamins were determined by AOAC official methods: vitamin B_1 (thiamine) – AOAC 986.27, vitamin B_2 (riboflavin) – AOAC 970.65, vitamin B_3 (niacin) – AOAC 975.14.

Statistical analysis

Statistical analysis was performed with statistical program SPSS 23.0 package for Windows 10. Mean value and standard deviation were calculated. ANOVA analysis was applied in order to see if there are

significant differences between the mature and immature wheat kernels ($p<0.05$).

Results and Discussion

Changes in chemical characteristics during ripening of triticale and hull-less barley are reflected in the diagrams below. The content of vitamin B_1 in the immature and mature triticale and hull-less barley samples are shown in Figure 1. The highest content of vitamin B_1 was found in mature triticale (0.52±0.01 mg 100 g^{-1}).

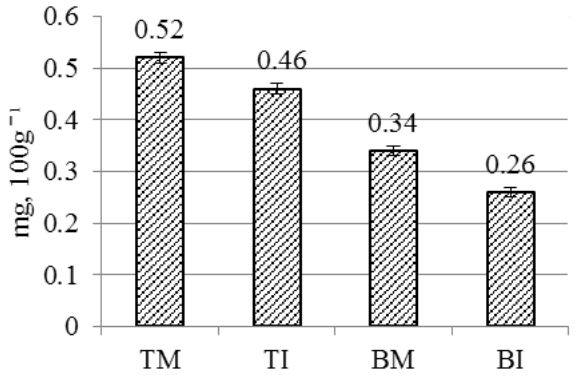

Figure 1. Content of vitamin B_1 (thiamine) in analysed grain samples

TM – triticale, mature grains, TI – triticale, immature grains, BM – hull-less barley, mature grains, BI – hull-less barley, immature grains.

The amount of vitamin B_2 was higher in immature triticale (0.40±0.01 mg 100 g^{-1}) compared with other grains mature or immature conditions in Figure 2. Difference between hull-less barley mature and immature grains is not so pronounced, however, immature grains has a higher vitamin B_2 content (0.25±0.01 mg 100 g^{-1}).

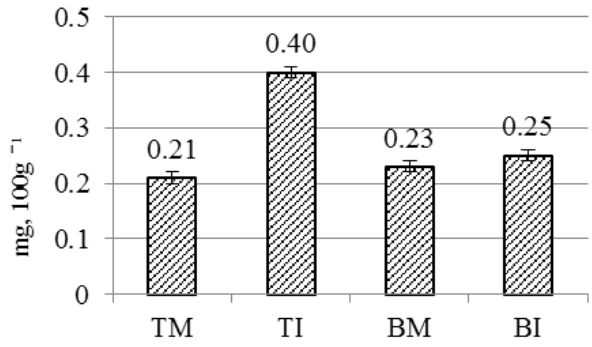

Figure 2. Content of vitamin B_2, (riboflavin) in analysed grain samples

TM – triticale, mature grains, TI – triticale, immature grains, BM – hull-less barley, mature grains, BI – hull-less barley, immature grains.

The results of vitamins B_1 and B_2 in mature triticale 'Ruja' presented by Kalnina et al. (2014) were lower (vitamin B_1 (0.36±0.20 mg 100 g^{-1}) and B_2 (0.10±0.02 mg 100 g^{-1})) than in this study. It could be explained by the annual variation in climatic conditions, also agricultural practices, soil, and technological practices applied. This only confirms that the chemical composition of grain depends on many factors, including the growing place, the weather, and the number of sunny days during harvest, and the storage of samples.

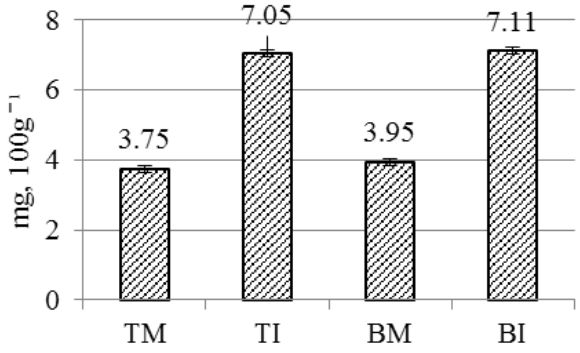

Figure 3. Content of vitamin B_3, (niacin) in analysed grain samples

TM – triticale, mature grains, TI – triticale, immature grains, BM – hull-less barley, mature grains, BI – hull-less barley, immature grains.

Vitamin B_3 (niacin) content was higher in immature hull-less barley (7.11±0.01 mg 100 g^{-1}). The vitamin B_3 content decreased more than by 46% for triticale, for hull-less barley 44%, findings clearly indicated that vitamin B_3 content decreased during the cereal ripening. In the immature grains are significantly higher (on basis of weight of the product) vitamin B_2 (riboflavin) (0.40±0.01 mg 100 g^{-1} in triticale; 0.25±0.01 mg 100 g^{-1} in hull-less barley) and vitamin B_3 (niacin) (7.05±0.01 mg 100 g^{-1} in triticale; 7.11±0.01 mg 100 g^{-1} in hull-less barley) content ($p<0.05$), but amount of vitamin B_1 (thiamine) in triticale and hull-less barley is higher in mature grains (0.52±0.01 mg 100 g^{-1} in triticale; 0.34±0.01 mg 100 g^{-1} in hull-less barley).

Conclusions

The amount of B group vitamins in triticale and hull-less barley is significantly affected by grain development stage at the time of harvest.

In the present research concentrations of B group vitamins (thiamine, riboflavin, and niacin) in immature triticale and hull-less barley compared to mature triticale and hull-less barley were established. The highest vitamin B_1 (thiamine) content is in mature triticale. Immature triticale and hull-less barley grains are good sources of the B group vitamins: B_2 (riboflavin) and B_3 (niacin), therefore could be representing a valuable ingredient for production of functional foods.

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