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Dear participants,

"FoodBalt 2008" conference is the first event dedicated to the 60th anniversary of Faculty of Food Technology at the Latvia University of Agriculture and addressed on fundamental and applied researches of food science. The conference covers a broad range or spectrum of subjects that relate to nutritional and safety aspects of food, approaches of modern technologies and application of new packaging materials for food products. Oral and poster presentations are chosen from competitive review of the proceedings.

On behalf of the Organizing Committee of the Conference and Faculty of Food Technology at the Latvia University of Agriculture we are proud and very pleased to welcome you in Jelgava to the "FoodBalt 2008" conference.

The Organizing Committee will wish you a happy and productive conference and looks forward to your active participation. We are convinced that your stay at Jelgava will be academically, educationally and socially rewarding.

Finally, to the readers of this issue, I hope that you will find the papers as valuable, stimulating and interesting for your scientific activities.

Inga Ciprovica
Dean of the Faculty of Food Technology
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WHAT MAKES RYE BREAD HEALTHY?

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Abstract

Rye bread baked of whole meal flour is a traditional element of the Finnish diet. The average consumption of rye bread in Finland is 66 g/d (women) and 100 g/d (men) (Findiet 2002). The rapidly expanding evidence from epidemiological studies about the protective effects of whole grain foods and cereal fibre against chronic diseases give a good background to study further the mechanisms by which rye bread may contribute to health.

In addition to its good and well balanced composition of macronutrients, rye features the highest dietary fibre content of all common grains. Rye is also rich in minerals, vitamins, sterols and phenolic compounds, such as phenolic acids, lignans and alkyresorcinols. These compounds, concentrated on the outer layers of rye grain, have many types of bioactivities (antioxidativity, anticarcinogenity etc) which may contribute to the health effects of rye bread. Our knowledge of their uptake and functions in the human body is quickly increasing.

Rye bread has in postprandial studies in healthy humans repeatedly shown to induce lower insulin responses that white wheat bread (Leinonen *et al*., 1999**,** Juntunen *et al*., 2002, 2003a). Intervention studies have shown that whole meal rye bread improves postprandial first-phase insulin secretion in oral glucose tolerance test, which is believed to be of importance for lowering the risk of type 2 diabetes (Juntunen *et al*., 2003b, Laaksonen *et al*., 2005). Most recently a diet rich in rye bread was shown to down-regulate gene expression in adipose tissue, including genes linked to insulin signalling and apoptosis, as compared to a diet rich in wheat and oat bread (Kallio *et al.,* 2007).

Rye bread is also known to improve bowel function (Gråsten *et al.*, 2000, 2007). Rye fibre is rich in arabinoxylan, which may positively affect the microflora of our gut, have prebiotic properties. Outer layers of rye bran also contain fructans, which are readily fermented by the gut bacteria and are known to be bifidogenic. The gut also acts as an important site for absorption of many of the phytochemicals of rye.

Key words: rye, health, dietary fibre, insulin response

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BARLEY AS A RAW MATERIAL FOR NOVEL FOOD PRODUCTS?

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Abstract

Barley is widely cultivated crop in Europe and North America and the total world production is around 140 million tonnes. Largest producers are Russian, Canada and Germany (FAOSTAT 2008). Vast majority of the barley crop is used as an animal feed. In addition the malting and brewing industry uses large quantities of barley and for example in Finland they account around 30% of the barley crop. Besides these traditional uses, barley has recentlygained popularity as a functional food ingredient.

The high beta-glucan content in barley makes it appealing for functional food concepts (Brennan and Cleary, 2005). In 2005 the US Food and drug administration, FDA, allowed the health claim for barley beta-glucan products. This allows labelling products with at least 0.75 grams of barley soluble fiber per serving having the ability to reduce risk of coronary heart diseases. The high level of beta-glucan and dietary fibre makes barley products also appealing for weight management products (Östman *et al.*, 2006). Besides beta-glucan and dietary fibre, barley contains also many other bioactive compounds. The nutritional role of these compounds is currently under extensive investigation (Bonoli *et al.*, 2004, Liu and Yao, 2007).

Processing of barley and barley ingredients requires different technological solutions than those adapted in wheat, rye and oat processing. The unpalatable hull attached to barley kernel needs to be removed from the barley that is aimed for food consumption. This dehulling process complicates the wholegrain definition of barley products and also effects the fractionation processing of barley. There is an extensive research activity to overcome the technological and sensory limitations related to barley baking technology. Gluten in barley is much weaker than in wheat and thus the availability of different barley bread types is more limited than wheat breads.

The presentation will give an overview of the current and potential food uses of barley and the research needs related to the exploiting the health promoting properties in barley products.

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THE CHEMICAL COMPOSITION OF ORGANIC AND CONVENTIONAL MILK IN LATVIA

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Abstract

According to the data in literature, when changing the agricultural system, animal keeping conditions and feed composition, the content of the product are influenced to a great extent. Therefore the aim of the present study was to investigate the chemical composition of Latvian organic milk.

A total 55 samples of raw organic milk, 20 of raw transitional period from conventional to organic agriculture, 20 of raw conventional milk samples were collected from different regions of Latvia. The mean content of protein, fat and lactose in organic milk was calculated and compared with the data from Latvia's State Agency "Agricultural data centre". The concentration of calcium, thiamine and riboflavin compared to conventional milk samples and the data from literature. The data was processed using the SPSS software package SPSS 11.0. and MS EXCEL.

The content of fat (4.98±0.08%) and lactose (4.85±0.04%) in organic milk samples was significant higher ($p<0.05$). In 32% of organic milk samples the content of urea was for 29.6 mg kg^{-1} lower than the minimum limit 150 mg kg^{-1}. The content of thiamin in organic milk samples was significantly lower ($p<0.05$) in comparison with the data from literature and conventional milk. Statistically significant difference between the organic and conventional milk samples ($p<0.05$) was found in the content of riboflavin.

The concentration of separate nutrients, as fat, lactose, thiamin and riboflavin in organic milk is significant different compared with conventional milk.

Key words: organic and conventional milk, chemical composition.

Introduction

Organic agriculture as an independent sector in Latvia exists from the 20th century nineties. The aim of organic agriculture is to create an integrated, human and environmentally friendly, economically well – balanced agricultural system, which rests on renewable raw materials of a local origin. Organic agriculture protects the cultivated plants from pests and illnesses, and provides agricultural animals with high quality feed. By developing of organic agriculture it is possible to reduce the negative influence of agricultural technology on environment and to improve the quality of obtained products, because the use of pesticides, organic compounds, exciters for growing, veterinary drugs and antibiotics are restricted in organic agriculture. During the decrease of public trust to genetically modified products, as well as due to animal diseases, the demands for organic food and the interest in them increase.

During the last years, the demand for organic food and the number of consumers, who assign more attention for high quality food and would like to know how it is produced, are significantly increasing. Organic agriculture is characterized by clear basic principles and the transparency of product origin, production and processing. There are all the necessary conditions in Latvia for production of qualitative livestock products for the internal market and as well as for export: land suitable for agriculture, multi–breed animal herds and ecological situation.

Scientists from different countries have very contradictory opinions about the chemical composition of organic milk. The complex evaluation of chemical composition of organic milk and the comparison it with conventional milk has not been performed in Latvia. According to the data in literature, when changing the agricultural system, animal keeping conditions and feed composition, the content of the product are influenced to a great extent. Therefore the aim of the present study was to investigate the chemical composition of Latvian organic milk.

Materials and Methods

The organic milk, conventional milk and milk obtained during transitional period (in further – the transitional period milk) were obtained from the farms "Lejasrembeni", "Jaunbiteni", „Kalna Gaurini", "Alejas", „Cemuri", which are located in Keipenes rural district, Ogres

region. Individual milk samples were taken from Riga, Cesis, Jelgava and Bauska region's farmers.

Milk samples from different breeds of cows were used for the research: 61% of *Latvian Brown*, 2% of *Holsteins Black* and 37% crosses of *Latvian Brown* and *Holsteins Black*. There are sufficient researches about the influence of cow's breed on the milk composition and quality; therefore the factor of breed was not taken into consideration in this research. The milk was obtained from healthy similar age cows. The scheme of taking milk samples was elected in way to eliminate a possibility to analyse colostrums, milk obtained during the late lactation, and milk obtained from mastitis cows.

A total 55 samples of raw organic milk, 20 of raw transitional period from conventional to organic agriculture, 20 of raw conventional milk samples were analysed.

The content of protein, fat, lactose, calcium, thiamin, riboflavin and urea were detected according to the standard methods (see Table 1).

Table 1

The standards of analysis

Indicators	Standard
The content of lactose	LVS ISO 5765–1:2003
The content of protein	LVS EN ISO 8968–5:2002
The content of fat	LVS EN ISO 8968–5:2002
The content of urea	LVS ISO 2446:1976
The content of calcium	LVS EN ISO 8968–4:2002
The content of thiamin	ISO 12081:1998
The content of riboflavin	AOAC 986.27

To evaluate the significant difference, the parameters were randomly arranged; parameters were detected for three duplications, the mean value of parameters was calculated. The mean content of protein, fat and lactose in milk was calculated and compared to the data from Latvia's State Agency "Agricultural data centre". The concentration of calcium, thiamin and riboflavin compared to conventional milk samples and the data from literature.

The data was processed using the SPSS software package SPSS 11.0. and MS EXCEL.

Results and Discussion

The content of lactose, protein and fat in organic milk and conventional milk is given in Table 2.

Table 2

The content of lactose, protein and fat in organic and conventional milk

Parameter	Organic milk			Mean value of conventional milk, %
	Mean value±standard error, %	Value, %		
		Min	Max	
Protein	3.30±0.04	2.24	4.99	3.34
Fat	4.98±0.08	3.50	7.69	4.42
Lactose	4.85±0.04	4.19	5.88	4.67

The mean content of protein in organic milk was 3.30±0.04%, which is not significantly different from conventional milk ($p>0.05$). The research results relate with many authors conclusions (Haggar, 1996; Byström, 2002; Mogensen, 2002; Toledo–Alonzo, 2003; Ellis, 2005), that the content of protein in organic and conventional milk samples had not significant difference. The research results contradict with Olivo (2005) statement that the content of protein is higher in conventional milk samples. The reason for lower content of protein can be lack of sugar–rich juicy feed, which stimulates production of butyric acid used for protein synthesis.

The mean content of fat was 4.98±0.08%; which is significantly higher than in conventional milk samples. The research results contradict with many authors (Mogensen, 2002, Toledo–Alonzo, 2003; Olivo, 2005) statement that fat content is higher in conventional milk.
The higher content of fat in organic milk could be explained with the differences in keeping and feeding conditions: high quality feed, well balanced and rich in cellulose, not chopped, in sufficient amount was available in organic farms; cows were always milked.
The mean content of lactose in organic milk samples was 4.85±0.04%, it significantly differs ($p<0.05$) from those of conventional milk. The results of research relate to Olivo (2005) statement that the content of lactose in organic milk is significantly higher. The higher content of lactose in organic milk can be explained by the higher concentrations of sugar in feed grasses of organic farms.
The content of urea in organic milk samples is shown in Figure 1. The urea content in organic milk samples ranged between 64.90 and 252.56 mg kg^{-1}. The mean content of urea in the analyzed organic milk was 167.43±9.64 mg kg^{-1}, which fitted in the common limits set for milk – from 150 to 300 mg kg^{-1}. In 32% of organic milk samples the content of urea was lower than the minimum limit – 150 mg kg^{-1}.

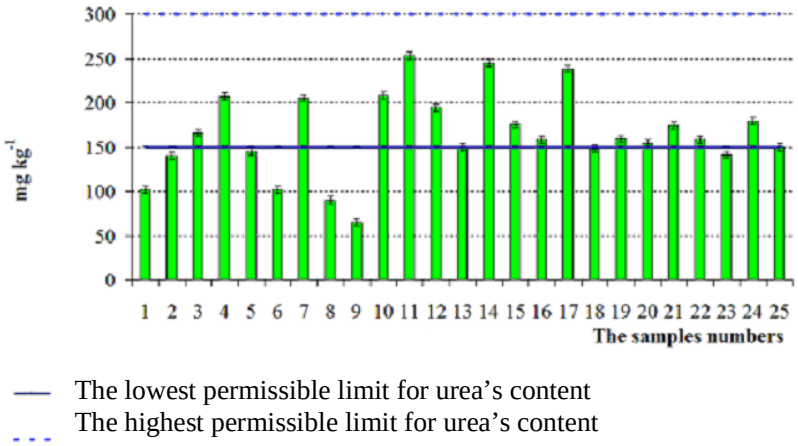

Figure 1. The content of urea in organic milk

The research results relate with Toledo–Alonzo (2003) results, where the author determines a significant difference in the content of urea in milk from different agricultural systems. The difference between the urea content in organic and conventional milk samples can be explained by stricter rules regarding to the amount of concentrate allowed in organic farms in comparison with the conventional farms.
The lower content of urea in organic milk samples could be explained by the influence of seasonal changes in milk. The lowest content of urea, according to Godden's (2001) published data, is from April to June.
The content of calcium in milk samples is shown in Table 3. The content of calcium in organic milk samples ranged between 20 and 25 mmol l^{-1}, the mean content of calcium was 21.90±0.22 mmol l^{-1}. However, a statistically significant difference in the content of calcium between the organic and the conventional milk samples was not found – 20.80±0.32 mmol l^{-1}.
The mean content of calcium in milk samples from different agricultural systems was significantly lower if compared with the data from literature ($p<0.05$)–30 mmol l^{-1}. Gorbatova (1997) mentions that the content of calcium in milk obtained in summer period, is lower in comparison with milk samples obtained in winter, it could explain the decreased calcium content in milk samples taken in summer and autumn months.

Table 3

The content of calcium in cows milk from different agricultural systems

Agricultural system	Number of samples	The content of calcium, mmol l^{-1}			
		Mean ±standard error	Min	Max	Neville *et al.*, 1994
Conventional agriculture	20	20.80±0.32	14.00	23.00	30.00
Transitional period agriculture	20	20.80±0.23	16.00	24.00	
Organic agriculture	20	21.90±0.22	20.00	25.00	

The cows kept in organic agriculture did not get mineral additives, so boosting of their immunity was connected only with facilitation of natural self–regulation processes. By means of that it is possible to achieve the same results as in the conventional agriculture, where animals are treated, without prophylaxis of any disease.
Within the framework of research the content of thiamin and riboflavin in milk was determined (see Figure 2).

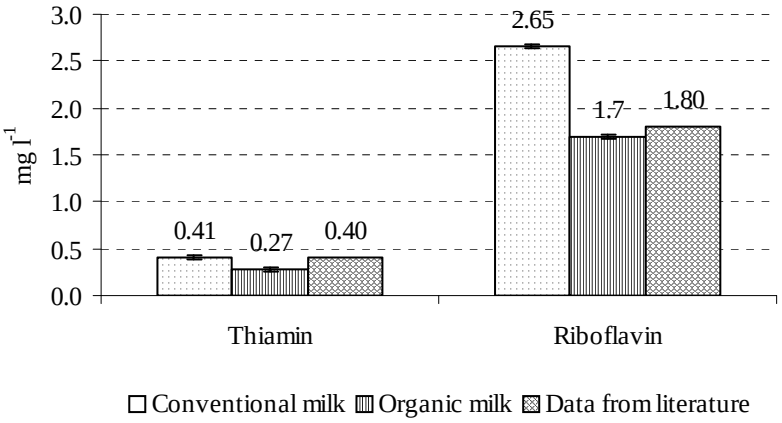

Figure 2. The content of thiamin and riboflavin in cows milk from different agricultural systems

The content of thiamin ranged between 0.20 to 0.32 mg l^{-1}. The content of thiamin in organic milk samples was for 34.1% lower if compared with the conventional milk and it was significantly lower ($p<0.05$) in comparison with the data from literature and conventional milk.
The content of riboflavin in organic milk ranged from 1.28 to 2.96 mg l^{-1}. The mean content of riboflavin in organic milk still was 1.70±0.10 mg l^{-1} and it was for 35.8% lower than in conventional milk, so a statistically significant difference between the organic and conventional milk samples ($p<0.05$) was found.
Many authors (Biesalski, 2002a; Biesalski, 2002b) have pointed that the content of thiamin and riboflavin do not vary in different seasons and that the feed composition has no significant influence on it. However there is still a possibility that feed can influence the content of thiamin and riboflavin in milk. While decreasing the dosage of concentrate allowed in organic farms, a lower concentration of thiamin and riboflavin in milk was established. Light contributes to decrease of the concentration of riboflavin in milk, therefore milking and pretreatment organization in organic farms is one of the most significant factors, which impact the concentration of this vitamin.

Conclusions

1. The concentration of separate nutrients in organic milk compared with conventional milk is different.
2. Statistically significant differences ($p<0.05$) between organic and conventional milk were found in content of largest milk component: fat and lactose.
3. The mean content of urea in organic milk was 167.4±9.6 mg kg^{-1}. In the 32% of organic milk samples the urea content was lower in comparison with the literature data – 150.0 mg kg^{-1}. It is explained with restrictions regarding to the amount of concentrate allowed, lower milk yields in organic farms and influence of season on milk chemical composition.
4. The concentration of thiamin and riboflavin was for 34.1% and 35.8% lower in comparison with conventional milk. It is explained with organisation of milking and milk storage process in farms and also with the low concentrate allowed used for feeding cows in organic herds.

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EFFECTS OF ENZYMES AND EXTRUDED WHEAT BRAN IN FIBRE–ENRICHED BREAD BAKING

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Abstract

Currently, increasing attention is given to enrichment of people's diet with dietary fibre. The aim of the present study was to improve the quality of fibre-enriched wheat bread by enzyme supplementation. The possible use of enzyme preparations of β-xylanase from *A.niger*, α-amylase from *A.oryzae* and glucoamylase from *A.niger* as bread improvers were tested in bread containing different amount (15–20 % of flour) of extruded wheat bran. Extruded bran was chosen for the positive nutritional and safety effects of the extrusion process. Extruded bran supplementation of wheat flour, in general, had pronounced effects on dough properties yielding higher water absorption and smaller extensibility in compare with those obtained without bran. The bran always contributed to a decrease of the bread volume and crumb porosity and changed the texture properties. Exploitation of enzymes considerably improved bread quality and prolonged shelf life. The tested multienzyme mix (β-xylanase, α-amylase and glucoamylase) was more efficient than individual β-xylanase in improving the bread quality. Enzyme addition allowed increasing the amount of bran up to 20 % without negative effects on bread acceptability.

Key words: wheat bread, extruded wheat bran, enzyme supplementation, dough properties, bread quality

Introduction

The importance of the dietary fibre is increasing due to the beneficial effects on the reduction of the cholesterol levels and the risk of colon cancer (Anderson, 1991; Tavani *et al.*, 2003). Health authorities, world-wide, recommend a decrease in the consumption of animal fats and proteins and an increase of cereal intake which is an important source of dietary fibre. Nevertheless, white bread is a commonly consumed type of bread. Therefore, the development of enriched bread with a higher dietary fibre content should be the best way to increase the fibre intake.

Bread can be enriched with dietary fibre, including bran, such as wheat (Sidhu *et al.*, 1999) and rye (Laurikainen *et al.*, 1998), β-glucans (Knuckles *et al.*, 1997), carob and pea fibres (Wang *et al.*, 2002). However the addition of fibres causes the neglected effect on the final bread quality. Bran supplementation usually weakens the structure and baking quality of wheat dough and decreases bread volume and elasticity of the crumb. The effect has been attributed to the dilution of gluten, which would affect the gas–holding capacity of the dough (Gomez *et al.*, 2003; Rosell *et al.*, 2006; Wang *et al.*, 2002). As the specific volume of bread is one of the important characteristics determining acceptability, different bran pre-treatment have been used to improve the volume of bread supplemented with bran. For example, washing the bran to remove harmful components, grinding the bran to obtain a smaller particle size, using various heat treatments to inactivate enzymes, or prefermentation of bran with yeast or with yeast and lactic acid bacteria have been successfully used to improve the quality of bread supplemented with bran (de Kock *et al.*, 1999; Salmenkallio-Marttila *et al.*, 2001). Also some of the negative effects of bran on gluten development can be compensated for by using of some additives such as gluten (Gan *et al.*, 1989) or baking enzymes (Laurikainen *et al.*, 1998; Shah *et al.*, 2006)

This study is dedicated to investigate the application of new sources of dietary fibre – extruded wheat bran to enriched wheat bread with a higher dietary fibre content. The beneficial effects of extrusion cooking on food safety and stability are well established in regard to inactivation of enzymes, some endogenous toxic compounds, and destruction of micro–organisms. The aim of the present work was to improve the quality of bran-enriched wheat bread by using carbohydrate degrading enzymes.

Materials and Methods

Commercial bakers' wheat flour (12.6% moisture, 12.4% protein, 28.5% wet gluten and 0.58% ash) was obtained from AB "Kauno grudai" (Lithuania). Extruded wheat bran (50.3% d.m. dietary fibre) produced using single-screw extruder was donated by a local flour mill Ustukiu malunas Ltd. (Lithuania). The enzyme preparations such as Bakezyme HSP6000 (β-xylanase from *A.niger*), Bakezyme P500 (α-amylase from *A.oryzae*), and Bakezyme AG800 (glucoamylase from *A.niger*) from DSM Food Specialties (The Netherlands) were chosen for the tests.

Dough characteristics. The effects of extruded bran and enzymes on dough rheology during mixing were determined by Brabender Farinograph (Brabender OHG, Duisburg, Germany) folowing the ISO 5530–1 (1993). All determinations were made at least in duplicate, and the average values were adopted.

Baking test. A straight dough breadmaking process was performed. Basic dough formula on 100 g flour basis consisted of salt (1.7 g), compressed yeast (1.5 g), and the amount of water required to reach a 500 FU of consistency. In the tested breads, the wheat flour was replaced by 15 and 20% of extruded bran. The effects of enzymes in breads with extruded bran were studied by adding β-xylanase (0.002 g 100 g^{-1} of flour) or multienzyme mix (0.002 g of β-xylanase , 0.0075 g of glucoamylase, and 0.0005 g of α–amylase of 100 g^{-1} of flour) to the dough. Each recipe mix was baked twice and three replicates were in each case taken for analyses.

Bread quality evaluation. Bread quality parameters included weigh, volume (determined by rapeseed displacement method [AACC Method 72–10, 1995]), specific volume, crumb porosity and texture. The crumb porosity was measured according to Lithuanian standard method LST 1442 (1996). Texture profile analysis (TPA) was performed using an Instron Universal Testing Machine Model 3343 (Instron Engineering Group, UK). Crumb slices of 1.3 cm were 40% compressed (compression rate 1 mm s^{-1}) and from the two bite force distance compression curve the texture parameters such as hardness, cohesiveness, springiness, gumminess and chewiness were derived (Armero and Collar, 1997).

Additionally acceptability test was carried out at the sensory laboratory of Food Institute of Kaunas University of Technology (Lithuania). A panel of 8 assessors was selected from the staff of the Food Institute according to ISO 8586–1 (1993). The overall acceptability of each sample was rated on a 150 mm hedonic line scale, where 0 means extreme dissatisfaction and 150 – extreme satisfaction.

Statistical analysis. Data obtained were analysed using statistical package SPSS for Windows (SPSS Ver.15.0, SPSS Inc., Il., USA, 2006). Significance of differences between control and treated samples was evaluated using Duncan's multiple range tests at a 5% level.

Results and Discussion

Influence of extruded bran and enzymes on dough properties. Extruded wheat bran suplementation (15–20 %) of wheat flour had pronounced effects on dough mixing behaviours measured by the farinograph (Table 1). Fibre addition mainly modified the water absorption. The 20% extruded bran increased the water absorption from 59.1 to 71.9 and decreased the dough softening from 88 to 59 FU. Similar effects on water absorption were observed when adding natural wheat bran or rye bran (Laurikainen *et al.*, 1998). Extruded bran addition did not modify the dough development time and stability, in opposition to the results reported by Laurikainen *et al.* (1998), who found a decrease of the stability when adding rye bran. The addition of β-xylanase decreased the water absorption of wheat dough with bran by 3%, approximately. The effect of β-xylanase on the other Farinograph characteristics was insignificant. Multienzyme mix had smaller effect on the degree of softening than that of the single β-xylanase used.

Table 1

Effect of extruded bran and enzymes on wheat dough mixing properties

Bread samples	Water absorption (%)	Dough development time (min)	Dough stability (min)	Degree of softening (FU)
Without bran	59.1±0.1	2.2±0.2	2.3±0.1	88±6.1
With 15 % bran	70.1±0.3	2.4±0.3	1.8±0.2	58±4.3
+ xylanase	67.2±0.2	2.0±0.7	2.0±0.1	57±5.2
With 20 % bran	71.9±0.3	2.5±0.4	1.9±0.4	59±3.1
+ xylanase	69.1±0.3	2.2±0.5	2.2±0.5	56±6.7
+ multienzyme mix	68.8±0.4	2.2±0.2	2.2±0.1	72±3.4

Influence of extruded bran and enzymes on bread quality. The effect of extruded bran supplementation on the bread quality characteristics is summarized in Fig. 1 and Fig. 2. The specific volume and crumb porosity always decreased as consequence of bran addition, which has been also reported by other authors (Knuckles *et al.*, 1997; Laurikainen *et al.*, 1998). The effect was more evident when levels of bran were increased from 15% to 20%. The 20% extruded bran reduced bread specific volume and crumb porosity by 31.4% and 7.2%, respectively, in compare with the control bread (Fig. 1). Carbohydrate degrading enzymes can be added to obtain larger bread volume and crumb porosity. The effect of multienzyme mix containing β-xylanase, glucoamylase and α-amylase was more notable than the effect of β-xylanase alone. In this case the specific volume of bread with 20% extruded bran increased by 44.4%, and crumb porosity by 6.8% in compare with bread without enzymes.

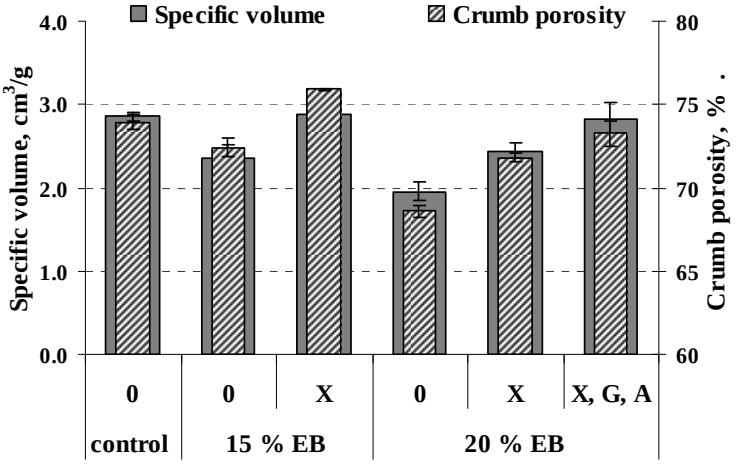

Figure 1. Effect of extruded bran (EB) and enzymes (X – β-xylanase, X, G, A – multienzyme mix, 0 – without enzymes) on bread quality parameters

Bread with extruded bran additions had significantly harder; less springy and cohesive crumb texture than the control bread (Fig. 2). The 20% extruded bran increased the crumb hardness by 2.3 times and decreased the springiness and cohesiveness by 45% and 57%, respectively, in compare with the control bread The changes in other texture parameters were not significantly different between the tested breads. The addition of enzymes modified texture parameters of wheat bread supplemented with bran. The bread made with β-xylanase as well as with multienzyme mix was rated less hard, but more cohesive than bread made without enzymes.

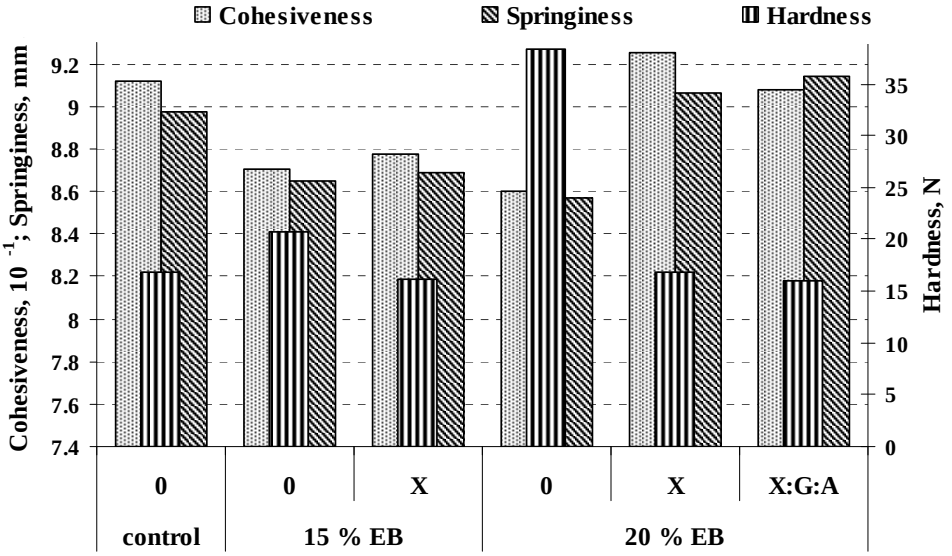

Figure 2. Effect of extruded bran (EB) and enzymes (X – β–xylanase, X,G,A – multienzyme mix, 0 – without enzymes) on bread crumb texture

Influence of extruded bran and enzymes on bread overall acceptability. Significant differences in acceptability were obtained with increasing the level of bran, which reduced the overall acceptability by 38% (Fig. 3). In general, panellists preferred bread without bran addition. Nevertheless, enzymes increased significantly the acceptability of bran supplemented bread. Panellists preferred bread with extruded bran and enzyme addition in compare with the control bread. Bread supplemented with 20% extruded bran and multienzyme mix obtained the highest score for acceptability.

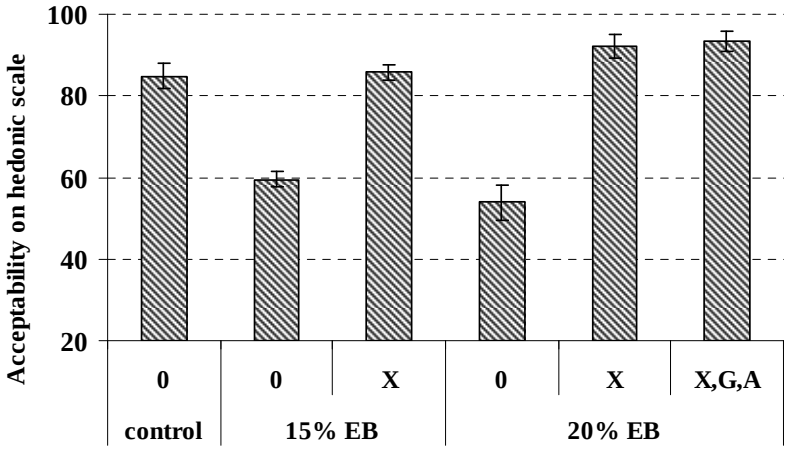

Figure 3. Effect of extruded bran (EB) and enzymes (X – xylanase, X,G,A – multienzyme mix, 0 – without enzymes) on bread overall acceptability

Conclusions

1. Extruded wheat bran supplementation (15–20%) of wheat flour had pronounced effects on dough properties yielding higher water absorption in compare with regular wheat flour dough.
2. The extruded bran decreased the wheat bread volume and crumb porosity and changed texture properties increasing the crumb hardness and decreasing the springiness and cohesiveness. The observed differences were more evident when levels of bran were increased from 15% to 20%.
3. Carbohydrate degrading enzymes can be added to improve quality of bread supplemented with bran. Multienzyme mix of β-xylanase , α–amylase and glucoamylase

showed better bread quality improving effects than β-xylanase alone. Enzyme addition allowed increasing the amount of extruded bran till 20% without negative effect on the bread acceptability.

Acknowledgements

This work is part of COST 928 supported by the Agency for International Science and Technology Development Programmes in Lithuania. The authors express special thanks to Ustukiu malunas Ltd. and DSM Food Specialties for supplying additives and Food Institute of Kaunas University of Technology for recruiting the sensory panel.

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HALF-FINISHED VEGETABLE PRODUCTS OF HIGH READINESS

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Abstract
Quality of production is of great importance in application of new technologies in catering enterprises. Foodstuff changes during preparation of food products take place. The perspective storage method for food is packaging in vacuum or modified gas atmosphere. Modified atmosphere packaging is the method to prolong storage time by days or even weeks, preserving high quality, original taste and texture appearance of the foodstuff, and improving overall cost-effectiveness. Packaged products have been stored in a chilled room in light at different temperatures. Qualitative changes of vegetables during storage have been investigated with instrumental methods. Regimes of storage time are determined, taking into account their influence on the structure-mechanical properties of the product. Elements of HACCP system are used.
Key words: vegetables, packaging, modified atmosphere, half-finished products.

Introduction
Quality of fresh vegetables is influenced by several factors:
1. internal factors (chemical content of food products, active acidity pH of the medium, water activity a_w, contamination level of raw material with micro-organisms);
2. external factors (pre-treatment and processing technologies, storage temperature, type and material of packaging, atmosphere in the package) (Soroka, 1995).

The most significant factor, characterising where and what food products consumer buys, is the safety and quality of the product which is provided by adequate packaging.
To comply with the current market demands, packaging has to provide: harmlessness, quality permanence of the food product, prolonged storage time, protection against mechanical and other external damages, protection against subordinate contamination, information availability about the packaged product and, the most important, it has to arouse consumer's interest. Packaging by use of modified atmosphere medium – MAP or vacuum is considered as potential methods in storage of food products without using preservatives (Hotchkiiss, 1999).
Therefore in order to determine the optimum type of packaging for salad mixes, the following objective is proposed: to investigate quality changes of fresh vegetable salad mixes during storage in packages with protective gasses – modified medium by applying HACCP principles.

Materials and Methods
Four different types of vegetable salad mixes from the farm "Ezerkaulini" are used in the research:
- red cabbage salad mix;
- kale salad mix;
- lettuce salad mix;
- cauliflower-celery salad mix.

Fresh vegetable salad mixes are used with the aim to estimate their quality and storage possibilities in the commercial network where their storage temperature changes from +2 ^{o}C to +6 ^{o}C, with the shelf-life of 7 days. Mixes are packed in a polypropylene box 190×140×50 mm, surrounded by a polypropylene bag of the size 260x190 mm, thickness 38 micrometers, applying gas mix: O_2 10%:CO_2 10%:N_2 80%. Polypropylene packaging is mechanically more durable and safeguards the packaged product against mechanical deformation, as well as its thermo stability from -30 °C to +140...+160 °C insures warming up of ready-made packed foodstuff for nutrition without removing it from the package. Net weight of the package is

300±0.5 g. All salad mixes are stored for 11 days at two different temperatures +2±1 °C and +6±1 °C, experiments carried out in three repetitions.

The following items are determined during storage:

1. Mass changes by using electronic scales *ACCULAB IV-600*;
2. Changes in active acidity pH level of the medium by using *INOLAB SELECTA pH 720 pH-meter*;
3. Determination of breathing intensity influence on the composition of protective gasses in the package during storage by using the analyzer OXYBABY ®V O_2/CO_2;
4. Colour changes by using the „ColorTecPCM/PSM" equipment in colour system CIE L*a*b*;
5. Content of ascorbic acid (vitamin C) in salads (mg l00 g^{-1}) is determined by iodine method (T-138-15-01:2002);
6. The total number of micro-organisms is determined according to *LVS ISO 4833:2003* with the method of dilution and sowings on Petri dishes.

Research is carried out at the research laboratories of Microbiology and Packaging material qualities at the Faculty of Food Technology, Latvia University of Agriculture.

Results and Discussion

Evaluating the technological process of making salad according to the requirements of HACCP system principles, as the possible stages in emergence of potential risk reasons turn out to be pre-treatment of vegetables, their storage before and after packaging and the very procedure of packaging. In all these stages of technological process the most important is the microbiological risk reason, but in the process of vegetable pre-treatment also the physical risk reason.

In order to prevent emergence of potential risk reasons, principles of good hygiene practice and principles of good production practice have to be strictly observed. Therefore attention was paid to the quality of drinking water used in the pre-treatment process, air pollution in the cooling chamber of the product, harmlessness of the packaging material and the very process of packaging.

As the storage process of packaged vegetables is one of the most important, changes of physical and microbiological quality indices are determined just in this stage of technological process.

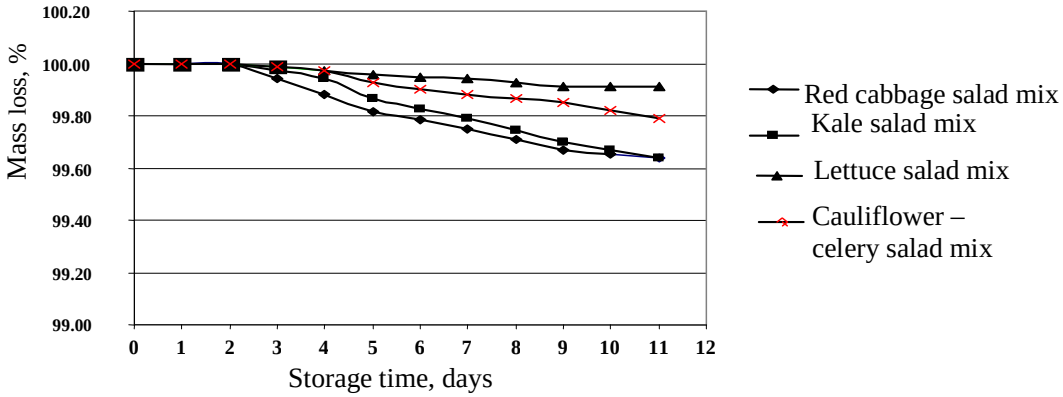

Figure 1. Changes of mass in salads during storage at the temperature 2±1 °C

Water vapour migration through the chosen packaging material is insignificant; during storage it provides slight mass loss. The packaged mass almost does not change till the third day of storage, mass loss ranges between 0.01–0.03% in comparison with the initial gross weight of the package.

The active acidity pH of vegetable cell sap essentially affects micro-organisms cell metabolism because bio-catalytic agents – enzymes of metabolism have pH interval of certain

activity. In sour medium, characteristic to the prepared salad mixes, cell sap prevents development of proteolytic bacteria, but facilitates development of yeast and mould.

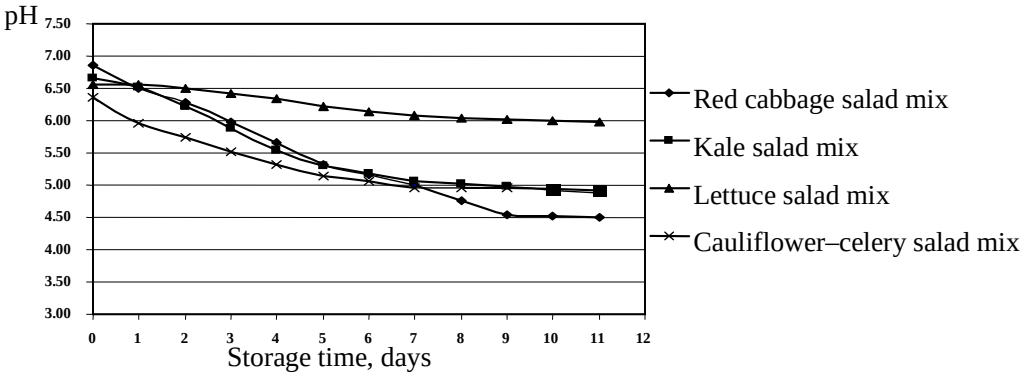

Figure 2. Changes in pH level during storage at the temperature 2±1 °C

Changes in active acidity pH indicate the development of micro-organisms and activity in the package during storage (Baumgart, 1993).

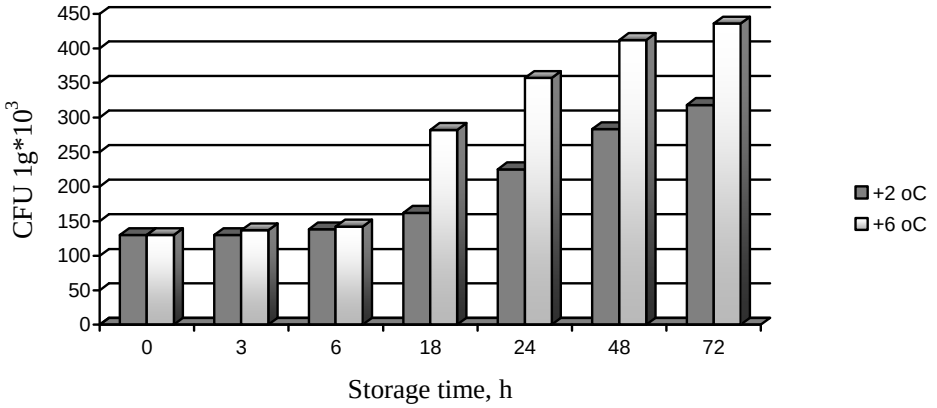

Figure 3. Changes in total number of micro-organisms in Lettuce salad during storage

By carrying out research it was ascertained that aerobic spore formers – bacteria of *Bacillus* family, were dominating. (These bacteria split organic compounds. The presence of *E. coli* form bacteria was not found in any of the prepared salad samples. The total number of bacteria starts to increase much faster after 24 hour long storage and during storage increases twice.

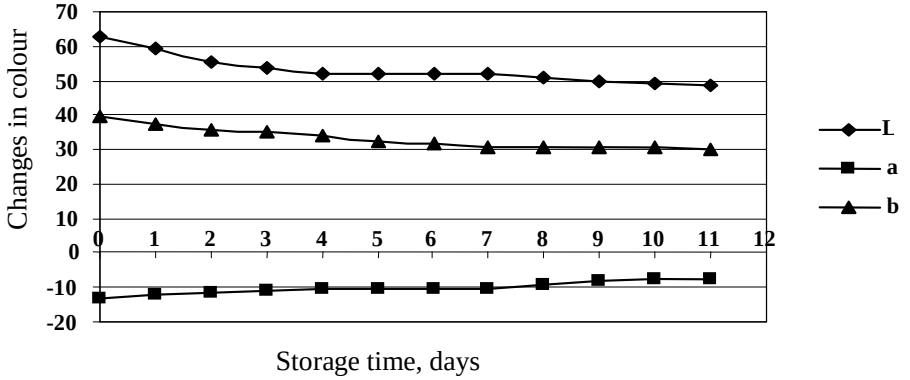

Figure 4. Changes in colour of „Lettuce salad mix" during storage at the temperature +2±1 °C

By estimating changes in colour of fresh salad mix with three-stimulus colorimeter, three quantities *L*, *a* and *b* are obtained. The quantity *L* denotes colour intensity, *a* – amount of red and green colour, *b* – amount of yellow and blue colour. Colour changes in package are estimated at two different temperatures. By comparing influence of temperature on stability of colour, it is observed that in higher temperature tissue colouring is faster, because during more intensive breathing process water vapour forms faster facilitating this process.
While storing lettuce salad mix at the temperature +2±1 °C it is observed that quantity *L* of colour intensity has changed from 62 to 49 at the end of the storage. During the first four days changes taking place are the fastest. Quantity *a* also changes from -14 to +2 and quantity *b* decreases from 40 to 30 at the end of storage. Similar results are obtained by storage of salad mix at the temperature +6±1 °C. Decrease of colour intensity could be explained by degradation of chlorophyll, which is facilitated by decrease of medium active acidity pH level (Zariņš, 2002).

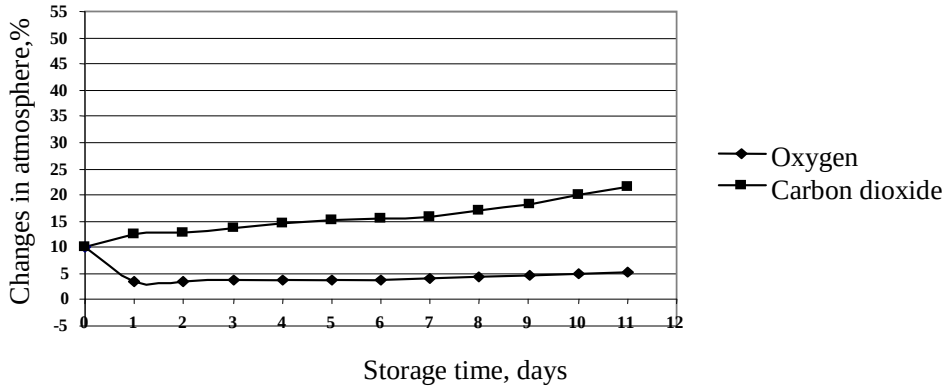

Figure 5. Changes of modified medium in the package during storage of Lettuce salad mix at +2±1 °C

Packaging is suitable for storage of Lettuce salad mix because composition of protective gasses changes slightly – carbon dioxide from 10% to 21% and oxygen from 10% to 6.2%. Oxygen concentration is sufficient to prevent anaerobic metabolism processes. The natural aroma of the product is well maintained during storage.

Discussion

Environmental temperature has an essential impact on number of products bacteria packed in modified medium because by increasing the temperature solubility of carbon dioxide decreases in the liquid stage of products wherewith it does not have so strong abilities to suppress micro-organisms.
Quality of products in modified atmosphere packaging is significantly influenced by temperature. The preferable storage temperature is in the range between 0±1 °C and +4±1 ^{o}C.
The natural freshness of vegetables and storage period are influenced by characteristic properties of the product as well as external factors. Thus the external factors influencing quality are the following: temperature; sanitary and hygienic conditions; gas atmosphere; production methods. The factors mentioned have crucial significance during the production and storage of the product.

Conclusions

1. The packaging used provides minimal mass losses.
2. Numerical quantity of pH decreases during storage what ascertains the fact that splitting of carbohydrates takes place and organic acids are formed.
3. The packaging material used for lettuce salad mix is suitable for storing salad up to 3 days because during storage changes in content of protective gasses O_2 are minimal, as well as

changes in total sum of micro-organisms taking place after 72 h storage period are admissible: from 10–6.2% and CO_2 from 10–21.6%.

4. The total number of bacteria increases for 2.2 times, when salads are stored for 72 hours at the temperature 2 °C, but for 3.2 times if stored at 6 °C.
5. When determining colour changes it is ascertained that colour intensity changes the most in Red cabbage and Cauliflower-celery salad mixes, but minimal changes take place in Kale and Lettuce salad mixes.

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SELENIUM AND CHANGES OF AMINO ACIDS CONTENT IN GERMINATED BARLEY GRAINS

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Abstract

The present work studies the effect of selenium (Se) on amino acids content in barley after soaking grains in solutions with different sodium selenate concentrations.

Barley (*Hordeum vulgare* L.) grains were soaked in sodium selenate solutions with selenium concentration 1, 5, 10, 25, 50, 100 and 200 mg l^{-1} for 5 days. Grains soaked in dionised water were used as control. Content of amino acids after germination was determined by Automatic Amino acid Analizator (AAA) T339 (Microtechna Praha).

Comparing content of separate amino acids after soaking grains in control solution and solutions with Se additives, we obtained that influence of Se depends on applied Se concentration. The highest increasing of amino acids content we observed regarding Valine – at Se concentration 50 mg l^{-1} and Methionine – at Se concentration 5 mg l^{-1}. Se concentration 100 mg l^{-1} gives increasing of 7 amino acids (Threonine, Serine, Glutamic acid, Glycine, Alanine, Leucine, Lysine). The highest applied Se concentration 200 mg l^{-1} promotes increasing of content of Tyrosine and Phenilalanine.

Selenium additives promote increasing of content of all investigated amino acids, excepted Isoleucine.

We can conclude that selenium influences the forming of amino acids in barley grains during germination and it depends on Se concentration in solution.

Key words: selenium, barley, amino acids, germination.

Introduction

The barley (*Hordeum vulgare L*) grain, like other cereals, contains different chemical compounds–carbohydrates (78–83 g 100 g^{-1}) proteins (8-15 g 100 g^{-1}), lipids (2–3 g 100 g^{-1}), minerals (2–3 g 100 g^{-1}), and small quantities of B-group vitamins, including thiamine (B_1), riboflavin (B_2), nicotinic acid, and pyridoxine (B_6), as well as panthothenic acid, biotion, folic acid, and vitamin E (5–6 g 100 g^{-1}) (MacGregor, Fincler, 1993). These components provide valuable nutrients required by humans and also domestic animals.

Barley is known for its high content of dietary fiber, which has been shown to lower plasma cholesterol, reduce glycemic index, and reduce risk of colon cancer (Anderson *et al.*, 1990; Jadhav *et al*., 1998, Slavin *et al.*, 2000).

Barley contains all the known tocols (tocopferols and tocotrienols) (Peterson, 1994). The concentration of α-D-tocotrienol, known as an inhibitor of cholesterol synthesis in liver of experimental animals, is higher in barley than in other grains. A second cholesterol inhibitor, α-linoleic acid, has also been found among the barley fatty acids. The recently recognized involvement of antioxidant compounds in preventing the formation of carcinogens from precursor compounds has directed attention to such barley compounds as phenolic acids, phytin, vitamin E, proanthocyanidins, and catechins (Slavin *et al*., 2000).

Barley is also a good source of B-group vitamins, especially thiamine, pyridoxine, pantothenic acid, as well as biotin and folacin. Phosphorus, potassium, and calcium predominate among the barley mineral components (Encyclopedia of grain science, 2004).

Cereal grains provided 45% of our total protein intake. Genotype, environment, and growing conditions affect the amount of protein in kernel. Protein quality is mostly dictated by amino acid content and digestibility. The apparent protein digestibilities in cereals range from 80–90% (Encyclopedia of grain science, 2004).

The distribution of various chemical constituents is not uniform throughout the component tissues of barley grains. The husk and pericarp, the two outermost and protective tissues of barley grain, consist primarily of cellulose, hemicellulose, lignin and lignans. The embryo is rich in protein (34 g 100 g^{-1}), lipids (14–17 g 100 g^{-1}), ash (5–10 g 100 g^{-1}), sugars (sucrose 15 g 100 g^{-1}) (Encyclopedia of grain science, 2004).

For all cereals, the most limiting amino acid is lysine. The next most limiting amino acids are tryptophan and threonine.

Amino acids are used both metabolically, as building blocks for protein biosynthesis, and catabolically, as energy sources. Catabolism for most amino acids proceeds through transamination pathways; the exceptions are lysine and threonine. Specific enzymes catabolize these nutritionally limiting amino acids: threonine dehydratase acts on threonine and lysine ketoglutarate reductase on lysine (Shewry *et al*. 1994, Encyclopedia of Grain Science, 2004).

Although selenium (Se) is not an essential element for plants, it is an essential micronutrient for both humans and animals. More then 20 different selenoproteins have been characterized, including glutathione peroxidases and thioredoxin reductase, which are involved in controlling tissue concentrations of highly reactive oxygen-containing metabolites (Forduce, 2005). The consumption of food provides the principal route of Se intake for the general population.

Average Se concentration in cereals amounts 0.024 mg kg^{-1} of grain dry matter, ranging from 0.006 to 0.122 mg kg^{-1}, for barley 0.06 mg kg^{-1}, for wheat 0.011 mg kg^{-1} on the average (Morris, Levander, 1970).

Low dietary ingestion of Se has been assumed to contribute to an increased risk of cardiovascular disease and cancer and to promote infectious viral diseases related to heart disease and AIDS. It is known that human body should contain 5–20 mg of Se (Combs, 2001). The Latvian reference nutrient intake for Se is 60 μg day^{-1} (Ministry of Welfare, 2001).

In the present work, the process of sprouting was used to enrich selenium in barley Sprouting additionally improves the nutritional value of seeds, for example, by a higher vitamin content, a better quality of protein, and some other parameters (Lintschinger et al., 2000). Sprouting grains causes increased activities of hydrolytic enzymes, improvements in the contents of total proteins, fat, certain essential amino acids, total sugars, B-group vitamins, and a decrease in dry matter, starch and anti–nutrients. The increased contents of protein, fat, fibre and total ash are only apparent and attributable to the disappearance of starch.

However, improvements in amino acid composition, B-group vitamins, sugars, protein and starch digestibilities, and decrease in phytates and protease inhibitors are the metabolic effects of the sprouting process.

The present study investigates the influence of selenium additives on changes of amino acids content during barley grains germination using solutions with different selenium concentrations.

Materials and Methods

The research was performed at the Laboratories of the Department of Chemistry at the Latvia University of Agriculture and at the Laboratory of Biochemistry and Physiology of Animals at the Institute of Biology of University of Latvia.

The barley grains were germinated for 5 days. Grains (100 g) were soaked in 500 ml of solutions containing 1, 5, 10, 25, 50, 100 and 200 mg l^{-1} of selenium in form of selenate at ambient temperature of 18±2 ºC for 6 h. The solution was then drained off and samples were germinated for 5 days under natural light conditions at 18±2 ºC. Every 24 h the grains were moistened with corresponding solution and carefully shaken. Control sample without selenium additives was prepared for comparison of obtained results.

After germinating all grains, which were soaked in selenium-containing solutions, 3 times were washed with 500 ml deionized water to prevent contamination of the surface of grains with the solution containing selenium. After that, the grains were put into plastic packs and stored at –18 ºC in a freezer for 24 h, then defrosted, dried and ground.

The content of amino acids was determined using AOAC Official Method 985.28 with Automatic Amino Acid Analyzator T339 (Microtechna Praha).

The germination was performed in duplicate and analysis was carried out in triplicate. The data given here are the mean values of the measurements.

Results and Discussion

Sprouts are rich in digestible energy, bioavailable vitamins, minerals, amino acids, proteins, beneficial enzymes and phytochemicals, as these are necessary for a germinating plant to grow. These nutrients are essential for human health.

Nutritive value of proteins is determined by amino acid content, especially the quantity of essential amino acids. Laboratory studies have showed that selenium additives have influence regarding the content of essential amino acids.

The data of changes of essential amino acids content in barley grains after germination using solutions with different selenium concentrations is shown in Figure1 and 2.

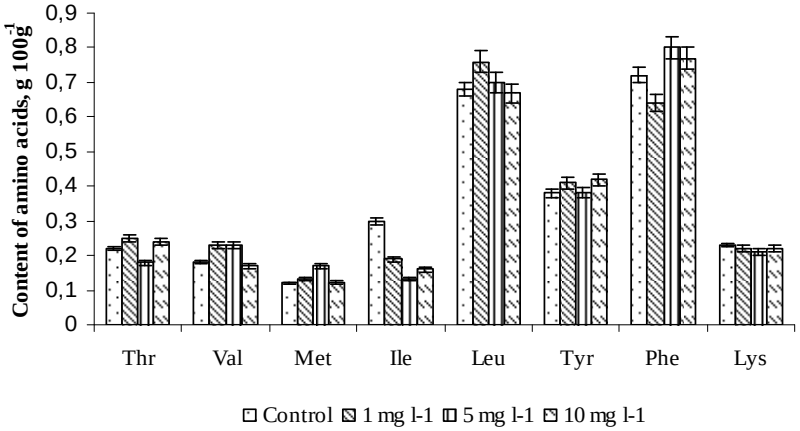

Figure 1. Content of essential amino acids at Se concentrations 1, 5 and 10 mg l^{-1}

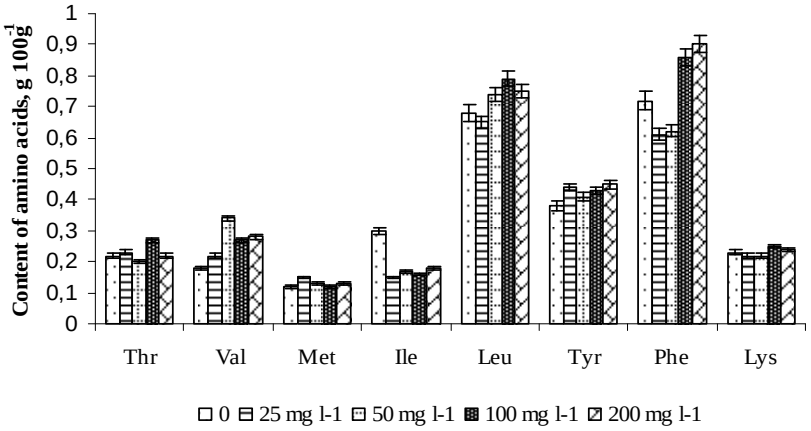

Figure 2. Content of essential amino acids at Se concentrations 25, 50, 100 and 200 mg l^{-1}

Analysing obtained results we can see that selenium additives promote changes of amino acids content in barley grains, and it depends on Se concentration. For, example, Se concentration 5 mg l^{-1} promotes the highest increasing of Methionine (Met) content. Se concentration 50 mg l^{-1} promotes increasing of Valine (Val), but concentration 100 mg l^{-1} promotes increasing of Threonine (Thr), Leucine (Leu) and Lysine (Lys) contents in barley grains. As mentioned above, the most limiting amino acids for cereals are lysine and threonine. After 5 days germination using solution with selenium concentration 100 mg l^{-1} it is possible to increase the content of these amino acids: for 16.2% (Lys) and for 22.7% (Thr).

The highest applied Se concentration 200 mg l^{-1} promotes increasing of Phenilalanine (Phe) and Tyrosine (Tyr) contents. Different concentrations of Se promote changes of all essential amino acids content except Isoleucine (Ile).

Figure 3 and Figure 4 show the changes of non–essential amino acids content in barley grains after germination using solutions with different selenium concentrations.

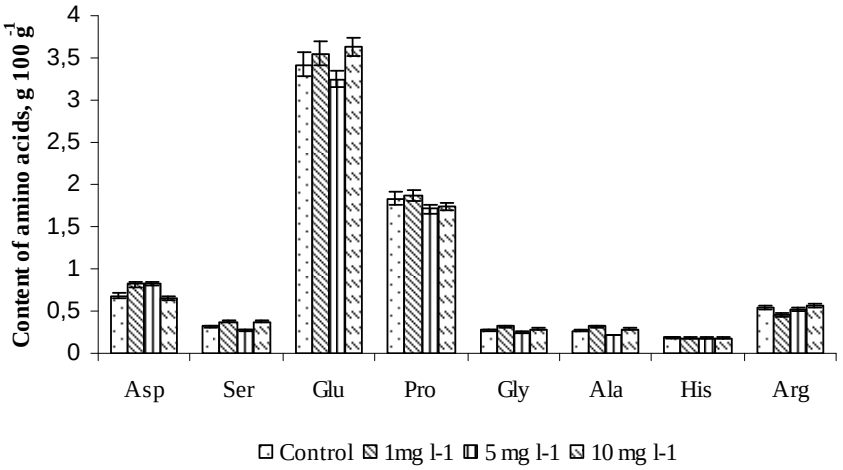

Figure 3. Content of non-essential amino acids at Se concentrations 1, 5 and 10 mg l^{-1}

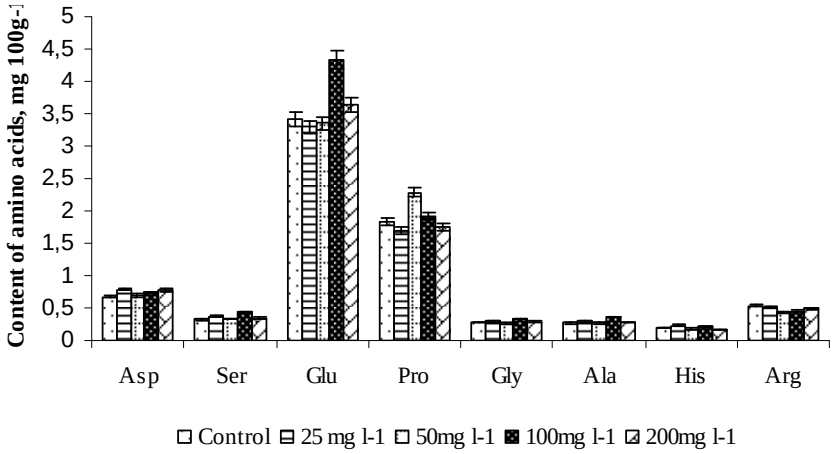

Figure 4. Content of non–essential amino acids at Se concentrations 25, 50, 100 and 200 mg l^{-1}

The obtained results are very similar regarding essential amino acids. As we can see, all applied Se concentrations promote increasing of investigated non–essential amino acids and it depends on Se concentration. For example, the one of the lowest applied Se concentrations – 5 mg l^{-1} promotes increasing of Aspartic acid (Asp), but Se concentration 10 mg l^{-1} promotes the highest increasing of Arginine (Arg). Se concentration 100 mg l^{-1} promotes the highest increasing of 4 non–essential amino acids: Serine (Ser), Glutamic acid (Glu), Glycine (Gly) and Alanine (Ala). Only the highest applied Se concentration 200 mg l^{-1} didn`t increased the content of investigated amino acids.

Conclusions

1. It is possible to change the content of amino acids in barley grains after 5 days germination using Se containing solutions.
2. Se additives promote increasing of content of all investigated amino acids except content of Isoleucine.
3. Se influences the forming of amino acids in barley grains during germination and it depends on Se concentration.

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SYNBIOTIC PROPERTIES OF FUNCTIONAL FOOD ADDITIVE FROM JERUSALEM ARTICHOKE TUBERS IN REGARD TO LACTIC ACID BACTERIA STARTER CULTURES FOR FERMENTED FOODS

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Abstract

Since health benefits of lactic acid bacteria and bifidobacteria consumption have been recognized extensive studies on specific health effects and technology aspects have been reported. Fructans are generally proved prebiotics and potent supplements for synbiotic foods, nevertheless the interaction of innovative prebiotic substances, starter cultures and involved probiotic strains, as well as food matrices should be evaluated for each one combination under consideration. A technology for production of fructan-containing Jerusalem Artichoke (*Helianthus tuberosus*) Concentrate (JAC) has been developed. The predominance of fructooligosaccharides (DP 3–8) as well as various macro- and micronutrients in the composition of JAC makes them different from a number of commercial fructan sources and provides good prebiotic properties, being generally suggested for this range of fructans. Supplementation of milk and oat- hydrolysate medium with JAC and subsequent fermentation with different probiotic dairy starters resulted in substantial stimulation of bacterial growth (probiotics *B. lactis* and *L. acidophilus* as well as *L. bulgaricus*) and acidification rate. The strain-specific responses of two general yogurt cultures *L. bulgaricus* and *S. thermophilus*, as well as probiotic strains by addition of JAC should have been considered during design of prebiotic starters and conditions of fermentations process. JAC is suggested to be perspective prebiotic fructan-containing additive for fermented synbiotic milks or oat-hydrolysate based products.

Key words: prebiotic, probiotic, fermented, milk, fructan

Introduction

The tenet "Let food be thy medicine and medicine be thy food," espoused by Hippocrates nearly 2.500 years ago, is receiving renewed interest. In particular, there has been an explosion of consumer interest in the health enhancing role of specific foods or physiologically-active food components, so-called functional foods. Clearly, all foods are functional, as they provide taste, aroma, or nutritive value. Within the last two decades, however, the term functional as it applies to food has adopted a different connotation - that of providing an additional physiological benefit beyond that of meeting basic nutritional needs (Hasler, 1998).

As scientific and technological advances develop in the field of health and nutrition, more and more focus has been directed toward the emerging field of functional foods. Biologically active components in functional foods may impart health benefits or desirable physiological effects. Functional attributes of many traditional foods are being discovered, while new food products are being developed with beneficial components. Consumer interest in the relationship between diet and health has increased the demand for information and research about functional foods. Rapid advances in science and technology, increasing healthcare costs, changes in food laws affecting label and product claims, an aging population, and rising interest in attaining wellness through diet are among the factors supporting worldwide interest in functional foods. Credible scientific research indicates there are many clinically demonstrated and potential health benefits from food components. A large body of credible scientific research is needed to confirm the benefits of any particular food or component. For functional foods to deliver their potential public health benefits, consumers must have a clear understanding of and a strong confidence in the scientific criteria that are used to document health statements and claims. The scientific community continues to increase its understanding of the potential for functional foods and their role in health (IFIC, 2006).

Products obtained by lactic acid fermentation processes therefore are of special importance for functional foods since the synbiotic (probiotic & prebiotic) properties could be obtained by an adequate combination of lactic acid bacteria LAB or bifidobacteria (Schrezenmeir *et al.*, 2001), besides with prebiotic substances such as oligo- and polysaccharides of plant and

microbial origin (Semjonovs *et al.,* 2007a;b). The use of any prebiotic substance for the enrichment of fermented products provides its delivery into human gastrointestinal tract (GIT) and, hence, a stimulation of beneficial probiotic bacteria regardless of their origin (native GIT inhabitants or delivered by functional products). The overall prophylactic and therapeutic qualities of fermented functional foods substantially depend on the content of viable probiotic cells. Therefore it is necessary to achieve a high cell count for probiotic bacteria (e.g. *Bifidobacterium lactis*) at the stage of lactic acid fermentation of product by an addition of potent prebiotic substances (Semjonovs *et al.,* 2004; 2007a;b;2008). Fructose polymers and oligomers (Flamm *et al.,* 2001) as well as exopolysaccharides of LAB (Semjonovs *et al.,* 2008) should be considered as most appropriate additives for this purpose. However, bifidogenic properties of prebiotics, being well manifested in GIT, remain not so evident at the stage of fermentation of synbiotic product with probiotics containing starters, particularly against a high background of basic carbon source, e.g. milk lactose. Besides, it is necessary to evaluate possible growth responses of basic starter cultures (for instance, *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus* for yogurt production) to the prebiotic additives during fermentation process since varied relations between them could substantially affect overall sensory properties and technological requirements.

The perennial vegetable plant *Helianthus tuberosus* (Jerusalem artichoke) which can be successfully cultivated in Latvia is a promissing plant as regards functional food constituents, as well as tubers and fresh tops can be used as fodder. The tuber flesh of this plant is a rich source for ß-2.1 fructooligo- and polysaccharides (e.g., inulin) (Bekers *et al.,* 2007b) acting as sweeteners that not affect blood sugar level after ingestion, as well as dietary fibre, blood cholesterol lowering agent, efficient prebiotics, stimulators of calcium absorption in the digestive tract etc. (Flamm *et al.,* 2001).

The aim of this study is to assess the influence of Jerusalem artichoke concentrate (JAC) and other fructans sources on the growth of common constituent cultures of probiotic starters in milk and oat substrates and, thereby to evaluate the suitability of JAC for development of fermented synbiotic foods.

Materials and Methods

Probiotic strains *Bifidobacterium lactis* Bb12, *Lactobacillus acidophilus* La5, as well as milk cultures *Lactobacillus delbrueckii ssp. bulgaricus Lb12 (L. bulgaricus)* and *Streptococcus thermophilus* ST (Chr. Hansen, Denmark) were used. Two percent of overnight culture grown in the MRS-lactose medium (Semjonovs, *et al.,* 2007a) was used as an inoculum for fermentations of individual strains, as well as for following compositions of mixed probiotic starters: (A): *L. bulgaricus, S. thermophilus, B. lactis, L. acidophilus*; (B): *L. bulgaricus, S. thermophilus, B. lactis*; (C): *S. thermophilus. B. lactis. L. acidophilus).* The equal cell concentration for each constituent strain in starter compositions (A, B, C) was monitored by OD_{550} measurements of individual cell suspensions). Fermentations were performed in 250 ml flasks containing 150 ml either milk- or oat- media, at 37 ºC in the semianaerobic environment (BD BBL™ GasPak™ Anaerobic System Envelopes, USA). Viable lactic acid bacteria and bifidobacteria were enumerated by use of plate-count method in accordance with IDF 177A:1988. Titrable acidity (ºT) was determined by alkaline titration (0.1 N NaOH) of samples, using phenolphthalein as an indicator (ISO 750:1998). Skim milk medium was prepared from reconsituted (75 g l^{-1} tap water) skim milk powder (Valmieras piens, Latvia). The thermal and enzymatic treatments of commercial rolled oats and the preparation of oat medium were performed in accordance with LV 12304 (Bekers *et al.,* 1999). Each experiment was performed at least in triplicate and data are presented as averages, where Standard deviations did not exceed 10% of the mean.

Results and Discussion

A technology for production of Jerusalem artichoke concentrate has been developed (Bekers *et al.,* 2007a;b). Jerusalem artichoke concentrate powder besides a short chain inulin and fructooligosaccharides (up to 70% from the total carbohydrates) contains other valuable components: proteins, plant lipids, as well as K, Mg, Zn, Cr and several other macro- and microelements *(*Mullin *et al.,* 1994). The employed technology includes washing of tubers, chipping, and dehydration, roasting and grinding to obtain powder with 5–7% of water content. The chemical composition of JAC obtained in accordance with originally developed procedure *(*Bekers *et al*., 2007a*)* is (% dry mass): solids, 94.6; total carbohydrates, 63.7 (including fructans, 45.0; sucrose, 8.5; fructose, 3.4; glucose, 0.8; others 4.9); proteins, 17.1; lipids, 1.9; nucleic acids, 2.1. The taste properties of JAC can be regulated by activating exo-inulase, the enzyme found in tubers, as well as by regulating dehydratation and roasting temperatures. The taste of JAC slightly reminds the taste of rye bread or malt. JAC can be used as additive to confectionary, bread, fermented milks and other products. Fermented milk or cereal hydrolysate products are excellent carriers for development of probiotic bacteria *(*Bekers *et al.,* 1999; Semjonovs *et al.,* 2007b; 2008) and can be enhanced by fructans or other prebiotic substances *(*Semjonovs *et al.,* 2004; 2007a;b; 2008*).* The dried concentrate of Jerusalem artichoke (*Helianthus tuberosus*) tubers containing 45–50% (d. w.) of fructans together with other biologically active macro- and micronutrients differed from commercial fructan sources in respect to both chemical (Bekers *et al.,* 2007b) and prebiotic properties (Table 1 and 2). The HPLC analysis of fructans from Jerusalem artichoke concentrate (JAC) displayed a prevalence of fructan oligosaccharides (FOS) of varied degree of polymerization (DP 3 – 8) as compared to the relative contribution of inulin (DP ~ 31), disaccharides and monosaccharides.

Table 1

The influence of fructan supplements (2g 100g^{-1}) on the growth of probiotics *Bifidobacterium lactis* Bb12 and *Lactobacillus acidophilus* La5, as well as general yogurt cultures *Lactobacillus bulgaricus* Lb5 and *Streptococcus thermophilus* ST in milk, after 12 h fermentation

Cultures	Fructan-containing supplements						
	Milk (Control)	+Inulin[a]	+FOS[b]	+Raftiline[c]	+Raftilose[d]	+JAC	+ Levan[e]
	Cell-count, cfu ml^{-1}						
B. lactis	2.03x10^7	2.08x10^7	2.82x10^7	2.87x10^7	2.89x10^7	3.04x10^7	3.12x10^7
L. acidophilus	5.27x10^7	6.26x10^7	6.98x10^7	8.19x10^7	6.42x10^7	8.72x10^7	6.24x10^7
L. bulgaricus	6.20x10^8	nd	1.272x10^9	nd	nd	1.31x10^9	nd
S. thermophilus	9.37x10^8	nd	9.53x10^8	nd	nd	9.68x10^8	nd

a) Inulin (inulin of analytical purity; Sigma- Aldrich, Germany);
b) FOS (NutrafloraFOS®- fructooligosaccharides; Twinlab, USA);
c) Raftiline ST ® (food-grade inulin, Orafti, Belgium);
d) Raftilose L60/75 ® (food-grade fructooligosaccharides, Orafti, Belgium);
e) Levan - high molecular weight (2000 kD) ß-2,6 polyfructan, produced by *Zymomonas mobilis*

The observed fructan composition of JAC therefore confirms the occurrence of possible shift towards short-chain fructans caused by thermal treatment and/or action of endo-inulinases during a processing of native Jerusalem artichoke tubers which contain about 50% of inulin (DP 11–30) (Semjonovs *et al.,* 2007b).

Table 2

The influence of JAC (2.0 %) on the development of titrable acidity during fermentation of milk by various starters

Starter composition	Medium	Time, h		
		3	6	9
		Titrable acidity, ºT		
A ***B. lactis*** ***L. bulgaricus*** ***S. thermophilus***	Milk (Control)	24.0	34.2	42.0
	+ JAC	36.6	68.0	85.0
B ***B. lactis*** ***L. acidophilus*** ***L. bulgaricus*** ***S. thermophilus***	Milk (Control)	20.0	32.0	37.5
	+ JAC	34.4	71.3	100
C (ABT-type) ***B. lactis*** ***L. acidophilus*** ***S. thermophilus***	Milk (Control)	22.4	32.0	42.0
	+ JAC	33.4	80.0	101.0

The predominance of oligosaccharides (DP 3–8) in the composition of JAC obviously makes them different from a number of other commercial components and could provide good prebiotic properties, being generally suggested for this range of fructan oligomers (Flamm *et al.* 2001).

Table 3

The total cell-count and titrable acidity in oat-medium after 8 h fermentation by various starters

	A ***B. lactis,*** ***L. bulgaricus,*** ***S. thermophilus***		**B** ***B. lactis,*** ***L. Acidophilus,*** ***L. Bulgaricus,*** ***S. thermophilus***		**C (ABT-type)** ***B. lactis,*** ***L. acidophilus,*** ***S. thermophilus***	
	cfu mg^{-1}	**ºT**	**cfu mg^{-1}**	**ºT**	**cfu mg^{-1}**	**ºT**
Oat medium (Control)	$1.12x10^9$	88	$3.96x10^9$	94	$3.66x10^8$	82
+ JAC, 2 %	$7.31x10^9$	99	$4.74x10^{10}$	108	$5.83x10^9$	97

Supplementation of milk with JAC fermented by three different probiotic starters *significantly* increased the yield of bacterial biomass and acidification rate. Besides, increased cell-counts of probiotics *B. lactis* and *L. acidophilus*, as well as *L. bulgaricus* were observed. A relatively wide choice of universally known prebiotic fructan supplements were compared to JAC during growth of *B. lactis* and *L. acidophilus* in milk media. The effect of JAC substantially (7–38%) exceeded those observed for commercial food-grade fructan sources such as Nutraflora FOS®, Raftiline ST® and Raftilose L60/75 ® in respect of *L. acidophilus.* Relative growth responses of *B. lactis* also indicated slight (5–8%) but significant advantages of JAC in comparison with other fructan additives. The growth of *S. thermophilus* was only negligible (1.02–1.08 times) affected by addition of JAC.

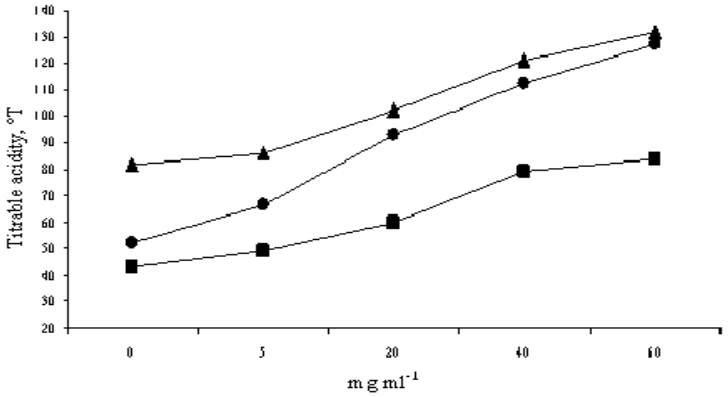

Figure 1. An increase of titrable acidity upon the various concentration of JAC in milk fermented by *L. bulgaricus* (▲), *L. acidophilus* (●) and *B. lactis* (■), after 12 h fermentation

The influence of JAC on the cell count of individual cultures involved as constituents in different starters during fermentation of milk or oat-hydrolysates (Table 2 and 3) showed the prebiotic properties upon *B. lactis* and *L. acidophilus*. The distinctions (Figure 1; Table 1) in responses of two general yogurt cultures *L. bulgaricus* and *S. thermophilus* to the addition of JAC, as well as another fructans (Table 1) could be of significance to design chemical, rheological and organoleptic properties of yogurts.

Conclusions

The above results suggest the good properties for application of JAC in the production of synbiotic oat- or milk-based fermented foods. The comparative evaluation of novel fructan-containing functional additive from Jerusalem Artichoke tubers showed very distinctive responses of probiotic cultures *B. lactis* and *L. acidophilus*, as well as two general yogurt cultures *L. bulgaricus* and S. *thermophilus* on the addition of fructan source to the food substrate under fermentation. An application of JAC for enhancement of fermented milks obviously requires an employment of certain food additives (starch, pectin etc.) for stabilisation of JAC particles and thus, an evaluation of possibly interacting stabilizer effects in regard to employed strains, as well as an overall accommodation of any particular fermentation process.The functional qualities of synbiotic fermented foods can be substantially improved due to enhanced cell-count of viable probiotics caused by addition of potent prebiotic substances (e.g. JAC or other fructan sources) to food substrates under fermentation. The prebiotic efficacy of JAC supplements shown to be of exceeding or equal worth as compared with other commercial fructan sources and, has been significantly manifested in fermentations performed by both, individual strains and starters.

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DEVELOPMENT AND ANALYSIS OF THE HAND ALCOHOL DISINFECTANT

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Abstract

Food cannot be exposed to the danger of pollution in its circulation. Hand hygiene is one of the simplest and most effective methods of preventing food poisoning by microbes. One of the most important parts of hygiene consideration is the hygiene of staff, including hand washing and disinfestations. It is connected with the right choice of disinfectant, actualizing the necessity to use low risk biocides which are completely biodegradable. Low risk biocide can't contain active substance which is bioacumulative, hardly degradable or classified as carcinogenic, mutagen and toxic to reproductive system or allergic. Low risk biocide is safe for environment and non-toxic to people. Ethanol is mentioned in the laws and legislations of the European Union as an active substance in the low risks biocides. According to European Union Biocide Directive (16.02.1998.) 98/8/EK alcohol hand disinfectants belongs to the first group of biocide as biocide for peoples hygiene. That is why a new formulation of a hand disinfectant on the base of ethanol has been made where besides the active substance, hand moisturizers and emollients are included. They help to keep the hand skin healthy, preventing its irritation. Alcohol hand disinfectant is a clear colorless gel, pH can vary from 7.0 to 7.7, the content of ethanol in mass is from 62% to 64%. It is important to ensure the bactericide and fungicide influence of the disinfectant. That is approved during research, according to LVS EN 1040:2006 (11.04.2006) and LVS EN 1275:2006(11.04.2006), use test microorganisms *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 15442, *Salmonella typhimurium* ATCC 14028, *Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 16404. A technology of manufacturing is made to secure the industrial produce of the disinfectant. A technical documentation for this disinfectant is made during this research, which includes the information required for the user of this product. Documentation includes the safety data sheet for this product, which informs about danger identification, specification, which presents information about the product stock qualities, product describing parameters, its terms of transportation and storage, its warranty period and technical task with information about the text on product label.

Key words: skin hygiene, hand disinfectant, microorganisms

Introduction

Skin hygiene, particularly of hands, is considered to be one of the primary mechanisms to reduce risk of transmission of infectious agents by both the contact and fecal- oral routes.

But depending on the product used, washing can raise the pH of the skin. Long-term changes in skin pH can cause skin damage, increased skin shedding, since some of the antibacterial characteristics of the skin are associated with its normally acidic pH (approximately 6.0). With prolonged soap contact, skin pH can reach 7.0–8.5 and remain high for 3–4 h. Soaps and detergents, particularly those that are anionic or cationic, are the most damaging of all substances routinely applied to skin (Larson, 1999)

Besides washing defeats the skin and the rate of lipid replenishment on the dorsum of the hands is only 20% after 1 h and 50% after 3 h. But fatty acids also have fungicidal and bactericidal activity important in modulating the skin flora (Larson, 1999)

It is important to recall that hand disinfection is significantly more efficient than standard hand washing with soap and water.

According to the field of application, strategies for the prevention of the transfer of microbial skin flora from the hands must consider the various categories of flora: transient, resident or infection flora. In contrast, resident skin flora is usually regarded as pathogenic only under certain circumstances such as in surgery. In the non-surgical field, only the transient and infection flora from the hands play a role (Pittet, 2001). Hands already contaminated may be rendered safe by procedures for the elimination of transients such as hand washing, hygienic hand wash.

Among all usable chemicals, ethanol, isopropanol and n-propanol (in the order of increasing efficacy) are the strongest and fasten agents. Most common used concentrations are: ethanol (60–90%), isopropanol (70–80%) and n-propanol (60–70%).The duration of treatment (between 30 and 60 s) significantly influences the achievable reduction of microbial release.

There exists a strong positive correlation of the reduction of microbial release and the hand treatment, between 1 to 5 min.
Alcohol preparations gives excellent spreading quality and rapid evaporation Alcoholic preparations are at least as tolerable for the skin as antiseptic detergents if they contain suitable emollients (Hygiene-Institute, 2001)
Other most popular active ingredients in hand disinfectants are: povidone-iodine, chlorhexidine gluconate and triclosan. But all of them are some specific negative properties compare to alcohol hand disinfectants.
The essential importance is antimicrobial efficacy of hand disinfectants witch are regulate by European Norms. A suspension test for the demonstration of bactericidal activity (EN 1040) is obligatory for hand disinfectants in all fields of application, but test to prove activity against yeasts applies only to hygienic hand rub (Division of Hygiene, 2006)
The purpose of work is to create the new hand disinfectant based on ethanol which is safe for environment.
Tasks:

1. To create prescription of alcohol hand disinfectant
2. To create manufacture technology of alcohol hand disinfectant
3. To define physical and chemical properties of alcohol hand disinfectant
4. To evaluate alcohol hand disinfectant – concentrate activity on test microorganisms: *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella thyphimurium, Candida albicans, Aspergillus niger*
5. To create a technical documentation of alcohol hand disinfectant

Materials and Methods
Characterize of raw materials included in alcohol hand disinfectant:
Monopropylenglycol. It is widely used in cosmetics and toiletries. Its emollient, solvent and non-toxic qualities are very important.
Glycerine. It serves as an excellent emollient, moisturizer and lubricant to prevent skin dryness in personal care products.
Neo-PCL. Consist of a mixture of fatty acid polyglycol ether and fatty alcohol polyglycol ether. As re-fatting and over-fatting agent, it makes skin soft, smooth and elastic. It is moisturizer and emollient. This ingredient regulates lipid balance in skin.
Acrylate polymer. It is used as thickening agent of hydroalcoholic hand disinfectants systems. Provides excellent skin feeling, isn't sticky and has very high clarity in the hydroalcoholic systems.
Trietanolamin. It is used as neutralizing agent.
Deionized water. Deionized water is specially prepared of drinking water in EUROWATER equipment. It is important because di-and multi-valent ions (like calcium and magnesium) will precipitate polymers. Using of deionized water essential influences clarity and viscosity of ready product (Table 1).
Perfume. **(***Citrone*)
Alcohol. It is used as active antimicrobial ingredient in appropriate concentration.
Bactericidal and fungicidal activity is tested according standards:

1. LVS EN 1040:2006 (11.04.2006.) „Chemical disinfectants and antiseptics – Quantitative suspension test for evaluation of basic bactericidal activity of chemical disinfectants and antiseptic – Test method and requirements.
2. LVS EN 1275:2006 (11.04.2006.) „Chemical disinfectants and antiseptics – Quantitative suspension test for evaluation of basic fungicidal or basic yeasticidal activity of chemical disinfectants and antiseptic – Test method and requirements.

Recommendation „Control of efficacy of disinfection in medical institutions", 1988.

Acording with standards alcohol hand disinfectant – concentrate is tested on test microorganisms:

1. *Staphylococcus aureus* ATCC 6538
2. *Pseudomonas aeruginosa* ATCC 15442.
3. *Aspergillus niger* ATCC 16404
4. *Candida albicans* ATCC 10231
5. *Salmonella typhimurium* ATCC 14028

Results and Discussion

As result prescription of alcohol hand disinfectant was made. It includes all raw materials in balanced concentrations. Concentration of active ingredient – alcohol is 62%.

A technology of manufacturing is made to secure the industrial produce of the disinfectant. To make alcohol hand disinfectant raw materials are put in reactor in following order:

Deionised water→ Acrylate polymer →Aroma →Alcohol → Neo-PCL → Glycerin → Monopropylenglycol → Trietanolamin.

After mixture of all ingredients as result is clear, transparent gel with small bubbles in mass (Table 2).

Table 1

Physical and chemical properties of water

Parameters	Water		
	Drinking water	**Specially prepared water in EUROWATER equipment 2nd level**	**Deionised water in EUROWATER equipment 3rd level**
Conductivity, µS cm^{-1} (25 °C)	615±1	612±1	5.9± 0.1
pH (20 °C)	7.32±0.01	7.5±0.01	5.66±0.01
Hardness, mmol l^{-1}	3.0±0.02	0.3±0.01	0.1±0.01
Fe^{2+} + Fe^{3-}, mg l^{-1}	1.06±0.1	0.05±0.1	0.03±0.1
Ca^{2+}, mg · l^{-1}	35±1	1	–
Cl^-, mg l^{-1}	12±1	12±1	–
SO_4^{2-}, mg l^{-1}	30±1	17±1	–
Turbidity, NTU	9.78±1	0.1	0.1

Table 2

Physical and chemical properties of Alcohol hand disinfectant

No.	Name	Parameters
1.	Appearance	Clear, transparent gel
2.	Colour	Colourless
3.	pH	7.0– 7.7
4.	Content of ethanol in mass %	62.0–64.0

According to results using this disinfectant in following concentrations 3 g and 7 g with exposure times 30 s and 1 min disinfectant can be declared as having a bactericidal effect on *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella thyphimurium,* and a fungicidal effect on *Candida albicans.* But it isn't effective against microorganism spore

according to research on *Aspergillus niger*. Alcohol gel shows only fungistatic effect on moulds (Table 3).

Table 3

Microbiological testing results of Alcohol hand disinfectant

Microorganisms suspension	Concentrate 3 g, 30 s	Concentrate 3 g, 60 s	Concentrate 7 g, 30 s	Concentrate 7 g, 60 s
Staphylococcus aureus **ATCC 6538**	Negative after 5 days incubation	Negative after 5 days incubation	Negative after 5 days incubation	Negative after 5 days incubation
Pseudomonas aeruginosa **ATCC 15442**	Negative after 5 days incubation	Negative after 5 days incubation	Negative after 5 days incubation	Negative after 5 days incubation
Aspergillus niger **ATCC 16404**	Positive after 48 h incubation	Positive after 48 h incubation	Positive after 48 h incubation	Positive after 48 h incubation
Candida albicans **ATCC 10231**	Negative after 5 days incubation	Negative after 5 days incubation	Negative after 5 days incubation	Negative after 5 days incubation
Salmonella typhimurium **ATCC 14028**	Negative after 5 days incubation	Negative after 5 days incubation	Negative after 5 days incubation	Negative after 5 days incubation

A technical documentation for this disinfectant is made during this research, which includes the information required for the user of this product.
Documentation includes the safety data sheet for this product, which informs about danger identification. Specification, which presents information about the product stock qualities, product describing parameters, its terms of transportation and storage, its warranty period and Technical task with information about the text on product label.
Product specification declares, that: Alcohol hand disinfectant contains skin softening and moistening substances. The cure creates pleasant refreshment feeling. It is meant for use in medical institutions, food turnover enterprises, and other public institutions. Biocide meant for humans hygiene. It does not constitute every day washing of hands. Inventory number of biocide is 19022007/1722.

Conclusions

Alcohol hand disinfectant is:

1. Fast-acting bactericidal and fungicidal agent with optimal anti-microbial spectrum
2. Low risk biocide which is safe for environment and non-toxic to people. Completely biodegradable.
3. Alcohols with the addition of appropriate emollients and moisturizers are less irritant on skin than any antiseptic or non-antiseptic detergents.
4. During complex hand hygiene providing procedure isn't possible biocide active substances neutralization by anionic surfactants.
5. It requires less time to put preparation on hands which is really important in status with high intensity of work
6. Alcohol hand disinfectant provides excellent hand care in conditions when water sources aren't available.

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MANUFACTURE OF MEAT PRODUCTS WITHOUT ADDED NITRITE OR NITRATE – QUALITY AND SAFETY ASPECTS

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Abstract

There is increasing interest in meat products processed with less or no additives. This applies particularly to 'organic' meat products. The use of nitrite in the processing of organic meats has been discussed controversially for many years. It is a synthetic chemical with toxicological concern but because of its effect on appearance and flavour of meat products, marketing of nitrite-free sausages and hams proved to be difficult. Moreover, nitrite contributes to the safety and shelf life of several meat products. However, this (antimicrobial) effect of nitrite strongly depends on the type of product and may be compensated, in most products, by adapting the processing conditions (e.g. by lowering the ripening temperatures for raw sausages; increasing the F_0 values for shelf-stable cooked sausages). The inhibitory effect of nitrite on oxidative deterioration of fats may be – within limits - compensated for by selection of the raw material and by limiting the access of oxygen. In dry sausages and raw hams with long aging times, an acceptable red colour which has been shown to consist of zinc protoporphyrin IX ("Parma Ham Pigment") is obtained without curing agents. This paper summarizes data of recent studies, in particular on the effect of nitrite on product safety and stability, and gives recommendations on the safe processing of meats with little or no nitrite added.

Key words: nitrite; curing; meat products; safety; quality

Introduction

The use of curing agents (nitrite, nitrate) in meat processing has been a controversial issue since the 1970s because toxicological concerns must be weighted against the benefits of curing agents to appearance, flavour, stability and safety of meat products. The last few years saw an increasing demand for meats with less additives. The "organic" meat sector (meat produced and processed according to Regulation (EC) 2092/91 as amended by Regulation (EC) 780/2006) grew rapidly in Germany and other countries, and even many manufacturers of "conventional" meat products look for possibilities to reduce the list of additives to these products. The Regulation (EC) 780/2006 only permits the use of nitrite and nitrate at reduced input levels and only if "no technological alternative giving the same sanitary guarantees and/or allowing to maintain the specific features of the product is available".

This paper discusses the role of curing agents in the manufacture of several meat products, and alternatives to their use.

Curing agents and colour of meat products. The mechanism of curing colour formation is well known and has been recently summarized by Honikel (2008). Since consumers associate pink colour of meat product with "freshness", marketing of greyish meats is difficult (Hamm, 2007). Recent discussion focused on the following two alternatives:

(1) In situ-formation of zinc protoporphyrin IX: During ripening of raw meats salted without nitrite, Fe^{2+} ions in the porphyrin ring are slowly replaced by Zn^{2+} ions, and the resulting reddish colour is reasonably stable (Wakamatsu *et al*., 2004). This explains the red colour of raw hams with extended ripening times. However, this alternative is, at present, not applicable to cooked meats and raw meat products with short ripening times.

(2) In situ-formation of nitrite from nitrate occurring naturally in components of plant origin: Some vegetables (e.g. celery) tend to accumulate nitrate even under conditions of limited nitrogen supply during cultivation. They could be added, in a dried form, to sausage mixtures. If these are pre-incubated at elevated temperatures (around 40 °C) in the presence of a suitable nitrate-reducing starter culture, small amounts of nitrite are formed that are sufficient for the formation of a pink colour during heating of the sausages (Fischer *et al*., 2005; Sebranek&Bacus, 2007). This method makes use of the fact that current food legislation normally does not require labelling of added enzymes, microbial starter cultures and compounds formed by these *in situ*. However, use of this method without proper labelling may mislead the consumer, especially buyers of organic meats.

Curing agents and flavour of meat products. The known effect of nitrite on the flavour of cured meats is mainly due to its ability to bind to Fe^{2+}. In this form, iron can no longer initiate oxidative changes of polyunsaturated fatty acids present in lipids. Attempts to replace this effect of nitrite by use of synthetic antioxidants had little if any success. To minimise undesired changes (in particular, rancid or warmed-over flavours), it is advisable to (i) use pork from animals fed a diet rich in tocopherol and poor in polyunsaturated fatty acids, (ii) exclude oxygen during the preparation and filling of the sausage mix as far as possible (Klettner & Troeger, 2000), (iii) use spices with high antioxidative capacity (e.g. rosemary), and (iv) limit the "best before" date of the products. The levels of nitrite formed *in situ* from "natural" nitrate from vegetables (see above) may also bring about an acceptable level of curing flavour (Fischer *et al.*, 2005).

Antimicrobial effects of curing agents: general remarks. European legislation on food additives (Directive 2006/52/EC) classifies curing agents as preservatives, i.e. as compounds inhibiting micro-organisms. This decision is mainly based on an expert opinion published by the European Food Safety Authority (EFSA, 2003) and focusing on the effect of nitrite on *Clostridium botulinum*. Indeed, nitrite has been shown in model systems and challenge studies (predominantly with products related to cooked hams or luncheon meat) to contribute to the inhibition of *Clostridium botulinum.* Nitrite also affects undesired Gram-negative bacteria in the early stages of sausage fermentation (Hechelmann *et al.*, 1974; Sanz *et al.*, 1997) and possibly *Listeria monocytogenes* (Duffy *et al.*, 1994). However, Grever & Ruiter (2001) and the EFSA (2003) pointed out that the antimicrobial effect of nitrite depends on many factors such as the pH and the levels and forms of iron in the product. For a comprehensive risk assessment, the probabilities of process failure and of non-detection of unsafe products during distribution and consumption, as well as epidemiological data and industrial experience must be taken into account.

Nitrite and product safety: some recent studies. Recent data of Stegeman et al (2006; see Table 1) confirm that use of nitrite at levels above 50 mg $NaNO_2$/kg delays growth of *Clostridium botulinum* in pasteurised Bologna-type sausages at 15 °C.

Table 1

Growth of *Clostridium botulinum* (inoculation level: ca. 100 spores/g; mixture of proteolytic and non-proteolytic strains) in canned Bologna-type sausages (boterhamworst) cooked to P_{70} =55 minutes and stored at 15 °C. No growth was observed at 10 °C or below. Data from Stegeman *et al.* (2006)

Batch	Water activity	$NaNO_2$ added (mg/kg)	Weeks until growth of clostridia
1	0.973±0.02	0	2–4
2		54	8–12
3		108	8–12
4	0.970±0.03	0	6–8
5		54	>12
6		108	>12

Using the approach of Hauschild (1982), Lücke & Hechelmann (1986; see Lücke & Roberts, 1993) calculated the probability of toxin formation during unrefrigerated storage of canned sausages heated to F_0 values of 0.3–0.4. This mimics the situation in traditional home-scale canning of meats which was identified as a major risk factor for botulism (see Lücke, 2003). The results (Table 2) show a marked effect of nitrite only for Bologna-type, not for liver sausages. They also indicate that the effect of nitrite may be compensated for by reducing the water activity. Alternatively, the heat treatment should be intensified by

increasing the F_0 value by about 0.6–0.8 units (resulting in a reduction of P by 3–4 units, assuming a D_{121} value of 0.2 for spores of proteolytic *C. botulinum* strains).

Table 2

Probability (P) of formation of botulinum toxin from a single spore present in sausage mixtures heated at 99 °C to about F_0 =0.34 and stored at 21 °C for 3 months. Data from Lücke & Hechelmann, 1986, quoted by Lücke & Roberts, 1993

Product	Water activity	$NaNO_2$ added (mg/kg)	$\log_{10}$ P
Bologna-type sausage	0.972	~80	-6.8
		0	-6.1
	0.979–0.982	~80	-6.9
		0	-3.9
Liver sausage	0.972	~80	-5.2
		0	-5.3
	0.979–0.982	~80	-2.5
		0	-2.2

Nitrite had only a small effect on growth of *Listeria monocytogenes* on cooked sliced meat products (Table 3). The effect of water activity was much more marked.

Table 3

Effect of water activity and nitrite on the growth of *Listeria monocytogenes* on cooked meat products. Data from Stegeman et al (2007)

Product	Water activity	$NaNO_2$ added (mg/kg)	Doublings of *Listeria monocytogenes* at 7 °C	
			within 14 days	within 21 days
Bologna-type sausage	0.965	79	<1	<1
		40	<1	~1.3
Cooked ham	0.973	79	11–12	11–12
		40	12–13	12–13

Nitrite and spoilage by micro-organisms. We determined the shelf life of six batches of cooked sliced vacuum-packed bologna-type sausages at 8 °C that had been prepared by one manufacturer without nitrite and with 80 mg $NaNO_2$/kg, respectively (Lücke *et al*., 2007). The products were spoiled by recontaminants (lactic acid bacteria, *Brochothrix thermosphacta*; see Borch *et al*., 1996). Judged by sensory evaluation, by pH decrease and by microbiological data, the vacuum-packed sausages analysed had a shelf life of about 5–10 days at 8 °C after being sliced without the usual precautions taken to avoid recontamination. Spoilage indicators were off-odours and aromas described as “sour” and “rancid”. In uncured sausages, these deviations were more pronounced and observed some days earlier, as also found by Graubaum *et al*. (2003), but this effect may be due to differences in the slicing process leading to higher initial counts of spoilage bacteria. Since „rancidity“ was observed in both cured and uncured sausages, it was rather due to short-chain fatty acids formed by *Br. thermosphacta* than to fat oxidation.

Psychrotrophic *Enterobacteriaceae* in the recontaminant flora were found to cause pink spots on a greyish product, probably by reducing traces of nitrate present in uncured sausages. This deviation will further reduce shelf life and acceptability.

Conclusions

It is difficult to compensate for the effect of curing agents on colour and flavour of meat products. On the other hand, the effect of curing agents on product safety and shelf life differs considerably according to the type of product. Under practical conditions, added nitrate has no antimicrobial effect, so it is difficult to understand why it is categorised legally as "preservative". Whether and how the manufacture of cooked meats should be modified if nitrite is omitted or if its ingoing level is reduced to 50 mg $NaNO_2$/kg or less may be summarised as follows (see also Lücke and Roberts, 1993; Lücke, 2003):

Perishable cooked meats not cooked in sealed containers are normally spoiled by recontaminants and may be expected to be stored under refrigeration. Thus, maintenance of the chill chain becomes even more important but no specific measures are necessary to compensate for any antimicrobial effect of nitrite.

Meat products pasteurised in sealed containers are often stored under insufficient refrigeration. Irrespective of nitrite addition, sausages containing liver or blood should be cooked to F_0 values above 1.5. Other sausages prepared without nitrite should be cooked to F_0 values above 1.0. Alternatively, the formulation should be modified to attain a water activity below 0.96 (for liver and blood sausages) or below 0.97 (for other products) and the products should be cooked to F_0 values above 0.4.

From literature data (see e.g. Lücke, 2003; Hummerjohann, 2004), the following recommendations for the safe manufacture of raw meat products can be derived:

Raw sausages without added nitrite should be fermented at 18 °C or below (such as traditional varieties prepared with saltpetre). At fermentation temperatures between 18 and 22 °C, use of appropriate starter cultures and sugars is necessary to ascertain a rapid pH decrease. Undried fermented sausages without nitrite addition should not be produced.

Raw hams can be safely produced by keeping the temperature of the meat below 5 °C until sufficient salt has penetrated into all parts of it to attain a water activity below 0.96. This is essential, irrespective of the use of curing agents.

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EVALUATION OF CHEMICAL COMPOSITION OF BIOLOGICALLY ACTIVATED DRIED RYE GRAINS

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Abstract

Cereal products are the important part of human diet, because those contain high amount of proteins, carbohydrates, B group vitamins and dietary fibre. Germinated seeds, grain and grain germs have been used in food for long time with the purpose to improve bread nutritive value.

The albumin content in rye grain composition is smaller, while maintenance of essential amino acids is by 1.5 times higher (lysine and threonine) compared with wheat grain. The biggest part of rye grain albumin dissolves in water and low-concentration salt dilutions. The increase of glutenin amount promotes strengthening of gluten. The amount of riboflavin and vitamin E in rye grain is higher.

The research was accomplished on grains of rye (variety 'Puhovchanka') harvested in Latvia in 2007. The grains were biologically activated in order to achieve the maximum biological value, and then dried in several conditions with a purpose to prolong grain storage time, to facilitate technological processes than to use such grains in wheat bread technology for increasing wheat bread nutritive value.

Results of our experiments shove that the optimal drying parameters of biologically activated rye grains are temperature +60±3 ºC and time approximately three hours. The content of vitamin E decrease in biologically activated rye grain during drying 1.2 times, of vitamin C – 1.3 times, of vitamin niacin – 1.8 times. The changes of albumen, fat, dietary fibre and Ca content in biologically activated rye grain during drying are not relevant. Increase content of oleic, linoleic, cys-11-eucosan, eruc and α-linolenic acids 1.5 times during grain drying process. The content of total aminoacids was lower comparing with initial grain aminoacids composition.

Key words: drying, biological activation, rye grains

Introduction

Rye – the Latin name is *Secale cereale* – is still generally regarded as the typical rye bread cereal. Nevertheless, there is a continuous decline in rye consumption. In the case of this bread cereal, too, it is chiefly winter rye that is used in bread. Although over 90% of the world's rye is growing in Europe, the cereal is by no means a uniform product. The main growing areas and breeding centres are in eastern, central and northern Europe, although rye is also grown and bred in North America, Australia and other regions (Popper *et al.,* 2006).

Rye's nutritional characteristics are similar to the other cereal grains, however rye is higher than wheat in fiber, vitamin E, riboflavin, folacin and pantothentic acid. And unusual for a cereal grain, rye contains twice as much of the amino acid, lysine as wheat. This is especially significant because lysine's the limiting amino acid in wheat and most other cereal grains which necessitates food mixing to develop a complete protein. This isn't a problem with rye as eating rye by itself gives you a well rounded protein. Rye's high fiber content, higher than the wheat, also aids in fighting heart disease (Bushuk, 2001; Rye, 2008[1]).

Although rye does have some gluten, it doesn't contain enough to make good bread and must be used with other high gluten flours. Because of this, rye bread is generally heavier than wheat bread and has a darker color, a reflection of the grain it comes from. The more wheat flour is used, the lighter and milder the bread. Pumpernickel is one of the breads on the rye heavy side of this spectrum, prized by many for its rich, dark brown color and strong flavor. Gluten is the main component characterizing dough quality. Gluten is a strong hydrolyzed gel, which mainly consists of albumen and carbohydrates, fats and minerals. The amount of gluten components depends on the variety of grain, flour type, preparing stage, and dough mixing and rinsing time.

Wet gluten of wheat grain consists of *gliadins* and *glutenins* (their ratio is 1:1), rye grain – of *gliadins* and *glutenins* (their ratio is 2:1), *barley* – of *prolamine*, *glutenins* and *hordeins* fractions. *Gliadins* decrease dough mixing time, whereas *glutenins* – increases it (Казаков, Кретович, 1989; Ruža, 2001; Казаков, Карпиленко, 2005; Rye, 2008[1]).

[1] Rye. 2008. Source: http://waltonfeed.com/self/rye.html, resource used on 28.01.2008.

The activity of enzymes increases during germination (biological activation): endohydrolase enzymes (α; β-amylases), proteolytic enzymes, diphenoloxsydase, and catalyse were activated. Stability of gluten depends on the amount of formed *disulfide* bonds *(-S-S-)* and *disulfide* bonds correlation with *sulfhydryl* group *(-SH-)* (Казаков, Кретович, 1989; Hugh Cornell *et al.*, 1998).

Optimal grain biological activation parameters are: relative air humidity – 80±2%, temperature – +34±1 °C, time – 24 hours. Gluten is not found in activated rye grains. Intense biochemical processes occur during the grain activation time, as a result grain biological value increases – the content of vitamins B_2, E and niacin, total sugar, dietary fibre and glycosamin increase; vitamin C is synthesized, and the content of irreplaceable amino acids is increased during the process of protein hydrolysis. Rye grains were biologically activated with the purpose to add such grain to wheat dough and to increase biological value of wheat bread. Biologically activated grain application in white bread technology is a new direction. As results of previous experiments show, the wheat bread with biologically activated grain additive had a higher biological value: higher content of vitamin B_2, niacin, E and C, dietary fibre, irreplaceable amino acid, total protein, total sugar, and glycosamin comparing with the test bread sample (Rakcejeva, 2006; Rakcejeva, Skudra, 2006; Rakcejeva *et al.*, 2007).

As practice show, the application of biologically activated rye grain in wheat bread production technology is very difficult and laborious process, because it is necessary to use biologically activated grains in bread production technology immediately; it is not possible to store such grains longer then six to eight hours, as a result the irreversible biochemical reactions will occur. The technology of rye grain drying will be developed for optimizing bread production technological process.

Drying process of biologically activated rye grains is not the same that malt production technology. In the malt production technology the grain germination time is from seven to nine days. For the bread production it is very long time for grain activation, because the gluten quality becomes unsatisfactory and it is not possible to produce high quality bread with such grain additive. In malt production technology, the temperature of germinated grain drying is from +80 to +85 °C and drying time is approximately to one day (Техноло–гия…, 2008[1]). We suppose that such temperature will be high for preserving of maximal quantity of vitamins in grains.

The aim of the current research work was set as follows – to study the changes of chemical composition of biologically activated rye grains during drying.

The following **objectives** are advanced to achieve the set aim:

- to determine experimentally the optimal parameters for biologically activated rye grain drying – temperature, and drying time;
- to investigate the changes of chemical composition of grain during the drying process.

Materials and Methods

For the research the following materials were used: rye grain (variety *Puhovchanka*), harvested in Latvia in 2007, drinking water for grain rinsing and steeping in compliance with Regulations No. 235 of the Cabinet of the Republic of Latvia „Compulsory Requirements for Harmlessness of Drinking Water", 2003[2].

Grain was washed (H_2O t=+20±1 °C) and wetted (H_2O t=+20±1 °C, τ=24±1 h). Grain biological activation was performed in the climatic chamber at temperatures (t) +35±1 °C at constant relative air humidity (φ) of 80±1% for up to 24 hours (τ).

The drying of biologically activated rye grain was accrued in thermo chamber on bolter with diameter d_b=0.185 m, the diameter of holes d_h=0.002 m, the square of bolter was S_b=0.030 m^2

[1]Технология производства пива. 2008. Source: http://www.beermarket.ru/beer/technology.htm, resource used on 23.01.2008.

[2] Dzeramā ūdens obligātās nekaitīguma un kvalitātes prasības, monitoringa un kontroles kārtība. 2003. Source: http://www.likumi.lv/doc.php?id=75442, resource used on 29.01.2008.

with loading capacity S=6.700 kg m^{-2}. Grain was dried till constant moisture content 13.78±0.50% for storage. The parameters of grain drying process were: t=+60±3 °C, τ=2.75 h; t=+70±3 ºC, τ=1.92 h; t=+80±3 °C, τ=1,60 h and t=+90±3 ºC, τ=1.40 h.
For determination of the chemical parameters changes in biologically activated rye grains during drying, standard methods were used:

Grain moisture (%) was determined under the standard method *LVS 272*.

Total protein content (%) was determined under the standard method *ISO 5983*.

Fat content (%) was determined under the standard method *ISO 6492*.

Wood-fibre content (%) was determined under the standard method *ISO 5498*.

Vitamin niacin content (mg kg^{-1}) was determined under the standard method *AOAC 961.14.*

Vitamin E content (mg kg^{-1}) was determined under the standard method *AOAC 971.30.*

Vitamin C content (mg kg^{-1}) was determined under the standard method *AOAC 985.33*.

The content of amino acids (g 100 g^{-1}) was determined under the standard method *AOAC 985.28.*

Content of Ca (%) was determined under the standard method *ISO 6490/2*.

The content of fatty acids (% of total fatty acids) – extract of biologically activated hull-less oats / wheat bread was analysed with GC-FID. GC conditions: column DB–WAX (30 m×0.32 mm×0.25 μm), carrier gas: nitrogen, constant flow rate 1.2 ml min^{-1}, temperature program: +60 °C (1 min), +10 °C (1 min), +250 °C (10 min), injector: +230 °C, split 1:1, detector: +280 °C. The samples were prepared as follows: a mix of hexane and acetones was added to 5 g of a homogenised sample; after extraction, 100 mg of fats were used for the analysis; cyclehexane and H_2SO_4 in methanol were added to the sample, the reaction was completed at a temperature +60 °C for 14–16 h; followed by cooling and centrifuging.
The testing included three reiterations.

Results and Discussion

Dynamics of absolute moisture content. The changes of absolute moisture content in biologically activated rye grain during drying process depending on temperature are shoved on Figure 1. As results of our experiments shove, decreases of moisture content are more intensive if the drying temperature is higher then +60±2 °C.

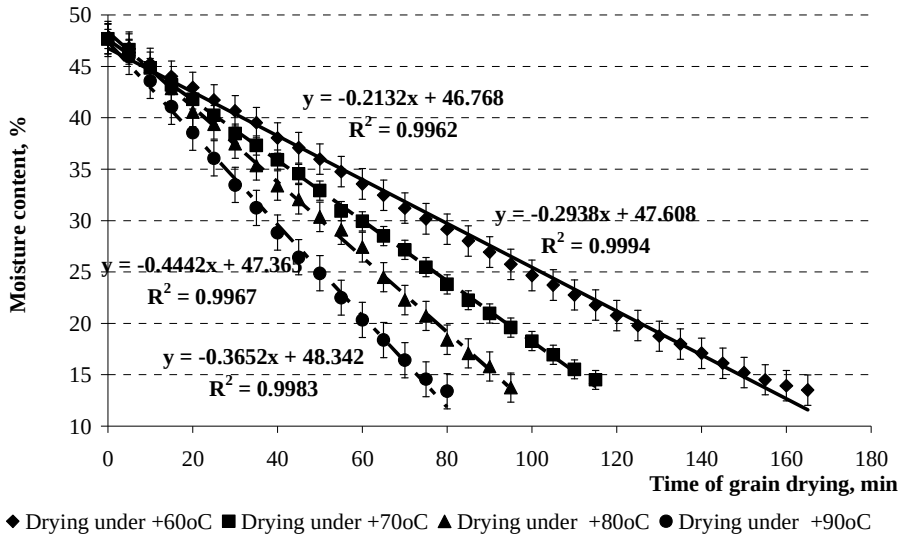

Figure 1. Dynamics of moisture content in biologically activated rye grain during drying

Dynamics of vitamin content. One of the tasks of our research was to found the optimal grain drying parameters, with the purpose maximally maintain quantity of vitamins in grains. In biologically activated rye grain the decreases of vitamins E, C and niacin was established. In unactivated rye grain content of vitamin E is 27.0 mg kg^{-1}, vitamin C – 0.0 mg kg^{-1} and niacin –54.0 mg kg^{-1}. As a control sample the rye grain biologically activated for 24±1 h was used in the research. The changes of vitamin content are showed in Table 1.

The results of our research prove, that the optimal drying parameters for biologically activated rye grain will be as follows: the temperature t=+60±3 ºC and the time τ=2.75h, because the changes of vitamin content are not so considerable; the decreases of vitamin E are ~1.2 times, vitamin C–~1.3 times and niacin–~1.8 times. Therefore, the biologically activated rye grains dried at temperature t=+60±3 ºC for τ=2.75h were use for future experiments.

Table 1

Changes of vitamins C, E and niacin content during grain drying, mg kg^{-1}

Vitamin	Changes of vitamin content				
	0	**t=+60±3 ºC τ=2.75 h**	**t=+70±3 ºC τ=1.92 h**	**t=+80±3 ºC τ=1.60 h**	**t=+90±3 ºC τ=1.40 h**
E	32.5	27.5	27.8	25.4	23.6
C	42.8	33.6	29.8	25.1	19.8
Niacin	66.7	37.5	33.3	32.5	30.1

Dynamics of total protein, fat and wood-fibre content. Activity of enzyme threacetilglycerolipase and lipoxygenase increase during the grain biological activation to 24 hours, as a result the changes of *fat* content are not so relevant, the content of fat increase from 1.37% to 1.4% in dry matter. Such changes will be explained with fat synthesise in grain shells during biological activation. During the biologically activated rye grain drying process at temperature +60±3 ºC and time 2.75 hours the content of fat is not changed.

Total albumen content could not increase during the grain biological activation, because the albumens are splited under the influence on proteolytic enzymes. As a result the content of albumins decreases from 11.0% to 10.53% in rye grains during biological activation to 24 h. The changes of total albumen content during drying process are not so relevant: the decreases were observed from 10.53% to 10.42%.

The content of wood - fibre increases from 2.80% to 3.73% during the rye grain activation time. It will be explained with amylolytic enzyme activity; as a result the cellulose and hemicellulose will be splited. The decrease of wood - fibre content during drying process was determined as not relevant (from 3.73% to 2.69% in dry matter).

Ca content. The increases of Ca content during rye grain biological activation were not so relevant: from 0.05% to 0.07%. Content of Ca during grain drying time was not changed.

Dynamics of fatty acid composition. The increase of oleic, linoleic, cys-11-eucosan, eruc and α-linolenic acids content during grain drying process was observed by 1.5 times. Such changes will be explained with the fat synthesise in grain shells during biological activation and not so high drying temperature which could not negative influence on grain chemical composition.

Dynamics of amino acids composition. The content of total amino acids decreases from 11.6% to 5.94% in dry matter during rye grain biological activation time. It could be explained with albumen splitting under the influence on proteolytic enzymes. During the drying process of biologically activated rye grain the increases of total content of amino acids was determined (from 5.94% to 7.15% in dry matter). Such changes will be explained with intensive biological process accruing in rye grain during first drying period, as a result the amino acids synthesize.

Conclusion

1. Optimal drying parameters of biologically activated rye grain are: temperature +60±3 ºC and drying time approximately three hours.
2. The content of vitamin E in biologically activated rye grain during drying decreases ~1.2 times, vitamin C – ~1.3 times, niacin – ~1.8 times.
3. The changes of albumen, fat, wood - fiber in biologically activated rye grain during drying are not relevant.
4. The increases of oleic, linoleic, cys-11-eucosan, eruct and α-linolenic acid content at the grain drying process is 1.5 times.
5. During the biologically activated rye grain drying process the increases of total amino acid content was determined (from 5.94% to 7.15% in dry matter).

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THE USE OF TRANSGLUTAMINASE IN FOOD PROCESSING

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Abstract

Enzymes play an important role in the food producing of both traditional and novel products. The ancient processes of brewing and cheese-making rely on enzyme activity at various stages of manufacture. But the traditional products like yoghurt and fermented beverages are performed by endogenous enzymes, that occur naturally in the plant and animal tissues or in the microorganism's cells. The idea of isolated, exogenous enzymes adding to improve existing reactions or to initiate new reactions, dates from the start since second part of past century in the USA led to the development of enzymes for the leather industry and started the commercial production of papain for use in the beer industry. Now there are many enzymes for food processing available that originate from different sources. Majority of applied in food industry enzymes are hydrolases such as glycosidases, and in part proteases used for the meat tenderizing. The new direction is the use of enzymes as a tool for modificaton of protein structure. For this aim are used microbial transglutaminase. It produces the both inter- and intra-molecular isopeptide cross-linking bonds in the proteins. We investigate its substrate specificity to attempt to develop the combined products consisting of the proteins from different sources.

Key words: fish, flour, processing, transglutaminase

Introduction

Enzymes play an important role in the food industry in both traditional and novel products. The ancient processes of brewing and cheese-making rely on enzyme activity at various stages of manufacture. Traditional products like yoghurt and fermented beverages owe their character to enzyme reactions but these are performed by whole organisms rather than isolated enzymes. These changes in traditional products are due to enzymes that are endogenous; that is they occur naturally in the tissues of the plant or animal or in the micro-organism. The activity of endogenous enzymes can be manipulated by optimizing the conditions for enzyme activity (pH, temperature) or by altering the genetic control of enzyme expression. However, there are limitations to the degree of manipulation that can be achieved by these means. The idea of adding enzymes from other sources (exogenous enzymes), to improve existing reactions or to initiate new reactions, dates from the start of past century when was started the commercial production of papain for use in the beer industry. Now there are many enzymes for food processing available that originate from different sources.

When enzymes are considered for use in a food processing, it is essential to ensure that they will confer some commercial benefit. The availability of enzymes on a large scale, and at reasonable prices, the food industry reconsidered the use of enzymes in food processing. At present time, advances in biotechnology in the field of genetic manipulation created new perspectives and the new technology were made. Despite of these technological advances and the numbers of potential applications, the use of enzymes in the food industry is limited: about ten enzymes accounted for 65% of the total revenue and 20 others – for rest 35%. Usually used enzymes are hydrolytic that possessed with splitting action, such as proteases (46%) and carbohydrase (47%) being the most common. We are interested in a new enzyme of controversial action. It is microbial transglutaminase (protein-glutamine γ-glutamyltransferase, EC 2.3.2.13; TG) catalyzes acyl-transfer reactions introducing covalent cross-linkages between proteins, creating high molecular weight polymers.

In the early stages of TG research, attention was focused on the cross-linking of proteins involved in blood coagulation. In a later stage cross-linking of other proteins received more attention. Guinea pig liver TG was the most used enzyme for these cross-linking studies. In addition, partially purified transglutaminase was used from bovine blood (factor XIII) or human placenta. Guinea pig liver TG and factor XIII are calcium dependent, which plays a very important role in the conditions necessary for the cross-linking reactions. The discovery of a Ca^{2+}-independent TG isolated from *Streptoverticillium mobaraense* enhanced TG research and utilization in food products, because of the better availability of this enzyme.

In addition to plasma TG, mammal blood contains two other types of this enzyme. One of them is from blood platelets, which is very closely related to plasma TG. The other one is found in erythrocytes and because of that location is called erythrocyte TG. Purification of this TG has been performed from human erythrocytes. However, the relatively small quantities of human blood and its origin make this enzyme uninteresting for large scale applications in food. On the contrary, the large availability of bovine and pig blood from slaughterhouses would make it more practical to isolate TGs from these sources (Govardus de Jong *et al.*, 2001). Using the two types of animal blood TGs and bacterial TG, cross-linking experiments with seven proteins (α-lactalbumin, β-lactoglobulin, BSA, casein, hemoglobin, myosin, glycinin) these authors showed large differences in substrate recognition and in cross-linking rates.

Bacterial TG showed the lowest substrate specificity, as it was able to cross-link all seven proteins tested. However, cross-linking of BSA and α-lactoglobulin is observed only after reduction of the disulfide bridges by dithiothreitol (DTT), which will promote the unfolding of the protein. The unfolding of BSA and α-lactoglobulin will increase the accessibility of glutamine and lysine residues for the cross-linking reaction.

Erythrocyte TG was able to cross-link BSA, casein, and glycinin, indicating a higher substrate specificity for this enzyme. The presence of DTT was necessary for these cross-linking reactions, which was confirmed in the fluorescence assay. However, the latter assay uses monodansylcadaverine and dimethylcasein as substrates, so no disulfide bridges are present. Consequently, not only should the role of DTT be ascribed to that of an agent reducing disulfide bridges, but DTT plays a role in the reduction state of cysteine in the active site of erythrocyte TG. Plasma TG does not need DTT for its activity, as can be deduced from the observed cross-linking of casein, hemoglobin, and myosin in the absence of DTT. In some cases such as α-lactalbumin and glycinin, the presence of calcium in the cross-linking buffer caused a solubility problem. α-Lactalbumin and glycinin were much more quickly cross-linked by bacterial TG without calcium than in the presence of calcium. This means that cross-linking of these proteins by the two blood TGs can be inhibited by the decreased solubility in the presence of calcium. This study shows that the erythrocyte TG has the highest substrate specificity, whereas the bacterial enzyme has the lowest specificity. An intermediate specificity is exhibited by the plasma TG.

These differences between types of TGs should be ascribed to the roles of these enzymes in their concomitant natural processes. The fact that these enzymes are able to cross-link proteins different from their natural substrates means that they can be used in several applications for which enzymatic protein cross-linking is desired instead of chemical cross-linking. Applications may be aimed toward the development of protein polymers with modified functional properties but also to direct applications in complex systems, such as foods. Depending on the number and types of proteins in an application and the need for specific cross-linking of particular proteins in such an application, one can select the most suitable TG.

With respect to applications related to food products or protein ingredients, the cross-linking of the described substrates looks promising, especially as it is known that cross-linking of protein can have substantial effects on functional properties, for example, gelling capacity, emulsifying capacity, and solubility. The possibility of using different types of TGs for the desired effect can be interesting, especially because the rates and numbers of cross-links produced will differ depending on the type of enzyme used. The problems concerning the erythrocyte TG regarding self cross-linking, the necessity of using a reducing agent, and the difficulties in the purification process will narrow the possibilities of this enzyme. Plasma TG offers better possibilities, although purification to a homogeneous enzyme preparation may not be necessary. A good example of the use of a partially purified plasma TG is in the area of meat processing, for which plasma TG is used in combination with fibrinogen to form a system that enables cross-linking of meat parts. Bacterial TG shows the lowest substrate

specificity and offers the greatest possibilities in cross-linking of protein ingredients. Cross-linking of proteins with this enzyme is favored because of its independence from calcium, which can be beneficial when proteins are to be cross-linked because the solubility is negatively influenced by the addition of calcium.
The substrate specificity of TGs for primary amines was investigated to incorporate various functional groups into proteins and peptides. In next study, microbial and guinea pig liver TGs were used. For the primary amines to be incorporated into benzyloxycarbonyl-L-Gln-Gly (Z-Gln-Gly), they were required to have more than four carbon chains without side chains between the functional groups. These results suggest that with appropriate primary amines as spacers, various functional groups, carboxyl groups, phosphate groups, saccharides, and so on, can be incorporated into proteins by using TGs (Ohtsuka *et al.*, 2000).
Generally, protein substrates of TG are classified into four groups: (1) Gln-Lys-type, in which both Gln and Lys residues are available for crosslinking; (2) Gln-type, in which only the Gln residue is available for reaction; (3) Lys-type, in which only Lys residues are available; and (4) a nonreactive type, in which both Glu and Lys residues are unavailable for reaction (Ikura et al., 1984). This classification is mainly based on the accessibility of Lys and Gln residues located on the protein's surface. According to the above classification, a mixture of two Gln-Lys-type substrate proteins or a mixture of Gln-type and Lys-type substrate proteins should be able to form heteroconjugates in TG-catalyzed reaction. However, in addition to the availability of Lys and Gln residues, another factor that could potentially affect cross-linking of two different macromolecular protein substrates is the thermodynamic compatibility of mixing of the protein substrates at the enzyme's active site (Xiao-Qing Han and Srinivasan Damodaran, 1996).

Materials and Methods
As a fish raw material it has been used by a fillet and farce of bream, pikes, a pike perch and small fry and, as vegetable additives - a texturized flour of peas, rice, a buckwheat and corn. The range of a mass fraction of the brought vegetable component from 5% up to 15% was considered. The used enzyme was TG preparation “Activa EB " manufactured by firm Ajinomoto Co's, Japan. Processing by enzyme spent addition of a solution of TG preparation to fish farce at a stage of farce formulation (the mass fraction of enzyme made 0.2–0.5 %, therefore, for its careful distribution in a product the solution was used). As basis of a composition the fish farce received by crushing of a fish fillet or mechanical deboning of fish raw material used. Additives entered in various percentage parities to weight of fish farce. There were following kinds of products and control variants of each kind have been investigated. Samples of half-finished products with entering of 5%, 7.5%, 10%, 12.5% and 15% of a flour to weight of fish farce and samples with simultaneous entering flours and 0.2, 0.3, 0.4 or 0.5% of enzyme. The farce maturation was for 6 hours by +4 °C. Definition of the module of elasticity was made by means of automatic consistency gage. Action of this is based on measurement of a degree of compression (squeezing) of a punch on sample by constant loading (100 g) during certain time (5 s). Measurements were spent three times, thus the things in common of a punch with a fish each time were displaced. The final result was calculated as an average arithmetic of three significances. Calculation spent to within 0.1 mm.

Results
We have investigated the application of TG in the production of fish cutlets with vegetable flour. Experimental party of cutlets is got, each of which contained from 0.2 to 0.4% of TG preparation. Control samples, unlike proper experimental, did not contain TG. After bringing of all necessary additives both control and experimental samples were maintained at 0° C over day. Control samples had a friable pastelike consistence, and experimental - dense, elastic. Deformation of the investigated samples decreased proportionally to concentration of enzyme preparation: a maximum in 3.8 times. The best results have been received at use of a pea flour

as the vegetable component. The result no significantly depends on a kind of the used fish. The strongest influence of enzyme on samples with a pea flour speaks about more close affinity of peas amino acids to enzyme than of other flours. The optimum quantity in such compounds makes 0.25% of enzyme and 15% of a pea flour. In the lead experiments the tendency to increase in elasticity of a product and the general hardening of a consistence is traced at increase in TG mass fraction. The increase in TG mass fraction in a product promotes condensation of its structure, increase in percentage of fiber in a finished article and to increase in food value of a product. However, after achievement of the certain value of elasticity (in this case - at a mass fraction of TG equal 0.25%), the further increase of TG is inexpedient in view of significant growth of the price of a ready product. Considering all half-finished products with TG concerning the control sample, the increase food and sensor values of these products are noted. From all points of view, at observance of optimum quantity TG, a product more racks to deformation, the consistence and appearance is better, than at control samples. From the economic point of view, addition of TG slightly increases the cost price of finished products.

Conclusion

One of the problems of meat and fish processing is utilization of crushed waste. Application of TG, which creates more large molecules from proteins by their cross-linking, allows to solve this problem. TG forms from the crushed meat a monolithic piece of the set form which after freezing and thermal processing gives the juicy product which is not differing on the parameters from a product, made from an integral piece of a beef or fish. The same enzyme preparation is used for reception of a “fillet” from fine scraps of a red fish. By means of TG is delivered also fermentative processing of proteins waste of an animal origin for giving cohesion and durability to a product, for example by manufacture of animal and vegetable row materials. By our results TG is more effective in connection of fish to pea proteins and it may be used in the next research work. Thus, TG application in food technology promotes improvement of their quality indicators, and also can serve for manufacturing of new kinds of food products. Use of TG in manufacture of fish products enables to receive from fish steady systems with the expressed elastic-plastic properties, to lower quantity of production wastes and to increase an yield of finished goods. Purposeful application TG in a complex with protein-containing additives, promoting the re-structuring of disperse food systems and output of food products with the set properties, is perspective and demands the further studying.

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YEASTS RESISTANCE TO PLANT AND BERRY EXTRACTS

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Abstract

Undesirable in food products eight yeasts species *Debaryomyces hansenii, Trichosporon cutaneum, Kluyveromyces marxianus var. lactis, Sacharomyces cerevisiae, Candida parapsilosis, Torulaspora delbrueckii, Pichia kluyveri, Rhodotorula rubra* were used in the test cultures. Antimicrobial effect of various extracts and essential oils against yeasts was determined by diffusion in agar method.

Tarragon, savory and parsley essential oils solutions at concentration of 0.5% inhibited the growth of yeasts. *T. cutaneum* and *R. rubra* were more sensitive to these essential oils than other yeasts cultures. The essential oil of long curcuma inhibited only before mentioned yeasts cultures. The extract of parsley also possessed inhibitory effect against all yeasts. The extracts of black cumin, cayenne pepper and red bell pepper did not influence the growth of yeasts. The inhibitory effect of green paprika and chilly pepper extracts was weaker comparing to the extracts of parsley. The extracts of leaves and seeds of coriander, leek and basil possessed inhibitory effect and inhibited growth of yeasts.

Yeast shows minimal sensitivity to the ethanol extracts from cranberry, black currant and bilberry berries and berry press cakes. Only *T. cutaneum* and *S. cerevisiae* makes bigger transparence zone. Yeast shows resistance to berry juice and water extracts from berry press cakes.

The main objective of this study was to evaluate antimicrobial activity of spices extracts obtained by carbon dioxide and ethanol extracts of cranberry, bilberry and black currant berry and their press cakes against selected yeasts strains.

Key words: yeasts species, essential oils, extracts

Introduction

Spices have been widely consumed throughout history in the human diet not only as flavoring substances but also as antimicrobial agents. Numerous reports demonstrate an inhibitory effect of essential oils and extracts, as well as purified compounds isolated from various plants on the growth of microorganisms; such substances have been used for preserving foods and drinks (Liu *et. al*., 1996; Azzouz *et. al*., 1982; Ozean *et al*., 2001).

Since the very first scientific experiments on the antimicrobial properties of spices, herbs and their components, which were performed carried out in the end of 19th century, the interest in this topic has not diminished. Hoffman and Evans (1911) were the first who performed a laboratory study on the effect of spices in food preservation. They found that cinnamon, mustard and clove were useful in preserving apple juice (Zaika, 1988).

Several studies have reported that garlic bulb extract can inhibit the growth of bacteria, fungi and viruses in culture media and food systems. It has also been shown that the antimicrobial activity of garlic bulbs is due to allicin (diallyl thiolsulfinate), ajoene and other sulfur compounds (Yin *et al.,* 1998; Conner *et al.,* 1984). It is well established that such compounds as thymol, anethole, menthol as well as essential oils and extracts from Jamaican pepper, cinnamon, clove, garlic, oregano, sage and thyme can inhibit the growth of pathogens and yeast found in foods. These substances were strong agents in terms of their capability to reduce the number of various microorganisms (Marino *et al.,* 1999).

Materials and Methods

The yeast strains were isolated from dairy products, equipment washing liquid and the air of industrial premises: *Debaryomyces hansenii, Trichosporon cutaneum, Kluyveromyces marxianus var. lactis, Sacharomyces cerevisiae, Candida parapsilosis, Torulaspora delbrueckii, Pichia kluyveri, Rhodotorula rubra.*

Fruit juices of cranberry, bilberry and black currant were pressed out in a conventional juicer and the press cake was stored in a freezer until extraction. The ethanol extractspigments were

extracted from 3 g of frozen berries or berry press cakes with 10 ml 95% (v/v) food grade ethanol acidified with 0.1 N HCl.

Spice (curcuma (*Curcuma longa*), tarragon (*Artemisia dracunculus*), savory (*Satureja hortensis*), parsley (*Petroselinum crispum*), horseradish (*Armoracia rusticana Gaerttn.*), garlic (*Allium sativum*), rosemary (*Rosmarinus officinalis*), lemon (*Citrus limon*), caraway (*Carum carvi*), coriander (*Coriandrum sativum*), chilli pepper (*Pimenta dioica*), fenugreek (*Trigonella foenumgraecum*) and marjoram(*Origanum vulgare*)) extracts were extracted with liquid carbon dioxide at ambient temperature and 60 bar pressure. Essential oils were hydrodistilled from the extracts and plant in a Clevenger type apparatus.

The antimicrobial properties were evaluated by the agar well diffusion method (Zaika, 1988). Yeasts were grown on a slant potato – dextrose agar (LAB 98, LAB M), respectively. Yeasts were cultivated 18 hours at 37 °C and 24 h at 25 °C, respectively. After cultivation, test culture cells were washed with saline and mixed using an MS 1 minishaker (Wilmington, USA). The yeasts cell suspensions were diluted according to McFarland No 1, respectively (Delaquis *et al.*, 2002). A suspension of cells was introduced into a dissolved medium cooled to 47 °C, 10 ml of which was pipetted into a 90 mm diameter Petri plate. After cooling wells nine-millimeter in diameter were pushed in the agar and filled with 50 µl of ethanolic solutions of extracts. The plates with yeasts were incubated overnight at a temperature of 30 °C.

After 24 h of incubation, the inhibition zones were measured with callipers to an accuracy precision of 0.1 mm and the effect was calculated as a mean of three replicate tests.

Results and Discussion

Tarragon, savory and parsley essential oils solutions at concentration of 0.5% inhibited the growth of yeasts. *T. cutaneum* and *R. rubra* were more sensitive to these essential oils than other yeasts cultures (Table 1).

Table 1

Inhibitory effect of essential oils on yeasts

Plants essential oil	Concentration, %	Inhibition zone, cm							
		D. hansenii	*T. cutaneum*	*K. marxianus var. lactis*	*T. delbrueckii*	*S. cerevisiae*	*C. parapsilosis*	*P. kluyveri*	*R. rubra*
Essential oil of curcuma from plant extract	50	0.0	1.8±0.0	0.0	0.0	0.0	0.0	0.0	1.5±0.0
	10	0.0	1.3±0.1	0.0	0.0	0.0	0.0	0.0	1.4±0.0
	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0±0.0
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Essential oil of tarragon from plant extract	50	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0
	10	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	3.8±0.0	4.0±0.0
	5	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	2.7±0.1	4.0±0.0
	1	4.0±0.0	4.0±0.0	4.0±0.0	2.6±0.0	3.0±0.7	1.6±0.1	2.0±0.0	4.0±0.0
	0.5	4.0±0.0	4.0±0.0	4.0±0.0	2.4±0.0	2.9±0.1	1.7±0.1	1.5±0.0	4.0±0.0
Essential oil of savory from plant	50	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0
	10	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0
	5	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0
	1	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0
	0.5	2.5±0.1	4.0±0.0	1.6±0.1	1.7±0.1	2.0±0.0	1.8±0.0	1.6±0.0	3.0±0.0

Plants essential oil	Concentration, %	Inhibition zone, cm							
		D. hansenii	*T. cutaneum*	*K. marxianus var. lactis*	*T. delbrueckii*	*S. cerevisiae*	*C. parapsilosis*	*P. kluyveri*	*R. rubra*
Essential oil of savory from plant extract	10	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0
	5	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0
	1	2.9±0.1	4.0±0.0	3.9±0.1	3.2±0.0	3.1±0.1	2.1±0.1	2.6±0.0	4.0±0.0
	0.5	1.8±0.0	4.0±0.0	2.0±0.0	2.4±0.0	1.8±0.0	1.8±0.1	1.5±0.0	4.0±0.0
Essential oil of parsley from plant extract	10	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0
	5	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0
	1	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	3.9±0.1	4.0±0.0
	0.5	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	2.8±0.0	4.0±0.0

The essential oil of long curcuma inhibited only before mentioned yeasts cultures. The combined effect of different pH of media and extracts was also investigated and it was found that pH did not have significant effect on antimicrobial properties of extracts.

After investigation yeast sensitivity on various plant extracts, it was found that yeasts are resistant on most of them. Only parsley leaves extract inhibit test cultures growing – *D. hansenii*, *T. cutaneum*, *C. parapsilosis* and *R. rubra* made inhibition zone in 4.0 cm diameter. Better resistance showed *K. marxianus* var. *lactis*, *T. delbrueckii*, extracts of concentration 10 and 5 % made lesser inhibition zone, their diameter was from 1.6 to 3.5 cm. The extract of parsley also possessed inhibitory effect against all yeasts. The extracts of black cumin, cayenne pepper and red bell pepper did not influence the growth of yeasts. The inhibitory effect of green paprika and chilly extracts was weaker comparing to the extracts of parsley.

In literature it is mentioned, that composition of several essential oils some times shows stronger inhibition effect compared to single component. After mixing extracts of savory, coriander and tarragon in proportion 1:1:1, obtained composition showed antimicrobial effect, but it was not very strong (Table 2).

Table 2

Sensitivity of yeasts on extracts compositions

Plants extract and their composites	Concentration, %	Inhibition zone, cm							
		D. hansenii	*T. cutaneum*	*K. marxianus var. lactis*	*T. delbrueckii*	*Sac. cerevisiae*	*C. parapsilosis*	*P. kluyveri*	*R. rubra*
Horseradish, garlic, rosemary	50	2.9±0.1	2.0±0.1	1.8±0.1	2.3±0.1	1.8±0.0	2.0±0.0	1.6±0.1	4.0±0.0
	10	1.9±0.1	1.6±0.1	1.6±0.0	1.7±0.0	1.6±0.0	1.8±0.0	1.4±0.1	2.8±0.0
	1	1.2±0.0	0.0	0.0	1.0±0.0	1.2±0.0	0.0	0.0	1.1±0.0
Extract of horseradish	50	2.3±0.3	1.6±0.2	1.5±0.0	1.4±0.1	2.3±0.1	1.5±0.1	1.5±0.0	2.7±0.2
	10	2.0±0.1	1.5±0.1	1.4±0.1	1.3±0.0	2.0±0.0	1.6±0.1	1.9±0.0	2.3±0.4
	1	1.3±0.0	0.0	0.0	1.1±0.0	1.2±0.0	0.0	1.2±0.0	1.2±0.0
Extract of garlic	50	2.6±0.1	2.4±0.1	3.2±0.3	1.8±0.3	2.6±0.1	2.8±0.4	1.7±0.0	3.2±0.3
	10	2.0±0.1	2.0±0.0	2.6±0.1	1.7±0.1	2.0±0.1	2.1±0.9	1.3±0.1	2.4±0.0
	1	1.4±0.1	1.3±0.0	1.3±0.0	1.0±0.0	1.1±0.0	0.0	1.1±0.0	1.2±0.1
Extract of rosemary	50	1.5±0.5	1.8±0.0	1.7±0.3	1.4±0.1	2.0±0.0	1.6±0.1	1.8±0.1	4.0±0.0
	10	1.6±0.0	1.8±0.0	1.6±0.1	1.3±0.1	1.3±0.0	0.0	1.6±0.0	3.0±0.3
	1	1.2±0.0	1.2±0.0	1.1±0.0	1.2±0.0	1.1±0.0	0.0	1.1±0.0	1.3±0.1
Peel of	50	1.9±0.1	1.8±0.0	1.2±0.0	1.3±0.0	1.4±0.0	1.6±0.1	0.0	0.0
	10	1.3±0.1	0.0	0.0	1.1±0.0	0.0	0.0	0.0	0.0

Plants extract and their composites	Concen-tration, %	Inhibition zone, cm							
		D. hansenii	*T. cutaneum*	*K. marxianus var. lactis*	*T. delbrueckii*	*Sac. cerevisiae*	*C. parapsilosis*	*P. kluyveri*	*R. rubra*
lemon. caraway, fenugreek	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Extract of lemon peel	50	0.0	1.2±0.0	1.1±0.0	1.1±0.0	1.5±0.0	1.2±0.0	0.0	2.0±0.0
	10	0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.3±0.1	0.0	0.0	1.5±0.0
	1	0.0	0.0	1.0±0.0	1.0±0.0	1.1±0.0	0.0	0.0	1.1±0.1
Extract of fenugreek *Trigonella foenum-graecum*	50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Savory, coriander, tarragon	50	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0 ±0.0	4.0±00
	10	4.0±0.0	3.8±0.0	4.0±0.0	3.8±0.0	4.0±0.0	3.8±0.1	3.5±0.1	4.0±0.0
	1	4.0±0.0	1.8±0.0	4.0±0.0	1.8±0.0	4.0±0.0	3.6±0.0	0.0	4.0±0.0
Extract of starragona-vory	50	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0
	10	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0
	1	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0
Extract of coriander	50	1.3±0.07	4.0±0.0	1.9±0.0	1.7±0.0	1.9±0.1	2.9±0.1	1.2±0.3	4.0±0.0
	10	1.3±0.0	4.0±0.0	1.6±0.1	1.3±0.0	1.6±0.0	2.1±0.1	1.3±0.0	4.0±0.0
	1	1.1±0.0	4.0±0.0	0.0	1.2±0.0	1.1±0.1	1.4±0.1	0.0	4.0±0.0
Extract of tarragon	50	1.50±0.0	3.3±0.1	2.9±0.1	2.7±0.4	3.2±0.0	2.4±0.1	2.2±0.1	4.0±0.0
	10	1.20±0.0	3.3±0.1	2.4±0.0	2.4±0.0	2.8±0.0	2.2±0.0	2.0±0.0	4.0±0.0
	1	0.0	2.8±0.0	1.5±0.0	1.2±0.0	2.0±0.0	1.8±0.0	1.2±0.0	4.0±0.0
Chilli pepper, marjoram, curcuma	50	0.0	1.2±0.0	1.3±0.0	1.2±0.0	1.6±0.0	1.1±0.0	0.0	2.0±0.0
	10	0.0	0.0	0.0	0.0	1.1±0.0	0.0	0.0	1.4±0.1
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9±0.0
Extract of chilli pepper	50	1.3±0.0	0.0	1.0±0.0	0.0	1.3±0.1	1.3±0.1	0.0	1.8±0.1
	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2±0.0
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0±0.0
Extract of marjoram	50	2.0±0.0	4.0±0.0	3.4±0.3	4.0±0.0	4.0±0.0	3.0±0.0	1.5±0.1	4.0±0.0
	10	1.3±0.0	1.7±0.0	2.0±0.0	1.6±0.0	2.8±0.0	2.3±0.3	1.3±0.0	4.0±0.0
	1	1.0±0.1	1.0±0.0	1.3±0.0	1.0±0.0	1.2±0.1	1.0±0.0	0.0	4.0±0.0
Extract of curcuma	50	1.3±0.0	1.9±0.0	1.3±0.2	1.3±0.1	1.3±0.1	1.1±0.0	1.5±0.0	1.7±0.2
	10	0.0	1.4±0.1	1.6±0.4	1.2±0.0	1.3±0.0	1.1±0.0	1.2±0.0	1.4±0.0
	1	0.0	1.3±0.0	1.3±0.1	1.1±0.0	1.1±0.1	1.0±0.0	1.1±0.0	1.2±0.0

Extract of 50, 10 and 1% concentration inhibit only *D. hansenii*, *K. marxianus*, *S. cerevisiae* test cultures. Other yeast made smaller inhibition zones, especially with extracts of concentration in 1%. After mixing extracts of horseradish, garlic and rosemary, increase of affectivity was not observed too. The extracts of leaves and seeds of coriander, leek and basil possessed inhibitory effect and inhibited growth of yeasts.

Yeast shows minimal sensitivity to the ethanol extracts from cranberry, black currant and bilberry berries and berry press cakes (Table 3). Only *T. cutaneum* and *S. cerevisiae* makes bigger transparence zone. *K. marxianus* var. *lactis* was the most sensitive to ethanol extracts of bilberry and black currant berry press cakes than other yeasts cultures.

Yeast shows resistance to water extracts from berry press cakes. (the results are not shown). Berry juice has weak inhibitory effect, in this case only cranberry juice and black currant juice inhibit growing of *T. cutaneum* and makes transparence zone in 0.8 and 1.0 cm respectively (the results are not shown).

Table 3

Inhibitory effect of berry and berry cakes ethanol extracts on yeasts

Ethanol extract	Inhibition zone, cm							
	S. cerevisiae	*C. parapsilosis*	*P. kluyveri*	*P. kluyveri*	*D. hansenii*	*T. cutaneum*	*K. marxianus var. lactis*	*T. delbruecki*
Cranberry berries	1.1±0.0	0.9±0.0	0.8±0.0	0.9±0.0	1.0±0.0	1.5±0.1	1.2±0.0	0.9±0.0
Bilberry berries	1.2±0.2	0.9±0.0	0.1±0.0	1.0±0.0	0.8±0.0	1.3±0.1	1.0±0.0	0.9±0.2
Black currant berries	1.2±0.2	0.9±0.0	0.9±0.0	0.9±0.0	0.8±0.0	0.9±0.0	0.9±0.4	0.9±0.0
Cranberry press cakes	1.3±0.2	0.9±0.0	0.9±0.0	0.9±0.0	0.8±0.2	1.2±0.0	1.0±0.0	1.0±0.0
Bilberry press cakes	1.2±0.4	0.9±0.0	0.9±0.1	0.9±0.1	0.8±0.2	1.2±0.2	1.9±0.1	1.0±0.1
Black currant press cakes	1.2±0.0	0.9±0.0	0.8±0.0	0.8±0.0	0.9±0.2	1.3±0.2	1.9±0.1	1.0±0.0

Conclusions

1. Essential oil of savory, tarragon and parsley showed strong inhibitory effect on yeast cultures. Effective concentration was from 0.5 to 50 %.
2. After investigation of various plants extracts mixtures, synergistic effect was not observed.
3. Yeast shows minimal sensitivity to the ethanol extracts from cranberry, black currant and bilberry berries and berry cakes. Only *T. cutaneum* and *S. cerevisiae* makes bigger transparence zone. Yeast shows resistance to berry juice and water extracts from berry cakes.

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THE EFFECT OF EXTRUSION CONDITIONS AND CEREAL TYPES ON THE FUNCTIONAL PROPERTIES OF EXTRUDATES AS FERMENTATION MEDIA

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Abstract

Extrusion cooking is used world wide for the production of expanded snack food, modified starch, ready-to-eat cereal foods. Recently a considerable interest has arisen in the appliance of extruded products, such as raw materials with specific physical properties, for the production of fermented products. Product quality can vary considerably depending on the extruder type, screw configuration, feed moisture, temperature profile in the barrel, screw speed, feed rate and die profile. In this study, the effect of feed moisture content on functional properties (water solubility index, water absorption index and degree of gelatinization) of different extrudates was investigated. Different extruded samples were prepared using wheat (flour and meal), rye (flour, meal and wholemeal), barley, triticale, maize and rice wholemeals, pursuant to selection of fermentation media. Cereal products were passed through a one-screw extruder using moisture contents of 30% and 50%.

The performed study revealed that functional properties of extrudates are strongly related to the cereal type and feed moisture content. The higher feed moisture content (50%) influenced the higher water solubility index (WSI), yet the lower water absorption index (WAI) and degree of gelatinization (DG). The highest values of WSI, WAI and DG were demonstrated by barley, triticale and rye flour extrudates and were as follows: 10.1%, 2.31 g g^{-1} and 100%. Meanwhile, the lowest values of functional properties were observed on triticale, rice and wheat extrudates, and were in the values of 1.8%, 0.63 g g^{-1} and 6.1%, respectively.

Key words: extrusion, feed moisture content, WAI, WSI, DG.

Introduction

Extrusion is one of the most common industrial processes used to make snacks, and it is among the most versatile technological processes for making food products, usually from cereals. Cereals, in turn, are the customary, traditional snack ingredient due to their high starch content (Perez-Navarrete *et al.,* 2006). Extrusion technology has many advantages, including its versatility, high productivity, low cost, and the ability to produce unique product shapes and high product quality (Singh and Smith, 1998; Singh *et al.,* 1999; Koksel *et al.,* 2004).

Extrusion-cooking is a versatile and feasible alternative for manufacturing snacks and water reconstitutable foods, and it has been the object of studies to enhance the nutritional and functional properties of extrudates for the development of products (Sacchetti *et al*.., 2005; Shankar and Bandyopadhyay, 2005; Gonzalez-Soto et al., 2006; Hernandez-Diaz *et al*., 2007). In extrusion cooking, important parameters for product quality include moisture content of the material, residence time, which is influenced by feeding rate, screw speed and configuration, die geometry, temperature and time (Gogoi and Yam, 1994; Obatolu *et al.,* 2005). The results of extrusion are gelatinization of starch, denaturation of proteins, inactivation of many native enzymes and antinutritional factors, reduction of microbial counts, and improvement in digestibility and biological value of proteins (Martin-Cabrejas *et al.,* 1999; Milan-Carrillo *et al.,* 2002). The suitability of extruded foods for a particular application depends on their functional properties like water absorption and water solubility indexes, expansion index, bulk density and viscosity of the dough (Hernandez-Diaz *et al.,* 2007).

The objective of this study was to investigate the effect of feed moisture content as processing variables on functional and physical quality of extrudates from different cereal types.

Materials and Methods

The extruded samples: wheat (flour and meal), rye (flour, meal and wholemeal), barley, triticale, maize and rice wholemeals were used throughout this study. Cereal products were passed through a one-screw extruder using feed moisture contents of 30% and 50%.

Water absorption index (WAI) and water solubility index (WSI). WAI and WSI were determined in duplicate following the method described by Anderson (1982). Each sample (1 g) was suspended in 6ml of distilled water and stirred for 30 min at 30 ^{o}C temperature.

Subsequently, the dispersions were centrifuged at 4000 g for 20 min using Heraeus Labofuge 200 Centrifuge (Thermo Electron LED GmbH, Langenselbold, Germany). The supernatants were poured into dry test tubes and stored overnight at 110 ^{o}C for the process of evaporation. WAI and WSI were calculated using following equations:

WAI = weight of sediment / weight of dry solids

WSI = weight of dissolved solids in supernatant x100/weight of dry solids

Degree of gelatinization (DG). Degree of gelatinization was determined by comparing OD values of a particular extrudate to its control (non-extruded) sample. OD values of the samples in triplicate were measured using Spectrophotometer Genesys 10 (Thermo Electron LED GmbH, Langenselbold, Germany) at a wavelength of 608 nm. Calculations were made using the following equation:

DG=optical density of extrudate x100/optical density of control sample.

Results and Discussion

Water solubility and water absorption indexes. Water solubility index (WSI) is used as a measure for starch degradation; it means that at lower WSI there is minor degradation of starch and such condition leads to less numbers of soluble molecules in the extrudates (Hernandez-Diaz *et al.,* 2007). Higher moisture content in extrusion process can diminish protein denaturation and starch degradation. The experimental results show that WSI of all examined extrudates (excluding maize) decreased with increasing moisture levels. The effect of feed moisture content on WSI is illustrated in Figure 1 (A).

The highest WSI under 50.0% feed moisture content in extrusion process were determined in barley and maize extrudates, being 8.7% and 5.9%, respectively. Whereas the lower WSI under same conditions were measured in rye meal and triticale extrudates, with the values of 2.0% and 1.8%, respectively. In the case of 30.0% feed moisture content, barley extrudate had the highest WSI, yet in triticale extrudate was measured the lowest WSI, being 10.1% and 3.6%, correspondingly.

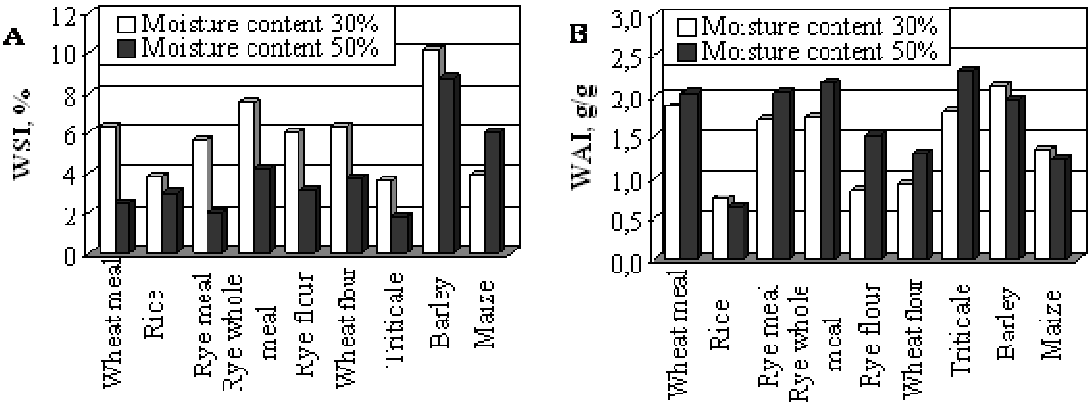

Figure 1. Effect of feed moisture content on WSI (A), WAI (B) and extrudates from different cereal types

WAI, an indicator of the ability of flour to absorb water, depends on the availability of hydrophilic groups which bind water molecules and on the gel-forming capacity of macromolecules. The results of the effect of feed moisture content on WAI of extrudates from different cereal types are demonstrated in Figure 1 (B).

The higher feed moisture content influenced higher WAI for all but rice, barley and maize extrudates. The highest WAI was determined in triticale extrudate (2.31 g g^{-1}) along with the lowest WAI in rice extrudate (0.63 g g^{-1}), both under 50.0% feed moisture content. Badrie and Mellowes (1991) and Hernandez-Diaz (2007) reported similar findings where WSI of several extrudates decreased and WAI increased with increasing moisture levels.

Degree of gelatinization. Degree of gelatinization was mainly affected by different cereal types. It was observed that lower feed moisture enhanced gelatinization in all except rye flour and wheat flour extrudates. The effect of feed moisture content on DG of different extrudates is illustrated in Figure 2.

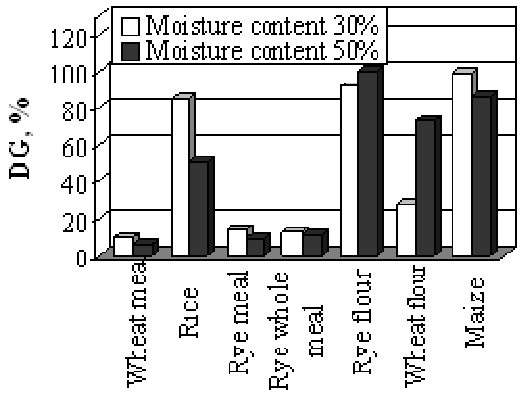

Figure 2. Effect of feed moisture content on DG and extrudates from different cereal types

Nevertheless the highest DG was examined under 50.0% feed moisture content in rye flour extrudate where all starch was gelatinized, yet in the wheat meal extrudate only 6.1% of starch gelatinization showing the lowest DG was achieved.

Conclusions

1. The effect of moisture content in extrusion process on functional properties of different cereal types was examined throughout this study. Experimental data indicate that the extrudate type had a significant influence on water solubility index (WSI), water absorption index (WAI) and degree on gelatinization (DG). Extrusion conditions have to be chosen considering cereal type of extrudate in order to get the preeminent functional properties for fermentation process.
2. Samples with higher degree of gelatinization resulted in lower water solubility and higher water absorbability. Therefore, extrudate samples with a higher degree of gelatinization have a higher gelling capacity and could be incorporated into foods as thickeners or in dough where retaining the moisture is important for the maintenance of the texture.

Acknowledgements

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STUDIES OF BIODEGRADABLE POLYMER MATERIAL SUITABILITY FOR FOOD PACKAGING APPLICATIONS

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Abstract

Since its invention in the 1930s, plastic packaging has posed two challenges: its dependence on petroleum and the problem of waste disposal. Over the past five years packaging suppliers have been introducing various forms of biodegradable plastics made from a variety of plants, in the main corn. The market of biodegradable polymers at the present is growing based on projections that consumers and recycling regulations will drive demand for environmentally-friendly packaging. Market introduction has started successfully all over Europe. Most important application sectors of biodegradable polymers today: organic food and service packaging, shopping bags, catering products, bio waste bags, mulch films, horticulture auxiliaries. At the Latvia University of Agriculture faculty of Food Technology the following tasks of some biodegradable polymer testing for food applications were carried out:

- plasticized PHB films were used for sour cream and salad with meat and mayonnaise packaging;
- PLA film influence on the quality indices of rye bread at the storage time and their suitability for bread packaging was evaluated;
- with the aim to check the published data of biodegradable PLA packaging film special suitability to provide longer shelf life of fresh fruits and vegetables, some in Latvia cultivated fruit storage were tested.

Key words: biodegradable films, food packaging, rye bread, fruits.

Introduction

Since its invention in the 1930s, plastic packaging has initiated two challenges: its dependence on petroleum and the problem of waste disposal. Most of today's conventional synthetic polymers are produced from petrochemicals and are not biodegradable. Theses stable polymers are a significant source of environmental pollution, harming organic nature when they are dispersed in the environment. The row materials such as fossil fuel and gas could be partially replaced by greener agricultural sources, which should also participate to the reduction of CO_2 emissions (Narayan, 2001). Over the past five years packaging suppliers have been introducing various forms of biodegradable plastics. These materials are made from a variety of plants, in the main corn. The market of biodegradable polymers at the present is growing based on considerations that consumers and recycling regulations will drive demand for environmentally-friendly packaging. Some of the biodegradable polymers are already competitive alternatives to conventional food packaging, polylactate (PLA) is being one most important of them (Haugard, Martensen, 2003).

State of the art of biodegradable polymer packaging. According to the Biodegradable products Institute (BPI) a biodegradable plastics is one in which degradation results from the action of naturally occurring micro-organisms such as bacteria, fungi or algae. This takes place in two-steps: degradation/defragmention initiated by heat, moisture, or microbial enzymes, and second step – biodegradation – where the shorter carbon chains pass through the sell walls of the microbes and are used as an energy source. The packaging is certified compostable and biodegradable according to European standard EN-13432, which is the internationally recognized standard for compostable packaging in Europe in 2000[1]. Biodegradable polymers are a growing field **(**Kaplan, *et al.*, 1993; Van de Velde, Kiekens, 2002; Rouilly, Rigal, 2002). Some micro organisms and enzymes capable of degrading them have been identified (Chandra, Rustgi, 1998; Kaplan, 1998). Depending to the evolution of

[1] Drachman F. // Development in Biodegradable Plastics for packaging, Industry Insights, Intertech Pira 2007. Source: www.intertechpira.com; resource used on 10.02.2008.

the synthesis process, different classifications of biodegradable polymers have been proposed. There are 4 different categories of biodegradable polymers; only 3 of them have been obtained from renewable resources. Production capacity for biodegradable polymers worldwide has grown dramatically since the middle of 1990s. Demand for bioplastics in Europe experienced its first boom in 2006, according to a survey by the European Bioplastics Association. Currently bioplastics account only for less than one percent of the European plastics market. Prospective amount of biodegradable packaging market at 2010 could be about 10%[1]. Renewable resource based biopolymers such as starch and PLA account for around 85% of the total production capacity with synthetic biopolymers accounting for the remaining 15%. Biodegradable polymers market introduction has started successfully all over Europe (Platt, 2006). European Bioplastics Association estimates the global production capacities of bioplastics to increase six times until 2011. The shares of the three material classes: synthetic biodegradable, biobased biodegradable and biobased non-biodegradable are expected to change significantly towards biobased non-biodegradable bioplastics. Their share is about 12 percent in 2007 of a total production capacity of 262000 tones in a year; in 2011 the share of biobased non-biodegradable plastics will be almost 40 percent of total capacity[2]. Most important application sectors of biodegradable polymers at the present time are mainly for organically produced foods packaging, conventional fruit and vegetables as well as bread and bakery products, ready-to-eat foods, service packaging, shopping bags, catering products, bio waste bags, mulch films, horticulture auxiliaries. Nets, trays and flow pack – from PLA, cellulose and starch materials – are being used as well. Not only the range of biodegradable products has widened but the number of those manufacturers, distributors and users has also increased. At present PLA is the most widely used biodegradable polymer for fresh-food applications[3]. A new study from Pira Intl. Ltd. estimated that biodegradable packaging will grow at a compound annual growth rate (CAGR) of 22 percent by introduction of lower-cost polyhydroxyalkanoate (PHA) in 2011[3]. Until today the poor barrier properties of uncoated biodegradable materials have prevented their use for products requiring a long shelf life. Currently Hycail Finland Oy have developed a new generation biodegradable PLA material – Hycail ® XM 1020[4], which is ovenable and microwavable and can withstand temperatures over 200 °C. Compostable PLA trays to improve shelf life for meats and other food products by absorbing any liquids exuded during storage have been developed[5]. Biodegradable lidding film Alcan's CERAMIS®- PLA with high-barrier properties to seal food trays (for fresh meat, sausages, cheese and pasta packaging) has been introduced[6]. Presently, biopackaging can be found almost everywhere on the shelves in European supermarkets. Supermarket – Sainsbury in the UK was first who recognized the opportunities for compostable plastics packaging. Supermarket chains such as Delhaize (Belgium), Iper (belonging to the Carrefour group; Italy), Albert Heijn (Netherlands) and Migros (Switzerland) are actively placing their trust in biopackaging[7]. As an industry leader in research and development of biodegradable films is Treofan Company offering one of the broadest product lines for food packaging. Biophan

[1] 1st European Bioplastics Conference, 21-22 November 2006, Crowne Plaza Hotel, Brussels. 2006. Source: http://european-bioplastics.org/; resource used on 09.02.2008.

[2] 2nd European Bioplastics Conference, 23 November 2007, Established as the place to be of bioplastics industry - Berlin/Paris. 2007. Source: http://european-bioplastics.org/index.php?id=646; resource used on 08.02.2008.

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[4] Hycail launches first transparent, microwavable and ovenable biopolymer. Source: http://www.hycail.com/pages/engels/nieuwsen.html; resource used on 11.02.2008.

[5] Compostable tray devised for meat packaging. 2008. Source: http://www.foodproductiondaily.com/news-by-product/news.asp?id=82700&idCat=0&k=Biopak--meat-tray-Polylactic-Acid; resource used on 12.02.2008.

[6] CERAMIS® PLA High-Barrier films Biodegradable packaging Films. 2007. Source: http://www.publications.alcan.com/sustainability/2007/en/pages/review_7_innovation_casestudies_9.html; resource used on 12.02.2008.

[7] Packaging. Source: http://european-bioplastics.org/index.php?id=133; resource used on 08.02.2008.

films (thickness of 20 µm up to 50 µm[1]) have excellent product features: high transparency, exceptional surface gloss, high stiffness, resistant to oil, fat and alcohol, low water vapour barrier, high water transmission rate. The headlines of PLA properties and application for cheese packaging have been studied and presented in the EC funded framework project "Biopack" (Plackett, *et al.*, 2006). The market development of biodegradable plastics has been hindering by their high price. Since 2003 the gap between conventional petroleum-based plastics and biodegradable plastic prices has narrowed considerably due to the price jump of crude oil and energy as well as growing of biodegradable polymer production capacities. NatureWorks produced PLA (to compete directly with PET) price is 2.2–1.5 € per kg, while for PHB Metabolix it is foreseen 1.85 € per kg in 2008[2;3].

The aim of this work is to batch information in general of the state of the art of biodegradable polymers for food packaging and to capsule the news about the studies carried out in Latvia on suitability of biodegradable polymers. In the laboratories of Department of Food technology as well as in the Latvia State Institute of Fruit-Growing, Dobele, the following tasks of some biodegradable polymer testing for food applications were performed:

- plasticized PHB films were used for sour cream and salad with meat and mayonnaise packaging;
- PLA film influence on the quality indices of rye bread at the storage time and their suitability for bread packaging was evaluated;
- with the aim to check the published data of biodegradable PLA packaging film special suitability to provide longer shelf life of fresh fruits and vegetables, some in Latvia cultivated fruit storage were tested.

Materials and Methods

All published data on the studies of biodegradable packaging materials for food performed in Latvia University of Agriculture (LUA) were analyzed and accordance with them consequences were drown. In details the quality of fresh strawberries, black currents and raspberries at the storage time were tested. Two strawberry varieties: 'Tenira 'and' Pegasus', which have been commercially grown in Latvia, black current as well as late-bearing raspberries, were used for our experiments. For quality studies at the storage time the fruits were packed in polypropylene (PP) trays (210x148x35 mm) and sealed on the sealing equipment *Pratica* with oriented polypropylene (OPP) film with thickness 40 µm, as well as the PP trays and Carton boxes as control packaging with berries were enclosed into pouches size of 200x300 mm made from biodegradable PLA films thickness of 25 (Treofan company) and 40 µm *(MaaG company).* All samples were stored in alight showcases at temperature +5±2 ºC. To evaluate the packaging material influence on the ambience in packaging head space the gasses composition was measured by gas analyzer "OXYBABY" ECO. The moisture dynamics of the berries at the storage time was determined by sample mass change weighing on the scales at each day of analyzes. The weight losses were calculated as % of the initial weight.

Results and Discussion

In the experiments performed at LUA several kinds of plasticized PHB were tested. The impact of plasticized PHB comparing with Lean Pouch, PE covered with light protective graphite layer films and PS cups of volume 250 ml usually used for dairy product packaging were evaluated on the quality indices of sour cream. It has been noticed that significant

[1] European Bioplastics Member. Source: Treofan GmbH http://www.european-bioplastics.org/index.php?id=356; resource used on 10.02.2008.

[2] Techno-economic Feasibility of Large-scale Production of Bio-based Polymers in Europe, European Commission, Directorate-general Joint Research Centre, EUR 22103 EN, December, 2005. Source: http://www.biomatnet.org/publications/1944rep.pdf; resource used on 06.02.2008.

[3] Drachman F. // Development in Biodegradable Plastics for packaging, Industry Insights, Intertech Pira, 2007. Source: www.intertechpira.com; resource used on 06.02.2008.

differences of L^*, a^* and b^*– colour values during storage time for 18 days exist among all sour cream samples packed in different kinds of materials; kind of PHB plasticizer slightly influences L^*, a^* and b^*– values during storage. Significant difference of secondary oxidation products acetaldehyde, pentanal and 2-methyl-1-propanol content among samples storage was observed as well. In general established that PHB based polymer films with various plasticizers (Dioctylsebacate or Bisoflex) might be suitable for different packaging technologies of dairy products (Muizniece-Brasava, 2006; Muizniece-Brasava *et. al*., 2006). The shelf life extension of meat salad in mayonnaise (cooked beef, potatoes, eggs, pickled cucumbers, salt, and mayonnaise) packed in pouches made from biodegradable commercially in Brazil produced plasticized PHB (poly–β-hydroxybutyrate) films thickness of 60±5 µm was determined. pH of samples packed in plasticized PHB film under vacuum significantly decreased after 10 days refrigerated storage at +4 ºC. A favourable impact of biopolymer packaging material – plasticized PHB film on total color changes of salads at the storage time has been observed. The growth of micro organisms in meat salad in mayonnaise demonstrated that storage time of salads in a vacuum packaged pouches made from plasticized PHB could be not more than 10 days (Muizniece-Brasava *et al*., 2007). Treofan Company as producer of biodegradable PLA films suggest their Biophan films for use in bread packaging because it is transparent, it allows preserve freshness and crispiness of bread products, what is provided by high water vapour permeability of the material. Our experiments showed that after 21 days of storage rye bread samples packaged in environmentally friendly PLA material films loosed 22.01–22.80% of their initial moisture content, as well as moisture loss from sweet-and-sour rye bread within 28 storage days reached respectively 30.05% and 33.11%. The mentioned moisture loss is too high and bread becomes stale – unacceptable for Latvian consumer. The results show the optimum shelf life for rye bread packaged in PLA film is up to one week, when moisture loss is still negligible. For longer term storage materials with better barrier properties should be used (Straumite *et al*., 2007). In the same way, vacuum-packaged cakes in PLA film due to its high water vapor permeability rapidly loses moisture and hardens faster then control sample in air ambiance, therefore can not be recommended in packaging technologies for shelf life extension of cakes (Muizniece-Brasava *et. al*., 2007).

As an example the dynamics of oxygen and carbon dioxide in the MAP packaged black currents container headspace at the storage time is shown in Fig. 1.

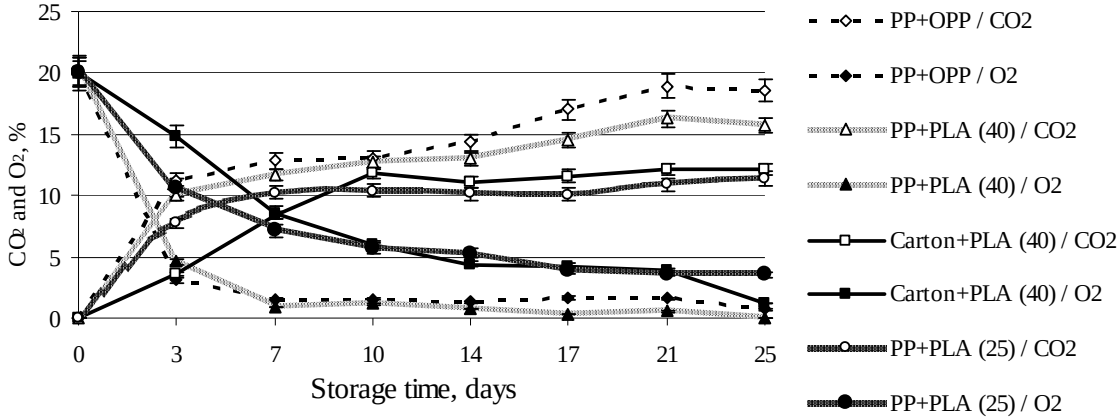

Figure 1. The dynamics of oxygen and carbon dioxide at the storage time in the headspaces of different containers with packed black currents (Latvia State Institute of Fruit-Growing, Dobele)

As the sealing of packages was performed at atmospheric ear ambiance, the initial content of CO_2 was presumed closely to zero and O_2 according as in atmosphere – 21%. The gas composition in packages established at the storage time depends from the barrier properties of films used for packaging. As a result of berry's breathing the CO_2 content in the head space of

packages has been raised accordingly O_2 content – decreased. The highest CO_2 content 18% have been observed in the PP trays sealed with OPP film after 25 storage days. It could be explained by low CO_2 permeability of OPP film, which promotes CO_2 accumulation in the packages. The more acceptable concentration of CO_2 for storage of berries has been observed in the carton boxes inserted in PLA pouches thickness of 40 μm (MaaG company) – 11 to 12% and O_2 – 4%, which could be assessed as adequate to equilibrium modified atmosphere (EMAP) for minimal breathing of fruits at the storage time. In the PP trays enclosed into pouches made from biodegradable PLA films thickness of 25 μm (Treofan company) the content of CO_2 was acceptable, whereas O_2 content decreased close to zero, the oxygen free ambiance could not provide the fruit quality at the storage time. The mass of berries packed in PP trays and sealed with OPP film accordingly to OPP inherent moisture barrier properties does not changes for 21–25 storage days.

Conclusions

A rapid growth of biodegradable packaging materials in European countries started since 2006, the global production capacities of bioplastics will increase six times until 2011. At present PLA is the most widely used biodegradable polymer for food packaging.
The experiments performed in LUA proved about the suitability of biodegradable packaging materials for food application, for all that the experiment should be followed up.

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PASTEURIZATION EFFECT TO QUALITY OF SALAD WITH MEAT IN MAYONNAISE

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Abstract

Experiments have been carried out at the Latvia University of Agriculture Department of Food Technology. The aim of the present study was to investigate effect of pasteurization regime for salad with meat and mayonnaise to maintain its quality. Salad samples were packed in vacuum using conventional packaging films as well as environmentally friendly plasticized poly-β-hydroxybutyrate (PHB) and polyactic acid (PLA) films foreseen for food packaging, applying pasteurization (*Sous vide*).Vacuum packed salad samples were placed in pouches size of 200x300 mm, PHB (thickness 75 μm), and PLA (thickness 40 μm). Mass of sample 200±1 g in each pouch from witch substantially all air was removed prior to final sealing on *MULTIVAC A 300/16*. Following-up by thermal treatment in water bath *Clifton Food Range* (*Sous vide*) in different temperature +60±0.5 ºC, +65±0.5 ºC, and +70±0.5 ºC, including warming up 15 or 10 min, different holding time, and following different cooling time. The cooling occurred in two steps: by water artesian well at +10±1 ºC temperature following by ice water cooling at +1–+2 ºC. The quality of meat salad in mayonnaise was defined by measuring of total plate count (CFU/g) of micro-organisms; from it were determined optimal pasteurization temperature and all treatment process in time. A control sample without any preservatives was packed in conventionally used polypropylene (PP) trays covered with non hermetical. Mild *Sous vide* treatment of meat salad in mayonnaise at ambient temperature in water bath +65±0.5 ºC in total thermal treatment time within 50 min, including warming up 15 min, holding time 20 min and core temperature of sample +63±0.5 ºC and following total cooling time 15 min does not have influence on the consistency of salads and cut down the total microbial colony count. Environmentally friendly packaging films plasticized PHB and PLA could be successfully used for *Sous vide* thermal food treatment at the temperature not higher than +63±0.5 ºC

Key words: salad with meat and mayonnaise, *Sous vide*, packaging, shelf life.

Introduction

The market for ready-to-eat products has become global. Consumers expect and demand safe, high quality fresh produce every day. An innovative approach to packaging that can increase shelf life of ready-to-eat products is necessary. The produce industries for ready-to-eat products are looking for increasingly to sophisticated plastic films packaging to ensure consistent high quality, protection form injuring, ensuring cleanliness, and reducing disease with ever increasing shelf life (Church, Parsons, 2000).

The market for different kinds of salads with mayonnaise has become popular in the recent years in the Baltic States. Last century in the 80's that kind of salads were popular only in the coffee-bars, in the 90's it was possible to find just several kind of no packed salads on the market, but in 1996 the first salads in the packaging boxes came at sight at the market showcases. Packaging boxes were plastic PE or PP trays with non hermetical lids. The main idea of salad packaging in the plastic trays comes from Europe. In 2000's Europe's idea was adapted in the Baltic States. Now, in 2008 it is possible to find in the commercial network salads in different forms packaged in plastic containers with non hermetical lids, as well as salads packaged in vacuum or modified atmosphere. Salad business in Baltic States at the time being is constantly growing and it is a very successful one. Assortment of salads in mayonnaise will continue to grow in the future. An innovative approach to packaging that can increase shelf life of salads is necessary. Ready-to-eat products need a preservatives or mild pasteurization to give them a commercially acceptable shelf life (Ghazale, 1998).

The term "*Sous vide*" means "under vacuum" and describes a processing technique whereby freshly prepared foods are vacuum sealed in individual packages and then pasteurized a time temperature combinations sufficient to destroy vegetative pathogens but mild enough to maximize the sensory characteristics of the product[1] (Galimpin-Johan, 2007; Jang,

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Lee, 2005). General processing steps involved in *Sous vide* are preparation of raw materials, vacuum packaging, pasteurization, chilling/cooling, chilled storage[1]. *Sous vide* has long been used as a method for cooked catering products and enhancing quality compared with conventional methods. The *Sous vide* technologies for ready-to-eat meals developed over the last 20 years as food service industry's need to become more efficient while simultaneously satisfying the consumer's growing demand for higher quality in food and food services (Nissen *et al*., 2002). Products treated with *Sous vide* are being increasingly used in the retail market. Using *Sous vide* the products offers greater microbiological safety, longer shelf life and flexible storage logistics. The *Sous vide* economic benefits include better use of labour and equipment through centralized production and extended shelf life due to vacuum packaging, which by excluding oxygen inhibits oxidative processes and growth of spoilage organisms (Paik *et al.*,1999; Rhodehamel, 1992). The shelf life of a *Sous vide* product can be as long as 42 days (Schaffner, Labuza, 1997).

Literature studies concluded that the safety of *Sous vide* products needs to be carefully evaluated product by product. Time-temperature combinations used in thermal treatments should be re-evaluated to increase the efficiency of processing, and the use of additional antibotulinar hurdles, such as bio preservatives, should be assessed (Wang *et al*., 2004). The effects of *Sous vide* packaging upon the sensory characteristics of chicken breast and of sliced potatoes in cream both immediately after cooking (70–80 °C) and following subsequent chilling, chilled storage and reheating – higher hedonic scores were associated with higher flavors and juiciness scores for chicken and with higher flavor and moisture scores for potato (Church, Parsons, 2000). Salads in mayonnaise belong to group of ready-to-eat foods with high risk and relatively few data have been published on the survival and growth of spore-forming bacteria in those products packed by several packaging technologies. Several guidelines give interpretation of microbiological analysis of some ready-to-eat foods[2] (Ohosone, 1997), but there is not mentioned, that those products are salads in mayonnaise. A novel process for preparing a pasteurized meat and vegetable containing salad in mayonnaise having a long shelf-life under refrigerated storage was proprietary[3]. This process includes essential steps of acid treatment to pH 4.5 and +65 to +75 °C temperature-short time bulk heat treatment before packaging. US Patent 5114733 (1992)[4] relates to a process for preparation of salad mixture with oil emulsion not requiring any preservatives. Prepared salad is placed in container and air tight closed, pasteurized under increased pressure, refrigerated and storage time achieved several weeks. Another finding is US Patent 5320856 (1994)[5] which informs about separate independent ingredients specific thermal stabilization treatments, cooling and after combination into the desired complex food article which is finally sealed in a package.

The aim of the present study is to investigate the effect of pasteurization for each of 21 variants of salads with meat and mayonnaise to find out the best treatment regime to prolong the shelf life without hazardous influence on the quality.

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[3] United States Patent 4191787 1980. // Process for Preparing a Pasteurized Meat-Containing Salad. 1980. Source: Available at: http://www.freepatentsonline.com/4191787.html; resource used on 29.04. 2006.

[4] United States Patent 5114733 1992. // Process for Preparing a Salad Product and an Emulsion Therefore. 1992. Source: http://www.freepatentsonline.com/5114733.html; resource used on 29 04.2006.

[5] United States Patent 5320856 1994. // Method of Making Complex Food Articles Having Prolonged Shelf-life. 1994. Source: http://www.freepatentsonline.com/5320856.html; resource used on 29.04.2006.

Materials and Methods

Experiments have been carried out at the Latvia University of Agriculture Department of Food Technology. The object of research is salad with meat in mayonnaise. Meat salads in mayonnaise produced for a local market were used for experiments. The ingredients in the salads were potatoes, cooked beef, boiled eggs, pickled cucumbers, salt, and mayonnaise Provansa purchased on the local market. Twenty-one different types of *Sous vide* treated and control samples were evaluated. The details of experiment are described in the Table1.

Table 1

Performed experiments of salads with meat in mayonnaise

Sample No.	Packaging material	Packaging technology	Pasteurization temperature, °C	Heating time, min	Holding time, min	Cooling time, min	
						By artesian water (+10±1), °C	By ice water (+1–2), °C
1.	PA/PE	*Sous vide*	65	15	20	15	0
2.	PA/PE	*Sous vide*	65	15	20	5	10
3.	PA/PE	*Sous vide*	65	15	25	5	10
4.	PA/PE	*Sous vide*	65	15	15	5	10
5.	PA/PE	*Sous vide*	65	15	20	5	5
6.	PA/PE	*Sous vide*	70	15	20	15	0
7.	PA/PE	*Sous vide*	70	15	20	5	10
8.	PA/PE	*Sous vide*	70	10	15	5	10
9.	PA/PE	*Sous vide*	70	10	10	5	10
10.	PA/PE	*Sous vide*	70	15	20	5	5
11.	PA/PE	*Sous vide*	60	15	20	15	0
12.	PA/PE	*Sous vide*	60	15	20	5	10
13.	PA/PE	*Sous vide*	60	15	25	5	10
14.	PA/PE	*Sous vide*	60	15	30	5	10
15.	PA/PE	*Sous vide*	60	15	20	5	5
16.	PA/PE (control)	Vacuum	–	–	–	–	–
17.	PE containers	Air ambiance	–	–	–	–	–
18.	plasticized PHB	*Sous vide*	65	15	20	5	10
19.	PLA	*Sous vide*	65	15	20	5	10
20.	PLA	Vacuum	–	–	–	–	–
21.	plasticized PHB	Vacuum	–	–	–	–	–

Salad samples were *Sous vide* packed in vacuum using conventional PA/PE film and environmentally friendly in Brazil commercially produced plasticized poly-β-hydroxybutyrate (PHB) film pouches, thickness of films were 75±2 μm. Polylactic acid (PLA) film foreseen for food packaging thickness of 40±1 μm, was used as well. Salad with meat in mayonnaise was placed in PA/PE (thickness 20/45 μm) pouches with barrier properties size of 200x300 mm and sealed by chamber type machine MULTIVAC A 300/16. As a control the meat salad in mayonnaise without preservatives was packed in traditionally in retail used PE containers covered with non hermetical lids. Mass of each sample was 200±1 g following-up by thermal treatment (*Sous vide*) in water bath Clifton Food Range in different temperature +60±0.5 ºC; +65±0.5 °C and +70±0.5 °C, including warming up 15 or 10 min, different holding time (10; 15; 20; 25; 30 min), and following by different cooling time (10; 15 min). The cooling occurred in two steps: by water from artesian well at +10±1 °C temperature following by ice water cooling at +1 to +2 °C. After processing, samples of each salads were

stored at +6±0.5 ºC. Microbial population in salads with meat in mayonnaise was assessed in the next day after packaging and pasteurization. Microbiological quality of selected samples was evaluated by the methods of the Guidance Note No. 3 of Ireland (2002)[1]. They conform the prepared mixed vegetable salads as well as cooked meat belonging to category D, accordingly acceptable till CFU10^6–<10^7. The principal spoilage mechanisms that limit the shelf life of cooked and processed meat products as ingredient of salad in mayonnaise is microbial growth. The results were processed by mathematical and statistical methods. Data were subjected using The Friedman's test using the statistical analysis software SPSS 16.0 for Windows, significance was defined at $p<0.05$.

Results and discussion

The remaining CFU, g^{-1} of micro organisms in pasteurised samples of meat salad in mayonnaise (cooked beef, potatoes, boiled eggs, pickled cucumbers, and mayonnaise) accordingly Table 1. is showed in the Figure 1.

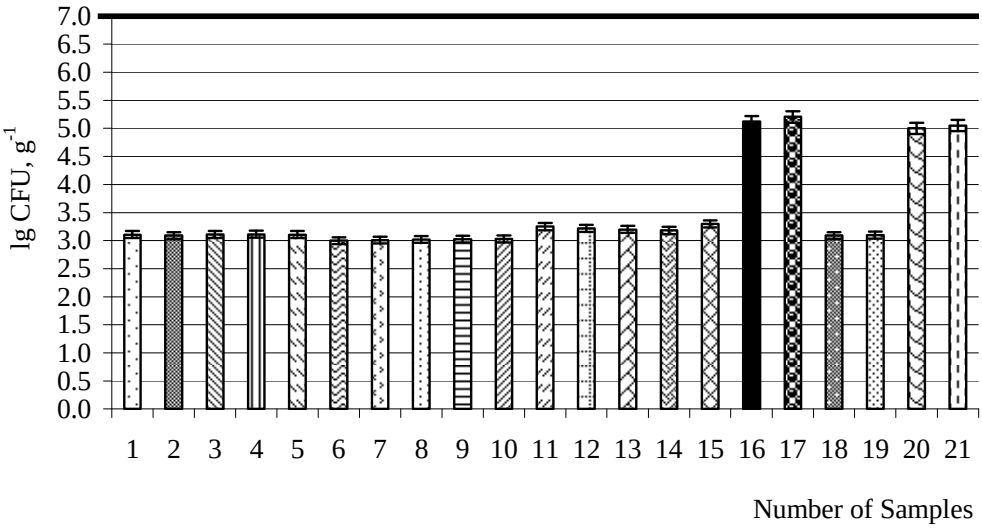

Figure 1. The total plate count values of salads

The horizontal line in the Figure 1, according to the guidelines from the Note No. 3 of Ireland (2002)[7] represents the acceptable maximum number of bacteria (lg CFU, $g^{-1} \leq 7$).

The results of Friedman's test showed that there are not a significant difference between the samples 1 to 15; 18 and 19 ($P>0.05$), while the difference exists between previously mentioned and samples16; 17; 20 and 21 ($P<0.05$). The lowest CFU could be reached applying the *Sous vide* treatment at the temperature in the water bath +70±0.5 ºC (samples 6 to 10, Table 1), nevertheless consistence and colour of product has been changed, and it is not attractive point for consumers. Applying pasteurisation temperature +60±0.5 ºC in the water bath the remaining CFU is higher (samples 11 to 15). The best *Sous vide* thermal treatment regime could be suggested using ambiance temperature in the water bath +65±0.5 ºC (samples 1 to 5, Table 1). In this case the consistence of salads has remained similar to fresh prepared product before pasteurisation. This process includes the essential steps of meat-containing salad with mayonnaise packaging in individual pouches, from which substantially all air has been removed prior to final sealing of containers, following-up by thermal treatment of hermetically sealed pouches in water bath at ambient temperature +65±0.5 ºC in total treatment time within 50 min, including warming up 15 min, holding time 20 min at pasteurization temperature in the core of sample +63±0.5 ºC and following cooling within 15

[1] Food Safety Authority of Ireland. // Guidelines for the Interpretation of results of Microbiological Analysis of some Ready-to-eat Foods sample at Point of Sale/ Guidance Note No.3, 2001. Source http://www.fsai.ie/publications/guidance_notes/gn3.pdf; resourse used on 30.01.2008.

min. The cooling occurs in two steps: by water from artesian well at +10±1 ℃ following by ice water cooling at +(1–2) ℃. Experimental data proved, that environmentally friendly packaging films plasticized PHB and PLA (samples 18 and 19) could be successfully used for *Sous vide* thermal food treatment at the temperature not higher than +65±0.5 ℃ and the pasteurization effect is similar than using conventional packaging films. The total count of micro organisms in salads packed in not hermetically closed PE containers at air ambiance as well as in vacuum packed pouches was lg CFU, g^{-1} >5 (samples 16 and 17).

Conclusions

Mild *Sous vide* treatment of meat salad in mayonnaise at ambient temperature in water bath +65±0.5 ºC in total thermal treatment time within 50 min, including warming up 15 min, holding time 20 min and core temperature of sample +63±0.5 ºC and following total cooling time 15 min does not have influence on the consistency of salads and cut down the total microbial colony count. Environmentally friendly packaging films plasticized PHB and PLA could be successfully used for *Sous vide* thermal food treatment at the temperature not higher than +65±0.5 ºC.

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OVERVIEW OF READY-TO-EAT OSTRICH MEAT PREPARATION METHOD WITHOUT DECOMPOSITION OF CONSTITUENTS

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Abstract

Ostrich commercial rearing is new in Latvia agriculture. The first several ostriches were introduced in Latvia ten years ago. Presently the largest ostrich's farms are located in east part of Latvia, mainly in region of Jekabpils. The task of this study was to start with batching of the information on several methods of ostrich ready-to-eat making and secondly – to develop *sous vide* mild cooking method with the aim to apply it for ostrich meat ready-to-eat preparation with better quality and prolonged shelf life. The information in the scientific literature as well as in website proved that *sous vide* cooking technology for the time being has not been applied for ostrich meat ready-to-eat preparation. Bullocks' tenderloin purchased at the local market as cheaper and more similar to ostrich meat was chosen to specify the preliminary experiments. In our experiments has been found that *Sous vide* thermal treatment improved the microbial stability of bullocks' tenderloin during 20 days storage at +4.0±0.5 °C.

Key words: ostrich meat, *Sous vides* pasteurisation.

Introduction

Ostrich commercial rearing is new in Latvia agriculture. The first several ostriches were introduced in Latvia ten years ago. Presently the largest ostrich's farms are located in east part of Latvia, mainly in region of Jekabpils (Horbacuks, 2005; Zdanovska, 2005). Ostrich meat is lean, tasty and healthy a highly nutritious red meat of the future. The global demand for ostrich meat has escalated with the international trend towards healthier eating, as it is virtually fat free and low in calories and cholesterol but very rich in protein. The ostrich meat is low in cholesterol (just 0.062 g per 100 g meat), low in fat (less than 0.2%), low in Joules (387000 J per 100 g of meat), reach in protein (20.5%), iron (3.2 mg per 100 g meat) and, absolutely reach in taste. The world's foremost supplier of quality ostrich meat in South Africa and internationally Klein Karoo offers to the wholesale trade and to consumers a wide range of raw semi-manufactured ostrich meat. Their range includes prime fillet, steak, goulash, mince, sausages, kebabs, marinated ostrich products, spice sprinkled ostrich meat products, smoked fillet and a variety of cold deli meats Cuts are vacuum sealed in bags of 500 g in each pack before being packaged. Meat is exported fresh, frozen as well as pre-cooked to accommodate out-of-season demand (Karoo). Klein Karoo ostrich meat is ideal choice for the weight conscious. Sportsmen, sportswomen and people with an active lifestyle benefit greatly from the high ostrich meat iron content. The low fat content of ostrich meat results in slightly drier meat. Salt and seasoning draws moisture therefore the meat could be seasoned after sealing. The perfect ostrich fillets or steak is pink to rare, moist and tender. Ostrich meat will become dry if overcooked or well done (Karoo). As a new industry there is a lack of supporting data as it relates to ostrich, but the principles are the same as for other comparable mainstream meat specie (Khalifa). Ostrich "ham" and a control pork ham product were prepared by a traditional ham cure, and Polish sausage products were formulated with either all-pork or a 67% ostrich/33 % pork blend and processed with a commercial spice blend. Results of experiments indicate that value-added ostrich meat products were more acceptable when they were more finely ground, spiced, and combined with pork in a sausage product than when they were prepared as a "ham" product (McKenna *et al.*, 2003). Previously frozen ostrich meat was evaluated over 28 days to determine the refrigerated shelf life and consequence has been drown that, vacuum-packaged ostrich meat stored under refrigerated conditions should be used within 10 days (Otremba *et al.*, 1999). Quality characteristics and storage stability of three types of burgers prepared with ostrich meat (alone or mixed with pork or beef meat) were evaluated. Burgers formulated with ostrich and pork meat had a

faster oxidation rate and became more oxidized than the others (Fernández-López *et al*, 2006). Three different formulations for sausages were prepared, two of them from different ostrich muscles, one – from beef meat. Physical, chemical, and sensory analyses improved that the ostrich meat formula reached the highest general quality scores in the sensory evaluation (Fernández-López *et al.*, 2003). An ultra-high pressure treatment was used to modify the commercial ostrich meat product "Yor" (Thai sausage) as Ostrich meat sausages (yor) were subjected to ultra-high pressures of 300, 500 and 700 MPa for 40 and 60 min at 40 and 60 °C. Subsequently the physical properties of the products, colour, released and expressible water, gel strength and stress relaxation as well as their thermal characteristics (by differential scanning calorimeter, DSC) were determined. The effects of pressure, temperature and holding time significantly influenced the L^*, a^* and b^* values well (Chattong *et al.*, 2007; Supavititpatana *et al.*, 2007). In July 2002 in Turkey an Ostrich meat processing plant was started to build with a capacity to process 65 tonnes per day – the assortment foreseen to produce are steaks, sausages, salami, roasted meat and pressed spicy meat (Ostrich…, 2002). Nowadays consumer demand refrigerated convenient meals, processed using a brief/mild heat treatment. This demand has led to a growth in the application of *Sous vide* and cook-chill processing technologies to extend the shelf life and to keep the quality of raw material. *Sous vide* and cook-chill pasteurized, refrigerated ready-to-eat foods were introduced in about 1970 by Georges Pralus in France as a more convenient food option than frozen food for the food market. Raw food maintains good quality for a few days, while pasteurized foods are acceptable for up to 90 days, or longer if preservatives such acid and salt are used. Translated directly from French, *sous vide* means "under vacuum". In the culinary world the term refers to a French cooking method in which fresh ingredients are cooked in air tight (vacuum-sealed) plastic bags in hot water bath (Sous vide…, 2008). The food maintains maximum flavour because it is slow cooked for an extensive period of time at a relatively low temperature below water boiling point. From an analysis of the conventional process for cooking Korean seasoned beef, a *sous-vide* processing method was developed that offers convenience and storage stability. *Sous-vide* packaging resulted in better sensory quality and storage stability compared with the conventional method (Jang JaeDeok *et al*, 2005). The *sous-vide* packaging was effective in protecting the product from microbial, physical, and sensory quality degradation. In most countries, food industry and retail food establishments are required to comply with the published guidelines/recommendations for microbiologically safe production, distribution, and sale of ready-to-eat, refrigerated foods (Juneja *et al*, 2007). In Europe, recommendations, guidelines, and codes of practice (ACMSF, 1995; ECFF, 1996; Martens, 1997) have been developed to ensure the safe production of *Sous vide* foods with respect to preventing growth and toxin production by nonproteolitic *C. botulinum*. The assurance of microbiological safety is a key factor in the success of cook-chill and *Sous vide* processed food products. The time and temperature combinations recommended by the European Chilled Food Federation (ECFF 1996) were 80 °C for 270.3 minutes, 85 °C for 51.8 minutes, 90 °C for 10.0 minutes, 95 °C for 3.2 minutes, and 100 °C for 1.0 minute. Cook chill, *sous vide* and vacuum packaging are common forms of reduced oxygen packaging (ROP) that occur in retail food establishments. The task of this study was to start with batching of the information on several methods of ostrich ready-to-eat making and secondly – to develop *sous vide* mild cooking method with the aim to adapt it for ostrich meat ready-to-eat preparation with better quality and prolonged shelf life.

Materials and Methods

Bullocks' tenderloin purchased at the local market as cheaper and more similar to ostrich meat was chosen to specify the preliminary experiments. The *sous vide* process is a pasteurization step that reduces bacterial load but is not sufficient to make the food shelf-stable. The process involves the following steps: preparation of the raw materials (this step may include partial cooking of some or all ingredients); packaging of the product in plastic

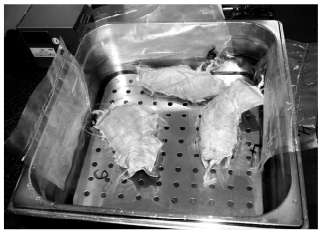

Figure 1. Water bath "Clifton Food Range" with samples

shrink film pouches thickness of 60 μm, 250 gram in each, application of vacuum, and sealing of the package on chamber type machine MULTIVAC A 300/16, vacuum level 99%; pasteurization of the product for a specified and monitored time/temperature water bath "Clifton Food Range" (Figure 1) at ambiance temperatures 90 °C for 45 to 60 minutes, by using full water immersion; rapid and monitored cooling of the product at or below 4 °C and reheating of the packages to a specified temperature before opening and service.

All vacuum packed and pasteurised samples were stored in Commercial Freezer/Cooler ELCOLD at temperature +4±0.5 ºC controlled by MINILog, Gresinger electronic for 20 days. Samples were analyzed before packaging (on 0 day) and after 8 and 20 storage days. pH values of the samples were determinate by METTLER TOLEDO MP120 pH-meter, measurement ranges pH 0.00 to 14.00. Cooking losses were determined by weighing the samples before and after cooking at each experimentally chosen treatment. The tenderness of cooked meat was characterised by compressibility of samples in N before and after cooking as well as at the storage time was determined by the Stable Micro Systems TA.XTplus Texture Analyser, test method compression: pre-test speed 1 mm/s, test speed 1 mm/s, post test speed 10 mm/s, target mode– distance 10 mm, trigger type – auto (force), trigger force 0.2 N. At the storage time microbial Growth dynamics of total aerobic mesophilic bacteria was investigated in the row, marinated and pasteurised meat samples. Microbiological testing was carried out using colony count method in accordance with Latvian standard Ltd. LVS ISO 7218:1996 and LVS ISO 21528-2:2004.

Results and Discussions

The information in the scientific literature as well as in website proved that *sous vide* cooking technology for the time being has not been applied for ostrich meat ready-to-eat preparation.

Microbiological and sensory changes are the main causes of quality decrease of cooked meat and poultry products during cold storage. Heat processes for *sous vide* or cook-chill operations should be designed so that, at a minimum, all vegetative pathogens are destroyed by a pasteurization process. Special labelling of these products is necessary to ensure adequate warning to consumers that these foods must be refrigerated at 5 °C and consumed by the date required by the Code for that particular product (U.S. Department …, 2001). The initial total microbial count of bullocks' tenderloin was $1.3x10^5$, which decreased till $3.1x10^3$ during marinating 12 hours. Changes in the number of micro organisms during storage of *sous vide* processed bullocks' tenderloin are shown in Fig. 2. In our experiments has been found that *sous vide* thermal treatment improved the microbial stability of bullocks' tenderloin with little microbial growth during 20 days storage at +4.0±0.5 °C. The cooking time in the water bath influenced the intransient microbial count as well. The low temperature storage gave the *sous vide* processed product the enhanced storage stability. In future more extensive studies would be needed to obtain a clearer image of bacterial stability and safety for the ostrich meat as a function of added ingredients, storage temperature and heat processing conditions. Initial meat pH value average was 5.643±0.04. The pH of product at the marinating process by the presence of citric acid was decreased slightly to 5.226±0.04, still at the cooking time it again reached the initial level. The pH value at the storage time did not change noticeably due to time of storage.

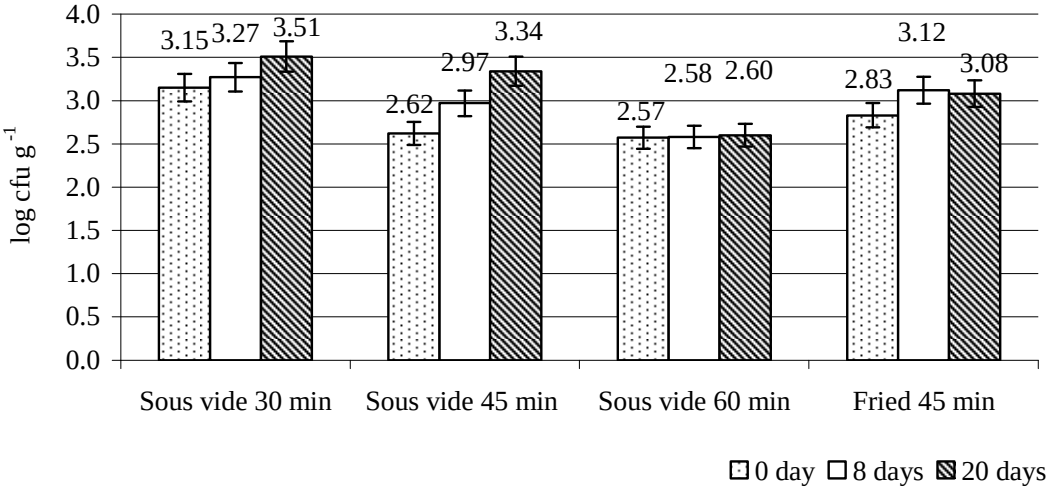

Figure 2. The effect of *Sous vide* cooking time on total aerobic bacterial count of bullocks' tenderloin during 20 days of storage at +4.0±0.5 °C, the temperature in water bath 90 °C

The data obtained in this study revealed the increase in duration of cooking time increases cooking losses from 30.5% if cooking time was 30 minutes to 37% – cooking time 45 to 60 minutes. It has been observed that cooking losses decrease of 15 to 20% during storage time as a result of juice absorption at low temperature. The cooking losses at the frying or rousting process reached about 50% and remained constant at the storage time.

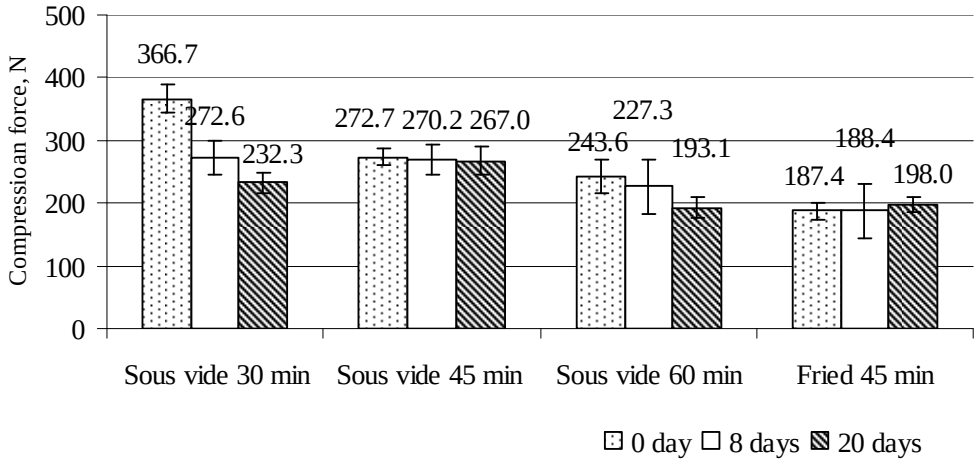

Figure 3. The effect of *Sous vide* cooking time on the compression force of bullocks' tenderloin during 20 days of storage at +4.0±0.5 °C, the temperature in water bath 90 °C

The changes in tenderness characterised by compression force (N) as indicator of readiness of the meat is shown in Figure 3. It is concluded that cooking time at temperature 90 °C at the water bath significantly decreased the compression force from 367.7±23.0 to 243.6±27.0. The least compressing force is found for fried meat, however in this process the cooking losses are highest and meat is not juicy.

Conclusions

Sous vide cooking technology for the time being has not been applied for ostrich meat ready-to-eat preparation.

In future more extensive studies would be needed to obtain a clearer image of bacterial stability and safety to use the *sous vide* technology for the ostrich meat ready-to-eat preparation as a function of added ingredients, storage temperature and heat processing conditions.

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THE RESEARCH OF HEAT TRANSFER PROCESS DURING FREEZING OF BERRIES

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Abstract

Good quality is one of the main problems 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. Raspberries, red currants and black currants were used in the research. 10.0 cm thick bulk frozen berry layer was chosen for studying the freezing process. The freezing dynamics of berries is characterised by temperature measurements in a layer and on its surface. As a result of the research the developed equipment for the measurements of heat flow are approbated. It is necessary to investigate suitability of different cultivars to freezing and influence of freezing to quality of final product. Experimentally verified results will help to explain and predict physical processes in berries during freezing.

Key words: freezing, temperature, berries, heat transfer

Introduction

Freezing as a physical process is connected with products inner moisture transforming into ice as the result of the temperature diminishing under the freezing point. With the decrease of temperature the chemical and microbiological processes in the product slow down. 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 diminishing to the minimum the harmful influences on their quality.

Freezing has to be done quickly. But one should not overestimate the benefical influence of a high rate of freezing on the quality of the product. Experience shows that only few products demand very quick freezing. They are fruit and berries whose quality is largely influenced by the freezing rate (Kampuse, 2003). The freezing rate, in its turn, is essentially influenced by the temperature of heat dissipation into environment, the thickness of the product layer and the heat release coefficient. The research devotes the most attention to the heat release coefficient. With the decrease in temperature of heat dissipation into environment, freezing time shortens almost proportionally but the freezing expense increases. The heat release coefficient at a relatively thin product layer diminishes the freezing time.

Water disappearing in the biological system during the temperature decrease changes the thermo-physical properties of the product. The main and almost the only reason for the changes of thermo-physical properties in the product is the transformation of the free water into ice, because water and ice possess different thermo-physical properties.

When freezing foodstuff, changes in their physical-chemical and biochemical properties are individual and depend on the nature of the product and the freezing process conditions but basically there are great similarities. The aim of the research is to study the heat transfer processes during freezing of berries grown in Latvia.

Materials and Methods

Research object: fresh and frozen raspberries, red currants and black currants grown in Latvia. Specific cultivars were chosen with already stated chemical content. Two cultivars of red currants were used in the experiments (currant-1, currant-2).

Actual berries freezing temperature was –2…–30 °C, the layer thickness was 100 mm. For the determination of the layer density weight-volume method was used. For experimental heat flow research, the equipment for heat flow measurement at the Department of Physics of Latvia University of Agriculture (*LLU*) was used. It consists of a horizontal freezing chamber

with a steady temperature (-30 °C) and a computerized wireless measuring system with heat flow sensors and thermocouples for temperature measurements (Figure 1).

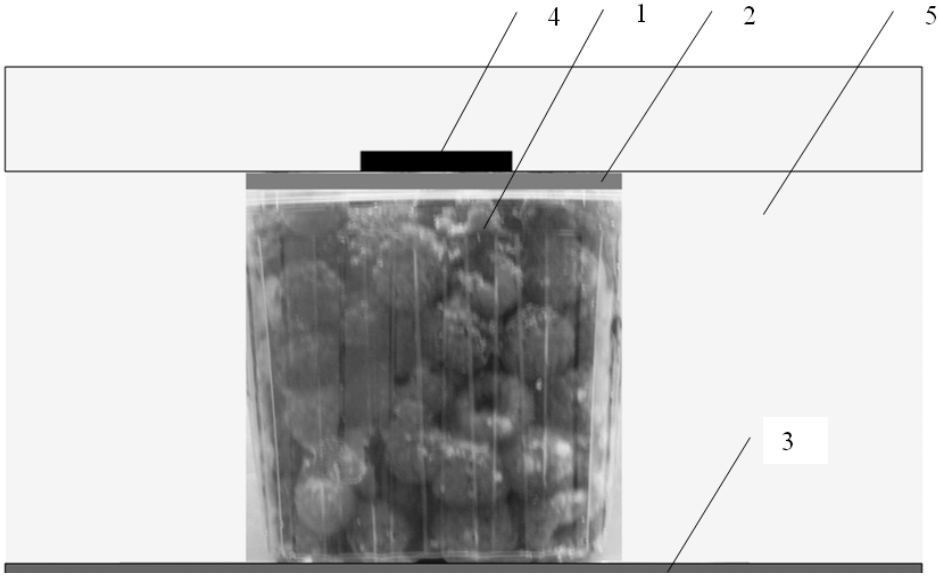

Figure 1. Scheme of the experiment for determining the heat conductivity coefficient of frozen berries

1 – berry sample, 2 and 3 – metal plates, 4 – sensor for heat flow measurement, 5 – thermo-isolating material

During the project, the machine was adjusted to measure the heat conductivity parameters of frozen berries samples. For this task, a sample holder of thermo-isolating material was made. The measurements are performed, placing frozen berries into a foam sample holder which is placed inside of the cold chamber. Under the sample of berries, there is a 0.3 mm metal plate placed for the insurance of the continuity of the temperature T_1. It is fixed by the thermocouple. Straight above the sample, another metal plate is placed for the keeping and the fixing of the continuity of the higher temperature T_3. On the warmer metal plate, symmetrically to the sample, the heat flow q measuring sensor is placed. Above the "warmer" plate and the q sensor, an additional plate of thermo-isolating material is placed to supply a negative temperature for the sample.

Results and Discussion

The research shows that the influence of the temperature has to be differentiated – if one can ignore the influence of the teperature on the specific heat and heat conductivity of fresh fruit, then regarding the enthalpy of frozen products, the temperature is one of the main factors. After the placement of the sample, fast change of temperature (Figure 3) and heat flow (Figure 4) in time period can be observed. It stabilizes in approximately 1000 minutes (~16 hours). With this, we can consider that stationary temperature distribution in the sample is set. At the stationary temperature distribution its change takes place in one dimension. In figure 2 heat flow through the sample is described by the Fourier equation:

$$q = \lambda \frac{\partial T}{\partial x}, \qquad (1)$$

where:

q – heat flow, W/m^2;

λ – heat conductivity coefficient, W/(m·K);

$\frac{\partial T}{\partial x}$ – temperature gradient, K/m.

This leads to the heat conductivity coefficient of the layer:

$$\lambda = \frac{q\partial x}{\partial T} = \frac{qd}{T_3 - T_1} \qquad (2)$$

where;

d – thickness of the sample, m,
T_1 – the lowest temperature, ^oK,
T_3 – the highest temperature, ^oK.

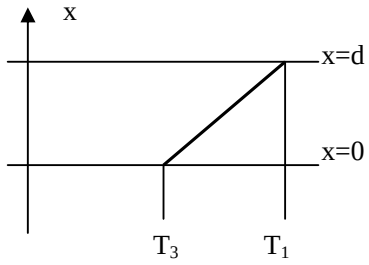

Figure 2. Distribution of temperature in the sample in a stationary situation

Data of the temperature distribution and heat flow after the set of stationary distribution with sample thickness d=0.1 m are used in the calculations. Results are summarized in the Table 1.

Table 1

Thermo-physical parameters of berries

No.	Sample type	p, kg/m^3	λ, W/(m K)
1	Currant-1	558	0.084
2	Currant-2	610	0.087
3	Black currant	438	0.072
4	Raspberry	419	0.079

The research proved that the increase of the product thickness over 20 cm and the increase of the heat release coefficient above 90 W/(m^2 K) almost do not influence hastening of the freezing process.

As the research showed (Kampuse, 2003), cell destruction is obvious in raspberries, i.e. berries with thin parenchyma walls and big intercellular space. These berries contain 84–89% water and 5–10% sugar of the total weight. It promotes the formation of big ice crystals. It is especially important to take into consideration the supplying of reversibility of the technological process. It was observed that in the heat processing of whole cells, the most important change of initial consistency is in the penetrability of the cells membrane.

The products temperature conductivity increases with ice crystals forming. So simultaneously specific heat diminishes and heat conductivity increases. The decreasing temperature of the products and growing of temperature conductivity stops with ice forming finishing. Analyses of professional literature (Skrede, 1996) and the results of the experiment enable to conclude that the transformation of water into ice is one of the most important aspects of freezing.

All these factors are taken into consideration in the research. The most attention is paid to the heat release coefficient. With the decrease of temperature of the heat dissipation into environment, the length of freezing shortens almost proportionally but production expenses increase. The determination of the precise freezing temperature for each product type allows one to create conditions for the effective use of technological process in temperature ranges where the most unfavourable quality changes affecting the berries take place. In temperatures

below the freezing point, its influence on fruit heat physical indicators cannot be taken into consideration.

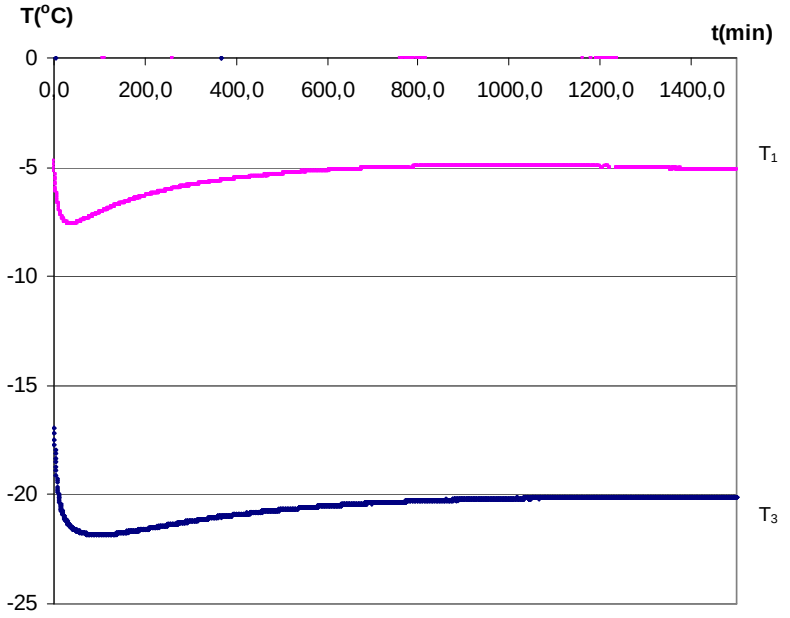

Figure 3. Change of temperature of the raspberry sample surfaces

In the heat calculations of the freezing process, the determined heat of the frozen products is used with the latent heat of ice formation (Wang, 1990). In technical calculations, the ice heat conductivity coefficient is asummed 2.22 – 2.33 W/(m K).

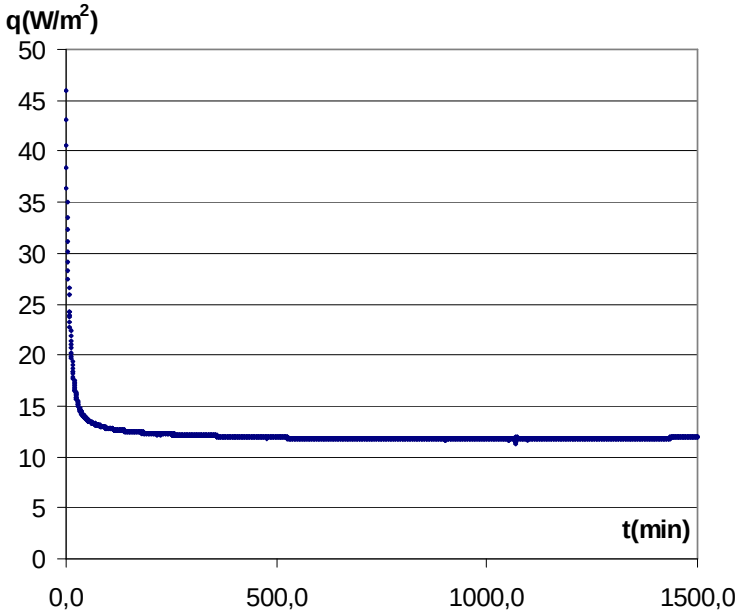

Figure 4. Change of heat flow through the raspberry sample

The heat conductivity coefficient of foodstuff containing 70–80% water including fruit and berries is determined by the general formula. During the research, evidence for the hypothesis on the ruling role of the temperature in the freezing processes was established. Also discoveries and new information was gained on the freezing processes that are mutually interconnected. It secures scientifically practical evidence for some technological developments of qualitative improvement in berry freezing methods and their usage range.

Conclusion

1. The lower the temperature of the heat dissipation into environment and the higher is heat release coefficient, the shorter is freezing time.

2. The rate of freezing is essentially influenced by the temperature of the heat dissipation into environment and the heat release coefficient.

3. The increase of the heat conductivity of the product by the decrease of the temperature is practically over when the water begins to turn into ice.

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AROMA COMPOSITION OF BLACKCURRANT BUD EXTRACTS ISOLATED BY SIMULTANEOUS DISTILLATION/EXTRACTION

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Abstract
The aim of this study was to compare chemical composition of aroma extracts of blackcurrant buds. Dormant buds of six blackcurrant (*Ribes nigrum* L.) cultivars grown in Lithuania were collected in the experimental field of Lithuanian Institute of Horticulture in February 2006. Aroma extracts were isolated from the frozen buds in a Likens-Nickerson micro-steam distillation/extraction apparatus using cosmetic fluid CF-61 as an extraction solvent. The extracts of volatile compounds were analysed by gas chromatography with flame ionisation and mass spectrometry detectors. The aroma extracts were mainly constituted of aliphatic and oxygenated terpenes 39-46 % and 35-38 %, respectively. Blackcurrant cultivars according to the main essential oil compounds, namely sabinene, δ-3-carene and terpinolene were classified into the three chemotypes. Other quantitatively important compounds detected in aroma extracts were β-caryophyllene, β-phellandrene, *cis*-β-ocimene, γ-terpinene, terpinen-4-ol and limonene. (+)-2-Carene, β-2,3-epoxycarene, 1,9-decadiyne, cyclohexylethylacetate, *cis*- and *trans*-1-methyl-4-(1-methylethyl)-2-cyclohexen-1-ol, *p*-mentha-1,4-dien-8-ol, β-selinene, 4-phenyl-1,3-thiazole, germacrene D-4-ol and aromadendrene oxide were reported in blackcurrant buds for the first time.
Key words: *Ribes nigrum* L., volatile compounds, cosmetic fluid

Introduction
Blackcurrant (*Ribes nigrum* L.) is a shrub growing wild in the cold and temperate climatic zones in Asia, Australia and Europe. The most important industrial product of blackcurrant are berries, however leaves and buds have also found some applications. They are used as a raw material for the food and cosmetic industries due to the characteristic colour and excellent flavour (Del Castillo *et al.*, 2002; Piry *et al.*, 1995).

Berries are the most important products of blackcurrant shrub. They are used to prepare juice, jams, liquors, sorbets, ice cream, etc. (Del Castillo *et al.*, 2002; Le Quere *et al.*, 1990). The volatile fraction of berries consists of more than 150 aroma compounds (Varming *et al.*, 2004); therefore berries have also been used as a perfume enhancer (Le Quere *et al.*, 1990; Griffiths *et al.*, 1999). However, the most important raw materials of blackcurrant for the isolation of flavour substances are dormant buds, which are harvested from blackcurrant canes during the dormancy period (Piry *et al.*, 1995; De Toro, 1994).

The aim of this study was to compare chemical composition of aroma extracts of blackcurrant buds isolated from six plant cultivars in Lithuania grown by Likens-Nickerson micro-steam distillation/extraction apparatus. Simultaneous steam distillation/extraction method has been applied for the isolation of aroma compounds since 1964 (Likens and Nickerson, 1964), by using various solvents, particularly such organic low boiling chemicals as diethyl ether and pentane. In our study we selected quite new solvent, so-called cosmetic fluid CF-61 (methoxynonafluorobutane), which is clear, colourless, fast drying substance with boiling point of 61 °C. Cosmetic fluids possess weak odour, they are environmentally favourable fluids offering a unique balance of properties.

Materials and Methods
RAW MATERIAL. Dormant buds of six blackcurrant (*Ribus nigrum* L.) cultivars (*Joniniai, Almiai, Gagatai, Ben Alder, Ben Nevis* and *Ben Lomond)* were collected in the experimental field of Lithuanian Institute of Horticulture (LIH) on 21st February, 2006. The buds were

stored in a freezer before extraction. Cosmetic fluid CF-61 (methoxynonafluorobutane) (3M, Saint Paul, Minnesota, USA) was used as an extraction solvent.

STEAM DISTILLATION-SOLVENT EXTRACTION (LIKENS-NICKERSON). Approx. 5 g of dormant buds were placed in a Likens–Nickerson micro-steam distillation/extraction apparatus together with 500 ml of glass-distilled water; 60 ml of CF-61 were used as the extraction solvent. The samples were extracted for 2 h. Volatile compounds were concentrated to 0.2 ml in a Vigreux column by purging gentle nitrogen steam. Two replicate samples were extracted from each cultivar ant the extracts were stored in a freezer before a further analysis.

GAS CHROMATOGRAPHY ANALYSIS

Gas chromatography and mass spectrometry (GC-MS). The GC-MS system consisted of a Clarus 500 gas chromatograph (PerkinElmer, USA) equipped with a mass selective detector Clarus 500 (PerkinElmer, USA) and automatic injector. The separation was performed using a non-polar fused silica capillary column Elite–5 (30 m×0.25 mm i.d. 1.0 μm film thickness). Mass spectra were obtained by EI at 70 eV. Oven temperature was programmed from 60 °C to 250 °C (5.0 min hold) at 3 °C/min. Injection volume was 0.5 μl at 1:100 split. The temperatures of the injector and detector were 250 °C. The samples were analyzed in duplicate.

Gas chromatography with a flame ionization detector (GC–FID). GC analysis was carried out on a VARIAN 3900 gas chromatograph (Palo Alto, California, USA) equipped with a flame ionization detector (FID) and automatic injector. The separation was performed using a non-polar fused silica capillary column DB-5 (50 m×0.32 mm i.d. 0.52 μm film thickness). Oven temperature was programmed from 100 °C to 250 °C (5.0 min hold) at 2 °C/min. Injection volumes were 1.0 μl at 1:10 split. The temperatures of the injector and detector were 250 °C. The samples were analyzed in triplicate. The amount of the individual compounds was expressed as a GC peak area percentage.

Results and Discussion

The compounds were identified by using GC–MS, while the content of separated components was measured by GC–FID. The identified in aroma extracts compounds constituted more than 93 % of the total integrated GC peak area of each extract. The compounds were identified by comparison of their KI relative to C_5-C_{18} n-alkanes, obtained on a non-polar DB-5 column with those provided in the literature (Adams, 2001); by comparison of their mass spectra with the data provided by NIST data system and literature sources (Le Quere *et al.*, 1990, Piry *et al.*, 1995).

In general, it was found that aliphatic (39–46 %) and oxygenated terpenes (35–38 %) were major fractions in blackcurrant bud aromatic extracts. The content of aliphatic mono and sesquiterpenes was 26–30% and 11–17%, respectively; while the amount of oxygenated mono and sesquiterpenes was 25–28% and 9–11%, respectively.

The most abundant volatile compounds in blackcurrant buds were monoterpenes sabinene, δ-3-carene and terpinolene. It was observed that blackcurrant cultivars analyzed in this study according to the main compounds in aroma extracts may be classified into the three chemotypes. *Joniniai, Almiai* and *Gagatai* were defined as cultivars biosynthesising sabinene (34–48%) as a major essential oil constituent; *Ben Alder* and *Ben Nevis* were assigned to a second group, which is characterised by a high amount of δ-3-carene (approx. 35%) and terpinolene (21–23%); while *Ben Lomond* contained remarkable amount of the all three major terpenes, sabinene, δ-3-carene and terpinolene (35%, 16% and 12%, respectively); therefore this cultivar was attributed to a separate chemotype (Figure 1).

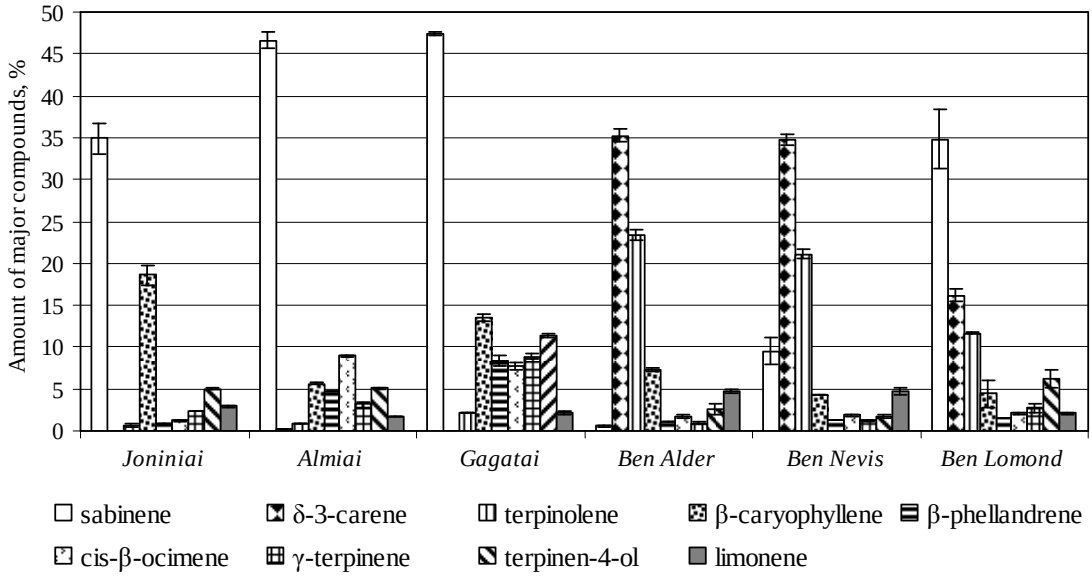

Figure 1. Major compounds of blackcurrant bud aroma extracts

Other quantitavely important compounds in aroma extracts of buds were β-caryophyllene (4–19%), β-phellandrene (1–9%), *cis*-β-ocimene (1–9%), γ-terpinene (1–9%), terpinen-4-ol (2–12%) and limonene (1–5%). The highest amounts of all compounds listed above were detected in *Almiai* (2–6%) and *Gagatai* (2–14%) cultivars (Figure 1).

α-Thujene, α- and β-pinene, myrcene, (+)-2-carene, α-terpinene, *trans*-β-ocimene, α-terpineol, α-humulene, germacrene D and several other compounds were identified in the blackcurrant bud aromatic extracts (Table 1). Sabinene, δ-3-carene, terpinolene, α- and β-phellandrene, γ-terpinene, β-caryophyllene, terpinen-4-ol, germacrene D, bicyclogermacrene and spathulenol were reported previously as the major components in dormant buds of blackcurrant (Piry *et al.*, 1995). All these compounds were found in the buds analysed in our study, except for bicyclogermacrene. To the best of our knowledge, (+)-2-carene, β-2,3-epoxycarene, *cis*- and *trans*-1-methyl-4-(1-methylethyl)-2-cyclohexen-1-ol, 1,9-decadiyne, cyclohexylethylacetate, p-mentha-1,4-dien-8-ol, β-selinene, 4-phenyl-1,3-thiazole, germacrene D-4-ol and aromadendrene oxide were not previously reported in blackcurrant buds (Le Quere *et al.*, 1990, Piry *et al.*, 1995).

Table 1

Composition of blackcurrant bud aromatic extracts of 6 cultivars, %

Compounds	***Joninininiai***	***Almiai***	***Gagatai***	***Ben Alder***	**Ben Nevis**	***Ben Lomond***
α-thujene	1.34±0.08	1.08±0.06	2.82±0.24	0.13±0.01	0.34±0.02	0.71±0.20
α-pinene	0.87±0.08	2.37±0.02	1.18±0.04	0.51±0.08	0.96±0.04	0.81±0.06
β-pinene	1.71±0.07	3.20±0.04	2.37±0.55	2.67±0.05	3.06±0.10	3.20±0.23
myrcene	0.93±0.06	2.31±0.01	1.27±0.06	0.14±0.01	0.89±0.03	0.55±0.06
(+)-2-carene	nd	nd	nd	0.36±0.11	0.36±0.01	0.17±0.10
α-terpinene	1.22±0.03	1.68±0.20	4.31±0.11	0.67±0.04	0.07±0.04	1.74±0.52
***trans*-β-ocimene**	1.23±0.03	1.15±0.02	1.20±0.03	2.75±0.04	2.79±0.17	1.32±0.07
α-terpineol	0.13±0.01	0.10±0.02	nd	0.11±0.01	0.25±0.02	0.08±0.02
α-humulene	4.53±0.34	2.33±0.08	4.93±0.15	2.34±0.08	0.54±0.07	1.96±0.85
germacrene D	3.31±0.23	1.15±0.03	3.87±0.15	3.27±0.07	1.44±0.13	1.27±0.60
spathulenol	2.17±0.00	0.48±0.01	1.53±0.13	1.50±0.04	0.17±0.03	0.46±0.12
camphene	0.05±0.00	0.34±0.00	nd	0.12±0.01	0.13±0.01	0.06±0.01
α-phellandrene	0.15±0.00	0.81±0.02	0.65±0.02	0.45±0.14	0.42±0.01	0.38±0.03

Compounds	*Joninininiai*	*Almiai*	*Gagatai*	*Ben Alder*	Ben Nevis	*Ben Lomond*
p-cymene	nd	nd	0.53±0.02	0.12±0.00	nd	0.21±0.06
***cis*-sabinene hydrate**	0.70±0.02	0.43±0.05	0.67±0.05	0.09±0.01	0.22±0.02	0.61±0.12
***trans*-sabinene hydrate**	0.84±0.03	0.51±0.06	1.24±0.62	0.20±0.01	0.16±0.01	0.67±0.11
β-2,3-epoxycarene	nd	nd	0.92±0.11	nd	0.06±0.02	0.03±0.00
***trans*-1-methyl-4-(1-methylethyl)-2-cyclohexen-1-ol**	0.59±0.02	0.55±0.03	0.76±0.10	0.27±0.00	0.35±0.06	0.57±0.09
***cis*-1-methyl-4-(1-methylethyl)-2-cyclohexen-1-ol**	0.26±0.03	0.37±0.02	0.55±0.08	0.56±0.03	0.70±0.11	0.40±0.09
p-mentha-1,5-dien-8-ol	0.07±0.00	nd	nd	0.51±0.01	0.42±0.04	0.12±0.01
p-cymen-8-ol	0.28±0.01	0.34±0.01	0.53±0.03	0.20±0.03	1.20±0.19	0.26±0.05
***cis*-piperitol**	0.08±0.03	0.14±0.02	nd	nd	0.07±0.02	0.11±0.03
***trans*-piperitol**	0.04±0.00	0.08±0.01	nd	0.11±0.01	0.04±0.01	0.07±0.01
1,9-decadiyne	0.04±0.00	0.16±0.01	nd	0.58±0.06	0.18±0.03	0.18±0.02
Cyclohexylethyla-cetate	nd	0.05±0.00	nd	0.27±0.01	0.39±0.15	0.15±0.02
bornyl acetate	0.13±0.02	1.06±0.07	2.22±0.22	0.12±0.00	0.27±0.07	0.19±0.03
p-mentha-1,4-dien-8-ol	0.07±0.02	0.15±0.01	nd	0.24±0.01	0.34±0.19	0.09±0.01
terpinyl acetate	0.56±0.03	0.17±0.00	0.75±0.00	0.50±0.02	0.33±0.10	0.31±0.06
citronellyl acetate	0.20±0.01	0.13±0.00	0.67±0.31	0.20±0.02	0.36±0.16	0.17±0.03
neryl acetate	0.15±0.01	nd	nd	nd	0.28±0.10	0.07±0.01
β-elemene	1.10±0.09	nd	0.74±0.13	0.38±0.01	0.10±0.03	0.25±0.09
γ-elemene	0.17±0.00	nd	0.34±0.01	nd	0.03±0.00	0.14±0.03
allo-aromadendrene	0.04±0.00	0.08±0.01	0.33±0.01	nd	0.03±0.00	0.07±0.00
β-selinene	0.46±0.02	0.04±0.00	nd	nd	0.03±0.01	0.56±0.24
germacrene B	1.06±0.12	0.15±0.01	2.33±0.11	0.20±0.04	1.64±0.64	0.16±0.12
4-phenyl-1,3-tiazole	1.02±0.07	0.18±0.01	1.50±0.38	0.54±0.07	0.08±0.04	0.19±0.15
germacrene D-4-ol	0.32±0.07	0.12±0.00	nd	0.17±0.06	0.10±0.03	nd
caryophyllene oxide	0.04±0.00	nd	1.15±0.13	nd	0.17±0.02	0.46±0.13
aromadendrene oxide	0.77±0.05	0.23±0.01	0.40±0.05	0.64±0.02	1.12±0.55	0.21±0.06
τ-cadinol	0.53±0.03	0.22±0.05	0.42±0.08	0.52±0.12	0.36±0.02	0.19±0.10
α-cadinol	0.47±0.02	0.14±0.05	0.95±0.13	0.51±0.03	0.37±0.11	0.04±0.02
6,10,14-trimethyl-2-pentadecanone	nd	0.06±0.01	0.34±0.00	0.12±0.01	0.05±0.01	0.07±0.02

nd–not detected

Conclusions

Six blackcurrant bud cultivars were analyzed in the present study and identified compounds constituted more than 93 % of total integrated GC peak area of each extracts. The major compounds of aromatic extracts were sabinene, δ-3-carene, terpinolene, β-caryophyllene, β-phellandrene, *cis*-β-ocimene, γ-terpinene, terpinen-4-ol and limonene. Blackcurrant cultivars according to the main essential oil components (sabinene, δ-3-carene and terpinolene) were separated into the three chemotypes: 1st with sabinene as a major compound (*Joniniai, Almiai* and *Gagatai*); 2nd with high amounts of δ-3-carene and terpinolene (*Ben Alder* and *Ben Nevis*); and 3rd with remarkable amount of all three terpenes (*Ben Lomond*).

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CHARACTERIZATION OF RYE SOURDOUGH MICROFLORA

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Abstract

Preparing of sourdough is one of the oldest biotechnological methods, but the research is still going on and is crucial. In Latvia the spontaneous sourdough is used in traditional rye bread baking which microflora is determined in flour and in microorganisms cultures presented in external environment. Literature data proved that spontaneous sourdough presents several lactic acid bacteria (LAB) and yeasts. Lactobacilli present in sourdough are both homofermentative and heterofermentative and depending on temperature can be presented as well as mesophylic and thermophilic. The latter present in scalding are essential to ensure technologocal process of rye bread. Metabolites of thermophilic LAB are responsible for providing sufficient dough acidity. Besides LAB sourdough contain yeasts including *Saccharomyces cerevisiae, Pichia saitoi, Candida crusei, etc*. The aim of the research was to analyze growth dynamics of microflora in three-stage spontaneous rye flour sourdough fermentation process and to isolate some of its representatives. One of the basic tasks for applying the research was to acquire methods of micro-organism identification. Results of experiments show predominance of LAB reaching 6.06 log10 cfu ml^{-1}, high amount of yeasts reaching 5.22 log10 cfu $ml^{-1}$and a final pH value 3.83 representing that this sourdough has desirable properties for preparation of rye flour sourdough starter. For identification of LAB cultures API CH 50 test was acquired while ID 32 C for yeasts. Results of the experiments reveal heterofermentative LAB *Lactobacillus Brevis* and *Saccharomyces cerevisiae* yeast presence in sourdough. These microorganisms are typical members of sourdough microflora with reference to foreign scientific publications.

Key words: sourdough microflora, lactic acid bacteria, yeasts

Introduction

Sourdough is essential in rye bread making and the tradition of rye sourdough fermentation correspond to the rye-growing areas in north, central and eastern European countries including the Baltic states, where rye bread constitutes a considerable amount of the bread consumption (Rocken, 1996). Traditional sourdough bread technology is based on a spontaneous fermentation process from LAB and yeast occurring naturally in flour. Classic sourdough preparation is a multiple stage process that starts with a mixture of flour and water left for a specific period of time. Every next stage is prepared with fresh flour and water added to the previous stage (Linko *et al.,* 1997, Kariluoto *et al.,* 2004). In the first stage of sourdough fermentation the temperature vary form 25 ℃ to 26 ℃, which is optimal for yeast development. In the second and third stage of sourdough fermentation an average temperature of 32 ℃ is applied – optimal for lactic acid bacteria (Kramer, 2002).

The character of the process results from growth of microorganisms in different environmental conditions. Temperature, dough consistence and dough resting time at each stage determine development of active microflora (Javanainen and Linko, 1993, Muller *et al.,* 2001).

In addition to environmental influence, flour is largely responsible for the properties and quality of spontaneously fermented sourdough. Rye flour naturally contains a wide variety of yeast and bacteria – *Candida crusei, Erwinia herbicala, Bacillus spp.*, moulds, *Saccharomyces spp.,* heterofermentative LAB and acid – tolerant yeasts (Kramer, 2002).

Genera of LAB identified from sourdoughs are *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus*, and the majority of the sourdough LAB belongs to the genus *Lactobacillus.* The taxonomy of LAB is still under revision. *Lactobacillus* present in sourdough has been divided in three groups according to their carbohydrate fermentation patterns: Obligate homofermentative LAB - *L. acidophilus, L. delbrueckii* spp. *bulgaricus, L. farciminis* etc.; Facultatively heterofermentative LAB: *L. casei, L. curvatus, L. plantarum* etc.; Obligately heterofermentative LAB: *L. brevis, L. fermentum, L. fructivorans etc*. (Kandler and Weiss, 1986).

The most frequently isolated yeast species from rye and wheat sourdoughs are *Saccharomyces cerevisiae* which are able to ferment glucose, galactose, maltose and raffinose, but not lactose.

Other yeast species often isolated from sourdoughs are *S. exiguus, Candida milleri (C. holmii), C. krusei*. The latter are able to ferment glucose only. The yeast species *Pichia sitoi, P. norvegensis* and *Hansenula anomala* and some *Saccharomyces* spp. have occasionally been isolated from sourdoughs (Manyenm *et al.,* 1999).
Further the LAB of the sourdough have a synergistic effect with yeasts and inhibit the growth of molds and of rope (*Bacillus mesentericus*) (Reed and Nagodawithana, 1995).
There are relatively few investigations regarding LAB and yeast interaction in sourdough. *Lactobacillus spp.* is the main producer of organic acids in spontaneously fermented sourdough. Although the decrease of pH may cause metabolites (organic acids, ethanol, etc) of *Enterobactericae,* moulds or acid – tolerant yeasts present in spontaneous sourdough (Kramer, 2002).
Bacillus spp. represents undesirable microflora in spontaneous sourdough. For example if *Bacillus subtilis* has expanded in dough, it produces proteolases and hydrolyse proteins resulting in increasing of dough pH and preventing of LAB and yeast activity. Besides the metabolites of *Bacillus subtilis* generate undesirable taste and aroma of dough (Kramer, 2002).
Scientific publications show that application of spontaneous sourdough in rye bread production may cause unstable quality of rye bread. Selected LAB starter cultures should be used in Latvian bakeries to provide controlled sourdough fermentation. Though LAB starters selected in Europe frequently does not satisfy Latvian bakers. Therefore dynamics of spontaneous rye flour sourdough microflora development in every fermentation stage was investigated and some of its representatives were isolated.
When the above is clarified it is possible to promote viability and development of desirable microflora accommodating technological processes – length of every stage, temperature of fermentation and flour – water proportion in favour of it. Desirable microflora in this case refers to LAB and yeasts providing the highest acidity and preferable sensory properties.

Material and Methods

Current research was carried out in Latvia University of Agriculture in the Department of Food Technology in Scientific Laboratory of Microbiology in 2008.
The rye flour from stock company "Jelgavas dzirnavas" (ash content 1.45%, moisture content 14.5%) and water was used in all samples. There were three stages of sourdough preparation totally 72 hours; the renewal of sourdough was realized each 24 hours (Figure 1).
10 grams of sourdough in 90ml 0.5% sterile physiological liquid were mixed in BagMixer® at speed 7 for 60 seconds.
Plate counting method was used for microbial detection. The samples for investigation in two reiterations were taken in: 0, 4, 8, 24, 28, 32, 48, 52, 56, 72 hour of fermentation.
Total plate count was investigated on Nutrition agar (dilutions 1:100; 1:1000; 1:10000). Yeast plate count was investigated on Malt extract agar (dilutions 1:100; 1:1000). Lactic acid bacteria plate count was investigated on MRS agar (dilutions 1:100; 1:1000). Incubation was performed at 35 °C (for total plate count and LAB) and 27 °C (for yeasts) for 24 hours to develop the colonies.
Counting of colonies formed and calculating the number of CFUs was accomplished by Acolyte colony counter.
Changes of pH (Jenway 3250 pH meter) in sourdough were observed using standard methods with reference to "Standard - Methoden für Getreide, Mehl und Brot" (Spicher, 1993).

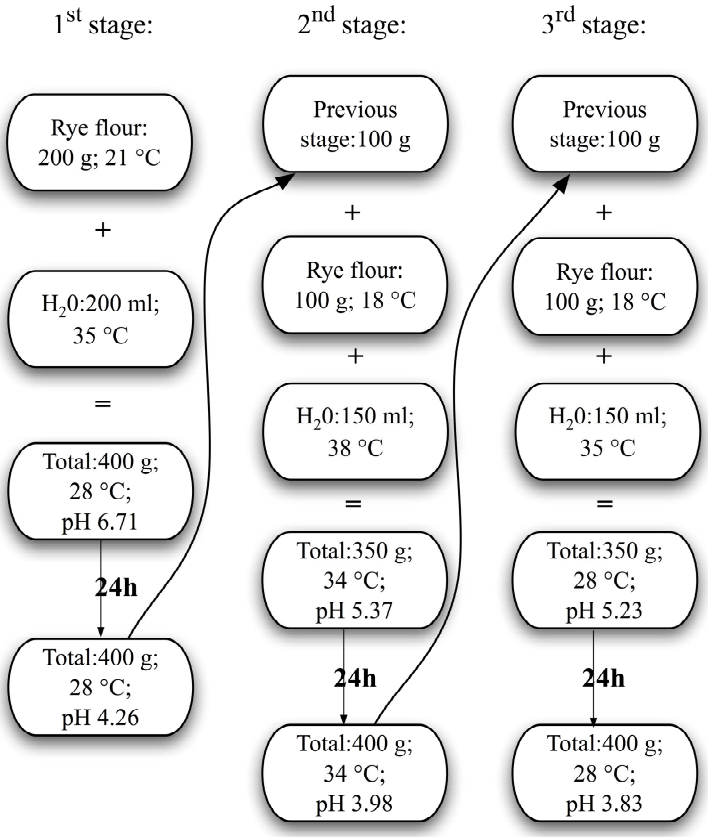

Figure 1. Three-stage technological process of spontaneous rye flour sourdough preparation

Dilution and Lindner methods were applied to obtain pure cultures. After isolating pure cultures API identification method was applied to identify microorganism cultures. For identification of LAB cultures API CH 50 test was used while ID 32 C for yeasts.

Results and Discussion

Three-stage method was used in spontaneous rye sourdough preparation (Figure 1). At the end of each stage, dynamics of microflora development was investigated.

Results shown in Figure 2 represent growth dynamics of spontaneous sourdough microflora during fermentation process and changes of pH value. The initial rates of plate count were very close – 4.27 log10 cfu ml^{-1} (LAB), 4.54 log10 cfu ml^{-1} (yeasts), 4.72 log10 cfu ml^{-1} (total plate count).

At the first four hours of fermentation changes in total amount of microorganisms and pH value were not relevant – LAB and yeasts remained in lag – phase and adapted to the new nutrients available. After four hours LAB and yeasts started an intensive exponential phase although at the end of the first stage of sourdough fermentation LAB and particularly yeast plate count started to decrease caused by limitation of nutrients.

Amount of yeast cells became 28% lower than initial rate supposedly because of activity of LAB. Generally, in the first stage of fermentation pH value decreased substantially as a result of intensive development of microorganisms – from initial rate pH 6.7 to pH 4.26.

Immediately after the first renewal of sourdough, pH value increased rapidly to pH 5.37 but after four hours it returned close to a previous level to pH 4.36. At the same time LAB started a new lag-phase whereas amount of yeasts increased by 31% in four hours after renewal. At the end of the second stage of spontaneous sourdough preparation LAB were developed

rapidly in exponential growth phase by 26% and reached 6.02 log10 cfu ml^{-1}. Equally, growth of yeasts increased in exponential growth phase during the second stage of fermentation. Decrease of pH value was not significant but remained stable and reached pH 3.98.

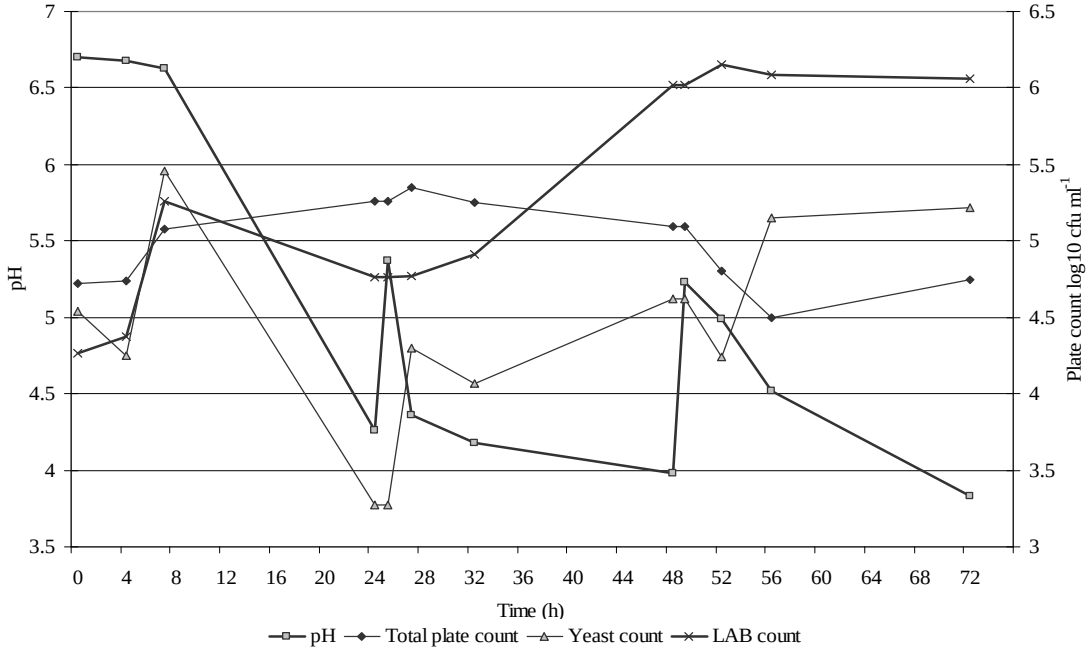

Figure 2. Development of microorganisms and changes of pH value in spontaneously prepared sourdough from rye flour during 72 hours

The second renewal at the beginning of third stage of fermentation had insignificant influence on development of LAB. It is possible that LAB cells were ageing and metabolites present in dough were inhibiting its regeneration. Opposite results were observed in the changes of yeast content – it increased by 23% during the third stage of fermentation and reached 5.22 log10 cfu ml^{-1}.

Convincing predominance of LAB, high amount of yeasts and a final pH value 3.83 represent that current sourdough has desirable properties for preparation of rye flour sourdough starter.

Results of API tests reveals microorganisms that are typical members of rye flour sourdough microflora - *Lactobacillus brevis, Lactobacillus fermentum, Saccharomyces cerevisiae.* With reference to "Handbook of food science, technology and engineering" (Y. H. Hui 2006): heterofermentative LAB *Lactobacillus brevis* are found in rye bread sourdough from Russia, Germany and Sweden; *Lactobacillus fermentum* are found in German, Austrian and Swedish rye bread sourdough; *Saccharomyces cerevisiae* yeast present microflora of rye flour sourdough from Germany, Finland, Poland, Germany and Denmark.

Conclusions

1. During 72 hours of rye flour sourdough preparation process, amount of LAB and yeasts increased by 42% and 15% respectively, though activity of these microorganisms increased significantly considering pH value changes from pH 6.7 to pH 3.83.
2. Predominance of LAB reaching 6.06 log10 cfu ml^{-1}, high amount of yeasts reaching 5.22 log10 cfu ml^{-1}and a final pH value 3.83 represent that this sourdough has desirable properties for preparation of rye flour sourdough starter.
3. Identification of microorganisms using API test method was successfully acquired. LAB and yeast cultures isolated and identified from current sourdough: *Lactobacillus brevis, Lactobacillus fermentum, Saccharomyces cerevisiae* are also typical members of German, Russian, Swedish etc. traditional rye flour sourdough.

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CARBOHYDRATE COMPOSITION OF MONOFLORAL WILLOW (*SALIX ALBA* SPP.) HONEY

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Abstract

The main task of this study was to determine the carbohydrate composition of willow honeys. The fructose, glucose and the minor oligosaccharide content of seven monofloral willow honeys collected in Lithuania in 2006 during flowering season were analyzed by gas chromatography with flame ionization detector (GC-FID) after the trimethylsilylation of carbohydrates. Fructose, glucose, sucrose, maltose, isomaltose, turanose, trehalose, palatinose, celobiose, raffinose and panose were identified and quantified in all samples. Glucose was predominant in 6 out of 7 samples. The mean values of fructose and glucose varied form 32.92 to 38.88 and from 35.27 to 42.29%, respectively. The ratio of fructose/glucose varied from 0.78 to 1.10. The amount of sucrose was 0.12–0.25%. Data obtained was thoroughly compared with previously published results and it was found that the characteristics of Lithuanian honeys in most cases meet international requirements for natural honey. Some correlations between sugar concentration and the content of willow pollen in the honey were established. However, the information on honey sugar composition is not sufficient for the reliable determination of the botanical origin of honey.

Key words: honey, carbohydrates, willow, gas chromatography

Introduction

In general, honey is a supersaturated sugar solution; sugars are the main constituents of honey accounting for about 95% of dry matter. Fructose and glucose are the major sugars, the former one being a major component almost in all honey types, except for some honeys of rape (*Brassica napus*), dandelion (*Taraxacum officinale*) and blue curls (*Trichostema lanceolatumi*) origin, when glucose is present in higher amounts (Cavia *et al.*, 2002). In addition, disaccharides, trisaccharides and other oligosaccharides are present in honey in small concentrations. The concentration of fructose and glucose as well as their ratio are useful indicators for the classification of unifloral honeys (Persano Oddo *et al.*, 1995; Persano Oddo and Piro, 2004).

It is known that there are compositional differences between honeydew and blossom honeys. Honeydew honey is characterised by a higher concentration of oligosaccharides, mainly trisaccaharides melezitose and raffinose, which usually are not found in blossom honeys (Bogdanov *et al.*, 2004).

Honey has been produced in Lithuania from the ancient times. Detailed carbohydrate composition of Lithuanian honey was not analyzed until now, except for routine measurements of glucose, fructose and saccharose for a standard quality assessment. The main honey plants in Lithuania is spring rape (*Brassica napus* L. ssp.) and in honey from those plants spring rape pollen are over-represented in honeys, but monofloral willow honeys also are collected. Monofloral willow honey medium are found in Spain, Croatia and Scandinavia countries (Persano Oddo and Piro, 2004). Moreover, there is a lack of the data in the literature about willow honey. Therefore, willow honey was selected for the analysis. The main task of this study was to comprehensively characterize carbohydrate composition of monofloral willow honey samples and to determine if there is any dependence between pollen content and the amount of corresponding carbohydrate in the honey. Such data may facilitate faster characterization of honey botanical source; it may also provide the information for the identification of adulteration and incorrect labelling.

Materials and Methods

Honey samples. Seven honey samples of willow origin were collected during 2006 years flowering season in Kėdainiai district, except one sample – in Vilkaviškis district (Lithuania).

The botanical origin of the samples was analysed by melissopalynology method (Baltrušaitytė *et al.,* 2007). Willow pollen content from total content of pollen in honey samples varied from 54.10 to 92.90%. In tested samples small amounts of rape, dandelion, white and red clover, cornflower, raspberry pollen also presented.

Gas chromatography analysis. Honey samples were diluted with ultra-pure water to a final Brix value 5–6. 140 µl of this solution and 10 µl of internal standard were transferred to a GC autosampler vials and freeze-dried in a Maxi-Dry Lyo (Heto-Holten, Allerød, Denmark) for 4 h. *β*-Gentiobiose was selected as an internal standard, because it was not found in honey. Freeze-dried samples were derivatized by the addition of 150 µl of 1-(trimethylsilyl) imidazole and 1 ml of pyridine. The vials were capped and the solutions heated at 80 ºC for 1 h. Trimethylsilylated carbohydrates are sufficiently volatile compounds and can be analyzed by gas chromatography. The stock solutions of reference compounds were prepared in the same way as the samples, from 5.0% (w/v) standard solutions in water.

Trimethylsilylated carbohydrates were analyzed on a GC 8000 series gas chromatograph with a flame ionization detector (Fison Instruments, Milan, Italy) by injecting 0.2 µl of the mixture to the capillary column ZB-5 (30 m×0.25 mm id×0.25 µm) coated with 5% phenylmethylpolysiloxane. The injector and detector temperatures were 260 ºC and 300 ºC, respectively. After testing several programs the most efficient separation was achieved when the temperature was raised from 100 ºC to 180 ºC at 4 ºC/min (5 min hold), increased to 215 ºC at 2 ºC/min and finally raised to 325 ºC/min at 3 ºC/min (10 min hold).

All reference compounds were analyzed in the same way. Identification of honey carbohydrates was achieved by comparing their retention times with those of reference compounds. The concentration was calculated using internal standard and expressed in % (w/w). The analyses were repeated three times.

Materials. All chemicals and solvents were of analytical grade. Ultra-pure (18.2 mΩ) water was used (Millipore, Simplicity, Canada). Pyridine and 1-(trimethylsilyl) imidazole, fructose (99.0%), glucose (99.5%), sucrose (99.5%), maltose (98.0%), isomaltose (98.0%), turanose (98.0%), trehalose (99.5%), *β*-gentiobiose (98.0%), palatinose (99.0%), celobiose (99.0%), raffinose pentahydrate (99.0%) and panose (98.0%) were from Sigma-Aldrich (Steinheim, Germany).

Statistical analysis. Standard deviations were calculated using spreadsheet software (Excel®). Correlation coefficients (R) to determine the relationship between several carbohydrates and the amount of the willow pollen in the honey, as well as between different sugars were calculated using SPSS statistical software.

Results and Discussion

GC-FID chromatograms indicate that sugar composition in the tested willow honey samples is similar, however some variations in the content of individual carbohydrates were observed. Three regions, representing mono-, di- and trisaccharides may be distinguished in the chromatographic profile of honey sugars (Figure 1).

In total, eleven carbohydrates were identified in the studied samples. Some separated peaks were not identified because reference compounds were not available. The amounts of the quantified sugars were within the limits established by Codex Alimentarius Commission (2001).

As it was expected (it is usual to the honey) fructose and glucose were dominant in willow honeys. As it was mentioned above, there are only few kinds of honey (honey from rape, dandelion and blue curl) when glucose is dominant (Cavia *et al.,* 2002). Our results do not totally coincide with this finding, because glucose was dominant in six out of seven samples. The mean values of fructose and glucose varied from 32.92 to 38.88 and from 35.27 to 42.29%, respectively (Table 1). Honey was intensively analysed for sugars, particularly in Southern European countries. For instance, 34.3–39.4% of fructose and 25.8–35.2% of glucose were reported in Spanish honeys (Mateo and Bosch-Reig, 1998); 31.4–39.8%

(fructose) and 27.4–36.3% (glucose) in honeys from Portugal (Mendes *et al.*, 1998). It was found that the percentage of carbohydrates also depends on the bee species (*Apis dorsata, A. cerana, A. millifera*) and concentration of the main sugar may vary in the wide range: 42.3–54.2% of fructose and 33.1–52.2% of glucose were determined in the honeys from Nepal (Joshi *et al.*, 2000).

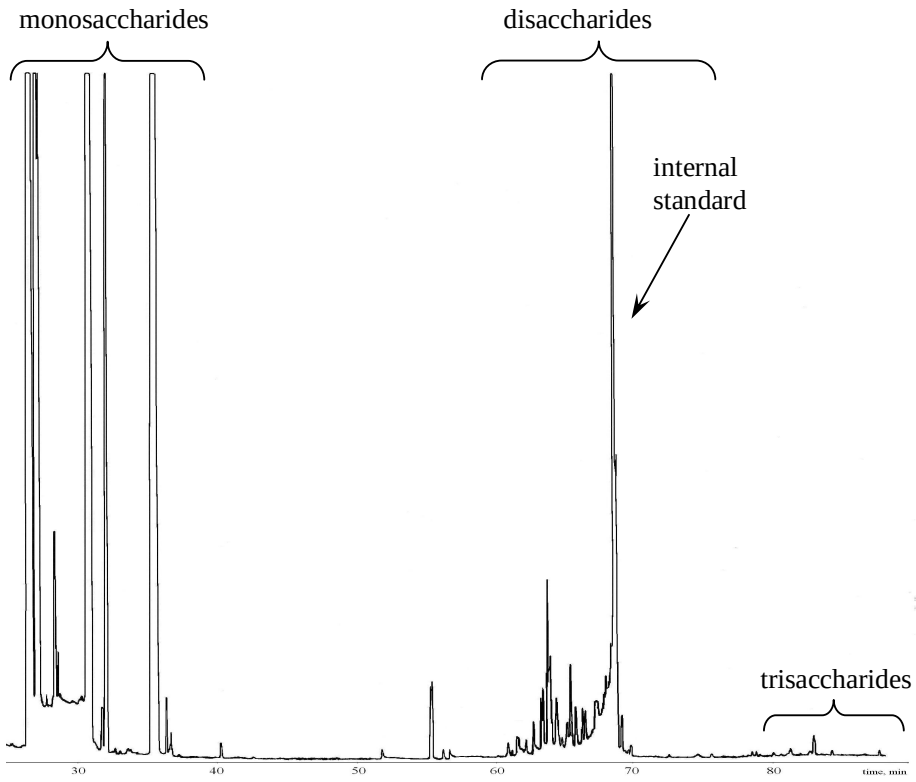

Figure 1. Typical chromatogram of the trimethylsilylated carbohydrates in willow honey

Table 1

Amount of pollen and the main carbohydrates in the tested willow honeys and fructose/glucose ratio

Pollen amount, %	Fructose, %	Glucose, %	F/G
92.90	34.27±0.27	41.55±0.03	0.82
75.80	32.92±0.22	42.29±0.08	0.78
73.20	34.74±0.14	42.23±0.18	0.82
70.20	38.88±0.24	35.27±0.09	1.10
65.60	34.12±0.10	42.21±0.06	0.81
57.50	36.11±0.06	37.80±0.18	0.96
54.10	34.67±0.11	41.86±0.23	0.83

Due to the low fructose value, the fructose/glucose ratio (F/G) was below 1. The ratio of F/G was 0.78–1.10; for comparison, in other studies of honey it was reported 0.84–1.89 (Persano Oddo *et al.*, 1995), 0.86–1.6 (Horváth and Molnár-Perl, 1997), 0.78–1.77 (Costa *et al.*, 1999), 0.99–1.77 (Mateo and Bosch-Reig, 1998).

Seven disaccharides and two trisaccharides were identified in the tested willow honey samples (Figure 2). Maltose was the most abundant disaccharide constituting from 1.14 to

1.85%. Maltose, which is usually present in honey in low quantities (to 3%), was suggested as a marker of natural honey (Persano Oddo *et al.*, 1995; Joshi et al., 2000; Cotte *et al.*, 2003).

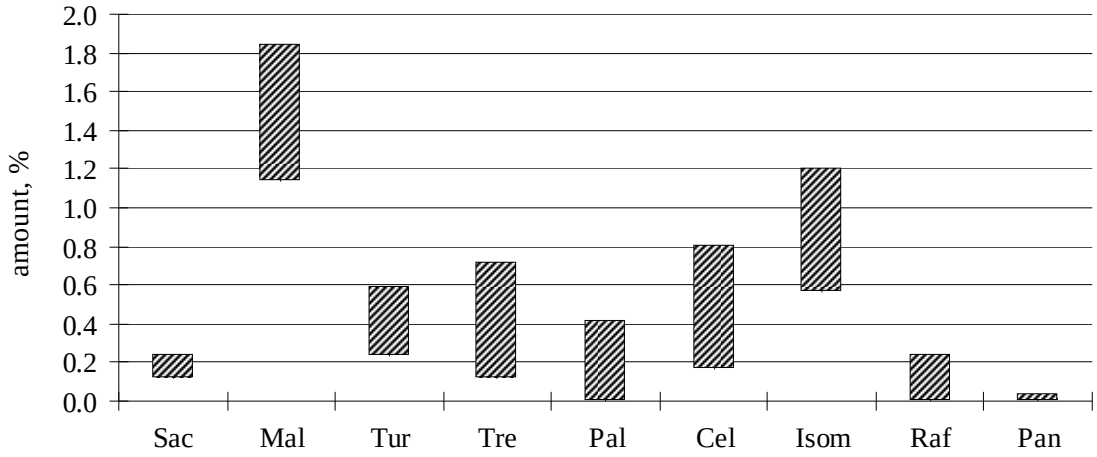

Figure 2. Distribution of disaccharides and trisaccharides in willow honey (Sac–saccharose; Mal–maltose; Tur–turanose; Tre–trehalose; Pal–palatinose; Cel–cellobiose; Isom – isomaltose; Raf – raffinose; Pan – panose)

The content of sucrose (which is the main source of the adulteration of honey) in the all tested samples was in conformity with the limits established by the European Codex Honey Standards, which are ≤5 g 100 g^{-1} for honeys in general and up to 10 g 100 g^{-1} for *Citrus* honeys (Codex Alimentarius, 2001).

Small amounts of two trisaccharides, panose and raffinose were found in the tested willow honeys (Figure 2). Raffinose was not find in one sample, while panose – in two samples. The origin of raffinose in floral honey is not clear; it is suggested that raffinose could be nectar constituent or could get in with honeydew contamination (Da Costa Leite *et al.*, 2000).

The total GC area percent of unidentified compounds in the chromatogram varied from 2.04 to 3.01%. The majority of the unidentified peaks eluted from the column at the end of GC run, i.e. in the region characteristic to trisaccharides (Figure 1). Therefore, it is likely that the majority of these peaks are representing various trisaccharides or other oligosaccharides.

The correlation coefficients between willow content and corresponding sugar, as well as between individual sugars were calculated. The correlation between willow pollen content in the honey and identified carbohydrates was weak; the calculated coefficients were not significant statistically. In the willow honey the correlation between glucose and fructose was strongly negative (R=–0.94, significant at the 0.01 level), while the correlation between some disaccharides was strongly positive: saccharose and maltose (R=0.88, significant at the 0.01 level), turanose and trehalose (R=0.93, significant at the 0.01 level). Medium correlation was noticed between saccharose and turanose (R=0.76, significant at the 0.01 level), and saccharose and trehalose (R=0.76, significant at the 0.01 level). It can be seen, that botanical source of honey cannot be concluded from honey sugar. All observed data were normally distributed (Gaussian distribution law).

Conclusions

The mono-, di- and trisaccharide composition of 7 honeys from willow origin were studied by GC–FID, after the trimethylsilylation of the carbohydrates. Fructose and glucose are dominant in all samples, their amount varied from 32.92 to 42.29% of honey. Fructose glucose ratio was below 1 in six samples, due to the higher amount of glucose in these samples. The amount of sucrose, like monosaccharides, coincides with the recommendations of European Codex Honey Standards; it varied from 0.12 to 0.25%.

The strong correlation between the willow pollen content in the honey and identified carbohydrate was not observed.

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PHENOLIC COMPOUNDS IN BASIL, OREGANO AND THYME

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Abstract
Among various abundant sources of phenolic compounds are spices such as oregano, thyme and basil. The aim of this research was to determine phenolic compounds in oregano, thyme, basil. In spices six phenolic compounds were identified – caffeic acid, rosmarinic acid, eriodyctiol, luteolin, naringenin, apigenin. The main phenolic compound in spices was rosmarinic acid, other compounds were present in less than 4% (from total phenolics). Caffeic acid was identified only in thyme. Compounds from two flavonoid classes were identified in spices: flavons (apigenin, luteolin) and flavonons (eriodictyol, naringenin). Apigenin and luteolin were detected in oregano and thyme. Eridyctiol was present in all spices, with the highest concentration determined in oregano, while naringenin was present in oregano and thyme. Flavonons eriodyctiol and naringenin were present in spices in higher concentrations compared to flavons apigenin and luteolin. Total amount of identified phenolic compounds was the highest in thyme.
Key words: phenolic compounds, aromatized oil, spices

Introduction
Several thousand molecules having a polyphenol structure (ie, several hydroxyl groups on aromatic rings) have been identified in higher plants, and several hundred are found in edible plants. Phenolics behave as antioxidants, due to the reactivity of the phenol moiety. Source of polyphenols as mentioned above are ubiquitous. Literature, however, shows that researchers have become interested in phenolic compounds in spices. Different amounts of phenolic acids and flavonoids have been detected in spices depending on growing conditions, plant part analyzed (leaves, flowers), and/or extraction conditions Spices are used for oil aromatisation either for oil enrichment or expanding the commercial advantages. Among spices frequently used for oil aromatisation are basil, thyme, oregano. Literature data show that these spices contain different amounts of phenolic compounds (Jayasingne *et al.*, 2003; Javanmardi *et al.*, 2002; Grayer *et al.*, 1996). Rosmarinic acid is a powerful antioxidant (Javanmardi *et al.*, 2002) and was identified as a main phenolic compound in oregano (Exarchou *et al.*, 2002, Pizzale *et al.*, 2002). Other phenolic compounds in oregano are caffeic acid, luteolin, apigenin, eriodictyol, dihydroxicampherol, dihydroxiquercitine (Škerget *et al.* 2005, Pizzale *et al.*, 2002, Kulevanova *et al.*, 2001). In thyme the main phenolic compounds are glycuronids of apigenin, luteolin, eriodyctiol, luteolin glycosides, rosmarinic acid, quercitine (Justesen, 2000; Guillen and Manzanos, 1998). The main phenolic compounds in basil are rosmarinic acid, lithospermic acid, vanillic acid, coumarinic acid, hydroksibenzoacid, syringic acid, ferulic acid, protocatheuic acid, caffeic acid (Jayasingne *et al.*, 2003, Javanmardi *et al.*, 2002). Plant phenolics are one of the most important primary antioxidants, and during aromatization process they could migrate from spices in oil and protect oil from oxidation. The aim of research was to detect phenolic compounds in the three spices (basil, oregano and thyme) that will be used for oil aromatisation and to evaluate two methods for extraction of phenolic compounds from spices.

Materials and Methods
The plant material for the analysis was obtained from the plant collections of Faculty of Agriculture, Latvia University of Agriculture and from Santa Maria (producer – AS Paulig Baltic, Estonia, licence – Santa Maria AB, Sweden, further – commercial sample). The following samples were analysed: commercial basil, commercial oregano, commercial thyme, basil Green (*Ocimum basilicum* L.), Greek oregano *(Oreganum vulgare* L.), thyme *(Thymus vulgare* L.). Commercial samples were supplied dry in hermetically sealed packaging. Samples from LUA Faculty of Agriculture were air dried (30 °C temperature) and packaged.

Phenolic compounds from spices were extracted using two methods.

Method 1.

500 mg of dried leaf material was pulverized (particle size 0.125–0.250 mm), suspended in 5 ml of methanol (High Performane Liquid Chromatography (HPLC) grade), and left overnight at 4 °C under dark conditions. All supernatants were decanted and filtered using syringe filter (Javanmardi *et al.*, 2002) and transferred into HPLC vials. This method is mainly used for the extraction of phenolic acids.

Method 2.

500 mg dried leaf material was pulverized, suspended in 10 ml diethyl ether (HPLC grade) and left to extract overnight at room temperature. The diethyl ether (HPLC grade) was evaporated in rotavapour (till dryness, temperature of water bath 18±2 °C). The dried residues was redissolved in 1 ml of 80% MeOH, filtered using syringe filter and transferred into HPLC vials (Grayer *et al.*, 2003).

Reversed-phase HPLC and mass detection were performed in an Agilent 1100 LC-MSD system controlled by Agilent software v. A.09.03 (Agilent Technologies, Waldbronn, Germany). A Phenomenex C18 (ODS, Octadecyl) security guard and a Phenomenex Luna C18 (2) 100 Å column (4.6 mm i.d. x 250 mm; particle size =10 µm), maintained at 35 ^{o}C, were used. Elution was performed at a flow rate of 1.0 mL/min, using as mobile phase a mixture of 0,05% formic acid (HPLC grade) in water (solvent A) with pH=3.1, acetonitrile (HPLC grade) (solvent B). The solvent gradient is changed according to the following conditions: 1) 0–5 min, B: 10–35%; 2) 5–20 min, B: 35–70%; 3) 20–40 min, B: 70–90; 4) 40–41 min, B: 90–50%; 5) 41–42 min, B: 50–25%; 6) 42–43 min, B: 25–5%; 7) 43–44 min, B: 5–0%; and 8) 44–46 min, B: 0–10% Detection was done at 280 nm and 320 nm. The mass detector was an Agilent G1946D (SL) ion-trap mass spectrometer (Agilent Technologies, Waldbronn, Germany) equipped with an electrospray ionisation (ESI) system. Nitrogen was used as nebulizing gas at a pressure of 50 psi and the flow was adjusted to 13 l/min. The full scan mass spectra of the phenolic compounds were measured from m/z 100 up to m/z 1000. Mass spectrometry data were acquired in the negative ionization mode. For identification of phenolic compounds, HPLC retention times, UV spectra, mass spectra were compared with those of standards or those phenolics identified in spices previously. Analysis of variance was performed using SPSS 11.0 for Windows. Significant differences between means were determined a level of $p<0.05$.

Results and Discussion

Total amount of quantified phenolic compounds in analysed spices differed significantly ($p<0.05$) (Fig.1). Thyme was shown to have the highest amount of phenolics whereas basil had six times lower concentration of phenolics. There were some differences between commercial samples and those obtained from the University plant collection. Besides, the obvious influence of the solvent on the amount of extracted phenolics was observed.

Out of diethylether and methanol, the latter was shown to contribute better to the recoveries of polyphenols from the matrix. Literature studies showed different methods that could be used for extraction of phenolic compounds (Lee, 2000, Jayasingne *et al*., 2003). Methanol is widely used to extract antioxidants from plant material which can be applied also for the extraction of antioxidants from spices (Kim *et al*., 2005, Jayasingne C. *et al*., 2003, Pizzale *et al*., 2002). On the other hand, for extraction of flavonoids also diethyl ether has been used (Grayer *et al*.., 2003; Grayer *et al.*, 1996).

In the present study more phenolics were extracted using methanol. Extracted amount with diethyl ether was 8.8–9.1 times lower. The highest amounts were in commercial thyme, Latvian thyme and Greek oregano, but the lowest amount in both basil samples. Phenolic compounds of spices belong to different clases: phenolic acids, flavonoids, anthocyanins.

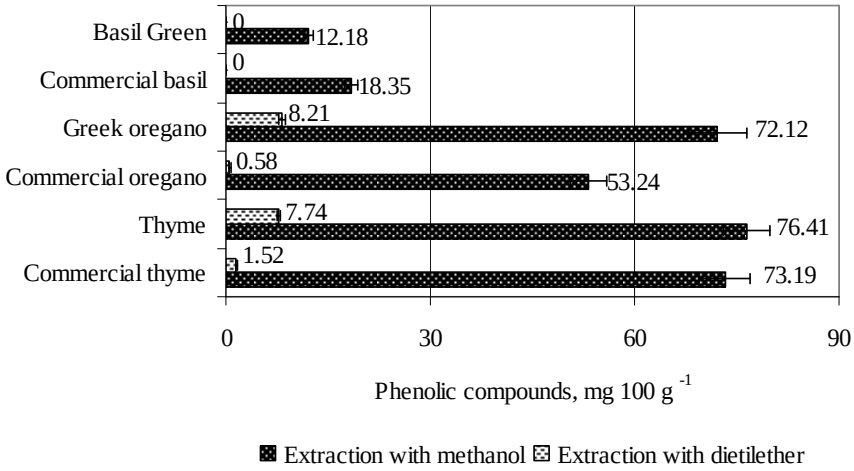

Figure 1. Total phenolic compounds in spices

In the analysed spices, 6 phenolic compounds, belonging to different classes were identified: phenolic acid (caffeic acid), phenolic acid derivative (caffeic acid dimmer – rosmarinic acid), flavons (apigenin, luteolin) and flavonons (eriodictyol, naringenin). Rosmarinic acid is the main compound in all analysed spices and content of other phenolic compounds is very low (till 4%).

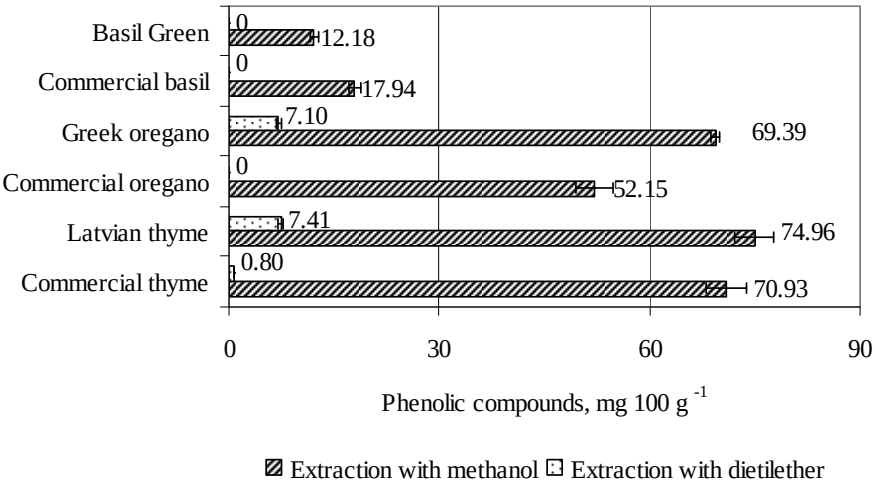

Figure 2. Rosmarinic acid in spices

The highest amount of rosmarinic acid was in Latvian thyme, commercial thyme and Greek oregano (Fig.2), but the lowest amount in basil samples. Rosmarinic acid was the only phenolic in basil Green. Extracted amount of rosmarinic acid was dependant on the used solvent, and higher amounts were obtained using methanol. There are big differences between chromatograms of samples obtained with both methods (Fig.3).

In the chromatogram of Greek oregano samples extracted with methanol (Fig.3.a) the main peak is rosmarinic acid, others are significantly smaller. On the other hand, in the chromatogram of sample extracted with diethyl ether (Fig.3.b) the main peaksare flavonoids (eriodictyol and naringenin) peaks, followed by rosmarinic acid peak. Of phenolic acids in spices, caffeic acid was the only one identified. This compound presented was present in both thyme samples: in the Latvian thyme 0.26 mg 100 g $^{-1}$, in the commercial thyme 0.38 mg 100 g $^{-1}$. Furthermore, four flavonoids were identified and their content differed significantly ($p<0.05$) between spices (Table 1). None of the flavonoids was identified in basil Green.

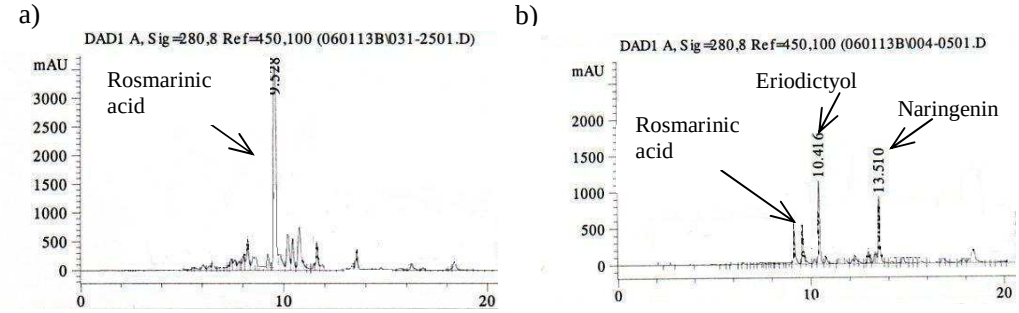

Figure 3. Chromatograms of Greek oregano phenolic compounds extracted with a) methanol, b) diethyl ether

Apigenin was identified in oregano and thyme with the highest amount in Greek oregano and Latvian thyme (Table 1) and higher amounts of apigenin were extracted with methanol. The second flavonoid – luteolin, was present in lower amounts, comparing to apigenin. It was not possible to identify apigenin and luteolin in basil samples.

Table 1

Flavonoids in spices, mg 100 g $^{-1*}$

Compounds	Extraction solvent	Spices			
		Greece oregano	Commercial oregano	Thyme	Commercial thyme
Apigenin	Methanol	0.282±0.014	0.105±0.006	0.376±0.019	0.209±0.01
	Dietilether	0.081±0.005	0.040±0.003	0.088±0.006	0.119±0.006
Luteolin	Methanol	n.d.	n.d.	n.d.	0.160±0.008
	Dietilether	0.027±0.002	0.064±0.004	0.047±0.003	0.043±0.003
Eriodictyol	Methanol	1.382±0.069	0.273±0.014	0.382±0.022	0.446±0.026
	Dietilether	0.518±0.037	0.104±0.007	0.059±0.004	0.122±0.006
Naringenin	Methanol	1.069±0.053	0.717±0.036	0.433±0.022	1.073±0.054
	Dietilether	0.481±0.024	0.372±0.019	0.124±0.001	0.432±0.022

*Results are given as mean±standard deviation
n.d. – not detected

This data are in accordance with those obtained for basil produced in Denmarks, where none of these compounds were identified (Justesen, 2001).
The highest amount of luteolin was in commercial thyme. Unlike apigenin, it was possible to extract higher amounts of luteolin with diethyl ether. Exception is the commercial thyme, from which luteolin was better extracted with methanol. The highest amount of eridyctiol was in Greek oregano, whereas in other analysed spices it was 3 to 5 times lower. Naringenin was identified in all oregano and thyme samples, with the highest amount in Greek oregano. Eridyctiol and naringenin were reported as characteristic compounds of thymes grown in Macedonia (Marin *et al.*, 2003) which is geographical close to Greece and Greece cultivars.

Conclusions

Total amount of identified phenolic compounds was the highest in the Latvian thyme, followed by the commercial thyme and the Greek oregano. In the spices six phenolic compounds were identified – caffeic acid, rosmarinic acid, eriodyctiol, luteolin, naringenin, apigenin. It is possible to extract more caffeic acid, rosmarinic acid, and apigenin using methanol as a solvent, whereas diethyl ether is a better solvent for extraction of – eriodyctiol, luteolin and naringenin. The main compound in all analysed spices was rosmarinic acid.

Literature data showed that this compound is a strong antioxidant, and therefore it can be suggested that these spices could contribute to the prevention of the oxidation of aromatised oils besides giving them nice aroma.

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AMINO ACID PROFILE IN LATVIAN POTATO VARIETIES PREPARED BY VARIOUS COOKING METHODS

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Abstract

Potato consumption in Latvia is taking significant part from total vegetable amount therefore it is an important to focus attention on potato nutritional changes during mostly used heat treatment methods. In fact potato contains small amount of protein but it is high in nutritional quality – contains considerable amount of crucial amino acids which change notably during frying/backing process.

Consequently the aim of the research was to estimate changes in amino acid profile among various potato varieties prepared using various heat treatment processes.

Following Latvian potato varieties were selected: 'Brasla', 'Imanta', 'Zile', 'Madara' and 'Lenora'. Potatoes were prepared using different heat treatment methods: shallow frying (150±5 °C), deep fat frying (180±5 °C) and baking in oven (210±5 °C). The highest total amino acid content was found in raw potatoes of the variety 'Brasla' and crucial amino acid content in 'Lenora' on dry matter (DM) basis while the lowest both total and crucial amino acid amount in the variety 'Imanta'. Among the baked potatoes in the variety 'Lenora' total amino acid content was found as the highest but crucial amino acids – in the variety 'Madara' like in shallow and deep fat fried potatoes. For their part, similarly the lower amount of total amino acids – in the variety 'Brasla' and crucial amino acids in the variety 'Imanta'. In addition, the effect of type of heat treatment and kind of potato variety on each amino acid was studied. Comparing heat treatment types significant changes were discovered in Arginine (Arg), Aspartic acid (Asp), Phenylalanine (Phe), Tyrosine (Tyr), Isoleucine (Ile), Histidine (His, Lysine (Lys) and Valine (Val) while among varieties in Asp, Ile, Val, Glutamic acid (Glu), Methionine (Met) and Tyr.

Key words: potato varieties, amino acids, heat treatment methods

Introduction

The potato (*Solanum tubersoum L.*) originated in the Andes Mountains of South America where it has been an important food for 8,000 years and know is spread through the entire World (Harris, 1992). Potatoes have an important role in a healthy diet, as they are a good source of complex carbohydrate, dietary fibre, vitamin C and protein. Concerning the protein amount, potatoes in average contain about 2% when quality is considered to be excellent, e.g. amount of lysine in potatoes is similar to that in typical animal protein (Salunkhe *et al.*, 1998), but the nutritional value of potato protein varies with variety, storage and environmental conditions as well as the type of cooking (Lisinska, 1989; Harris, 1992). Traditionally, potatoes are a central component of warm meal in many European countries (Wandel *et al.*, 2001) and are prepared using various methods of cooking from where the traditional ones and well known are boiling, frying and baking (Salunkhe *et al*, 1998). Frying, especially deep fat frying has become the most popular preparation technology during the last few decades. In the research by Nordic and Baltic countries' authorities on the type of potato cooking methods, it was discovered that in Latvia consumption level of fried potatoes comparing with neighbour countries has been one of the highest (NorBaGreen, 2003). Out of that, in Latvia other cooking methods like shallow frying and baking in oven are famous as well. One of the explanations could be formation of desirable flavour, colour, taste and texture during frying and baking processess which can be pointed out by occurrence of Maillard reaction (Sanibal, 2004; Amrein, 2003). Reaction is based on the high temperature during heating process where the accumulated reducing sugars react with free amino acids in the tuber cells (Mottram *et al.*, 2002) and nutritional changes in amino acid content occur. Several amino acids, like Asp, Asn, Gln, Glu, Val and Lys, which are present in relatively minor amounts had been shown to be the most active amino acids in the Maillard reaction (Ashoor *et al.*, 1984; Akiko *et al.*, 2005) Therefore, the aim of the research was to evaluate the changes in amino acid profile during various heat treatment processes in several potato varieties selected and cultivated in Latvia.

Materials and Methods

In the cooperation with State Priekuli Plant Breeding Institute (Latvia) five table potato varieties almost in the same size were chosen: 'Lenora', 'Brasla', 'Imanta', 'Zile' and 'Madara' which are selected and cultivated under the control of the institute. Selected potato varieties were analysed after short period (2 weeks during sizing) of storage at temperature 4–6 °C and relative air humidity (RH) 80 %.

Washed and hand-peeled potato tubers were cut in three ways: for shallow frying potatoes were sliced into 0.7x1.0 and 3–4 cm long strips while for deep fat frying – into 0.6×0.6 and 4–5 cm long strips but potatoes prepared for oven frying were cut horizontally into halves. Control sample is raw potato.

Sunflower-seed oil was used for frying. Potatoes were baked in oven (210±5 °C), shallow fried on the pan (150±5 °C), and deep fat fried in the deep fat fryer (180±5 °C), (Fig. 1). Throughout the oven and deep fat frying procedure time/temperature was recorded by USB TC-08 Thermocouple Data Logger PICO-Technologist equipment (Fig. 1).

Amino acids were determined by AOAC official method 994.12, total protein content was determined by the Kjeldahl method (LVS ISO 5983:1997) and dry matter (DM) content by LVS ISO 6496:1999 and XM120 Precisa moisture analyser.

Figure 1. Time/temperature control with TC-08 Thermocouple Data Logger equipment

Obtained data were analysed by statistical software S-PLUS 6.1 Professional Edition. By means of specifying the differences between independent groups *Two-Way Analysis of Variance* and for the multiple comparisons of the means *Tukey* test was applied. Conclusions were done at 95% significance level.

Results and Discussion

In the research the amount of protein, DM content and 16 amino acids including seven essentail: Thr, Val, Met, Ile, Leu, Phe and Lys were determined in five various potato varieties prepared applying several heat treatment methods. To compare obtained amino acid values data were calculated on DM basis. The protein content is presented in g per 100 g of product (Table 1).

Table 1

Protein content in potato varieties prepared by several cooking methods, g 100 g $^{-1}$

Potato variety	Control	Baked in oven	Shallow fried	Deep fat fried
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
'Brasla'	2.09±0.04	2.33±0.10	2.63±0.08	3.41±0.04
'Imanta'	1.68±0.07	2.11±0.11	2.13±0.10	4.76±0.13
'Lenora'	2.04±0.06	2.08±0.07	1.94±0.07	2.70±0.08
'Madara'	1.55±0.04	2.46±0.10	2.61±0.13	4.27±0.08
'Zile'	1.44±0.01	2.07±0.11	2.86±0.13	3.48±0.08

Among the potato varieties and cooking methods the highest protein content was found in the control samples of 'Brasla', among baked samples – 'Madara', shallow fried – 'Zile' and deep

fat fried – 'Imanta' while the lowest – in the control and baked samples of 'Zile' furthermore shallow fried and deep fat fried – 'Lenora'.
Changes of the protein content can be explained by occurrence of several reactions during pre-treatment and heat treatment at high temperature.
There are several amino acids which biological activity loses during heat treatment process therefore it is important to follow up the changes of the amino acids itself. Comparing the differences among samples by type of cooking methods (n=4) and potato varieties (n=5) per each type of amino acids (n=16) the differences was found $p<0.001$ within some of varieties and cooking methods (Table 2, 3).

Table 2

Differences of amino acid content among the potato varieties

Amino acid	'Brasla'	'Imanta'	'Lenora'	'Madara'	'Zile'
Aspartic acid (Asp)* **	A	A	B	B	AB
Glutamic acid (Glu)* **	A	B	B	B	AB
Valine (Val)* **	A	A	AB	B	A
Methionine (Met) **	A	A	B	AB	AB
Isoleucine (Ile)* **	A	A	B	B	AB
Tyrosine (Tyr)*	AB	A	AB	AB	AB

The same letter within each component is not significantly different at the 5 % level by the Tukey multiple-comparison test. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Table 3

Differences of amino acid content per DM depending on the heat treatment method

Amino acid	Control	Baked in oven	Shallow fried	Deep fat fried
Aspartic acid (Asp) **	A	AB	AB	B
Valine (Val)*	A	AB	AB	B
Isoleucine (Ile)*	A	AB	B	B
Tyrosine (Tyr)* **	A	B	B	B
Phenylalanine (Phe) **	A	AB	B	B
Histidine (His)* **	A	B	B	AB
Lysine (Lys) **	A	AB	B	B
Arginine (Arg)*	A	AB	AB	B

The same letter within each component is not significantly different at the 5 % level by the Tukey multiple-comparison test. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

In table 4 can follow up the changes in the amount per each amino acid on DM basis between potato varieties prepared by several heat treatments.

Table 4

Amino acid content depending on type of heat treatment and potato variety, g 100 g $^{-1}$ dry matter

Potato varieties: **B** – 'Brasla', **I** – 'Imanta', **L** – 'Lenora', **M** – 'Madara', **Z** – 'Zile'

Amino acids	Control					Baked in oven					Shallow fried					Deep fat fried				
Variety	**B**	**I**	**L**	**M**	**Z**	**B**	**I**	**L**	**M**	**Z**	**B**	**I**	**L**	**M**	**Z**	**B**	**I**	**L**	**M**	**Z**
Asp	1.32	1.34	1.85	1.57	1.57	1.20	1.10	1.61	1.67	1.22	1.11	1.08	1.56	1.72	1.41	0.88	1.03	1.17	1.41	1.35
Thr	0.28	0.30	0.33	0.34	0.29	0.33	0.27	0.29	0.31	0.26	0.30	0.23	0.25	0.35	0.40	0.26	0.23	0.20	0.28	0.20
Ser	0.29	0.28	0.31	0.28	0.29	0.25	0.21	0.29	0.28	0.29	0.21	0.20	0.29	0.31	0.40	0.22	0.21	0.22	0.26	0.20
Glu	1.05	1.28	1.88	1.61	1.28	1.00	1.54	1.57	1.25	0.97	0.93	1.51	1.52	1.75	1.32	0.79	1.45	1.27	1.36	0.77
Pro	0.20	0.23	0.19	0.22	0.21	0.25	0.24	0.21	0.21	0.23	0.18	0.23	0.22	0.28	0.37	0.26	0.26	0.16	0.23	0.16
Gly	0.19	0.22	0.20	0.18	0.16	0.19	0.18	0.16	0.21	0.16	0.15	0.16	0.18	0.22	0.28	0.18	0.17	0.14	0.19	0.12
Ala	0.21	0.24	0.22	0.27	0.22	0.22	0.18	0.25	0.21	0.23	0.21	0.20	0.25	0.25	0.37	0.22	0.21	0.22	0.23	0.14
Val	0.31	0.22	0.31	0.38	0.22	0.22	0.18	0.29	0.31	0.26	0.21	0.20	0.33	0.31	0.28	0.18	0.19	0.26	0.28	0.22
Met	0.09	0.07	0.12	0.12	0.09	0.07	0.08	0.13	0.12	0.09	0.09	0.08	0.14	0.11	0.11	0.08	0.07	0.09	0.09	0.09
Ile	0.17	0.15	0.21	0.21	0.18	0.14	0.12	0.16	0.21	0.19	0.12	0.14	0.18	0.19	0.15	0.13	0.13	0.14	0.19	0.16
Leu	0.38	0.42	0.46	0.34	0.41	0.36	0.33	0.33	0.42	0.45	0.33	0.34	0.29	0.41	0.34	0.33	0.30	0.26	0.35	0.37
Tyr	0.38	0.23	0.42	0.41	0.36	0.22	0.18	0.25	0.24	0.19	0.21	0.20	0.29	0.22	0.31	0.26	0.19	0.22	0.21	0.22
Phe	0.31	0.31	0.32	0.25	0.23	0.25	0.21	0.25	0.24	0.26	0.21	0.20	0.22	0.22	0.25	0.20	0.19	0.22	0.21	0.24
His	0.20	0.21	0.21	0.20	0.25	0.14	0.12	0.16	0.17	0.13	0.12	0.11	0.18	0.16	0.15	0.13	0.11	0.12	0.12	0.12
Lys	0.36	0.28	0.46	0.32	0.36	0.31	0.27	0.29	0.31	0.32	0.27	0.25	0.29	0.31	0.31	0.26	0.26	0.22	0.28	0.31
Arg	0.59	0.28	0.50	0.49	0.41	0.33	0.39	0.45	0.35	0.29	0.51	0.34	0.40	0.28	0.28	0.37	0.30	0.20	0.26	0.27
Essential	1.91	1.76	2.19	1.97	1.79	1.69	1.45	1.74	1.93	1.83	1.52	1.44	1.70	1.90	1.83	1.45	1.37	1.39	1.68	1.59
Total	8.26	6.07	7.98	7.20	6.55	5.50	5.57	6.69	6.53	5.53	5.14	5.47	6.60	7.10	6.71	4.77	5.29	5.11	5.94	4.95

Some of amino acids play an important role during Maillard reaction like Lys which is one of the most essential amino acids and is present in considerable amount in the potatoes. Another important amino acid is Asp which is one of the main precursors of acrylamide formation through the Maillard reaction. Losses of Tyr can be explained by the oxidation in the presence of oxygen during potatoes cutting prior to frying.

Conclusions

1. There are considerable differences in several amino acid profiles among potato varieties and type of heat treatment was found. Significance within the type of the potato variety was found in Asp, Glu, Val, Met, Ile and Tyr while within the type of heat treatment applied in Asp, Val, Ile, Tyr, Phe, His, Lys and Arg.
2. The change in Lys was discovered in type of heat treatment however differences within the varieties were not significant as well as Glu and Val while Asp changes were found both within the potato variety and type of heat treatment.
3. From the nutritional point of view, fair essential amino acid content in the control samples was in 'Lenora', in baked, shallow fried and deep fat fried potato samples of the variety 'Madara'.

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CHANGES OF BIOCHEMICAL COMPOUNDS IN SEABUCKTHORN MARC DURING STORAGE

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Abstract
Seabuckthorn fruits contain functionally active compounds: vitamins, minerals, fiber, organic acids, and phenolic compounds, important for human health. As a result of juice pressing marc is left which consists of fatty acids, carotenes, insoluble fiber in a relatively large amount. Since the tendency of using finely ground wheat bread is rising, it is possible to increase its nutritive value by adding seabuckthorn marc. The aim of this work was to determine the changes of different fatty acids, carotenes, phenolic compounds and vitamin E of seabuckthorn mark during storage. The research was carried out at the Experimental Fruit and Berry Processing Center of Latvia State Institute of Fruit Growing during 2005, Latvia. It was found that the content of fatty acids reduced for 4.3 to 21.6%, the content of vitamin E – for 54.5%, the content of phenolic compounds – for 44.9% but the content of carotenes – for 32.7%.
Key words: seabuckthorn marc, fatty acids, phenolic compounds

Introduction
Seabuckthorn are one of the most valuable plant crops because they contain vitamin, organic acids, fiber, pectic compounds, carotenes, polyunsaturated fatty acids and other components (Kawecki *et al.*, 2004; Jamyansan and Badgaa, 2005; Lebeda, 2003). In the process of obtaining seabuckthorn juice, as a leftover remains marc, which contains 8–10% biologically active compounds important for the human organism, including natural antioxidants – vitamin C and E, carotenoids, minerals and polyphenols (Tsybikova *et al.*, 2003; Novruzov, 2005). The marc is used for the production of pulp and seed oil (Singh, 2005; Singh and Mörsel, 2005). In Germany the marc after pressing is dried and the oil is extracted by hexane, liquid gas or similar way (Heilscher, 2003). In Mongolia oil is extracted from the dry marc of the seabuckthorn by Kazantsevis and Ohina method at 50–65 °C (Avdai and Chimed-Ochir, 2003).
The marc is possible to be utilized as a raw material for the production of palmitoleic acid methyl ester concentrate (Klaas and Meurer, 2004). A new use was researched in the Institute of Technology of Mogilev where fresh pressed seabuckthorn marc was used for the making beverages (Timofeeva, 1996). However in Russia the uses of marc are wider: in baking of bread and manufacturing of vitamin mixtures for farm animals and birds (Zolotareva, 2004; Tsybikova *et al*., 2003; Koshelev *et al*., 2003). Similar research was done in Estonia using seabuckthorn marc to increase the nutritive value of wheat bread (Lõugas *et al.*, 2005).
The seabuckthorn marc can be used in bread, confectionery production and in other cereal products to increase their nutritive value (Singh, 2005; Gailite and Strautniece, 2005).
The storage of seabuckthorn marc after juice pressing can be different: it can be dried immediately and stored dry or put into the refrigerator and stored at the temperature of -18 °C. Biochemical content of the dry marc changes during storage. The changes can significantly influence its nutritive value. Therefore the aim of this work was to determine the changes of different fatty acids, total carotenes, phenolic compounds and vitamin E during storage of dry seabuckthorn marc.

Materials and Methods
The research was carried out at the Experimental Fruit and Berry Processing Center of Latvia State Institute of Fruit Growing during 2005. The research material – fruits of different seabuckthorn (*Hippophae rhamnoides* L.) cultivars, widely grown in Latvia, was harvested at "Baltplant" Ltd, Dobele region and was stored frozen at minus 18 °C until analyzing. The

juice was pressed from thawing fruits with a press "Voran 60K". The marc was dried by compulsory air circulation for 24 hours at the temperature +4 °C; crushed in mill and stored in plastic bags in a dark place at temperature +5 °C for three months. The biochemical composition of seabuckthorn marc was analyzed during storage.

The content of vitamin E in samples was established by a modified method. The method is based on the extraction of carotenoides with petroleum benzene. The same solvent was used for the determination both of carotenoides and vitamin E.

Five (5) g of sea buckthorn berries were crushed and put into a 100 ml retort. Ten (10) ml of 96% ethanol were added and the solution was mixed for 5 minutes. After adding of twenty five (25) ml of petroleum benzene mixing was continued 4 hours. Then thirty (30) ml of H_2O was added and mixed for 15 minutes. The sample was filtered. The sediment was rinsed with ten (10) ml of 96% ethanol and two times with H_2O. After the separation of both layers the upper one was used for analyses. Yellow petroleum benzene layer was put in twenty five (25) ml retort and refilled till the mark.

Two (2) ml of petroleum benzene were taken and put into twenty five (25) ml retort. Ten (10) ml of ethanol were added and mixed. Then one (1) ml of 0.5% 2,2'-dipiridyl solvent was added in ethanol and mixed; one (1) ml of 0.2% $FeCl_3$ solvent added in ethanol and the retort was placed in a dark place. After 15 minutes ethanol was added to the retort till the mark. The absorbance of light was measured with a spectrophotometer at 500 nm. At the same time a control substance was prepared in the same way as the analyzed substance, only instead of the analysis two (2) ml of petroleum benzene were taken. The content of vitamin E was found by using a graduation curve and calculated by using the formula:

$$X = \frac{c \times 12.5 \times 100}{a},\ \text{mg } 100\text{g}^{-1} \qquad (1)$$

where:

c – the content of vitamin E by using a graduation curve, mg/25ml;
12.5 – factor of sample dilution;
a – weight of sample, g.

Analysis for determination of carotenoides:

Two (2) ml of petroleum benzene solvent were taken and put in twenty five (25) ml retort, refilled till the mark. The absorbance was read at 450 nm. Petroleum benzene was used as control.

For the analyzes of total phenolic compounds (mg $100g^{-1}$) the Folin-Ciocelteu method (Singelton, 1999) was used, for fatty acids (% of total fatty acids) standard method ISO 5508:1990 was used.

Dates were statistically elaborated using SPSS for Windows and MS Excel.

Results and Discussion

The biochemical compositions of seabucthorn pulp and seed oil are substantially different. The palmitic ($C_{16:0}$) and palmitoleic ($C_{16:1}$) acids dominate in the pulp oil, whereas linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acids dominate in the seed oil (Jamyansan and Badgaa, 2005). Since the seabuckthorn marc contains both oils, then its content of fatty acids depends of the proportion between these ingredient contents.

At the beginning of the research there were established higher contents of $C_{18:2}$ and $C_{18:3}$ fatty acids (respectively 32.3±2.3 and 25.8±1.8%) in the sample (Figure 1). However, the contents of $C_{16:0}$ and $C_{16:1}$ fatty acids were less than on average in pulp oil (respectively 15.4±1.1 and 12.5±0.9%). It means that the samples of marc contain a higher amount of seeds than the skin and pulp part. The samples contained 2.1±0.1% $C_{18:0,}$ 11.4±0.8% $C_{18:1}$ and 0.4±0.03% $C_{20:0}$ in average (Tab. 1), that conforms to data given in literature (Jamyansan and Badgaa, 2005; Korovina and Fefelov, 2005). After 60 days of marc storage the considerable losses were

established for $C_{16:1,}$ $C_{20:0}$ and $C_{16:0}$ fatty acids (respectively 21.6; 20.0 and 14.9%). The content of $C_{18:1}$ fatty acid decreased by 10.2%, but $C_{18:0,}$ $C_{18:3}$ and $C_{18:2}$ respectively by 9.5; 7.0 and 4.3%.

Table 1

Changes of fatty acids during storage

*Fatty acids	Storage time, days		
	0	30	60
Palmitic ($C_{16:0}$)	15.4 ±1.1	14.9±1.0	13.1±0.9
Palmitoleic ($C_{16:1}$)	12.5±0.9	11.7±0.8	9.8±0.7
Stearic ($C_{18:0}$)	2.1±0.1	1.8±0.1	1.9±0.1
Oleic ($C_{18:1}$)	12.7±0.8	12.0±0.8	11.4±0.9
Linoleic ($C_{18:2}$)	32.3±2.3	33.4±2.3	30.9±2.1
Linolenic ($C_{18:3}$)	25.8±1.8	24.6±1.7	24.0±1.7
Arahinic ($C_{20:0}$)	0.4±0.03	0.37±0.03	0.32±0.02

** % of total fatty acids*

The content of total carotenes in whole dried fruits is in average 20.43 mg 100 g^{-1} (Novruzov, 2005). It still substantially depends on the initial content of carotenes in the fruits, as well as on the processing technology (Heilscher, 2003). Several researches testify that the content of carotenes in seabuckthorn oil in a dark place at temperature +5 °C after three month storage decrease from 42.5 to 47.5% (Novruzov, 2005; Bekker *et al.*, 2005).

At the beginning of our research the content of total carotenes in seabuckthorn marc was on average 37.0 mg 100 g^{-1} (Figure 1).

This research did not show disparity in the content of total carotenes after 30 storage days ($p>0.05$). Disparity was established after 60 storage days ($p<0.05$) where the content of total carotenes decreased to 32.05 mg 100 g^{-1}. The content of total carotenes decreased for 32.7% during whole storage time (90 days), which is comprisable with the data in literature.

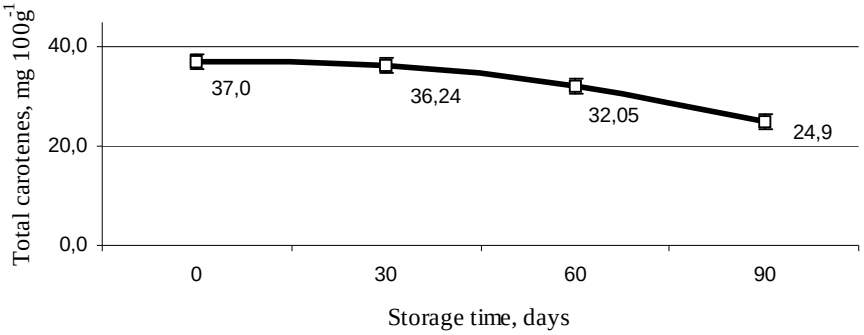

Figure 1. Changes of total carotenes content during storage

Fruits of seabuckthorn contain different phenolic compounds that are represented mainly by flavonols, leuoanthocyanins, catechins, chlorogenic acid etc. The total content of phenols depends on the cultivar and varies from 828.7 to 1099.6 mg 100 g^{-1} (Novruzov, 2005). As this researcher indicates, the content of phenolic compounds is influenced not only by cultivar but also species, grooving place and stage of ripeness. In the beginning of the research there were 2256.4 mg 100 g^{-1} of total phenolic compounds in dry seabuckthorn marc (Figure 2). With probability 95% there was established a disparity ($p=0.028<0.05$) after 30 storage days. Relevant decrease was established after three-month storage reaching 1243.3 mg 100 g^{-1}.

The content of total phenols in seabuckthorn marc decreased for 44.9% during the whole storage time.

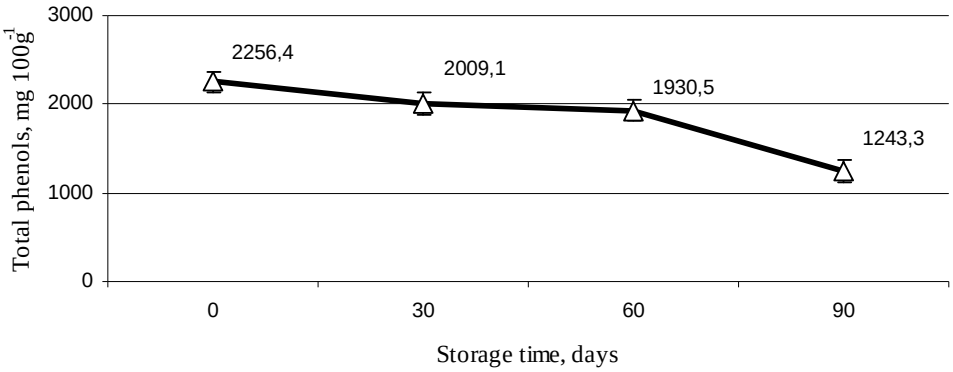

Figure 2. Changes of total phenolic content during storage

Summarizing the research data from China and Italy, the content of vitamin E in pulp oil may be 54.3–248.2 mg 100 g^{-1} (Rongsen, 2005; Antonelli *et al.*, 2005). Depending on the extraction way and technology the content of vitamin E is different, in addition it is significantly influenced by the growing environment of the crop. For example in *subsp. sinensis* (growing place Jiaoko County, Shaxi, China) in wet soil the content of vitamin E in pulp oil and seed oil was higher (respectively 220 and 688 mg 100 g^{-1}) than in dry badlands (respectively 150–170 mg 100 g^{-1}) (Wei and Guo, 1996). Similar results were shown by research from Mongolia: the pulp oil contained 330.4 mg 100g^{-1}, but seeds oil 260.0 mg 100 g^{-1} of vitamin E.

The beginning of our research there was 559.5 mg 100 g^{-1} of vitamin E in seabuckthorn marc. The content of vitamin E relevant by decreased during all storage time. In addition the decrease was on average about 85 mg 100 g^{-1} in first two months, but in the third month on average 134 mg 100 g^{-1} (Figure 3). At the end of research the content of vitamin E was decreased by 54.5% (to 254.39 mg 100 g^{-1}).

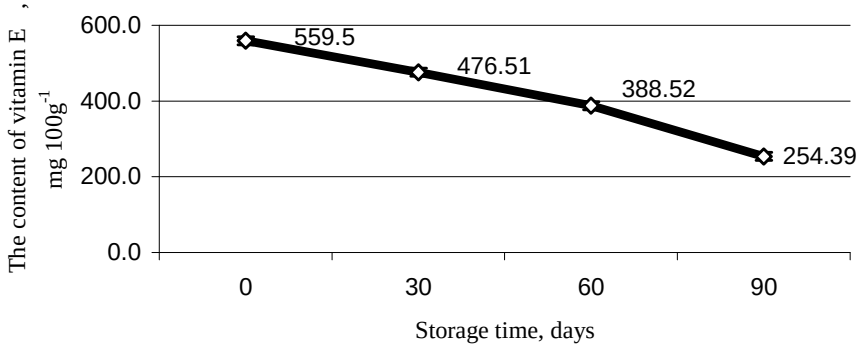

Figure 3. Changes of vitamin E content during storage

Conclusions

The obtained data show that it cannot be recommended to store dry seabuckthorn marc, because during storage its nutritive value significantly decreases, along with the possibility to use it for the nutritive improvement of other products, e.g., wheat bread. The highest losses of fatty acids during 60 storage days were established for $C_{16:1,}$ $C_{20:0}$ and $C_{16:0}$ fatty acids (respectively 21.6; 20.0 and 14.9%). The content of total carotenes in seabuckthorn marc decreased by 32.7%; the content of total phenolic compounds – by 44.9% but the content of vitamin E decreased by 54.5% during 90 days.

Acknowledgements

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RADICAL SCAVENGING CAPACITY AND CHEMICAL COMPOSITION OF *AGRIMONIA EUPATORIA* AND *AGRIMONIA PROCERA*

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Introduction

Aromatic and medicinal plants are a good source for bioactive components which may be used in foods, nutraceuticals agrimony (*Agrimonia eupatoria* L.) and fragrant agrimony (*Agrimonia procera* L.) are Rosaceae family plants growing wild in Europe. They have been used in folk medicine for blood, cardiovascular, gastrointestinal, genitourinary, inflammatory, liver, respiratory tract, skin and some other conditions. However, scientific information on their composition and properties is rather scarce. The aim of this study was to assess radical scavenging properties and composition of extracts and their fractions isolated from two *Agrimonia* species by different polarity solvents.

Materials and Methods

The plants were grounded before extraction and extracted with acetone or methanol in automatic extractor. The solvents were removed in a rotary vacuum evaporator. Aqueous extracts were obtained by shaking ground material with water and concentrating obtained extracts in a freeze drier. Crude acetone extracts were further fractionated by using two immiscible solvent systems in a separation funnel. The separation was based on different solvent polarity (hexane, *t*-buthylmethylether, butanol and water). Radical scavenging capacity (RSC) of extracts was measured in $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical reactions; their composition was determined by HPLC/UV/MS.

Results and Discussion

The RSC of *A. eupatoria* and *A. procera* extracts varied in a very wide range: 9.1–97.5% in $DPPH^{\bullet}$ reaction and 6.7–79.5% in $ABTS^{\bullet+}$ reaction, depending on the solvent polarity. The extracts isolated with hexane were not effective, due to a low solubility of polar antioxaidants. HPLC/UV/MS analysis of extracts resulted in several quantitatively important peaks; three of them were identified as luteolin-7-*O*-glycoside, hyperoside and apigenin-glycoside. It can be concluded that the results obtained encourage further more comprehensive studies of *Agrimonia* species, which may find applications in the preparation of health promoting functional ingredients.

COLLABORATION DUTCH PUBLIC FOOD SAFETY AUTHORITY AND PRIVATE CERTIFICATION BODIES

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Introduction

In the Netherlands, there is a long tradition in practices in the field of food safety. The obligation to have a good working HACCP-system in each place where food is being produced, stored or processed already exists since 1996. Another important driver for Dutch food producers to upgrade and improve the HACCP-system continuously were the specific requirements on food safety of some leading food retailers in the Netherlands. They claimed that each supplier not only had to fulfil the legal requirements of having a well working HACCP-system, but also had to have a HACCP-certificate. As a result of these requirements, nowadays 60% of the Dutch food producers/processors possess a HACCP-certificate which is a much higher number than in any other country, either in EU or in the rest of the world.
This claim could be set due to the fact that a Dutch HACCP scheme exists that is already accredited since 1997. This Dutch HACCP scheme ("Requirements for a HACCP based Food Safety system") results in a private certificate. The Dutch HACCP-scheme is compiled by the National Board of Experts – HACCP in The Netherlands and is owned by the (private) Foundation for Food Safety Certification. The Dutch HACCP scheme is GFSI approved since 2002.

Need for less duplication of audits.

Of course the Dutch Food Safety Authority (VWA = Dutch Food and Consumer Product Safety Authority) has a legislative task and responsibility to safeguard the food safety. This authority surveys, assesses and communicates the risks and makes them manageable in the society. They try to realize this task by monitoring the safety of food, feed and consumer goods, the health and welfare of animals and the alcohol and tobacco legislation. The VWA until now disregarded the HACCP-certificates which where achieved by the food businesses. The motivation for this was the own responsibility given by the EU and Dutch government. Certified food businesses were assessed and monitored on the same way as non-certified food businesses. Certification is a private initiative and is a company's choice. Of course this way of monitoring and assessing was criticised by the certified food businesses which put a lot of effort in gaining the HACCP-certificate.
Recently started a discussion between the public VWA and the private certification bodies to discuss how more confidence from the VWA could be achieved. A better collaboration was seen as a general interest for anybody. When the VWA could be convinced of the quality of these private certification audits, this could benefit all HACCP certified food companies and the VWA as well. So an intensive discussion started. A number of measurement were discussed which were already implemented since many years as qualification of auditors, harmonisation of audits and audit reports and making the information available for VWA. In return, the VWA is willing to share their specific knowledge and experience, e.g. by opening their unique database which gives a clear view of all possible risks in a certain sector of the food industry.

New public and private partnership

Very recently the certification bodies and the Food Authority and the food industry agreed in a unique cooperation which resulted in a change in the policy of the Dutch Food Safety Authority: a risk based policy and using private initiatives of the food industry. Transparency in the market and sharing food safety knowledge are important preconditions for this collaboration. Finally it has to result in less audits and an improvement of food safety throughout the whole food supply chain. This new way of monitoring and surveying food businesses by the VWA has already started in 2008 as a pilot. If the experiences are positive this cooperation will be extended to other sectors of the Dutch food industry.

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HETEROCYCLIC AROMATIC AMINES IN HEATED MEAT

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Abstract

It is well–known that cooking of various meats at high temperatures results in the formation of compounds that are not present in uncooked meats. Heterocyclic aromatic amines (HCAs) are an important class of heat-produced compounds. They were found to be mutagenic and carcinogenic for animals and even for humans. The aim of this study was to measure the amount of HCAs in beef during very long time and high temperature cooking. After sample extraction from the model system by using Chromabond XTR and Chromabond PS-H^+ cartridges the concentrations of HCAs[1] were separated by HPLC and measured by a mass spectrometry using mass selective detection mode. Six HCAs (IQ; MeIQ; 4,8-DiMeIQx; MeAαC; Trp-P-1; PhIP) were identified and quantified in the heated beef samples. The concentrations of these HCAs varied from undetectable levels to 50.4 ng g^{-1}. MeAαC and Trp-P-1 were present in heated beef extracts at the highest concentrations, while the values for the amino imidazoazaarenes were lower. PhIP which is one of the most abundant HCAs in conventionally heated meat was found at rather high concentration.

Key words: heterocyclic aromatic amines, heated beef, amino imidazoazarenes, amino carbolines

Introduction

Heterocyclic aromatic amines (HCAs) are formed during the cooking of meat (Keating *et al.*, 1999). Since the discovery of heterocyclic aromatic amines in foods a large number of scientific publications have been published on this subject until nowadays. Generally, there are two classes of HCAs: amino imidazoazarenes and amino carbolines. The amino-imidazo part of the amino imidazoazaarene molecule is formed from creatine (creatinine), while other substitutes come from Strecker degradation products (pyridines or pyrazines), which are formed in the Maillard reaction between sugars and amino acids. Amino-carbolines are formed during pyrolysis of amino acids or proteins at higher than 300 °C temperature. Both of these classes contain more than 20 different compounds. Some of them are strong mutagens and carcinogens as it was observed in the experiments with animals (Sugimura, 2002); moreover, some study models showed how HCAs may influence the formation of human cancer (Felton, 1995).

There are a lot of factors influencing the formation of HCA in meat. However, it seems that the most important factors are four: food composition, temperature, time and contact with heat source. Therefore, the increase of temperature and/or heating time may result in higher HAs concentrations. The aim of this study was to measure the amount of HCAs in beef during very long time and high temperature cooking.

Materials and Methods

Chemicals and materials

All HCAs references were purchased from Toronto Research Chemicals (Toronto, Canada); acetic acid, acetonitrile, methanol (all HPLC grade) and diethylene glycol were from Sigma-

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Abbreviations of HCAs:

PhIP	1-Methyl-6-phenyl-1H-imidazo[4,5-b]pyridin-2-amine CAS No.: 105650-23-5;
IQ	2-Amino-3-methylimidazo[4,5-F]quinoline CAS No.: 76180-96-6;
MeIQ	2-Amino-3,4-dimethylimidazo[4,5-f]quinoline CAS No.: 77094-11-2;
4,8-DiMeIQx	2-Amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline CAS No.: 95896-78-9;
Trp-P-1	3-Amino-1,4-dimethyl-5H-pyrido[4,3-b]indole, acetate CAS No.: 68808-54-8;
Trp-P-2	3-Amino-1-methyl-5H-pyrido[4,3-b]indole CAS No.: 72254-58-1;
AαC	2-amino-9H-pyrido[2,3-b]indole CAS No.: 26148-68-5;
MeAαC	2-amino-3-methyl-9H-pyrido[2,3-b]indole CAS No.: 68006-83-7;
Glu-P-1	2-amino-6-methyldipyrido[1,2-A:3',2'-D]imidazole, hydrochloride hydrate CAS No.:67730-11-4;
Glu-P-2	2-aminodipyrido[1,2-α:3',2-D]imidazole, hydrochloride CAS No.: 67730-10-3.

Aldrich (Germany); ethyl acetate from Chempur (Poland); sodium hydroxide, hydrochloric acid and ammoniumhydroxide (25%) from Merck (Darmstadt, Germany). Diatomaceous earth extraction Chromoband XTR (70 ml, 14500 mg, kieselguhr) and Chromoband PS-H+ (strong PS/DVB cation exchanger in H+ form) cartridges were provided by Macherey-Nagel (Duren, Germany).

Meat preparation and cooking

Beef was purchased from the local market. One kg of raw beef meat was homogenized, freeze-dried and stored in a freezer at -18 °C until use. One g of freeze-dried meat was heated with diethylene glycol (10 ml) in crucibles at 220 °C for 30 min using oven (grill with convection program). The crucibles with meat were immediately cooled on ice after heating.

Extraction of HCAs

The chromatographic separations of HCAs were performed using modified method (Messner & Murkovic, 2004). All samples were dissolved in 12 ml 1 M NaOH and homogenized for 30 min at 150 rpm. The alkaline solution was poured into Extrelut cartridges. Ethyl acetate (50 ml) was used as the extraction solvent and the eluate was passed into PS-H^+ cartridges. The cartridges were washed with 0.1 M HCl (2 ml) and MeOH (2 ml) and HCAs were eluted with 2 ml MeOH-concentrated ammonia (19/1, v/v). All samples were evaporated to dryness (using nitrogen) and extracts were dissolved in 100 mg methanol before measurement.

Identification and quantification of HCAs

All conditions were maintained similar as described previously (Messner & Murkovic, 2004). HCAs were identified and their amount was determined by HPLC-MS system using Waters ASSC-MS equipment (Waters, Milford, USA) equipped with Waters 1525 ASSC pump, Waters ZQ-2000 mass spectrometer on a reverse phase analytical column (Altima C18 5u, 150 mm, ID 2.1 mm). The data was analyzed using Mass Lynx 4.0 software (Micromas UR Ltd., England). Mass selective detector was equipped with an atmospheric pressure ionization electrospray (API-ES) using a fragmentation voltage of 45 V for positive ionization. Drying nitrogen was heated to 350 °C and the drying gas flow was 10 l/min.

The data were acquired in the selected ion mode for HCAs and calculated in the extract ion mode. The HCAs were quantified using calibration curve of each HCA in MeOH.

Results and Discussion

Preparation of beef samples, identification and quantification of HCAs were performed using new model system described elswhere (Messner & Murkovic, 2004). LC-MS chromatograms of the selected solutions of HCAs references in MeOH and beef meat samples heated for 30 min at 220 °C in crucibles are presented in Figure 1; six HCAs (IQ; MeIQ; 4,8-DiMeIQx; MeAαC; Trp-P-1; PhIP) were identified in beef samples.

LC-MS chromatograms of several solutions of HCAs references in MeOH (100 ng ml) and beef meat samples heated for 30 min at 220 °C in crucibles are presented in Figure 1.

The concentrations of HCAs in freeze dried beef samples are presented in Figure 2.

In general, the concentration of different HCA in meat varied from undetectable to 50.4 ng g^{-1}. MeAαC (50.4 ng g^{-1}) and Trp-P-1 (31.4 ng $^{-1}$) were present in highest amounts in beef extracts. It should be noted that unusually high amount of MeAαC was found in meat of our study comparing to some previously published results (Skog *et al.*, 1998; Toribio *et al*., 2000; Messner & Murkovic, 2004); the concentration of MeAαC in earlier studied real and/or model systems was more than 10 times lower. Comparatively small amounts of IQ (11.1 ng g^{-1}), MeIQ (14.3 ng g^{-1}) and 4.8-DiMeIQx (10.6 ng g^{-1}) were formed during thermal treatment of meat. PhIP which is one of the most abundant HCAs in conventionally heat treated meat was determined in our meat samples in a quite high concentration constituting 22.3 ng g^{-1}. It should be noted that this finding differs from the previously reported, when beef samples were heated using almost the same model system at the same time and temperature (Messner & Murkovic, 2004). It might be due, that in our experiment 10 times higher amounts of samples and reagents there were prepared and cooked.

Also, some role may play differences in a type of cooking oven as well as extraction cartridges, which were used in our and above mentioned study. Others available HCAs as a reference compounds were not found in cooked meat samples.

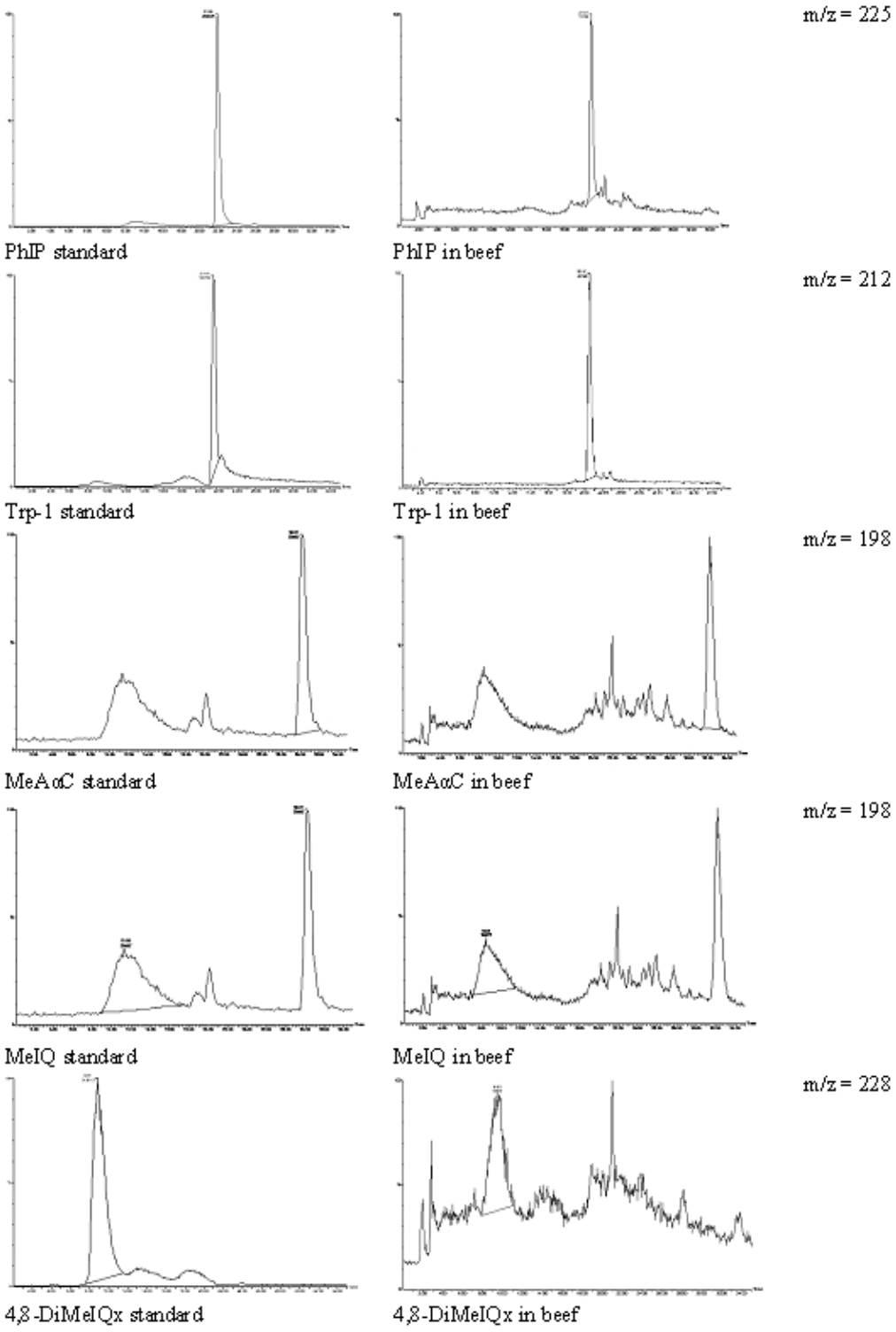

Figure 1. LC-MS chromatograms of several solutions of HCAs references in MeOH (100 ng/ml) and beef meat samples heated for 30 min at 220 °C in crucibles

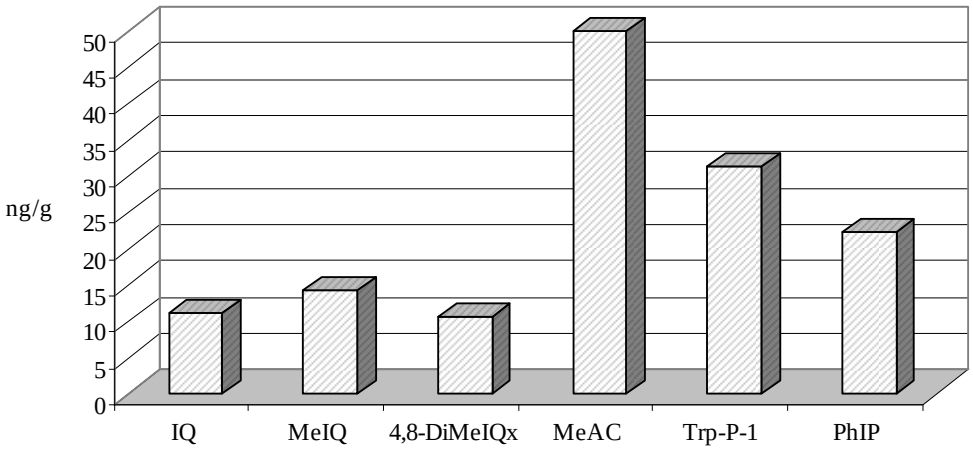

Figure 2. HCAs concentrations in freeze-dried beef samples after heating for 30 min at 220 °C temperature

The comparison of the data obtained on various HCAs indicates that there was a significant difference between the concentration of amino imidazoazaarenes and amino carbolines. The values for the amino imidazoazaarenes were lower. Most likely this finding can be explained by the fact that carbolines and their analogues usually form during treatment of meat at very high temperatures. Consequently, heating 1 g of meat for 30 min at 220 °C can be considered as a very long time and high temperature thermal treatment.

Conclusions

Five of six HCAs which were identified in beef samples possess strong mutagenic-carcinogenic properties. The concentrations of the identified HCAs in heated meat varied from undetectable levels to 50.4 ng g^{-1}. MeAαC and Trp-P-1 were present in heated beef extracts at the highest concentrations. The concentration of PhIP, which is one of the most abundant HCAs in cooked meat was also present at a quite high quantity. The values of IQ; MeIQ; 4,8-DiMeIQx in heated beef were lower, on average 12 ng g^{-1}. It can be concluded that higher amounts of amino-carbolines HCAs than amino imidazoazaarenes type HCAs are formed during long time and high temperature cooking of beef.

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CONTENT OF CARBOHYDRATES AND SPECIFIC ROTATION ANGLE OF HONEY

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Abstract

The main carbohydrates of honey are fructose, glucose, sucrose and maltose. Invertase hydrolyzes sucrose about fructose and glucose. Therefore low content of sucrose and high content of glucose and fructose in honey are parameters for characterization of honey quality. Several sorts of honey contain heightened content of maltose. Therefore it is possible to use this criteria for identification several honey sorts. Each carbohydrate has a specific angle of rotation of polarized light (specific rotation). It depends on relations and content of carbohydrates in honey. The aim of the present research was to establish the relationship between honey sorts and content of carbohydrates as well as specific rotation and possibilities of using these criteria (content of carbohydrates, specific rotation, and activity of invertase) for characterization of honey quality. Following parameters were determined with different physico – chemical methods: specific rotation – by method of polarimetry, content of sugars with high pressure liquid chromatography and activity of invertase – spectrophotometrically. The following results of honey analysis were obtained: the activity of invertase 4–30 (invertase number); content of carbohydrates (sucrose 0.5–3.0%, glucose 30–38%, fructose 35–42%, and maltose 1–6%); specific rotation $[\alpha]_D^{20}$ at -16° to -5°. The obtained results indicated that content of carbohydrates partially dependent on honey sorts. Content of sucrose depends from invertase activity in honey. Invertase is good parameter for honey characterization. Specific angle of rotation of polarized light is not available for identification of honey sorts.

Key words: honey, sugars, invertase, polarized light, identification.

Introduction

Honey is a complex natural product, containing more than 400 different substances, e.g. various carbohydrates, organic acids, proteins, amino acids, enzymes, aroma substances, mineral substances, pigments, waxes, etc (Belitz *et al.*, 2005).

The main carbohydrates of honey are fructose, glucose, sucrose and maltose. Invertase hydrolyzes sucrose about fructose and glucose, but enzymes amylases hydrolyzed starch about carbohydrate maltose, glucose and dextrose (Кашковский, Кузнецова, 2003).

The name of mix from sugars glucose and fructose is invert sugars. The content of glucose and fructose in honey average is 31.3% and 38%. In her turn content of maltose in honey is to 9%, but content of sucrose to 8%, in several honey sorts yet more. Proportions of glucose and fructose partially determine the crystallizations speed of honey. Fructose determines the hygroscopic features of honey, but glucose – the speed of honey crystallization. Honey with partially crystallization, the top liquid layer, basically contains fructose (Farmer, 2003; Belitz et al., 2005; Kasenburger, 2006).

The highest content of sucrose and lowest content of invert sugars (glucose and fructose) characterize the bad maturing of honey or else about bee feeding with sucrose (Шабаршов, 2002).

EU and Latvia are adopted the following standards of quality control for honey sugars: invert sugars in flower honey – no less than 60%, in honeydew honey – no less than 45%; sucrose – no more than 5%, in some exceptions – 10% (Council Directive 2001/110/EC, 2002; LR MK noteikumi Nr.522, 2003).

Several sorts of honey contain heightened content of maltose. Therefore it is possible to use this criteria for identification several honey sorts (Чепурной, 2002).

Each carbohydrate has a specific angle of rotation of polarized light (specific rotation). It is depending on relations and content of carbohydrates in honey. As now, that specific rotation of carbohydrate fructose is -92.4°, specific rotation of glucose +52.7°, specific rotation of sucrose +66.5°, but specific rotation of maltose 130.4^o (Чепурной, 2002).

One of the characterizations criteria of honey quality is activity of enzymes. Important enzyme in honey is invertase. Invertase is more sensitive to heat than amylases and loses activity during storage faster compared to amylases. That is why in few countries (Italy,

Switzerland) invertase is used as additional criteria to characterize honey quality. As a freshness indicator invertase is also used in honey standards of the beekeepers association in Germany, Belgium and Spain (Bogdanov *et al.,* 1999).
In conformity to EU recommendations it was proposed that fresh and unheated honey should have an invertase number (IN) higher than 10, but for honey with low enzymatic activity IN higher than 4 is recommended (Bogdanov, 1997).
The aim of the present research was to establish the relationship between honey sorts and content of carbohydrates as well as specific rotation and possibilities of using these criteria (content of carbohydrates, specific rotation, and activity of invertase) for characterization of honey quality. Research the relationship between invertase activity and content of sucrose in honey. Following parameters were determined with different physico – chemical methods.

Materials and Methods (Bogdanov, 2002)
In our work was analysed various honey sorts (various flowers, wild flowers, lime blossom flowers, dropwort flowers, phacelia flowers, sweet clower flowers, heather flowers, meadow flowers and buckwheat flowers) from different regions of Latvia.
Content of carbohydrates (glucose, fructose, sucrose, and maltose) in honey was determined by method of high pressure liquid chromatography (HPLC).
Principle. After filtration of the solutions, the sugar content is determined by HPLC with RI – detection. Peaks are identified on the basis of their retention times.
Calculation. Quantitation is performed according to the external standard method on peak areas or peak heights.
Parameters for method:
column: Altima Amino 100A 5 u,
flow rate: 1.3 ml min^{-1},
mobile phase: acetonitrile/water (70:30 v/v),
column and detector temperature: 30 °C,
sample volume: 10 µl.
Specific rotation was determined by method of polarimetry.
Principle. The angular rotation of a clear, filtered aqueous solution is measured by means of a polarimeter. The value is related to the carbohydrate composition.
Calculation. The specific optical rotation, $[\alpha]_D^{20}$ is the angle of rotation polarized light at the wavelenght of the sodium D line (λ=589.3 nm) at 20 °C of an aqueous solution of 1 dm depth and containing 1g ml^{-1} of the substance.

$$[\alpha]_D^{20} = \frac{\alpha \cdot 100}{l \cdot m}, \qquad (1)$$

where: α – angular rotation found,
l – length in decimetres of the polarimeter tube,
m – grams of dry matter taken.

The activity of invertase in honey samples was determined by method of spectrophotometry.
Principle. p-Nitrophenyl-α-D-glucopyranoside (pNPG) is used as substrate for the determination of the invertase number in honey. pNPG is split into glucose and p-nitrophenol by invertase. By adjusting the pH value to 9.5 the enzymatic reaction is stopped and at the same time nitrophenol is transformed into nitrophenolate anion, witch corresponds to the amount of converted substrate and is determined spectrophotometrically at 400 nm.
Calculation. Invertase activity is expressed in invertase units (IU kg^{-1}) or in invertase number (IN), where 1 IN=7.344732 IU kg^{-1}. One IU is defined as the number of micromoles of substrate destroyed per minute and expressed per kilogram of honey. One IN is defined as the number of gram of sucrose hydrolysed per hour and expressed per 100 grams of honey.

$$IN = 21.64 \times A_{400}, \quad (2)$$

where: A – the value of absorbtion,

21.64 – slope of the linear regression of IN (y axis) on ΔA_{400} (x axis).

Results and Discussions

Various honey samples from different regions of Latvia (gathered in 2007) were investigated. Analyses were done in laboratories of the Chemistry department of Latvian University of Agriculture.

Results of investigations of different kinds of honey are shown in Table1.

Table 1

Content of carbohydrate in honey and specific rotation

Kinds of honey	Regions of gathering	Content of carbohydrate in honey, %				Specific rotation, $[\alpha]_D^{20}$
		Fructose	Glucose	Sucrose	Maltose	
Various flowers	Ludza	38.64	33.13	2.32	1.04	-12
Various flowers	Jekabpils	37.10	37.94	1.92	3.92	-9
Various flowers	Cesis	40.17	36.13	2.28	3.02	-14
Wild flowers	Madona	36.40	32.67	2.31	1.26	-5
Wild flowers	Cesis	41.74	33.09	2.12	0.73	-16
Lime blossom	Riga	37.72	35.21	1.98	1.95	-7
Lime blossom	Talsi	38.04	36.31	2.17	2.11	-8
Dropwort flowers	Valka	40.50	38.17	2.14	0.87	-16
Heather flowers	Limbazi	37.97	33.20	2.40	4.29	-10
Meadow flowers	Riga	39.12	37.03	2.32	1.75	-14
Buckwheat flowers	Saldus	37.96	38.48	1.72	3.12	-16
Phacelia flowers	Jelgava	41.52	38.51	2.31	6.00	-14
Sweet clower	Riga	37.30	37.77	2.69	4.99	-8

From results of "specific rotation" it is possible to ascertain, that specific rotation of honey is not depending from a honey kinds. It means that the parameter of specific rotation cannot be used for identification of honey kinds.

It is necessary to note, that at all samples of honey, and a parameter of specific rotation is a negative size. Polarized light of all analyzed honey samples turn on left.

From results of content of carbohydrates in honey it is possible to ascertain, that the content of carbohydrates are not full depending from honey kinds. At analyzed honey samples the attitude fructose/glucose is in an interval 0.98–1.26.

It is known, that the crystallization of some honey kinds are faster, and the crystallization of some honey kinds are more slowly. Speed of crystallization in honey is defined with a proportion and the content of carbohydrates in honey.

It is known, that carbohydrate glucose promotes the crystallization of honey, and however carbohydrate fructose breaks crystallization of honey.

Crystallization of such kind as heather blossom honey is slowly. About the analysis of ours research can see, that the content of glucose in heather blossom honey one of the smaller.

It is necessary to note, what even in boundaries of one kinds of honey, honey can have a different speed of the crystallization.

The content of reducing sugars is differentiated in quality standards of flowers and honeydew honey. Standards of EU, *Codex Alimentarius* and Latvia require the following: in flower's honey it has to be $\geq$60 g 100 g^{-1}, in honeydew honey it has to be $\geq$45 g 100 g^{-1}. The same content ($\geq$45 g 100 g^{-1}) of these sugars has to be in blends of both flower and honeydew

honey (Codex Alimentarius Standart 12–1981, Rev. 2, 2001; Council Directive 2001/110/EC, 2002; LR MK noteikumi Nr. 522, 2003).

In both standards the norm of sucrose is required to be no more then 5%. In some sorts of honey the sucrose content can be higher. So in honey from lucerne and citric plant sucrose content can achieve 10%, and in honey from lavender even 15%.

The data of our analyses give evidence that content of reducing sugars and sucrose in explored honey's samples complies with requirements of quality standards of EU and LR for honey, as well as they correspond to data given in the literature (Belitz et al., 2005).

If to compare the content of carbohydrate maltose in honey between different kinds of honey, it is necessary to note, that despite of some kinds of honey with the raised content of carbohydrate maltose in honey (sweet clover and honey of phacelia), at other kinds of honey the maintenance of carbohydrate maltose approximately similar.

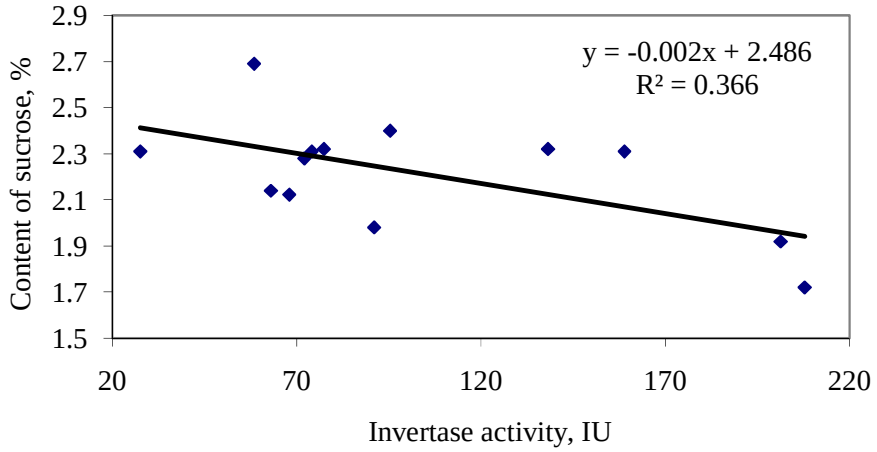

Figure 1. Influence of invertase activity in honey on the content of sucrose in honey

However it is possible to approve, that the content of carbohydrate maltose in honey partially depends on a kinds of honey.

It is know, that enzyme invertase hydrolyses sucrose about glucose and fructose. In our work also was determined activity enzyme invertase in honey samples. The correlation between activity enzyme invertase and the content of sucrose in honey was determined. Our observations are disclosed in Figure 1.

From the results of regression analysis (significance level 0.05) ascertained, that there is the some dependence between the content of sucrose in honey and invertase activity. As can see from Figure 1, the more the content of sucrose in honey, then less is activity invertase in honey. If to compare activity of invertase in honey between different kinds of honey, it is necessary to note, that lowest activity of invertase in honey are at such kinds of honey as honey from phacelia, meadowsweet honey and sweet clover honey. At other samples, activity of invertase in honey corresponds with criteria of "Interantional Honey Commission" discussions. Despite of rather low activity of invertase at some samples of honey, it is impossible to approve, that in these samples the lowered quality. About quality of honey it is possible to judge, estimating other parameters, which characterise quality of honey.

Conclusions

1. Specific rotation of light cannot be used for identification of honey kinds.
2. The content of maltose in honey partially depends on a kind of honey.
3. Content of invertsugars (glucose and fructose) and sucrose in honey correspond with quality criteria of EU and LR.
4. Content of sucrose in honey partially depends on a activity of enzyme invertase in honey.

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SENSORY PROPERTIES OF WHEAT BREAD WITH RASPBERRY MARC

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Abstract
The aim of the study was to determine the influence of raspberry marc on the degree of liking of the wheat bread and the intensity of the main sensory properties (colour of breadcrumb, aroma, flavour, porosity and sourness). The evaluation took place in the sensory laboratory at the Faculty of Food Technology, Latvia University of Agriculture. The berry marc used in the research was prepared according to the technology worked out in Latvia State Institute of Fruit Growing. The control sample and wheat bread samples with 3%, 5%, and 7% of raspberry marc (from the flour mass) were made and baked in experimental bakery. The intensity of the main sensory properties was determined by using the line scale. The nine point hedonic scale was used to evaluate the degree of liking for wheat bread with raspberry marc. The analysis of variance (ANOVA) and Tukey`s test were used to analyze the results of sensory evaluation. The results of the sensory analysis show that the use of raspberry marc in baking high milling wheat bread influence colour of breadcrumb, flavour, aroma and sourness but it does not affect porosity. The hedonic evaluation of the bread samples show that there do not exist any significant differences in the degree of liking among the wheat bread control sample and the new wheat bread samples with 3%, 5%, and 7% of raspberry marc.
Key words: wheat bread, raspberry marc, sensory properties

Introduction
Bread and its products are an integral part of nourishment for the human body. Notwithstanding the long history of bread it is one of the most unique foodstuffs for human bodies (Кострова, 2001).
Statistical data show that the tendency to eat wheat bread is increasing. (Consumption of Food Products.., 2004, 2005; Statistical Yearbook.., 2005), therefore it is essential to enrich bread with fiber, vitamins, mineral substances and other substances, which improve nutritional value of the bread.
Raspberries are a widely spread cultivar that is grown all over the world. These berries are popular because of their colour, lovely flavour, juiciness and sweetness (Laugale, 2002). 100 grams of raspberries contain 3–8 g of sugar (glucose, fructose), 0.9–1.4 g of organic acids (malic acid, citric acid, salicylic acid), vitamin A and vitamins of B group, P, E, C, 0.5 g of fat, 3.7 g of fiber, pectin substances (Strautina, 2005). Fresh and frozen raspberries are used for food, as well as they are processed to obtain juice, fruit salad and jam; raspberries are used as additive in yoghurt and confectionery production. After juice is obtained with the pressing method the berry marc is 36% of the berry raw material, therefore some fiber, sugar, organic acids, pectin and other substances remain in the marc (Deval, 1996).
Raspberry marc, that is a by-product remaining after juice is produced, is not used any further so the possibility to use this valuable product with the aim to enrich the nutritional value of wheat bread is being studied.
The aim of the study was to determine the influence of raspberry marc on the intensity of the main sensory properties (colour of breadcrumb, aroma, flavour, porosity and sourness) and on the degree of liking of the wheat bread.

Materials and Methods
Dried raspberry marc has been made in Latvian State Institute of Fruit Growing production site. When the juice is liquidized by using the pressing technology, the marc is dried in a dryer with forced air circulation; the temperature is suggested not higher than 40 °C, the content of moisture in marc should be 9%. Raspberry marc was ground in the grinder and sifted.

The control sample and wheat bread samples with 3%, 5%, 7% (of the flour mass) of raspberry marc was baked at the experimental bakery of S/C "Dobeles Dzirnavnieks" in accordance with the recipes and technological schemes of the bakery and by using the high milling flour and additives such as - pressed baker's yeast, sugar, salt, water, oil and raspberry marc.

Four wheat bread samples were presented for the sensory evaluation:

A – wheat bread (control);

B – wheat bread with 3% raspberry marc;

C – wheat bread with 5% raspberry marc;

D – wheat bread with 7% raspberry marc.

The line scale was used to evaluate the intensity of sensory properties (colour of breadcrumb, aroma, flavour, porosity and sourness) of the wheat bread. The degree of liking of the wheat bread was evaluated by using nine point hedonic scales (Poste *et al*., 1991, Strautniece, 2004)

The sensory evaluation was repeated twice in the sensory laboratory of food evaluation, Faculty of Food Technology, Latvia University of Agriculture. 25 panellists (6 men and 19 women, mean age 23) were involved in the assessing. Each panelist received four identical, coded bread samples. The sensory data were analyzed using the analysis of variance (ANOVA) and Tukey`s test (Meilgaard *et al*., 1999).

Results and Discussion

The results of line scale evaluation showing the intensity of the sensory properties (colour of breadcrumb, flavour, aroma, porosity and sourness) of the wheat bread are shown in Table 1.

Table 1

Results of sensory evaluation by line scale of the wheat bread with raspberry marc

Sensory properties	Samples			
	A	B	C	D
	Means value			
Colour of breadcrumb	5.60	6.90	8.60	10.80
Flavour	6.60	6.80	8.00	8.20
Aroma	6.50	6.60	7.60	8.50
Porosity	7.10	6.50	7.20	6.90
Sourness	3.90	4.80	6.80	8.60

The results of analysis of variance of bread with raspberry marc are demonstrated in Table 2.

Table 2

Results of analysis of variance of main sensory properties

Sensory properties	Variance ratio $F_{calculated}$	Variance ratio $F_{critical}$
Colour of breadcrumb	30.6	2.68
Flavour	4.98	2.68
Aroma	9.17	2.68
Porosity	0.84	2.68
Sourness	27.48	2.68

$\alpha \leq 0.05$

In accordance with the results of the analysis of variance – $F_{(calculated)}=30.6>F_{(critical)}=2.68$ ($n_1=3$, $n_2=72$), the conclusion is that there are significant differences in the breadcrumb colour intensity among the four estimated bread samples. The colour intensity of new bread samples is influenced by the amount of the added raspberry marc i.e. 3%, 5%, 7% (from flour mass). The results of Tukey`s test show that there is a significant difference between the

control sample (A) and the wheat bread samples (B, C, D) with raspberry marc. When raspberry marc, which is pink, is added to the wheat flour, the soft part of the bread has riched shade of raspberries.

The analysis of variance of flavour $F_{(calculated)}=4.98>F_{(critical)}=2.68$ reveal significant differences in flavour intensity exist among the four evaluated bread samples. The results of Tukey`s test demonstrate that sample (C) with 5% of raspberry marc and sample (D) with 7% of raspberry marc have the most intensive flavour, but the bread sample (B) with 3% of berry marc and control sample (A) have no difference in flavour intensity. When 5 and 7% of raspberry marc is added to the new bread samples, alongside with the bread flavour the flavour of raspberries can be felt.

The results of the analysis of variance of aroma ($F_{(calculated)}=9.17>F_{(critical)}=2.68$) show that there significant difference in aroma intensity among the evaluated wheat bread samples with raspberry marc and the control sample. Tukey`s test results show that bread sample (D) with 7% of raspberry marc has a distinctly marked difference in aroma, then it is followed by the bread sample with 5% of raspberry marc, but there is no difference in aroma in bread sample (B) with 3% of raspberry marc and the control sample (A). The amount of the marc added to the wheat bread increase the intensity of bread aroma, because the aroma of raspberries enrich aroma.

The consequently of the analysis of variance of porosity evaluation show that $F_{(calculated)}=0.84<F_{(critical)}=2.68$, it means that no significant differences were discovered among the four evaluated bread samples in the intensity of porosity. The porosity of the wheat bread is not influenced by raspberry marc added to the dough.

The results of the analysis of variance of sourness show that $F_{(calculated)}=27.48>F_{(critical)}=2.68$, it means there exist significant difference in sourness intensity among the evaluated four wheat bread samples. Raspberry marc contains organic acids and it influences the intensity of sourness of the new bread samples (Gailīte *et al.*, 2006). Tukey`s test results show that bread sample (D) with 7% of raspberry marc has the highest degree of sourness, then it is followed by the bread sample with 5% of raspberry marc, and bread sample (B) with 3% of raspberry marc. The control sample (A) has the least sourness.

The obtained results show that the hedonic evaluation is from 5.7 (neither like nor dislike) to 6.5. (like a little). The results of analysis of variance of bread are shown in Table 3.

Table 3

Results of analysis of variance of bread samples using hedonic scale

Source of variation	Degree of freedom, df	Sum of squares SS	Mean square MS	Variance ratio, F
Treatments	3	10.61	3.53	1.96
Panellists	24	46.72	1.95	1.08
Error	72	129.77	1.80	
Total	99	187.10		

$\alpha \leq 0.05$

The results of the analysis of variance show that $F_{(calculated)}=1.96$ does not exceed $F_{(critical)}=2.68$ therefore there do not exist any significant differences in the degree of liking among the wheat bread samples. That means that the panelists liked all the bread samples equally.

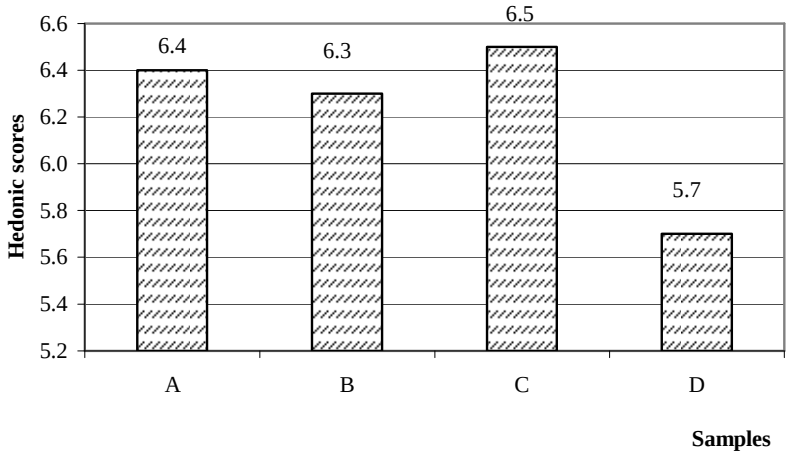

Figure 1. Hedonic scores for the four bread samples

A– wheat bread (control); B – wheat bread with 3% raspberry marc; C – wheat bread with 5% raspberry marc; D – wheat bread with 7% raspberry marc.

Figure 1 shows the degree of liking of the four estimated wheat bread samples, the results have been obtained by using hedonic scale.

Conclusions

1. The results of evaluating the sensory properties show that the use of 3%, 5%, and 7% raspberry marc in the wheat bread technology influence the colour of breadcrumb, aroma, flavour and sourness of the wheat bread differently but it does not influence its porosity.
2. The sample containing 3% of raspberry marc has the least changes in the intensity of sensory properties, when compared with the control sample wheat bread, which has only some differences in their color of breadcrumb, and sourness.
3. The hedonic evaluation of the bread sample show that there do not exist any significant differences in the degree of liking among the wheat bread control sample and the new wheat bread samples with 3%, 5%, and 7% of raspberry marc.

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ALUMINIUM ANALYSIS – A PROFICIENCY TESTING STUDY

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Abstract

Proficiency testing is an external analytical quality assurance (AQA) measure i.e. the quality of the analytical result is checked against criteria that are set independently of the laboratory carrying out the analyses. In a proficiency test (PT), the participants' results are used to derive the assigned value. Then, the difference between each result and the assigned value is compared to the target standard deviation. The end product of the performance assessment is a standardised statistic known as a z-score.

In addition to assessing performance of participating laboratories, proficiency testing highlights problems in laboratory analysis and can be used as an educational tool to help to improve data quality. The last three FAPAS® proficiency tests (0770, 0787, 0784) have highlighted problems with assessing aluminium.

The results from laboratories taking part in a typical chemical analysis will be normally distributed i.e. the majority of results will be centred on a mean value. In proficiency test 0770 (soya flour), multiple modes were observed rather than a normal distribution. As there was insufficient evidence to draw any conclusions it was not possible to set an assigned value or calculate any z-scores for this analyte. FAPAS® test 0784 (milk powder) had a bimodal distribution and 3 modes were observed in the FAPAS® test 0787 (soya flour). In these tests, the major mode was used as the assigned value.

FAPAS® investigated using a reference value to check the assigned value for one of the proficiency tests (0787). This reference value was compared to the results of the proficiency test and the methods used by participants. The reference value was close to the major mode and the results for ICP-MS methods were also similar to the reference value.

Key words: proficiency testing, aluminium, quality

Introduction

Plants can take up aluminium from the soil and from water. So some plants, such as tea, and some herbs and leafy vegetables, can build up high levels of aluminium naturally. Aluminium can also be added to food during processing and some food additives contain aluminium. These are used in foods such as bakery products, dried powdered foods and drinks, and processed cheeses to improve the texture (Pennington, 1988).

It is therefore important that materials, such as aluminium, that are added to food or come into contact with food, do not make food harmful and that it does not change the nature, substance or quality of the food.

Scientific judgements on food data quality are under continuous scrutiny. It is therefore important to demonstrate that adequate confidence can be placed on results obtained by laboratories. Laboratories need, therefore, to demonstrate their performance and reliability in such analyses. It is widely accepted in most areas of food analysis and regulation that there are a number of essential elements to laboratory quality assurance. These elements include the use of validated analytical methods, accreditation involving third party auditing and the participation in laboratory proficiency testing schemes (Wood, Nilsson and Wallin, 1998). Thus, laboratories take part in proficiency testing for different reasons, which include export regulation on contaminants, labelling regulations, customer requirements for quality and quality data for food databases.

There is an increasing demand for independent proof of competence both from regulatory bodies and customers. Individual laboratories need to know how well they perform against objective standards and how their analytical results compare with others.

By setting the acceptable allowable variation around the assigned value at a level that reflects best practice PT testing provides such objective standards. By expressing the participants' submitted results as z-scores they can be compared with each other, with those at the extremes of the overall distribution being clearly indicated.

Materials and Methods

All the schemes administered by FAPAS® follow a similar pattern. For the aluminium proficiency tests, suitable test materials are selected and tested to ensure sufficient homogeneity and then distributed to requesting participants. Laboratories usually have 6–8 weeks to analyse their samples and return their results. The statistical analysis is carried out on the submitted results and a report compiled for issue to laboratories. The suitability and quality of the test materials distributed are fundamental to the effectiveness of a PT scheme. The two main criteria for a suitable test material are that:

- It resembles, as closely as possible, the real samples with which a laboratory routinely deals.
- Variations in the composition of the samples of the test material distributed to participants are kept to the minimum. This is readily checked statistically (Fearn and Thompson, 2001).

After a stipulated closing date the submitted results are put through the statistical analysis. Deriving the best estimate of the 'true' value (the assigned value) must take account of the often far from normal distribution of the submitted results. A simple mean value is not appropriate as it is too easily influenced by the presence of extreme values hence FAPAS® uses a variety of other more sophisticated robust statistical procedures to derive the assigned value (Analytical Methods Committee, 1989; Thompson, Ellison and Wood, 2006).

Results and Discussion

Obtaining the assigned value for a proficiency test can be difficult if the distribution of results is not normally distributed. Proficiency testing providers examine the distribution of the participants' results prior to arriving at the consensus mean. Sometimes there may be a suspicion that laboratories are using different methods resulting in distributions that show multimodality, skewness or a large variance.

A graphical representation means that FAPAS can check result spread, central tendency, etc. Looking at distributions showing multimodality using bump-hunting means that the overall structure of the distribution can be observed (Lowthian and Thompson, 2002). Such problems with distribution of data for aluminium have been highlighted in three recent FAPAS® proficiency tests (0770, 0784, 0787).

A bump hunt of the data for PT 0770 (Soya Flour; FAPAS®, 2006) identified multiple modes (Figure 1). As there was insufficient evidence to draw any conclusions it was not possible to set an assigned value or calculate any z-scores for this analyte.

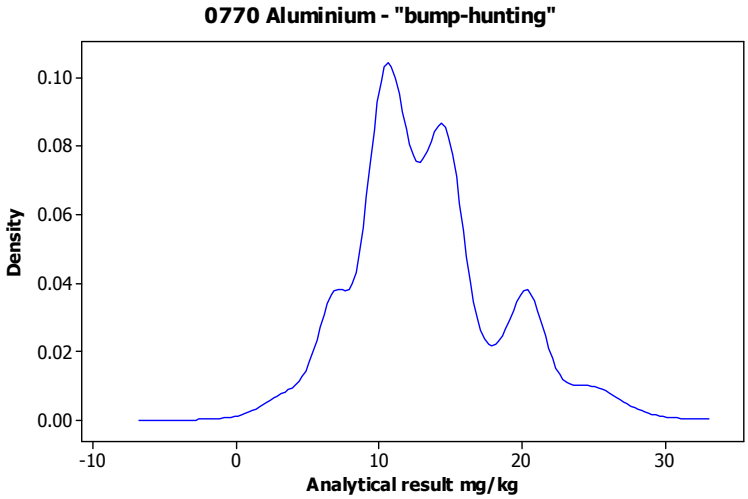

Figure 1: Bump-hunting Histogram of Aluminium in FAPAS® 0770 Proficiency Test

Bump-hunting for PT 0784 (Milk Powder; FAPAS® 2007a) revealed the data set to be bimodal (Figure 2). Although the major mode (1000 µg kg^{-1}) was used to set the assigned

value, the uncertainty of the mode was larger than expected and had a questionable effect on participants' z-scores. Hence the assigned value and z-scores were issued for information only. The homogeneity mean value was 973 µg $kg^{-1}$and was similar to the major mode.

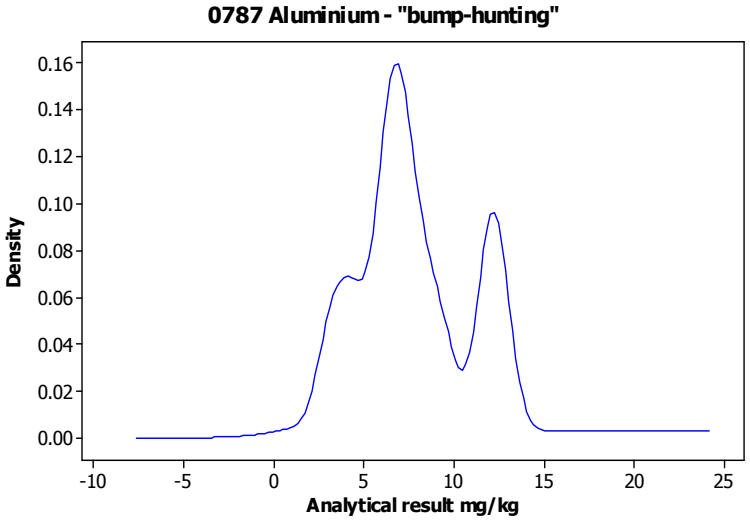

Figure 2: Bump-hunting Histogram of Aluminium in FAPAS® 0784 Proficiency Test

The recent soya flour PT (0787; FAPAS® 2007b) identified three modes (Figure 3). The major mode (6.81 µg kg^{-1}) was used to set the assigned value, but z-scores were again issued for information only. The homogeneity mean value for aluminium was 7.1 µg kg^{-1}.

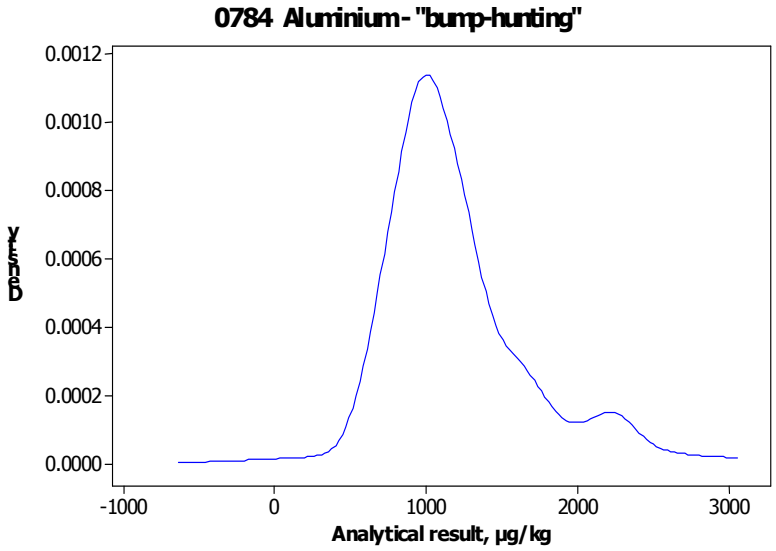

Figure 3: Bump-hunting Histogram of Aluminium in FAPAS® 0787 Proficiency Test

Figure 4 is a dot plot, from PT 0787, of the valid submitted results for aluminium, separated by participants' declared methodology. The seven ICP-MS results (5.98-9.496 µg kg^{-1}) were clustered around the assigned value (6.81 µg kg^{-1}), whilst data from other detection methods appear to be more widely scattered (between 3 and 35 µg kg^{-1})

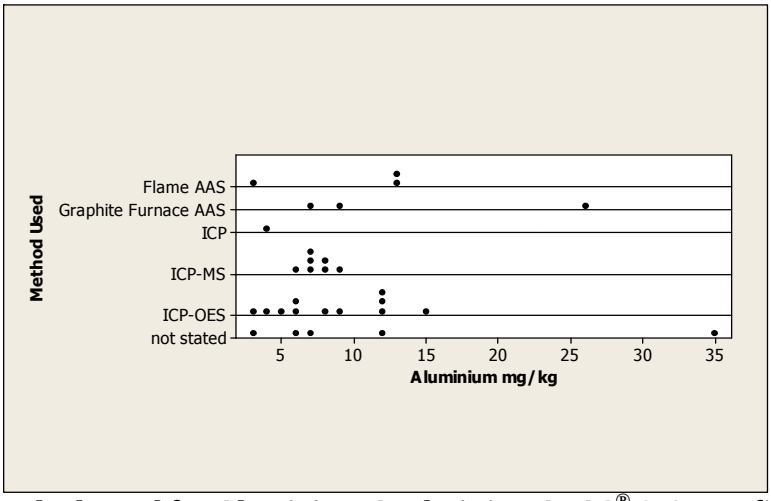

Figure 4: Methods used for Aluminium Analysis in FAPAS® 0787 Proficiency Test

A UK national reference laboratory for metallic contaminants undertook some research to determine the reference value for aluminium in soya flour (0787). The content of Al was determined using ICP-MS and was found to be 7.22 µg kg^{-1}. This value was close to the homogeneity mean value and the major mode, providing evidence that the major mode can be used as the assigned value for aluminium proficiency tests.

Conclusions

Aluminium analysis is of particular importance in monitoring foods to ensure that there are not harmful levels present or that the quality of food is not compromised. Thus, ensuring that data quality is luminium is prone to contamination from the laboratory environment e.g. from traces in acids, leaching from glassware and from powdered gloves. However aluminium can be difficult to solubilise when digesting a solid sample, so under reporting can occur which may be defit for purpose is important for end users. Proficiency testing is able to highlight problems in the quality of analytical results and consequently help to improve it. Analysis of apendent on matrix, method or aluminium species in the sample.

Acknowledgements

The UK Food Standards Agency funded research into obtaining the reference value for aluminium in soya flour.

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IMPACT OF MINERAL ELEMENTS (Ca, Mg AND Fe) ON MAILLARD REACTION IN MODEL SYSTEMS WITH CASEIN

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Abstract

Non-enzymatic browning, known as Maillard reactions (MR), occurs in many foods, including dairy products. The initial reactants are the amino compounds and reducing sugars. However, the MR in the real food systems proceeds in presence of other macro- and micro-components. The task of the work was to determine the influence of Ca, Mg and Fe on the browning characteristics of the MR model systems consisting glycine or casein and lactose or glucose, and to estimate the interaction of the elements with the high molecular weight (HMW) products formed during MR. The extent of browning in MR solutions was evaluated by measuring absorbance at 420 and 550 nm. The HMW melanoidins were separated by means of dialysis.

It was determined that Ca^{2+}, Mg^{2+} promote, and Fe^{2+} slows down the formation of colored MR compounds in the lactose–casein as well as in glucose–casein model systems by thermal treatment to 12 h at boiling temperature. The amount of Ca in the non-dialyzable part of the systems increased over a longer time of interaction from 28–38 to 62–71 %, while the amount of non-dialyzable Mg stays fixed about 26%. The peak of non-dialyzable Fe (~50%) was found in the first hour of interaction.

The results demonstrate the possible involvement of Ca, Mg and Fe in the thermal changes of dairy products and the impact of Maillard reactions on the solubility and supposed bioavailability of the elements.

Key words: casein, Maillard reaction, mineral elements, influence

Introduction

Non enzymatic browning, known as the Maillard reactions (MR), occurs in many foods, including dairy products. The initial reactants in the MR are the amino compounds (mainly free amino acids or proteins) and reducing sugars (as glucose, lactose and others). In the case of milk products, casein and lactose are particularly involved. I the early step, the free amino groups of the proteins (such as amino groups of lysine residues in casein) react non-enzymatelly with reducing sugars. In the advanced and final steps of the reaction proteins are modified into colored, fluorescent and cross-linked molecules, which form later the brown polymers – melanoidins. This reactions is of importance in many heated, dried and stored foods because the formation of flavoring ingredients, browning products, the loss of nutritive value (Ames, 1998, Van Boekel, 1998).

MR in the real food systems proceeds in presence of many others macro- and microcomponents. It is determined that the development of non-enzymatic browning in foods and the nature of compounds formed in different stages of MR depend on the presence of such minor substances as oxidizing or reducing agents (Bobbio *et al*., 1985; Swales, Wedzicha, 1992; Zidertman *et al.*, 1989), formaldehyde (Vasiliauskaitė and Wedzicha, 1997), urea (Braekman et al., 2001) and mineral elements, especially transition metals (Birglouez-Arragon, 1997; Cheng, Kowakishi, 1993; Hayase *et al*., 1996; Randleman and Inglett, 1990).

Diverse studies have suggested that melanoidins behave as anionic hydrophilic polymers, which can form stable complexes with metal cations. According to several authors, Ca, Mg, Fe, Cu and Zn are bound to some degree by soluble and insoluble melanoidins derived from different amino acids-sugar model systems (Rendleman, 1987; O'Brien and Morrissey, 1997). However, heated methionine-sugar mixtures had a little effect on Ca and Mg solubility (Delgrado-Andrade *et al.*, 2004). It was predicated, that the effects of browning products generated during food processing should be taken into account, particularly in trace element solubility and bioavailability (Morales, 2005). Heat treatment of reducing sugar-casein model system also affected the calcium bioavailability by in vitro and in vivo assay (Seiquer *et.al.*, 2001).

The results that appear in the literature give not sufficient information about the Maillard reactions in food systems near to realistic for milk products, where some metal present in the reaction media. The aim of this study is to define the influence of mineral elements Ca, Mg

and Fe on the browning characteristics of the model systems from glycine or casein and lactose or glucose solutions, and to evaluate the interaction of the elements with high molecular weight products formed during Maillard reactions.

Materials and Methods

Sample preparation. Glucose and lactose (Merk, Darmstadt, Germany), glycine (Sigma Chemical Co, St. Louis, MO, USA) and dried casein were used to prepare the samples. Mineral elements (Ca, Mg and Fe) were added as water solutions of the salts: anhydrous $CaCl_2$, $MgCl_2 \times H_2O$ and $FeSO_4 \times 7H_2O$.

The concentration of reactants in 100 ml model solutions was as followed: lactose or glucose – 0.25 mol; glycine – 0.25 mol; casein – 0.5 g; calcium – 120 or 240 mg, magnesium – 60 or 120 mg and iron – 20 mg. The model mixtures with casein were prepared in Na_2SO_3 solution (0.7 %). The concentrations of Ca and Mg were selected to simulate usual content of minerals in cow's milk or dairy products, and the concentration of Fe used similar as in fortified milk formulas.

Thermal treatment and dialysis of model samples. Prepared model mixtures were heated at boiling temperature in a flask under reflux for 6 hours and samples were taken after 1, 2, 3, 4, 5 and 6 hours to monitor the course of the reaction.

Maillard reaction products formed in each stage of heating were separated by means of dialysis into two fractions: (1) high molecular weight (HMW) compounds (>12 000 Da), and (2) dialyzable compounds. 10 ml of each solution was dialyzed for 72 hours at temperature 4–6 °C against 1000 ml bidistilled water, which was changed for first time after 8 h, and later for each 12 h.

Spectrometric analysis. Browning of samples was evaluated spectrophotometrically as absorbance at 420 nm (A_{420}) and 550 nm (A_{550}) using Spectrophotometer Varian Carry 1/3, USA. Browning index (BI) was calculated as: $BI=A_{420}-A_{550}$. For browning measurements, due to the insoluble nature of the brown pigments in proteins, the model samples (5.0 ml) were digested with 1 ml pancreatin solution (50 mg/ml) at 45 °C for 2 h. Trichloracetic acid solution (50% w/v) was added to stop the enzymatic reaction and the samples were centrifuged (8000 g for 10 min) and filtered trough filter paper (Davies *et al.*, 1998). For comparison, the direct measurement of absorbance carried out in the non-digested samples.

The results of measurements were calculated as average of triplicates.

Determination of mineral elements. The content of Ca, Mg and Fe in non-dialyzable fractions was determined by flame AAS method after dry ashing (550±25 °C) of non-dialysable parts of the model systems.

Results and Discussion

Calcium added in concentration corresponded its content in cow's milk (120 mg or 3.0 mmol/100 ml) stimulated the browning interaction in heated lactose-glycine system. The increase of absorbance at 420 nm was more rapid at longer heating time. When a higher content of calcium was added to the system (240 mg or 6.0 mmol/100 ml) the stimulating effect of Ca^{2+} on the accumulation of browning compounds was lower (Fig. 1*a*). This suggested that effect of Ca ions on the MR in lactose-glycine system become slight limiting.

Supposedly, in the presence of Ca ions in an appropriate amount the interaction of some charged advanced MR compounds eased due the formation of Ca bridges among reacting groups, and the rate of accumulation of brown melanoidins expand. However, at the abundance of Ca^{2+} ions in the system the blocking effect on the development of MR was observed. On the other hand, by higher content of calcium lactose-casein system (Fig. 1*b*) can lead to form of more HMW complexes, including protein-bound melanoidins, which availability to enzymatic digestion was badly, and a part of melanoidins can to be precipitated by adding of trichloracetic acid. Unfortunately, browning status of precipitates was evaluated in this work only visual, and a higher darkness of the precipitates of the samples with higher

Ca content was observed in this way. Both additives of Ca shortened an incubation time, after which measurable absorption values were obtained in the samples.

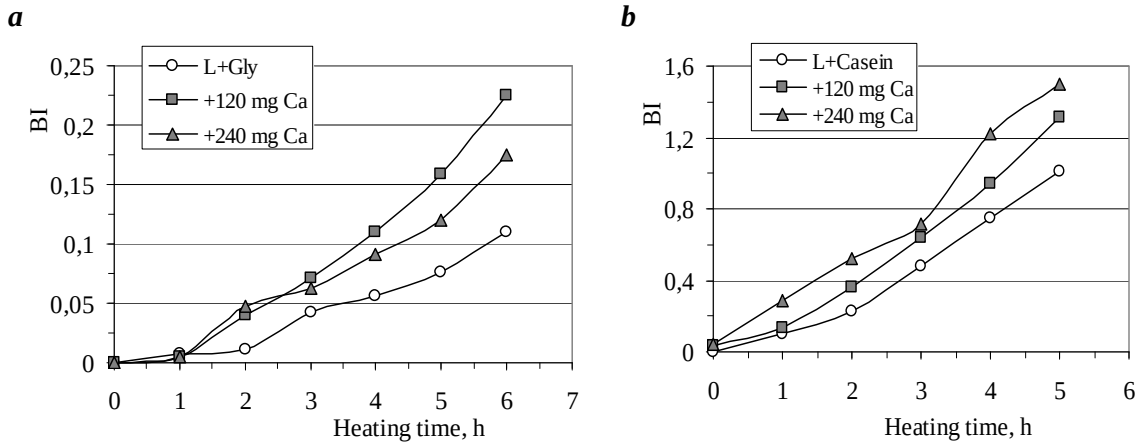

Figure 1. Change of browning index (BI) value in MR model systems by heating with added Ca: *a*) lactose-glycine (L+Gly) **and *b*) lactose-casein** (L+Casein)

The browning of test solutions with added Mg, was measured at 2 different means – without use of enzyme and after enzyme digestion. The absorbance A_{420} in the non-digested samples was directly proportional to the concentration of Mg added and reflected rather opalescence than absorption. However, the dependence of the accumulations of color compounds on the level of Mg in the digested samples (60 mg or 2.5 mmol/100 ml and 120 mg or 5.0 mmol/100 ml) was similar as in case of Ca addition (Fig. 2). Moreover, the browning rate in glucose-casein model systems was higher than in lactose-casein systems, but the effect of Mg^{2+} ions was similar, as it can see by comparison the data given in Fig. 1*a* -1*b*, and 2*a* - 2*b*

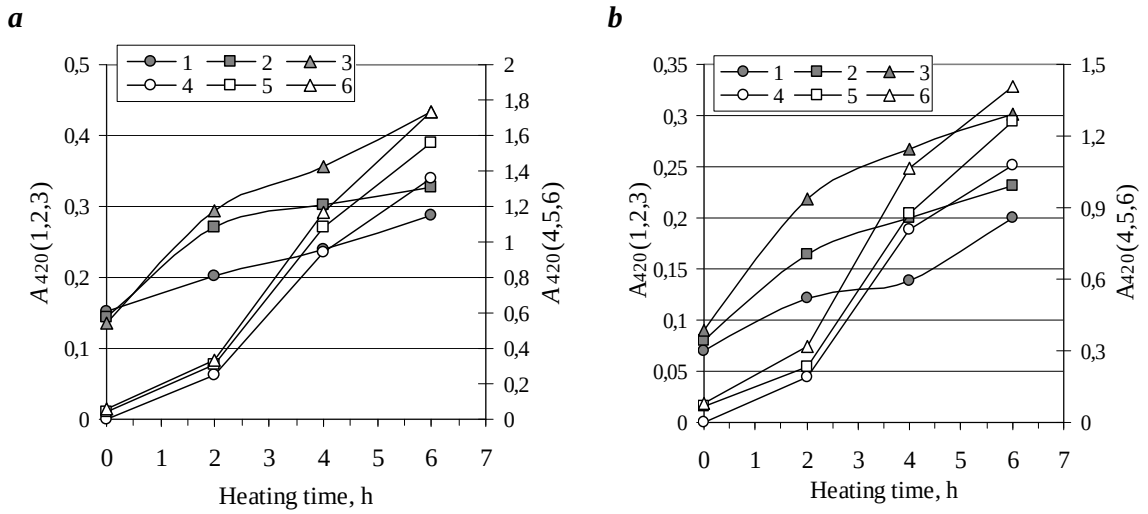

Figure 2. Change of absorbance in glucose-casein (*a*) and lactose-casein (*b*) model systems by heating: 1 and 4–no Mg added; 2 and 5–60 mg; 3 and 6–120 mg Mg. Filled and addle points show data without and after enzyme digestion, respectively.

The change of absorbance A_{420} and A_{550} in

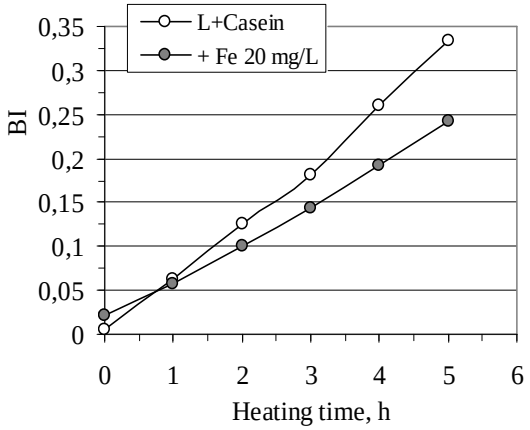

Figure 3. Influence of Fe^{2+} on the change of browning index (BI) in lactose-casein system (L+Casein) **by heating**

time, expressed as increase of browning index, showed that Fe added in concentration of 20 mg or 0.36 mmol/L inhibited the formation of melanoidins in the lactose-casein system (Fig. 3). It was previous determined (Cheng *et.al.*, 1991, Hayase, 1996) that iron involves in MR not only as complexing but also as oxidizing agent. On this reason, the oxidative effect on the brown chromophores can be proposed in this experiment.

According to the data obtained by analysis distribution of added Ca, Mg and Fe between dialyzable and non-dialyzable fractions of the heated model systems, a part of the elements is associated with HMW melanoidins. By progress of MR in sugar- casein solutions the relative amount of calcium incorporated into structure of non-dialyzable compounds, increased from 28–36% to 63–71% (Fig. 4). The binding properties of HMW compounds derived in the lactose containing model systems trended to be was similar as in glucose systems (the separate data is not given). As it was reported in the studies, Ca^{2+} ions are able to form complexes with melanoidins (Rendleman, 1987, Seiquer, 2001). The results of our experiments are in agreement with these findings and indicate that bioavailability of Ca in milk products trends to decrease by thermal treatment.

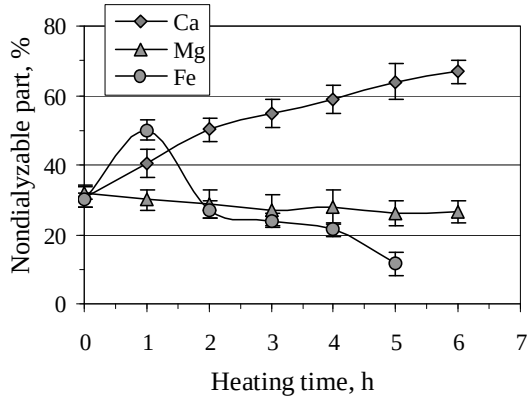

Figure 4. Percentage of non-dialyzable elements in both glucose-casein and lactose-casein model systems by heating (present as average of percentage values)

The percentage of Mg in non-dialyzable fractions of tested model systems remained near to constant during all heating time.

The complexation of Fe by non-dialyzable compounds was other than Ca and Mg. In the first hour of lactose-casein interaction, when the products from early and advanced stages of MR begin to form and when the concentration of protein-bound melanoidins is low, the percentage of Fe added, that remains in non-dialyzable fraction, increased approximately from 30% to 50%. As the reactions proceed and the products of final stage of MR accumulate, the amount of Fe^{2+} bound to HMW fraction suddenly decreased to 13.5%. It is proposed that metal binding ability of melanoidins formed in sugar-casein system decreased due the oxidative effect of Fe^{2+} theirself, incorporated into the structure of casein-bound melanoidins.

Conclusion

The results of the study demonstrate the possible participation of Ca, Mg and Fe in the thermal changes of foods containing casein and reducing sugars and the impact of Maillard reactions on the solubility and supposed bioavailability of the elements.

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EFFECT OF PACKAGING ON CHEMICAL COMPOSITION AND STORABILITY OF FRESH-CUT ICEBERG LETTUCE

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Abstract

The aim of this study was to evaluate the influence of the method of packing and storage conditions on sensory properties and chemical composition of the fresh-cut iceberg lettuce. There was investigated iceberg lettuce of cultivar 'Elena'. Chemical investigations of lettuce and their products were carried out according to the methods applied at the Biochemistry and Technology laboratory. Fresh-cut iceberg lettuce stored at the temperature of +1±1 and +6±1 °C in package in air ambiance was distinguished for the best taste properties. Fresh-cut iceberg lettuce stored at the temperature of +1±1 °C in vacuum package had the biggest amount of dry soluble solids. When products are stored at the temperatures of +6±1 and +9±1 °C, the bigger amount of dry soluble solids are left in the packages without vacuum. Fresh-cut iceberg lettuce stored at the temperature of +1±1 °C in vacuum package and package without vacuum has bigger amounts of ascorbic acid. When products are stored at the higher temperatures, more ascorbic acid is found in fresh-cut lettuce stored in packages without vacuum. Bigger chlorophyll amounts remained in cut lettuce, stored at the temperatures of +1±1 and +6±1 °C in vacuum packing.

Key words: chemical properties, fresh-cut lettuce, packaging.

Introduction

Iceberg lettuce (*Lactuca sativa* L. var. *capitata*) – one of the lettuces, which are grown in Europe and North America for a long time. Their popularity is determined by low cost and long period of storage. This lettuce is distinguished for dietetic properties. Their leaves accumulate considerable amount of folic acid, A and B group vitamins, calcium, iron and ascorbic acid (20–50 mg 100 g^{-1}). Because of good storage and taste properties iceberg lettuce is in great demand among users. Minimally prepared lettuce mixes are especially popular.

The quality of made up products depends on the method of their preparation, packing material and storage conditions. It was observed that even 20% more of ascorbic acid remains in the products, which are stored at the low temperature (Barry-Ryan, O'Beirne, 1999). Loss of marketable appearance and quality of products is most often observed due to the browning of cut lettuce. Water, which is used in the production and has much nitrogen and calcium, and suitably balanced composition of modified air slow down oxidation processes of ascorbic acid and polyphenolic substances in cut lettuce. Their quality and time of usage increase. Water temperature, when preparing lettuce, also considerably influences production quality (Martin-Diana *et al.*, 2005; Beltran *et al.*, 2005).

In order to slow down oxidation processes of ascorbic acid and to improve sensory properties of products, it is suggested to irradiate packs with γ-rays, applying 0.5–1 kGy doses (Xuetong *et al.*, 2003).

Packing materials also influence sensory properties of products, because lettuce respiration intensity and product colour changes depend on the composition of the used materials (Del Nobile *et al.*, 2006).

It was observed that cut lettuce, which were stored in vacuum packing and before that were soaked in 1% ascorbic acid solution, distinguished themselves with attractively intensive colour (Pospilšil, Kovacev-Granic, Kukec, 2003).

Lithuanian Institute of Horticulture together with joint stock company "Salpronė" started quality evaluation investigations of iceberg lettuce products, made according to the minimal preparation method.

The aim of this study was to evaluate the influence of the method of packing and storage conditions on sensory properties and chemical composition of the fresh-cut iceberg lettuce.

Materials and Methods

Investigations were carried out at the Lithuanian Institute of Horticulture, Biochemistry and Technology Laboratory. Iceberg lettuce were prepared and packed in the enterprise of vegetable processing joint stock company "Salpronė". The object of the research was iceberg lettuce of cultivar 'Elena'. Lettuce-heads were cut in belts (6 mm in width and 80–100 mm in length) and packed into packages of two types 200 g in each. Packages were made out of barrier PA/PE 80 mik and TOPLEX HB PE 45 film in two ways – using vacuum and without it, in the air.

Investigations of the sensory properties and chemical composition of the prepared products were carried out on the sixth day of storage. Chemical investigations of lettuce and their products were carried out according to the methods applied at the Biochemistry and Technology laboratory. Ascorbic acid was measured by titration with 2, 6-dichlorphenolindophenol sodium chloride solution (Ермаков *и др*., 1987); dry soluble solids – by digital refractometer ATAGO; amounts of chlorophylls a and b–spectrophotometrically according to Vernon (Гавриленко, Ладыгина, Хандобина, 1975). Investigations were carried out in three replications. Sensory properties significance was denoted by a confidence level of 5%. Statistical processing of chemical composition results was made with the help of MS EXCEL.

Results and Discussion

In the department of vegetable processing in joint stock company "Salpronė" the heads of lettuce cultivar 'Elena' were cut in belts. Before packing, there were carried out investigations of the cut lettuce chemical composition. There were established 3.6% of dry soluble solids, 64.0 mg 100 g^{-1} of ascorbic acid and 0.03 mg g^{-1} of chlorophylls in fresh-cut lettuce of cv. 'Elena' (Fig. 2, 3). Cut lettuce was packed into film of different types in two ways – with vacuum and without it, in air.

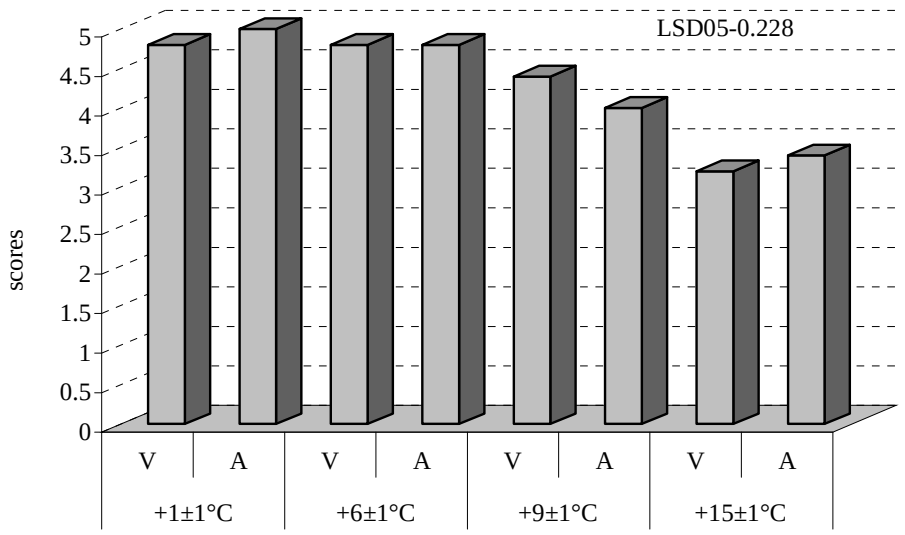

Figure 1. Sensory (total: external attractiveness, smell) evaluation of fresh-cut iceberg lettuce (V– vacuum, A – air), scores

It was established lettuce, stored at the temperature of +1±1 and +6±1 ºC, had good sensory properties (external attractiveness, smell); they were evaluated in 4.7–5.0 points (Fig. 1). Cut lettuce, stored at the temperature of +1±1 and +6±1 ºC in packing without vacuum distinguished themselves with the best aroma and taste, though storing products at the temperature of +6±1 ºC, there were observed "dew" drops in the packing. Marketable appearance of the cut iceberg lettuce stored at the temperature of +1±1 and +6±1 ºC in

vacuum packing was evaluated in 5 points, but their taste and aroma were worse that these of lettuce stored in packing without vacuum.

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During storage the amount of dry soluble solids in made up products decreased. The biggest amount of dry soluble solids (3.3%) was in cut lettuce stored at the temperature of +1±1 ºC in vacuum packing. When temperature of storage in packing without vacuum was +6±1 and +9±1 ºC, in iceberg lettuce there was established more dry soluble solids than in vacuum packing. When products were stored at the temperature of +15±1 ºC, the amount of dry soluble solids in lettuce in vacuum packing and in packing without vacuum essentially didn't differ (Fig. 2).

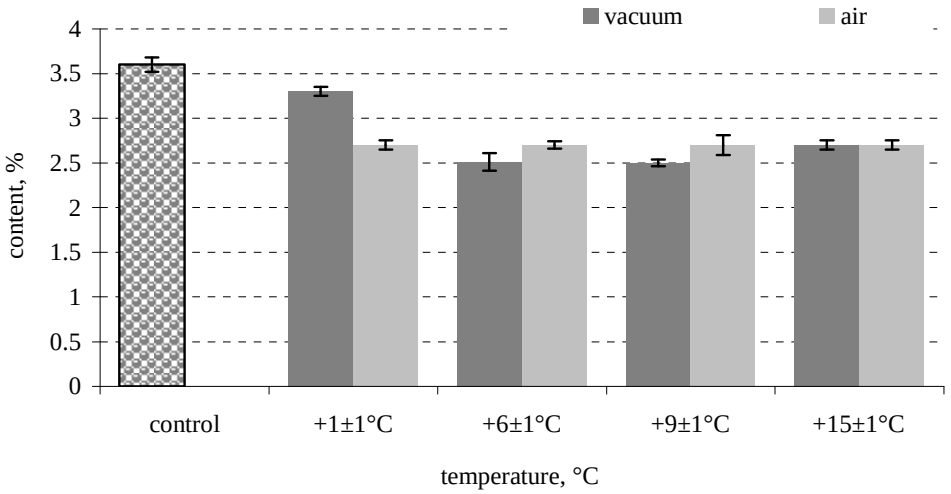

Figure 2. Dry soluble solids content in fresh-cut iceberg lettuce, %

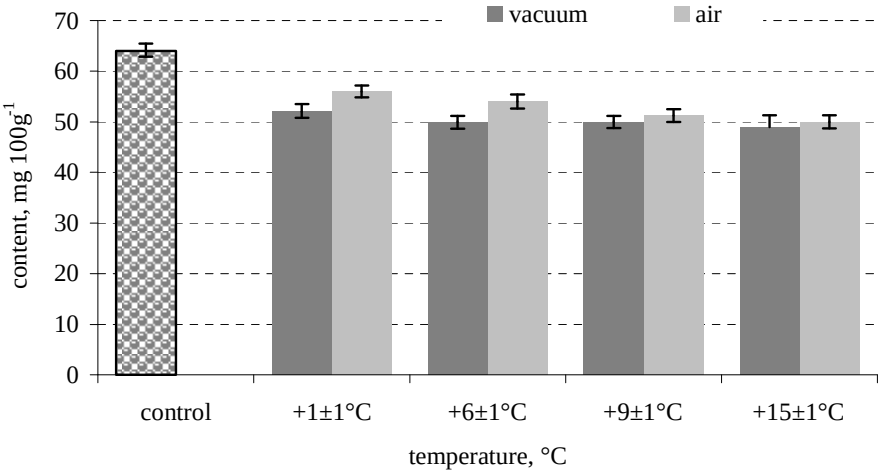

Figure 3. Ascorbic acid content in fresh-cut iceberg lettuce, mg 100 g^{-1}

The amount of ascorbic acid in cut iceberg lettuce decreased from 8.0 mg 100 g^{-1} to 15 mg 100 g^{-1}, dependending on storage conditions and packing method (Fig. 3). The bigger amounts of ascorbic acid were established when lettuce was stored in both packings at the temperature of +1±1 °C. When storing at the higher temperature, the amount of ascorbic acid in cut lettuce decreased from 5.7 to 10.7%, dependently on packing method. The bigger amounts of ascorbic acid remained in lettuce, stored at different temperatures in packing without vacuum.

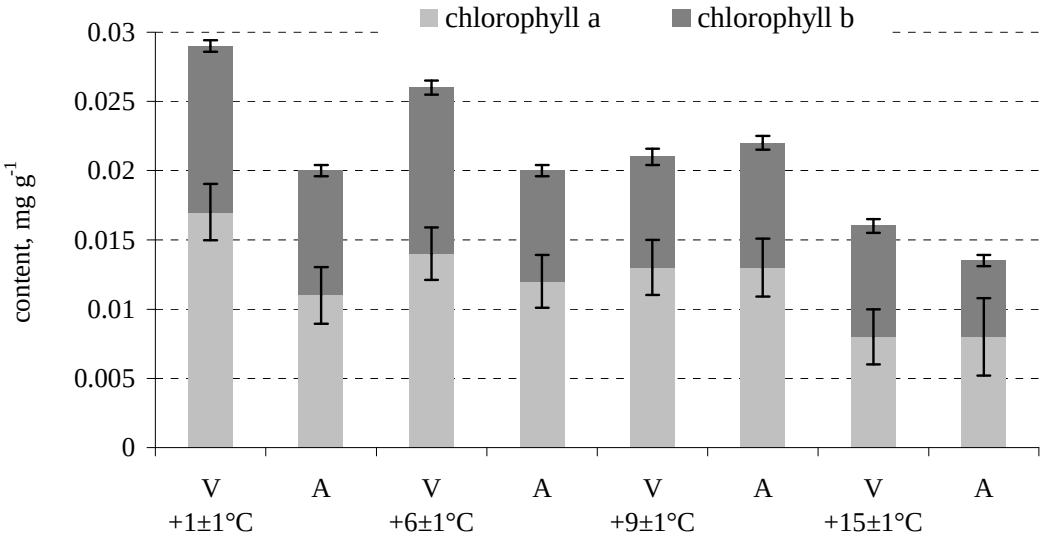

Figure 4. Chlorophylls a and b contents of fresh-cut iceberg lettuce (V– vacuum, A – air), mg g^{-1}

Amounts of chlorophylls a and b in cut lettuce essentially decreased during storage. Temperature of storage and packing method influenced pigment concentration in products. Bigger amounts were established in cut lettuce, stored in vacuum packing in vacuum packing at the temperatures of +1±1 and +6±1 °C (Fig. 4). The least amount of chlorophylls a and b remained in products, stored in vacuum packing and packing without vacuum at the temperature of +15±1 °C. When lettuce was stored in air, without vacuum, at the temperatures of +1±1, +6±1 and +9±1 °C, pigment concentration in them decreased, but differences among chlorophyll amounts in products weren't established.

Conclusions

1. Fresh-cut iceberg lettuce stored at the temperature of +1±1 °C and +6±1 °C in package without vacuum was distinguished for the best taste properties.
2. Fresh-cut iceberg lettuce stored at the temperature of +1±1 °C in vacuum package had the biggest amount of dry soluble solids. When products are stored at the temperatures of +6±1 and +9±1 °C, the bigger amount of dry soluble solids are left in the packages without vacuum.
3. Fresh-cut iceberg lettuce stored at the temperature of +1±1 °C in vacuum package and package without vacuum has bigger amounts of ascorbic acid. When products are stored at the higher temperatures, more ascorbic acid is found in fresh-cut lettuce stored in packages without vacuum.
4. Bigger chlorophyll amounts remained in cut lettuce, stored at the temperatures of +1±1 and +6±1 °C in vacuum packing.

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THE QUALITY OF PORK FROM VARIOUS PIG GENETIC LINES

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Abstract

The purpose of the study was to analyze some pork quality factors with regard to various pig genetic lines. The study was conducted in spring on the material collected from fatteners born from sows of Polish Landrace (P.L.) breed matted with a cross-breed boar (pietrain (pi) x duroc (du)) and synthetic Hypor sows with PIC 337 boars. Two types of muscles were analyzed: *m. longissimus lumborum* and *m. semimembranosus*. The measurements included: dry matter, total protein, fat and collagen content as well as physicochemical properties, such as: colour, acidity and drip loss. Besides, the effects of heat treatment on the sensory value of the raw material, meat colour, exudate and tenderness were studied.

The results of the study show that percent of meat in carcass of pigs under investigation amounted to 57%. As has been found, total protein content of the porcine muscles depended on the breed. Pork from the synthetic cross-breeds was higher in protein than that from P.L. x (pi x du) cross-breeds. Fat content of the muscles from the pigs of the synthetic line was lower than that from three-breed crosses pigs. *M. semimembranosus* from three-breed pigs were higher in collagen as compared to those obtained from the hybrids of Hypor x 337. The analysis of meat after heat treatment did not show any significant qualitative differences depending on the genetic lines of pigs. The sensory analysis showed a correlation between the sensory value and the genetic line only for *m. l. lumborum.*

Key words: quality, pork meat, cross-breed

Introduction

Today, when the supplies of meat and meat products on food markets are in abundance, the quality of the product is of great concern of the consumers. Currently, the competition in Polish meat industry is so high that manufacturers of slaughter animals have to be subjected to the requirements of consumers. Creation and improvement of quality traits of pork starts with animal breeding and ends with the culinary preparation of meat by consumer. The specific quality traits of pork depend on age, genetic lines, raising conditions and feeding.

The choice of animal for crossing is a very important factor which affects the quality of carcass (Florkowski *et al.*, 2006). Breeding should be aimed at creating optimal interracial crossing variants, which would enable us to obtain maximum profits from both increased quantity and improvement of meat quality. Polish pork has a good quality, but insufficient carcass meatiness. Crossing Polish breeds with high-yielding breeds can improve the quality of raw meat (Rybarczyk *et al.*, 2002, Wojciechowski *et al.*, 2000).

The purpose of the study was to analyze some pork quality factors with regard to various pig genetic lines.

Material and methods

The study was conducted on material collected from fatteners born from sows of Polish Landrace (P.L.) breed matted with a cross-breed boar (pietrain x duroc) and synthetic Hypor sows with PIC 337 boars. The meatiness of carcass under investigation amounted to 57%. The experimental material consisted of two types of muscles: *musculus semimembranous* (*m. s.*) and *musculus longissimus lumborum* (*m. l. l.*).

In samples of meat, the content of the following chemical components was analyzed: dry matter according to Polish standards of PN-ISO 662:2000; protein content by Kjeldahl's method, using Kieltec™ 2300; free fat [PN-ISO 1444:2000]; connective tissue by determining hydroxyproline content [PN-ISO 3496:2000]. The estimation of the physicochemical properties of muscles included a measurement of colour indices: L*, a* and b* using a Minolta Chromameter CR 200, acidity according to PN-ISO 2917:2001, drip loss following the method of Honikiel (1998). In the samples of muscles after heat treatment (0.8% solution of NaCl, 1:2 proportion meat to solution, time of heat treatment: about 30 minutes until final temperature in the sample centre reached will

obtain 74 °C), colour values, exudates as cooking loss, tenderness as texture measurement were analyzed using the Zwick/Roell type Z010 machine (meat samples were shaped as cuboids and measured 10x10x10 mm, with fibres running perpendicular to the cutting plane. Sensory evaluation after heat treatment was carried out by 6 panelists according to 5-point Tilgner's scale of acceptance (Baryłko-Piekielna, 1975). The overall acceptability, colour, flavor, taste and tenderness were selected as the sensory descriptors.
The muscles of meat under investigation were collected from three fatteners from each of the four series in the spring time.
The data were analyzed statistically, using STATISTICA 6.0 software. One-way analysis of variance was used to test the effect of genetic lines on the variables examined. Significant differences between the mean values were determined using Duncan's test (α=0.05).

Results and Discussion

The result obtained in the study (Table 1) showed that the dry matter content of *m.s.*, as well as *m.l.l.* from both researched breeds was similar (about 26 %). A majority of authors confirmed that the dry matter content of *m.l.l.* was within the range from 25 to 30 %, although in *m.s.* it was from 25 to 32%. The dry matter content depends on animal species, age, breed, raising conditions and feeding (Buczma, 1999; Litwińczuk et al., 2002). The results of the study were consistent with those reported by Rybarczyk et al.(2002) in crosses with pietrain, duroc and Polish Landrace breeds.

Table 1

Chemical components

Parameter [%]	Type of muscles			
	m. longissimus lumborum		*m. semimembranous*	
	P.L. x (pi x du)	Hypor x PIC 337	P.L. x (pi x du)	Hypor x PIC 337
Dry matter	26.10[a]	25.56[a]	26.11[a]	26.25[a]
Protein	23.04[a]	23.45[b]	21.73[a]	22.10[b]
Fat	2.91[a]	2.95[a]	3.85[a]	3.42[b]
Collagen	0.10[a]	0.11[a]	0.17[a]	0.16[b]

a, b – different letters in the same row for the same parameter means differences statistically significant ($P \leq 0.05$)

The pig genetic line proved to be a factor significantly differentiating the protein content of meat. Pork from the synthetic cross-breed was higher in protein than that from P.L. x (pi x du) cross-breed.
The estimation of the content of intramuscular fat demonstrated that its lowest content was observed in *m.l.l.* of three-breed crosses pigs (2.91%) but the differences were insignificant. In case of *m.s.*, the fat content was significantly higher for three-breed crosses pigs than that observed for the synthetic line (3.84% and 3.42% , respectively). Intramuscular fat content affects the technological, nutritional and sensory value of meat and meat products. The results of numerous authors indicate that its optimum content for those values should be about 1.5–3% in *m.l.l.* and 2.2–4% in *m.s.* (Litwińczuk *et al.*, 2002; Wood *et al.*, 1996, Daszkiewicz *et al.* 2005).
The measurements of collagen showed that breed was not a factor differentiating the content of this protein in *m.l.l.*, but it did have a significant effect in case of *m.s.* – a significantly higher quantity was observed in meat of P.L. x (pi x du) cross-breed. Collagen is a major protein present in connective tissue. Added to meat products, it improves water binding capacity, juiciness and tenderness, reduces thermal exudates. The content of this protein depends on intravital factors and should be within 0.1–0.2% (Purslow, 2005; Sadowska, 1992; Vanderhaeghe & Deroanne, 1989).
Table 2 shows the differences in values of acidity. The lowest value of pH was observed in the *m.l.l* of P.L. x (pi x du) cross-breed. In the second type of muscles, the lowest values were

recorded for synthetic line, but the differences were insignificant. The meat from fatteners under investigation was characterized by good quality (Borzuta, Pośpiech, 1999), there was not recorded any pig presenting PSE, DFD, ASE meats.

Table 2

Physicochemical traits

Parameter		Type of muscles			
		m. longissimus lumborum		*m. semimembranous*	
		P.L. x (pi x du)	Hypor x PIC 337	P.L. x (pi x du)	Hypor x PIC 337
pH		5.70[a]	5.80[b]	5.76[a]	5.70[a]
Water holding capacity [%]		2.78[a]	2.69[a]	2.64[a]	2.44[a]
Colour	L*	47.37[a]	51.73[a]	44.99[a]	50.59[a]
	a*	2.99[a]	2.66[a]	4.04[a]	4.74[a]
	b*	5.83[a]	4.99[a]	5.87[a]	5.67[a]

a, b – different letters in the same row for the same parameter means differences statistically significant ($P \leq 0.05$)

For the economy of meat processing, the value of water holding capacity is very important. Exudates of meat from both cross-breed were recorded on a similar level (P.L. x (pi x du): 2.78% *m.l.l.* and 2.63% *m.s.*; Hypor x PIC 337: 2.68% and 2.82%, respectively). The results of these analyses were in agreement with the earlier observation of Barton-Gade *et al.* (1993).
Table 2 also presents the results of colour traits analyses of raw meat. It was observed that the genotype had insignificant effect on CIE L*, a*, b* values.
Also, no significant differences in colour evaluation between the groups of fatteners were recorded in meat after heat treatment (Table 3).

Table 3

Analyses after heat treatment

Parameter		Type of muscles			
		m. longissimus lumborum		*m. semimembranous*	
		P.L. x (pi x du)	Hypor x PIC 337	P.L. x (pi x du)	Hypor x PIC 337
Drip loss [%]		25.57[a]	28.32[b]	28.55[a]	29.37[a]
Shearing force [N/cm^2]		58.90[a]	60.30[a]	51.26[a]	50.68[a]
Colour	L*	13.11[a]	13.25[a]	13.02a	13.21[a]
	a*	25.57[a]	28.32[b]	28.52[a]	29.37[a]
	b*	58.90[a]	60.30[a]	50.68[a]	51.26[a]
Overall acceptability		3.64[a]	3.81[a]	3.82[a]	3.60[a]

a, b – different letters in the same row for the same parameter means differences statistically significant ($P \leq 0.05$)

Meat samples from *m.l.l.* of synthetic line had higher loss of water during heat treatment than the samples from other cross-breed. No significant differences were observed in the second type of muscles. The results of the experiment showed that the genetic line was not a factor differentiating tenderness of muscles, expressed by the shearing force.
In general, there were no differences in the overall acceptability of meat after heat treatment between groups of fatteners. Higher flavour and tenderness acceptance of *m.l.l.* and taste of *m.s.* were found in meat from three-breed crosses than from the synthetic line. However, the differences were insignificant.

Conclusions

1. Total protein content of the porcine muscles depended on the genetic line. Pork from Hypor x PIC 337 cross-breeds was higher in protein than that from P.L. x (pixdu). The analysis of collagen content showed significantly lower content of this protein in the muscles from synthetic line.
2. Intramuscular fat content of the muscles from the pigs of the synthetic line was lower than from three-breed crosses pigs.
3. The genetic line was not a factor differentiating the physicochemical properties in muscles under investigation.
4. The results of the study showed no differences in the overall acceptability of meat after heat treatment between groups of fatteners.

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EFFECT OF THE PHOSVITIN ISOLATION METOD ON YIELD OF THE PROCESS

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Abstract
The aim of the present study was to compare two NaCl extraction methods of hen egg yolk phosvitin isolation, which differ in the use of organic solvents. The study was conducted on eggs from hens of Lohmann line and Green-leg breed, which were divided into 4 groups : I – eggs from Lohmann line fed without supplementation, II – eggs from Lohmann line fed with the addition of humokarbowit and humobentofet, III – eggs from Green-leg breed fed without supplementation, IV – eggs from Green-leg breed fed with the addition of humokarbowit and humobentofet. In the study, the yield of the phosvitin was examined and the following chemical components of yolk were analysed: dry matter, free fat and protein content.
The results of the experiment showed that the breed of hens is responsible for yield of phosvitin isolation from hen egg yolk. Green-leg breed had the highest yield of phosvitin isolation in comparison to Lohmann line. The highest yield of phosvitin isolation was recorded in groups fed with addition of humokarbowit and humobentofet (II, IV). The type of used method of phosvitin isolation from hen egg yolk had no significant effect on the yield of the process.
Key words: phosvitin, isolation method, egg yolk protein

Introducion
The avian egg is an encapsulated material indispensable for the development of the embryo. The chicken embryo develops into a chick by utilizing only the materials inside the shell at an adequate temperature. The protein, lipids, carbohydrate, vitamins (except vitamin C), and minerals contained in the egg are all necessary and sufficient for developing the chicken's body (Gutierrez *et al.,* 1997).
Egg proteins have an ideal balance of amino acids. The protein value of whole egg protein is considered to be 100. The World Health Organization, approved egg protein as a standard for measuring the nutrition quality of other food proteins (FAO/WHO/UNU, 1985).
Egg yolk is considered a potentially important source of energy because more than 65% of the contents of dry yolk is lipids. Egg yolk contains triglycerides, phospholipids, and sterols. Hen eggs are a rich source of linoleic acid, which is essential in human nutrition (Gutierrez *et al.,* 1997).
Egg yolk phosphatidylcholine contains important quantities of ω3 fatty acids as the non polar part. Choline, from the polar part is an important nutrient in brain development, liver function and cancer prevention (Gutierrez *et al.* 1997). Consumption of phosphatidylcholine increases plasma and brain choline level and accelerates neuronal acetylcholine synthesis. It has been demonstrated that consumption of yolk phospholipids tends to alleviate the symptoms of Alzheimer disease (Juneja 1997).
Hen egg yolk phosvitin represents about 11% of yolk protein (Ito *et al.,* 1962). Phosvitin has a molecular weight of about 35 kDa and contains 217 amino acid residues, of which 123 residues are serine residues (Byrne *et al.,* 1984, Clark *et al.,* 1971). Of the 123 serine residues, 118 are phosphorylated and 5 are "free" (Clarc *et al.,* 1985). Phosvitin, due to its peculiar physical and chemical characteristics, possesses a variety of functional and biological properties (Anton *et al.,* 2007).

Material and Methods
The study was conducted on two types of eggs from Lohmann line and Green-leg breed. The eggs were divided into 4 groups, depending on the line of hens and the feeding method. Two groups of hens were fed without supplementation and the remaining were fed with addition of humokarbowit and humobentofet. Humokarbowit and humobentofet contain polyunsaturated fat acids and their formula is exclusive.

The four groups were:
I – eggs from Lohmann line fed without supplementation,
II – eggs from Lohmann line fed with the addition of humokarbowit and humobentofet,
III – eggs from Green-leg breed fed without supplementation,
IV – eggs from Green-leg breed fed with the addition of humokarbowit and humobentofet.
In the study, the following chemical components of yolk were analysed: dry matter by thermal drying method in 105 °C according to PN-ISO 1442:2000, free fat by Soxlet's method according to Polish standards of PN-ISO 1444:2000 and protein content by Kjeldahl's method, using a Kjeltec^{TM} 2300 according to Polish standards of PN 75/A-0418.
The isolation of phosvitin was prepared with 15 eggs from each group.
The first method of isolation of hen egg yolk phosvitin was modification of the Losso and Nakai method (1994). Fifteen egg yolks were diluted with 0.5 l of cold distilled water at pH 5.0 and stirred at 4 °C for 1 h. The precipitated material was collected by centrifugation at 10,000g for 20 min at 4 °C. The pellet was dissolved in 200 ml distilled water, stirred for 1 h and centrifuged at 10,000 for 20 min at 4°C. The pellet was extracted with 400 ml of hexan: etanol (3:1, v/v) at 4 °C for 3 h and centrifuged. The resulting cake was dried with acetone and extracted with 200 ml of 1.74 M NaCl overnight at 4 °C. Next, the suspension was centrifuged at 10,000 g for 20 min at 4 °C and the supernatant was dialyzed against distilled water for 24 h at 4 °C and freeze dried.
The second method of isolation of hen egg yolk phosvitin was modification of the Pangborn method (1950). Fresh egg yolk from 15 eggs was diluted with 200 ml of 0.9 NaCl solution at pH 5.5 and centrifuged at 10,000 g for 1.5h. The granules were extracted in 100 ml of acetone, stirred for 1h and centrifuged at 10,000 g for 10 min at 4 °C. The precipitated material was extracted in 100 ml acetone, stirred for 1 h and centrifuged at 10.000 g for 10 min at 4 °C. Next, the pellet was extracted twice in 100 ml of etanol, stirred for 1 h and centrifuged (10.000 g, 10 min, 4 °C). The resulting cake was dried with acetone and extracted with 100 ml of 1,74 NaCl overnight at 4 °C and the supernatant was dialyzed against distilled water for 24 h at 4 °C and freeze dried.
The data were analyzed statistically, using 6.0 software. Two-way analysis of variance was used to test the effect of diet and breed on the variable examined. Significant differences between the mean values were determined using Duncan's test ($\alpha = 0.05$).

Results and Discussion
In the study, the following chemical components of yolk were examined: dry matter, free fat and protein content (49.57%, 16.19% and 30.16%) in research groups.
The results obtained in the study showed that the breed of hens and way of feeding was not responsible for dry matter content (Table 1). However, the breed of hens was responsible for protein content. Free fat content depend on the type of hen feeding, but it is significant only at $0.01<P<0.05$.

Table 1

Influence of hen line and the method of hen feeding on chemical components

Chemical components	Variability	F Value	Significance
Dry matter	Feeding stuff	2.118	0.1489
	Hen line	0.901	0.3551
Protein content	Feeding stuff	1.069	0.3038
	Hen line	10.220	0.0019**
Free fat	Feeding stuff	6,736	0.0147*
	Hen line	0.073	0.7922

*** $P\leq0.001$ and ** $0.001<P\leq0.01$ and *$0.01<P<0.05$ meant the significant difference between groups and isolation method.

The measurements of the dry matter content (Table 2) in hen egg yolk showed that the highest content was in eggs from Green-leg breed fed without supplementation. The remaining groups (I, II, IV) had similar content of dry mater and the differences were insignificant.
The highest content of protein (Table 2) was found in egg from both Green-leg breed fed with supplementation and without it. The way of feeding was not responsible for protein content.
Eggs from Green-leg breed fed without supplementation had the highest content of free fat (31.25%) (Table 2). Group I, II and IV had lower content of free fat, but the differences were insignificant.

Table 2

Yolk chemical components

Chemical components	Research group			
	I	II	III	IV
Dry matter[%]	49.41 a	49.92 b	49.61 ab	49.34 a
Protein content[%]	16.10 a	16.31 b	16.07 a	16.30 b
Free fat[%]	30.03 ab	31.25 c	30.19 b	29.17 a

a, b, c – different letters in the same row for the same parameter indicate statistically significant differences ($P \leq 0.05$)

The results obtained in the study show that the yield of phosvitin isolation did not depend on research groups and the isolation method (Table 3).

Table3

Influence of research group and isolation method on the yield of phosvitin isolation

Structure	Variability	F Value	Significance
Phosvitin	Group Isolation method	2.006 0.052	0.1369 0.8231

*** $P \leq 0.001$ and ** $0.001 < P \leq 0.01$ and *$0.01 < P < 0.05$ meant the significant difference between groups and isolation method.

Table 4 shows the yield of phosvitin isolation from eggs using Nakai- Losso and Pangborn method.

Table 4

Yield phosvitin isolation method from hen egg yolk [%]

Isolation method	Research group			
	I	II	III	IV
Nakai- Losso	2.92 a	3.62 ab	3.55 ab	4.00 b
Pangborn	2.36 a	3.74 ab	3.73 ab	4.73 b

a, b, c – different letters in the same row for the same parameter indicate statistically significant differences ($P \leq 0.05$)

The results of the experiment showed that the highest yield of phosvitin isolation using Nakai-Losso method was in eggs from Green-leg breed fed with supplementation. The lowest yield of phosvitin isolation was recorded in eggs from Lohmann line fed without supplementation. The highest yield of phosvitin isolation by Pangborn method was found in eggs from Green-leg breed fed with supplementation and the lowest was in eggs from Lohmann line fed without supplementation.

Table 5

Comparison of phosvitin isolation method from hen egg yolk [%]

Isolation method	Research group			
	I	II	III	IV
Nakai- Losso	2.92 ab	3.62 ab	3.55 ab	4.00 ab
Pangborn	2.36 a	3.74 ab	3.73 ab	4.73 b

a, b, c – different letters in the same row for the same parameter indicate statistically significant differences ($P \leq 0.05$)

Table 5 shows the yield of phosvitin isolation from all research groups isolated by Nakai-Losso and Pangborn method. The analysis of the results showed that the highest yield of phosvitin isolation was in eggs from Green-leg breed fed with supplementation, isolated using Pangborn method. The lowest yield of phosvitin isolation was recorded in eggs from Lohman line fed without supplementation, also isolated using Pangborn method.

Conclusions

1. The results of the experiment showed that the breed of hens is responsible for the yield of phosvitin isolation from hen egg yolk. The eggs from Green-leg breed had the highest yield of phosvitin isolation in comparison to Lohmann line.
2. Feeding with addition of humokarbowit and humobentofet, which contains polyunsaturated fat acids, is responsible for yield of phosvitin isolation.
3. The results obtained in the study show that isolation method was not responsible for the yield of phosvitin.

Acknowledgments

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EVALUATION OF NUTRIENTS AVAILABLE FROM DIFFERENT KINDS OF BREAD AND THEIR COVERAGE IN COMPARISON TO REFERENCE DAILY INTAKE IN ADULT GROUP

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Abstract

Bread is one of the most important staple food in almost all countries including Latvia and plays an important role in human nutrition but affect of time pressured lifestyle and changes in eating habits, it consumption significantly decreases and do not reach recommended amount need to be consumed. Therefore the purpose of the research was to find out and evaluate within the gender in adult group amount of nutrients covers reference daily intake (RDI) by consuming wheat bread, rye bread and sweet sour bread. Results show that by consuming rye bread female covers only 7.1 % of protein 1.2 % of fat and 11.1 % of carbohydrates from RDI while male – 5.9 % of protein, 1.0 % of fat and 9.3 % of carbohydrates which is a part of total 28.6 % of nutrients need to be taken in accordance with WHO recommendations. The same conclusion has been done in insufficient consumption of wheat bread and sweet-sour bread. Important deviations from RDI were found on mineral substances and vitamins as well.

Key words: Bread, nutritional value, RDI, adult group

Introduction

Since the earliest of times bread has been a staple food for mankind, with records of grain milling and baking stretching back over 7.000 years (Jefferson, 2000) and plays an important role in human nutrition (Al-Kanhal *et al.*, 1999; Isserliyska *et al.*, 2001) as well as is one of the most important staple foods in almost all countries us an excellent source of nutrition (Padamavathy, 2007). According to Department of Health and Food Standards Agency healthy eating guidelines, one third of our total calories should be in the form of starchy foods, such as bread, potatoes, pasta, rice and other cereals (MRC Human Nutrition Research, 2007). Dieticians suggest consuming cereal products in the same amount like potatoes which is 40 % from the total products are consumed. While changing eating patterns of the past 50 years have led to an overall fall in bread consumption (Jefferson, 2000). From the Norbagreen survey was found that large portion of the Nordic and Baltic populations do not reach the official and nonofficial guidelines for consumption of fruits, vegetables, fish and bread (Norbagreen, 2003). According to the Latvian Central Statistical Bureau (CSB) data during the last decade bread consumption is decreased by 24 kg per capita – while in 1996 bread consumed per capita was 76 kg than in 2005 it was only 55 kg. Within the type of bread most significant decrease – 15 kg was for rye bread while almost 9 kg – wheat bread but sour sweet bread decrease was not significant. Nutritional value of bread differ within the type of bread which is affected by the type of flour is used in bread preparation as well as another ingredients such us milk which is used instead of water, eggs, brains, sugar and fat. Wheat bread contains around 45–55 % carbohydrates, 6–10 % protein and 1–2 % of fat. Most rich in nutrients is wholemeal bread because containing both high amount of vitamins like thiamine, riboflavin, pyridoxine, folic acid, trace elements and fibre (Insel *et al.*, 2003). Therefore the purpose of the research was to find out and evaluate within the gender in adult group amount of nutrients covers RDI by consuming wheat bread, rye bread and sweet sour bread.

Materials and Methods

The research was divided into two stages consisting of literature review on bread consumption and nutritional value of different kind of bread as well as calculation part on recommended daily allowance on adults per gender on bread consumption and recommended amount need to be consumed. Calculations and analysis has been done based on direction about "Ieteicamās enerģijas un uzturvielas devas Latvijas iedzīvotājiem" Nr. 233 issued by Ministry

of Welfare of Republic of Latvia, 23 of January, 2003. Calculations have been done for adult group meant separately for female and male and average values were used for further results.

Results and Discussion

Latvia CSB data shows that in 2005 wheat bread consumed was 25 kg per capita while rye bread – 26 kg and sweet-sour bread – 6 kg (Table 1).

Table 1

Bread consumption per capita, CSB

Type of bread	Consumption, kg year $^{-1}$	g per day	Slices per day
Wheat bread	25	68.49	2.74
Rye bread	26	71.23	1.58
Fine rye-bred	6	16.44	0.41
Total	57	156.16	4.73

Data presented in Table 1 explains that consumption of bread in 2005 was insufficient because a bread consumption recommendation by World Health Organisation (WHO) suggests eating 250 g of bread per capita per day. To clarify the amount of basic nutrients are obtained by consuming 156 g of different type of bread calculation has been done based on direction "Ieteicamās enerģijas un uzturvielas devas Latvijas iedzīvotājiem" Nr. 233 issued by Ministry of Welfare, Latvia, 23 of January, 2003. Reference values of recommended daily allowance (RDI) are presented in Table 2 and are used for further calculations.

Table 2

Average amount of energy and nutrients recommended for adults per day

Gender	Average body mass, kg	Average high, cm	Energy (E), kcal day	Protein E%	Fat E%	Carbohydrates E%
Male (average)	75	175	2400	10-15 (12.5)	25-30 (27.5)	55-60 (57.5)
Female (average)	65	165	2000	10-15 (12.5)	25-30 (27.5)	55-60 (57.5)

Achieved results (Table 3) are compared with the amount of nutrients suggested by consuming bread (Fig. 1). Since RDI of nutrients for female is less than for male (Table 2) segmentation of the nutrients, within each type of bread consumed, automatically will be more for female than male.

Table 3

Nutrient segmentation in % from RDI per each type of bread per capita

	Protein, %		Fat, %		Carbohydrates, %	
	Female	**Male**	**Female**	**Male**	**Female**	**Male**
Wheat bread	8.3	6.9	1.3	1.1	11.1	9.3
Rye bread	7.1	5.9	1.2	1.0	11.1	9.3
Sweet-sour bread	1.8	1.5	0.3	0.2	2.9	2.4
Total	17.2	14.4	2.8	2.3	25.1	20.9

From the data presented in the Fig. 1 can be concluded that in 2005 till present time consumer do not reach recommended amount of nutrients from bread because results show significant deviation from recommended numbers.

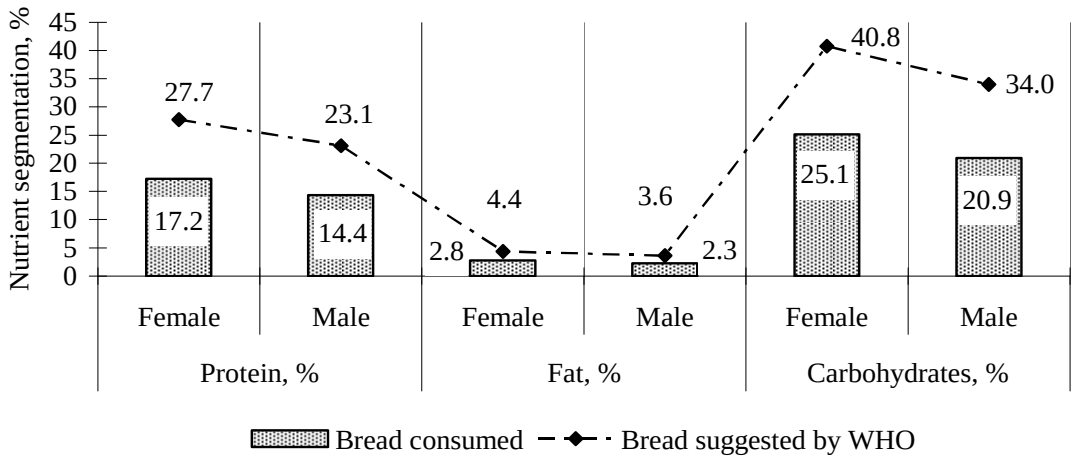

Figure 1. Nutrient segmentation in comparison between consumed and suggested amount of bread

Regarding to the fibre content, well known is statement that cereal products are good source of fibre. In average wheat bread contains 4 g 100 g^{-1} fibre whereas rye bread contains 8 g 100 g^{-1} and sweet-sour bread around 6 g 100 g^{-1} fibre. Based on the Latvia CSB data about the consumption of the bread by wheat bread was ingested 2.74 g of fibre however by rye bread – 5.70 g and by sweet-sour bread only 0.99 g of fibre. In comparison with suggested portion of fibre – 30 g per day with bread has been ingested 9.43 g (31%).

In addition to nutrients, as well as energy source nutrients, macro-elements, trace elements and vitamins play an important role in the human body. Since bread consumption is not reaching the amount of bread need to be consumed inorganic nutrient and vitamin intake will be less than it should be (Figure 2 and 3). RDI for Na, K and Mg both for female and male is the same therefore segmentation of mentioned nutrients will be the same, but for Ca, Fe and P it is different. Significant distinction was found on Fe segmentation within gender.

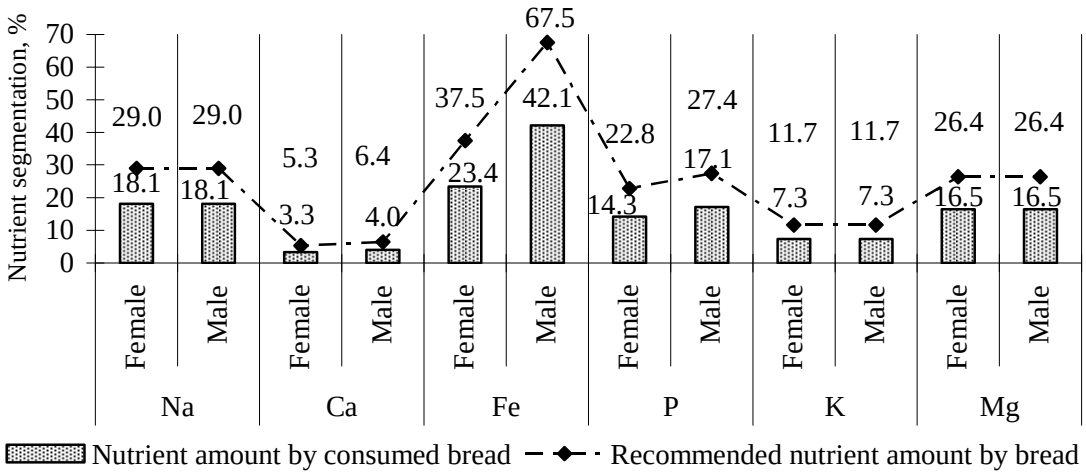

Figure 2. Inorganic nutrients consumed and recommended per day per capita

RDI of vitamins between female and male is the same; therefore segmentation is calculated in general.

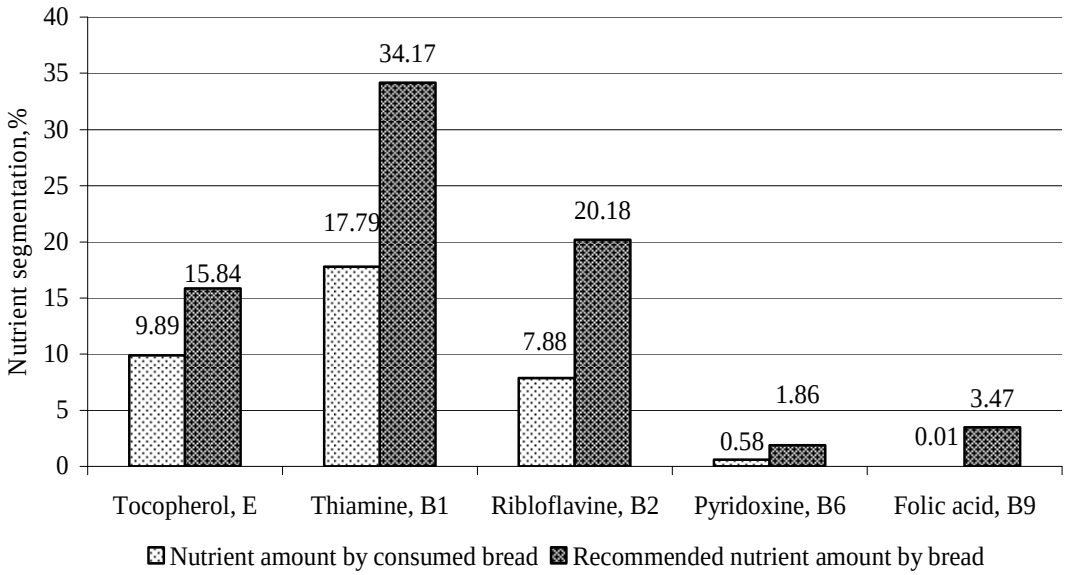

Figure 3. Vitamins consumed and recommended per day per capita

From Figure 3 can be concluded that significant differences are found within each vitamin group which means that bread consumption need to be increased to reach suggested amount of nutrients from bread.

Conclusion

Bread consumption needs to be increased to reach the amount of nutrients coming from suggested amount of bread by WHO. To raise consumption of bread, one of the fields would be suggested to pay attention is advertisement as well as popularisation of bread as good source of nutrients in schools by developing special nutritional/health education programmes where one of the topics would be bread and its importance in human health. It is already approved that bread as sandwich (e.g. bread, butter, salads, slice of meat, slice of cheese and vegetables) can be as one basic meal in one of four/five meal times.

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STERIGMATOCYSTIN PRESENCE IN DIFFERENT LATVIAN BREAD SAMPLES

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Abstract

29 samples of different types of bread purchased from the local Latvian supermarkets were analysed for sterigmatocystin (STC) content. STC is carcinogenic and mutagenic mycotoxin produced by fungi of many *Aspergillus species* and it is a precursor of aflatoxin B_1 in the biological transformation.

Analysis of STC were done using previously developed method. Method includes sample extraction with acetonitrile: water solution (84:16, v/v), extract clean up on Strata X solid phase extraction (SPE) column, extract concentrating by evaporation under nitrogen and further redissolving in mobile phase, separation on reversed phase C_{18} high performance liquid chromatography (HPLC) column and STC detection by high performance liquid chromatography – tandem masspectrometry with electrospray positive ionization (LC – ESI^+ – MS/MS).

17.2 % of analyzed samples were positive for STC with the concentration levels ranging from 2.4 to 7.1 µg kg^{-1}.

From 6 rye bread samples only one (16.7%) was contaminated with STC, from 9 rye-wheat bread samples only one (11.1%) was contaminated with STC and 3 (21.4%) of 14 wheat bread samples were contaminated with STC. Totally 4 (80%) of 5 contaminated samples contained whole grains as the main ingredient.

So, whole grain bread can be a possible source of STC, however positive samples contain quite low levels of STC.

Key words: Sterigmatocystin, mycotoxin, bread, *Aspergillus spp.*, *Aspergillus versicolor*, LC-MS/MS

Introduction

STC is a mycotoxin produced by fungi of many *Aspergillus spp.* (Atalla *et al.*, 2003). Its molecular structure is similar to aflatoxin B_1 (AFB). It is a precursor of AFB in the biological transformation (Betina, 1989).

STC is a carcinogenic compound that has been shown to affect various species of experimental animals (Purchase *et al.*, 1970) and it is classified as a 2B carcinogen by the International Agency for Research on Cancer (IARC) (IARC, 1976). There are many reports about toxicity, carcinogenity, mutagenity, and teratogenity of STC (Sweeney *et al.*, 1999; Tong-xi *et al.*, 2000). STC is an important contaminant of building materials and dwellings (Engelhart *et al.*, 2002; Nielsen *et al.*, 1999). Natural occurrence in food and food products appears to be infrequent although only a limited number of surveys have been carried out. There are reports about the occurrence of STC in grains (Scott, 1972; Shannon *et al.*, 1976; Mills *et al.*, 1986; Rao *et al.*, 2000), in rapeseeds (Mills et al., 1986), in soybeans (Shannon *et al.*, 1976), in cheese (Francis *et al.*, 1987; Lund *et al.*, 1995; Abdalla *et al.*, 1996; Scudamore *et al.*, 1996), in spices (red pepper, caraway, cumin and marjoram) (ElKady *et al.*, 1995), in cocoa beans (Hurst *et al.*, 1987), in vegetables (Thurm *et al.*, 1976), in pistachio nuts (Sommer *et al.*, 1976), in coffee beans (Purchase *et al.*, 1973) and in feed (Scudamore *et al.*, 1997; Domagala *et al.*, 1997).

Neither country has legislation for STC, however some countries have set already relatively low maximum levels for STC (e.g., Czech Republic and Slovakia at the level 5 µg kg^{-1} for rice, vegetables, potatoes, flour, poultry, meat, milk, and 20 µg kg^{-1} for other foods) (Stroka, 2004) and soon after STC was recognized as a highly toxic compound, the California Department of Health Services used TD 50 values from the Cancer Potency Database to produce “no significant risk” intake levels for humans. The level resulting was 8 µg kg^{-1} body weight/day for a 70 kg adult (European Mycotoxin Awareness Network).

The aim of this study is to research samples of different type of bread, that are available in local supermarkets, for STC content using sensitive LC-MS/MS method (Versilovskis *et al.*, 2007).

Materials and Methods

Bread samples

Bread samples (6 rye bread, 9 rye-wheat bread and 14 wheat bread) were purchased from local supermarkets.

Before the analysis, the samples were crushed and than homogenized.

Chemicals and reagents

Methanol (HPLC-grade) and acetonitrile (HPLC-grade) was purchased from Merck (Darmstadt, Germany). Deionized water was purified with Millipore Milli-Q Plus system (Millipore, Molsheim, France). STC standard was purchased from Sigma (St Louis, MO, USA). Argon (AGA, Latvia) was used as a collision gas in the mass spectrometry.

Preparation of standards

A stock solution of a concentration of approximately 500 μg ml^{-1} was prepared by dissolving 5 mg of STC in 10 ml of acetonitrile/methanol (50:50, v/v). An aliquot 500 μl of the stock solution was evaporated to dryness under oxygen-free nitrogen at ambient temperature and immediately redissolved in acetonitrile (5 ml).

The calibrated stock solution (50 μg·ml^{-1}) was used to prepare a standard stock solution of 5 μg/ml of STC, in acetonitrile/water (75:25, v/v). This solution was used to spike samples for recovery experiments, and to prepare working standards 0.25 μg ml^{-1}, 0.1 μg ml^{-1}, 0.05 μg ml^{-1} and 0.005 μg ml^{-1} as equivalents to 25 μg kg^{-1}, 10 μg kg^{-1}, 5 μg $kg^{-1}$and 0.5 μg kg^{-1}.

Sample preparation

An amount of 25 g of homogenized sample was extracted with 16% of water in acetonitrile (100 ml) for 30 min using a horizontal shaker. After filtering through a filter paper, 10 ml of the raw extract was diluted with 20 ml water and purified using Strata X (500 mg) SPE column (Phenomenex, Torrance, CA, USA). Purifying procedure: column was conditioned with 6 ml methanol, followed by 6 ml of water prior to use, then 30 ml of diluted extract was loaded in the column, then column was washed with 35% acetonitrile in water, then with 35% methanol in water and STC was eluted with 4 ml of 100% acetonitrile. The resulting eluate was evaporated to dryness under nitrogen at 60 °C and re-dissolved in 250 μl 25% water in acetonitrile. The calibrants were prepared by spiking the blank matrix with the standard and prepared in the same way as the samples.

LC-MS/MS –analysis

A Waters Alliance 2695 liquid chromatograph (Waters) was connected to a MicroMass Quattro LC triple-quadrupole mass spectrometer (Micromass, Manchester, UK). An electrospray ionization (ESI) probe in the positive mode was used in the analysis of STC. The mobile phase consisted of 0.01 % formic acid in acetonitrile and 0.01% formic acid in water (75:25, v/v) used in isocratic regime. The column used was a Phenomenex Luna C_{18}(2) (5 μm), 150x3.0 mm (Phenomenex, Torrance, CA, USA). The flow rate was 0.3 ml/min, column temperature was 30 °C and the injection volume was 50 μl. The parameters of the mass spectrometer were optimized using the STC standard. The best response was recorded with the following parameters: cone voltage 30 V, capillary voltage 3.5 kV, extractor 2 V, radio frequency (RF) lens 0.2 V, source temperature 120 °C and desolvation temperature 350 °C, cone gas flow 63 l h^{-1}, desolvation gas flow 553 l h^{-1}, collision energy 30 eV.

For the structural identification in multiple reaction monitoring (MRM) mode, the molecular ion [M+H] + (*m/z*=325) was fragmented within the MS to its daughter-ions (325>310 and 325>281) collision energy 30 eV, dwell 0.2 sec. Argon at pressure 3.5 bar was used as a collision gas. A calibration curve constructed using external standardization in matrix. The daughter-ion (*m/z*=281) was used for the quantification of STC. The ratio between peaks of STC obtained on two MRM channels (Peak area (325>310) / Peak area (325>281)) was used for confirmation of analyte. This ratio should be 0.69±0.14 for the compound to be confirmed.

Spiking for recovery studies

Spiked samples of different grains were prepared by adding 25 μl and 125 μl of the 5 μg ml^{1}

STC standard solution using a digital pipette to 25 g of sample in an 250 ml flask, whitch was left for 1 h at ambient temperature with occasional agitation to allow the acetonitrie to evaporate. These volumes of standard were equivalent to levels of 5 µg kg^{-1} and 25 µg kg^{-1} STC respectively. Six replicates at each concentration level were prepared from each commodity for recovery experiments. Recovery results for bread are shown in Table 1.

Table1

Recovery and precision results obtained from bread matrix

Spike level, µg kg^{-1}	Mean result (n=6), µg kg^{-1}	Standard deviation (SD), µg kg^{-1}	Relative Standard deviation (RSD), %	Mean recovery, %
5.0	4.8	0.2	4.6	96.0
25.0	25.8	1.6	6.2	103.1

Calibartion and Linearity

In LC-MS methods the matrix often causes the change of the response, because the matrix components disturb the ionization of the analytes (Tang *et al.*, 1993). Because of the matrix effect, the calibrants were always prepared in blank matrix.

The method was linear for STC from 0.5 µg kg^{-1} to 25 µg kg^{-1}. A tolerance of ±10% accepted for the separate calibration points for good linearity. The regression line without matrix was y=13656x+212 (R^2>0.999) and regression line in matrix was y=4477x-176 (R^2>0.999). On this basis, the method considered linear for the analysis of STC.

Results and Discussion

From the analysed samples only one (16.7%) rye bread sample, one (11.1%) rye-wheat bread sample and three (21.4%) wheat bread samples were contaminated with STC. Four (80%) of five contaminated samples were prepared using whole grains.

Totally, 17.2% (5 samples) of the analyzed samples were contaminated with STC (Table 2).

Table 2

STC presence in different bread samples

Sample No.	Bread sample name	Sample type	Bakery	Result, µg kg^{-1}
1	"Hanzas" rye bread	R	Hanzas	n.d.
2	Real brown bread	R	Lāči	n.d.
3	"Saules" dark whole grain bread	R	Iļguciema	n.d.
4	"Rīgas" brown bread	R	Iļguciema	n.d.
5	"Fazer" whole grain rye bread	R	Druva	n.d.
6	Rye bread	R	Sono	**2.4**
7	"Hanzas" wheat-rye bread	RW	Hanzas	n.d.
8	Real fine rye-bread	RW	Lāči	n.d.
9	"Fit Life" Bread	RW	Lāči	n.d.
10	Fine rye-bread	RW	Iļguciema	n.d.
11	"Meistara" Rye bread	RW	Druva	n.d.
12	"Meistara" lime-pit fine rye-bread	RW	Druva	n.d.
13	Dinaburga	RW	Dinella	n.d.
14	"Saimnieks" whole grain bread	RW	Sono	**7.1**
15	Fine rye-bread	RW	Sono	n.d.
16	Fitness	W	Hanzas	n.d.

Sample No.	Bread sample name	Sample type	Bakery	Result, μg kg^{-1}
17	"Hanzas" wheat bread	W	Hanzas	n.d.
18	"Sendviču" rain bread with barley and rye flakes	W	Hanzas	n.d.
19	"Sendviču" grain bread with rye flakes	W	Hanzas	4.4
20	"Sendviču" grain bread with oat flakes	W	Hanzas	n.d.
21	Real wheat bread	W	Lāči	n.d.
22	"Saules" grain bread	W	Iļguciema	**3.2**
23	City's wheat bread	W	Iļguciema	n.d.
24	"Fazer" King's grain tosterbread	W	Druva	**5.6**
25	"Fazer" wheat tosterbread	W	Druva	n.d.
26	"Spēkotava" bran bread	W	Druva	n.d.
27	"Zeltene" Wheat bread	W	Dinella	n.d.
28	"Autumn" wheat bread	W	Dinella	n.d.
29	"Kurzemes" wheat bread	W	Sono	n.d.

R – rye bread; RW – rye-wheat bread; W – wheat bread; n.d. – not detected

However the concentration levels were quite low from 2.4 to 7.1 μg kg^{-1} and did not exceed maximum levels for this toxin set in Czeh Republic and Slovakia (20 μg kg^{-1}) but exceed level set in these republics for rice, vegetables, potatoes, flour, poultry and meat – 5 μg kg^{-1}. So, results of our research give possibility to suppose that levels of STC in grains from which these bread samples were prepared, were highly contaminated with STC. So, in case that STC is carcinogen it is not recommended to use contaminated products, because of possible chronic effects. Nevertheless, as mentioned above (in introduction section) "no significant risk" intake level for humans is about 8 μg kg^{-1} body weight/day for a 70 kg adult – it is approximately 480 μg per day! In this case, concentrations that were found in bread during this investigation cannot seriously affect consumer's health.
There are no available comparable results in literature for so wide range of bread samples.

Conclusions

This is the first research on STC content in different types of bread in Latvia. This study indicates about the occurrence of STC in bread, especially in whole grain bread. Although found contamination of bread is quite low, bread still can be a possible source of investigated toxin.
So, in the aspect of all mentioned above, monitoring of the presence of STC in bread and other food products in Latvia is clearly necessary.

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APPARENT ENDOXYLANASE ACTIVITY IN RYE CULTIVARS FROM LITHUANIA

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Abstract

The efficiency of cereal-based biotechnological processes closely linked to both their chemical composition and selection of cell-wall degrading enzymes. Degradation of cell-wall polysaccharides during processing, by means of added or naturally occurring endogenous xylanases, may also affect the quality of the end-product. The aim of the present study was determination of the apparent activity of endogenous xylanases in four winter rye varieties and in two newly developed rye hybrids. The influence of variety and climatic conditions on apparent endoxylanase activity was investigated. Rye cultivars were grown on different soils at three Plant Breeding Stations of Lithuania in 2003 and 2004. The apparent endoxylanase activity was studied in the albumin type extracts from meal of the rye kernels (pH 4.5; 40 °C) by an assay based on dinitrosalicylic acid. The arabinoxylan used as substrate was isolated from wheat bran, and the reducing sugars were purified by cation-exchange chromatography. The results showed the significant influence of the genotype and agro-climatic conditions on the endoxylanase activity varying between 5.1±0.6 and 23.6±0.9 nkat/g grain. No significant correlations were found between Falling number (FN) and apparent endoxylanase activity in rye.

Key words: rye, endoxylanase, activity, genotype.

Introduction

The efficiency of cereal-based biotechnological processes and the quality of end- products are closely linked to the chemical composition of the fermented cereal raw material. Degradation of cell-wall polysaccharides during processing, by means of added or naturally occurring endogenous xylanases may also affect the quality of the end-product.

Rye is the second important crop in Lithuania next to wheat because of its production extent and is widely used in various fermentation processes. Rye is characterized as to have a higher amount (~16%) of the dietary fiber polysaccharides the most important constituent of which are arabinoxylans (AX) (Vinkx and Delcour, 1996). AX-fraction is of considerable importance for rye processing, bread-making quality and nutritional properties of food and feed (Vinkx *et al*, 1993; Åman *et al.*, 1997). Although, arabinoxylans together with β-glucans cause the low extract yields, high wort viscosity and decreased the rate of filtration or haze formation in beer (Antoniuou *et al.*, 1981; Izydorczyk and Biliaderis, 1995; Schwarz and Han, 1995). The presence of water-extractable AX constituting approximately 25–30 % of the total AX increases dough viscosity, bread volume, gas retention, crumb texture, colour and taste (Bengtsson and Åman, 1990; Courtin and Delcour, 2002).

In recent years, the interest in carbohydrate-active enzymes such as xylanases has increased due to their potential application in the food and feed industry. Endoxylanases are primarily responsible for the degradation of AX increasing the level of water soluble AX and affect the fermentation process, herewith the quality of the end-product (Poulsen *et al.*, 2002). However, efficiency of added commercial endoxylanases can vary depending on rye variety and growing location making the optimal dosage of enzyme difficult to determine. It may be due to the levels of endoxylanases and endoxylanase inhibitors in rye cultivars (Sørensen *et al.*, 2001; Dornez *et al.*, 2006). Similarly, evaluation of the action of endogenous xylanases is of a growing importance in case of increasing the efficiency of fermentation process of rye-derived stock in different applications. The optimization of enzymatic hydrolysis of rye causes the problems due to the lack of studies related to the endoxylanase activity and the influence of the growth conditions and genotype on the endoxylanase level.

The present work has the objective of determination the apparent activity of endogenous xylanase in winter rye crude extracts, which serve as a model system of fermentable raw material.

Materials and Methods

Rye samples. The samples of four rye varieties 'Joniai', 'Fernando', 'Matador', 'Picasso' and new developed hybrids LVA 426 and LVA 391 were obtained from Plant Breeding Stations (PBS) located in different parts of Lithuania after two growing seasons (2002–2003 and 2003–2004). The weather conditions in the summer of 2003 and 2004 were completely different as well as the precipitation levels at PBS I and PBS II locations (Figure 1). The summer of 2003 was warm and dry. Higher falling numbers (FN) for the rye varieties grown in 2003 were observed with an average of 266 s. In contrary, the summer of 2004 was cool and wet. Heavy rainfall before harvest increased the risk of sprouting and the microbial contamination of the rye kernels. This was evidenced by relatively low FN values with an average of 169 s.

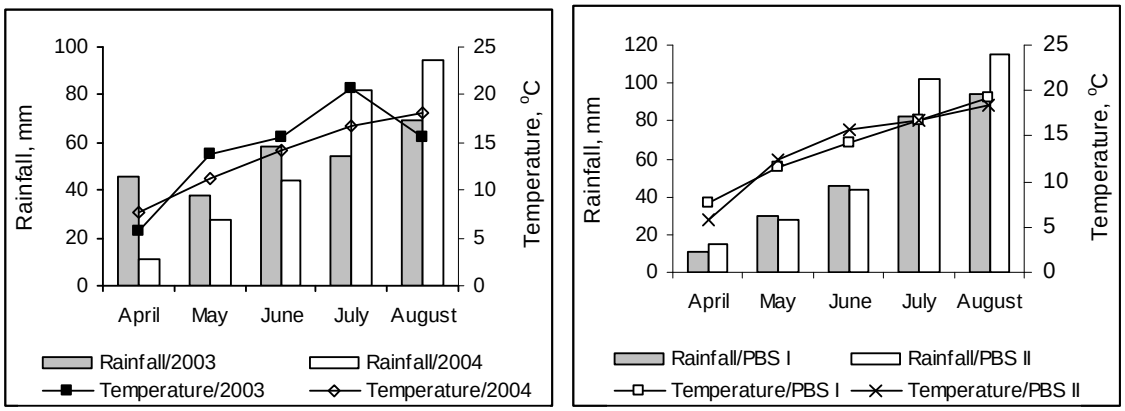

Figure 1. Average temperature (curve) and total rainfall (bars) in 2003 and 2004 (a) and at PBS I and PBS II locations (b)

Sample preparation

Rye crude extracts. The rye wholemeal (5±0.01 g) was homogenized in 40 ml of sodium acetate buffer (0.1 M; pH 4.5) on ice-bath for 10 min. The homogenate was centrifuged (10000 g; 4 °C; 25 min) and filtered.

Substrate solution. The wheat arabinoxylan (3.2 mg/ml) solution was prepared from wheat bran at the same manner as rye crude extracts. After homogenization and centrifugation procedures the solution was boiled for 15 min and cooled to 4 °C.

Stop solution (DNS reagent). 3.5-dinitrosalicylic acid (1±0.01 g g) and sodium potassium tartrate (30±0.01 g g) dissolved in 100 ml of 0.4 M NaOH.

Determination of falling number. Falling number were determined according Hagberg-Perten by ISO 3093:2004 method.

Endoxylanase activity assay. The endoxylanase activity in rye extracts was measured by an assay based on dinitrosalicylic acid according Miller (1959) with some modifications. One unit of enzyme activity is defined as the amount of enzyme required to releases 1 μmol of xylose equivalents per minute from the arabinoxylans under the assay conditions used (pH 4.5; 40 °C).

The reaction mixture (1 ml) containing cereal extract (200 μl)) and substrate (100 μl) in acetate buffer (0.1 M; pH 4.5) was incubated at 40 °C for 60 min. The reaction mixture prior to treatment by dinitrosalicylic acid was subjected to carbohydrate purification by CEC according to the principles described by Sørensen *et al*., (1999). The equal amounts of

carbohydrate solution and DNS reagent were mixed and incubated in a boiling water bath for exactly 5 min. After cooling, reaction solution was diluted (1:10) and the absorbance of the solutions was measured at 540 nm. The mean absorbance values of the triple determinations and the two blanks were calculated. The D-xylose standards (0–0.25 mM) were made up in acetate buffer (0.1 M; pH 4.5) and allowed the construction of a calibration curve. For the calculation of the endoxylanase activity (nkat/g grain) the slope from the xylose curve was used.

Carbohydrate purification by cation-exchange chromatography (CEC). CEC columns contained the strongly acidic cation-exchanger (Dowex 50WX8 H^+ 200–400 mesh) were washed with water to neutral pH. The reaction mixture (1 mL) was added, and the aqueous effluent contains neutral compounds including carbohydrates was washed with 10 mL of water.

Statistical analysis. The variations in the endoxylanase activity were analysed with the Statistical software Analyse-it for comparison of the means by one-way analysis of variance (ANOVA). The significant of the results from the data analysis was considered by $p<0.05$.

Results and Discussion

Endoxylanase variability. Table 1 presents the apparent endoxylanase activities in different rye varieties of 2003 and 2004. The measured endoxylanase activity values in the rye grain extracts after 1 hour of incubation varied from 5.1±0.6 to 23.6±0.9 nkat/g. The statistically significant differences between the apparent activities of rye varieties from PBS I and PBS II same as between varieties of 2003 and 2004 (PBS III) harvest were found. This verifies that the growing location influence ($p<0.05$) on the formation of the endoxylanase in rye cultivars. Also a strong correlations between apparent endoxylanase activities within mentioned groups was found ($R^2=0.74$ and $R^2=0.76$, respectively; $p<0.05$). The apparent endoxylanase activities in the rye varieties of 2004 were found to be higher on an average by 46% comparing to the activities of analogous cultivars of 2003. The results show the significant influence ($R^2=0.99$; $p<<0.05$) of the precipitation levels on the apparent endoxylanase activity.

The lowered endoxylanase activity values determined after 6 hours (Table 1) of incubation indicated the decrease in activity which can be due to the action of rye inhibitors which are able to inhibit microbial kernel-associated endoxylanases. The apparent endoxylanase activity of rye variety ‘Fernando’, ‘Picasso’ and LVA hybrids show the lowest changes during incubation. They may have the lowest activity of microbial endoxylanases and can be characterized as more resistant to microbial contamination than the other varieties.

Table 1

Apparent enoxylanase activity levels in winter rye cultivars

Rye variety	FN, s	Activity, nkat/g		$lnA=-k_Dx+lnA_o$	$T_{1/2}$, h
		after 1 h	after 6 h		
PBS I (2004)					
‘Joniai’	202	16.65±0.88	7.42±0.16	y=0.173x+3.049	4
‘Matador’	208	14.50±0.82	6.85±0.19	y=-0.156x+2.862	4
‘Fernando’	297	8.90±0.42	3.57±0.18	y=-0.170x+2.417	4
‘Picasso’	253	5.06±0.37	2.87±0.19	y=-0.138x+2.010	5
PBS II (2004)					
‘Joniai’	203	19.07±0.57	7.72±0.17	y=-0.157x+3.006	4
‘Matador’	248	23.55±0.92	9.22±0.28	y=-0.176x+3.193	4
‘Fernando’	232	10.59±0.39	6.21±0.22	y=-0.103x+2.461	7
‘Picasso’	262	8.29±0.50	5.73±0.19	y = -0.076x + 2.142	9

Rye variety	FN, s	Activity, nkat/g		$lnA=-k_Dx+lnA_o$	$T_{1/2}$, h
		after 1 h	after 6 h		
PBS III (2003/2004)					
'Joniai'/2003	244	8.54±0.35	5.28±0.16	y=-0.107x+2.327	6
'Joniai'/2004	184	16.72±0.96	8.41±0.18	y=-0.143x+2.944	5
LVA 426/2003	263	7.62±0.26	5.08±0.18	y=-0.082x+2.131	8
LVA 426/2004	164	14.50±0.92	7.97±0.29	y=-0.120x+2.794	6
LVA 391/2003	292	7.10±0.28	4.78±0.18	y=-0.061x+1.924	11
LVA 391/2004	160	12.26±0.82	7.60±0.29	y=-0.099x+2.623	7

The obtained activity values were found higher than those reported in literature by Autio *et al*. (1998). They measured endoxylanase activity of 1 and 5 nkat/g·grain in ungerminated and germinated rye kernels, respectively, using birchwood xylan as substrate. Either, the activities of endogenous β-D-xylanase were quantified in extracts from ungerminated rye grain by Rasmussen *et al*. (2001). The obtained activity of the endoxylanase against RBB-xylan was 11 pkat/g·grain. The higher activity of endoxylanase in our assay might to some extent be explained by the increased susceptibility of the soluble wheat AX to enzyme attack.
No significant correlations were found between FN and endoxylanase activities in rye (R^2=0.232). However, low reverse correlation (R^2=0.414) between FN and endoxylanase activity after 6 hours of incubation was detected. This suggests that the sources of endoxylanase and amylase activities in rye are quite different. Also the susceptibility of rye varieties to microbial infection could play a role.

Conclusions

The apparent endoxylanase activities are at least partially genetically determined, but the levels of endoxylanase largely depended on the climatic conditions prior to harvesting. The fact that not only sprouting kernels but also microorganisms on the cereal can produce endoxylanases may explain why no significant correlation could be found between FN and endoxylanase activity.
The functionality of commercial xylanases in different applications involving cereal processing may be influenced to different degrees by the relative quantities of cereal endoxylanase and endoxylanase inhibitors presented in cereal raw material and by the sensitivity of the endoxylanase to the inhibition. As endoxylanases strongly influenced on rye functionality, endoxylanase activity could be used as one of additional criterion for the selection of rye varieties suitable for applications required.

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CONTENT OF CAROTENOIDS AND PHYSICAL PROPERTIES OF TOMATOES HARVESTED AT DIFFERENT RIPENING STAGES

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Abstract

The aim of the research was to investigate dynamics of variations of carotenoids content (lycopene and β-carotene) and physical parameters (colour and texture) in two tomato hybrids 'Admiro' and 'Kassa' and one Lithuanian tomato cultivar 'Rutuliai' during the ripening process. Investigated tomatoes were grown in the greenhouses of Lithuanian Institute of Horticulture.

During the ripening process the amount of carotenoids, especially lycopene, in fruits increased. The highest lycopene and β-carotene content was determined in red-ripe fruits of cv. 'Rutuliai', 10.44 and 1.22 mg $100g^{-1}$ respectively. Lycopene content was 1.6 times higher in red-ripe fruits of 'Rutuliai' than in fruits of 'Admiro F_1 and 2 times higher than in fruits of 'Kassa' F_1 at the same ripening stage.

The colour of tomato fruits was evaluated by the CIE L*****a*****b***** system. The ratio of colour coordinates a***** and b*****, which is indicative of the red colour development in tomatoes, increased with maturity. The highest a*****/b***** value of 0.99 was determined in red-ripe fruits of cv. 'Rutuliai'. Strong positive correlation (r=0.86) was found between a*****/b***** and lycopene content in the present study.

The firmness of fruits was influenced by the degree of tomato ripeness. Turning stage tomatoes of Dutch selection 'Admiro' F_1 and 'Kassa' F_1 were the firmest, while fruit firmness of turning stage tomatoes 'Rutuliai' was less than that of Dutch selection tomato hybrids at the same ripening stage.

Key words: tomatoes, ripening, carotenoids, colour, firmness.

Introduction

Tomato is one of the most popular vegetables in the world. In Lithuania there are harvested approximately 18 000 t of tomatoes every year and about 25 000 t are consumed (Viškelis *et al.,* 2005). Tomatoes and their products provide an essential source of vitamin C, potassium, and antioxidants (primarily lycopene). Lycopene, present at high concentrations in tomatoes and tomato products, has attracted considerable attention because of epidemiological evidence that suggests this compound may provide protection against cancer and other degenerative diseases (Weisburger, 2002). Producing cultivars with big amount of lycopene has been a goal of breeders for a long time, primarily because of the increased red colour of such cultivars, but more recently because of the enhanced health benefit for humans.

The quality of fresh ripe tomatoes is influenced by growing conditions and genetic factors. The fruit ripening process results in biochemical changes that enhance fresh fruit quality, such as carotenoids accumulation and development of flavour volatiles. However, the ripening process also initiates degradative processes, such as fruit softening. The texture of the ripe fruit has significant effect on quality and influences consumer preferences, storability, shelf life, pathogen resistance and transportability.

Therefore the objective of this work was to establish the influence of fruit ripeness degree on the amount of carotenoids in tomato fruits, fruit texture and colour.

Materials and Methods

At the Laboratory of Biochemistry and Technology of the Lithuanian Institute of Horticulture there were investigated tomatoes of Lithuanian cultivar 'Rutuliai' and Dutch selection hybrids 'Admiro' and 'Kassa'. For the investigations tomatoes of four ripeness degrees (I – turning, II – pink, III – light-red and IV – red-ripe) were gathered during mass fruit yielding. Ripeness classification of tomatoes was based on external fruit colour (Saltveit, 2005). Tomato hybrids 'Admiro' and 'Kassa' were grown in the greenhouses covered with the double polymeric film ('Rovero 961'), in the mineral wool. Tomatoes of cultivar 'Rutuliai' were grown in the greenhouses covered with single stabilized film, in the soil.

The amount of carotenoids in tomatoes was established spectrophotometrically (Davies, 1976).

Tomato fruit surface colour was measured with a spectrophotometer MiniScan XE Plus (Hunter Associates Laboratory, Inc., Reston, Virginia, USA). CIEL*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). Data were presented as the averages of the three measurements. Colour parameters were processed with program Universal Software V.4–10.

Tomato texture was measured with texture analyzer (TA.XTPlus, Stable Micro Systems, England). For the penetration of the tomato fruit 2 mm diameter flat head stainless steel cylindrical probe was used. Penetration test was started when the probe got in contact with tomato surface, and finished when the probe penetrated the tissue to a depth of 8 mm. Probe speed during penetration test was 1 mm/s. For the analysis of texture three tomatoes of each ripening degree were taken and each tomato was punctured three times around the equatorial area of the fruit. Statistical analysis was performed using Texture Analyzer software.

Results and Discussion

Ripeness degree influenced the content of carotenoids in tomato fruits. Red-ripe fruits of investigated tomatoes had the highest β-carotene and lycopene contents. Depending on cultivar or hybrid, β-carotene content in red-ripe tomatoes was 1.9–3.3 times higher comparing to the turning stage fruits (Figure 1).

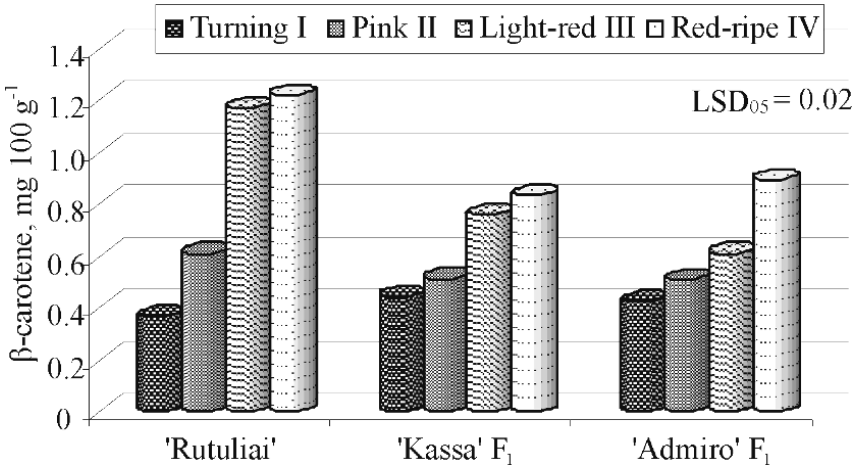

Figure 1. **Content of β-carotene in tomatoes of different ripeness degree**

The highest content of β-carotene was found in red-ripe fruits of cv. 'Rutuliai' (1.22 mg 100 g^{-1}). The content of β-carotene in red-ripe tomatoes of 'Admiro' F_1 and 'Kassa' F_1 was 0.89 and 0.83 mg 100 g^{-1} respectively.

The biggest amount of lycopene was accumulated in red-ripe fruits of cv. 'Rutuliai' (10.44 mg $100g^{-1}$) (Figure 2). Its content was 1.6 times higher than in fruits of 'Admiro' F_1 and 2 times higher than in fruits of 'Kassa' F_1 at the same ripening stage. The highest ratio of lycopene and β-carotene was also found in red-ripe fruits of cv. 'Rutuliai' (8.56). Thought in third ripening stage (light-red) the highest lycopene/β-carotene ratio was established in fruits of hybrid 'Admiro' F_1 (5.45).

Colour coordinate L*, characterizing fruit lightness, decreased during tomato fruit ripening (Table 1). At the beginning of ripening process these changes were not statistically significant, but when the red colour deepened, coordinate of lightness significantly decreased. The darkest were red-ripe tomatoes of cv. 'Rutuliai', 37.65.

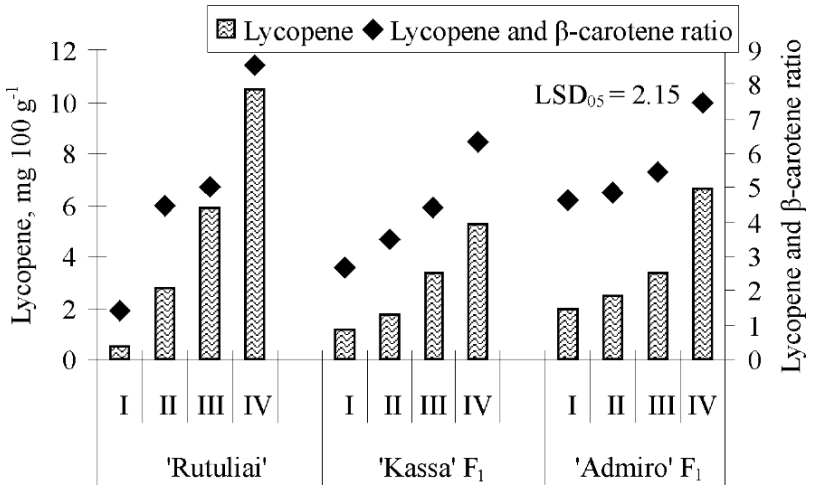

Figure 2. Amount of lycopene and lycopene/β-carotene ratio in tomato fruits of different ripeness degree

Table 1

Colour coordinates and indexes of tomatoes of different ripeness degree

Ripeness stage	L*	a*	b*	C*	h°	a*/b*
'Rutuliai'						
Turning I	46.78	5.79	26.38	27.00	77.60	0.22
Pink II	45.94	13.41	30.83	33.60	66.50	0.43
Light-red III	44.30	17.66	31.11	35.80	60.40	0.57
Red-ripe IV	37.65	21.57	21.53	30.50	44.90	0.99
'Kassa' F_1						
Turning I	46.07	3.69	21.89	22.20	80.40	0.17
Pink II	44.37	7.12	23.88	24.90	73.40	0.30
Light-red III	42.20	18.55	28.33	33.90	56.80	0.65
Red-ripe IV	39.46	19.20	25.67	32.10	53.20	0.75
'Admiro' F_1						
Turning I	48.68	2.52	20.99	21.10	83.20	0.12
Pink II	45.51	10.81	25.64	27.80	67.10	0.42
Light-red III	42.00	20.62	26.85	33.90	52.50	0.77
Red-ripe IV	40.95	21.20	25.37	33.10	50.10	0.84
LSD_{05}	2.14	1.58	1.44	1.98	1.90	

The positive a***** (redness) coordinate showed the most obvious change with maturity (Table 1). Generally, it increases as a consequence of both chlorophyll degradation and lycopene synthesis. The redness value a***** during tomato fruit ripening increased from 2.52 ('Admiro' F_1) to 21.57 ('Rutuliai').

The positive b***** (yellowness) value during tomato fruit ripening slightly increased from 20.99 ('Admiro' F_1) to 25.67 ('Kassa' F_1) (Table 1). Thought not significantly, b***** values were higher in light-red stage of the investigated tomatoes and in pink stage of 'Rutuliai' and 'Admiro' F_1 comparing to red-ripe fruits. This may be due to the fact that ζ-carotenes (pale-yellow colour) reach their highest concentration before full ripening, where lycopene (red colour) and β-

carotene (orange colour) achieve their peaks (Fraser *et al.,* 1994). The average value b***** of red-ripe tomatoes of the investigated cultivars and hybrids was 24.19.

The ratio of colour coordinates a***** and b*****, which is indicative of the red colour development in tomatoes, increased with maturity (Table 1). The highest a*****/b***** value of 0.99 was determined in red-ripe fruits of cv. 'Rutuliai'. Strong positive correlation (r=0.86) was found between lycopene content and a*****/b***** ratio. Additionally, strong positive correlation (r=0.78) was determined between lycopene content and coordinate a*****. Correlation between colour parameters and lycopene content in tomatoes was also reported by other authors (Arias *et al.*, 2000; Brandt, 2006).

Chroma C***** is an expression of the purity or saturation of a single colour. Colour purity C***** increased from turning to light-red stage of tomatoes, but in the fourth ripeness stage slightly declined (Table 1). The purest colour (the highest C^* values) was in the third stage of ripeness of all investigated tomatoes, the average – 31.9. Strong positive correlation (r=0.78) was found between b***** (yellowness) and colour purity C*****.

During fruit ripening of tomatoes the hue angle h° declined, because yellowness component decreased and redness – increased (Table 1). The average h° value of red-ripe tomatoes was 49.4°, it indicated that component of yellowness still had a big influence on the overall colour of tomato (90° would mean that tomato is yellow, and 0° – completely red).

Firmness of tomato fruit decreased with maturity (Figure 3). Fruits of 'Kassa' F_1 were the firmest ones, while fruits of cv. 'Rutuliai' were the softest. During fruit ripening of hybrid 'Kassa' F_1 their firmness decreased from 292.2 to 224.6 N/cm^2. Thought fruits of 'Kassa' F_1 in the fourth ripening stage (red-ripe) were almoust two times firmer comparing to the fruits of 'Rutuliai' at the same ripening stage.

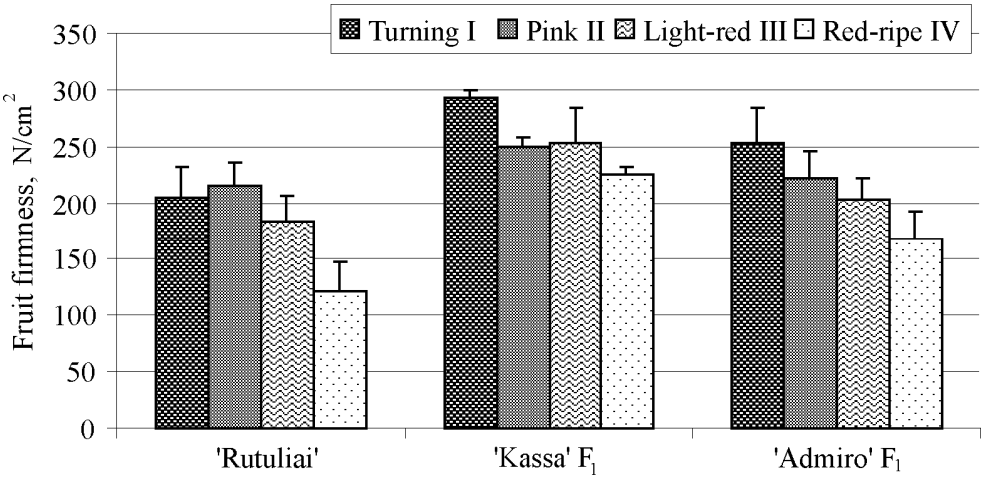

Figure 3**. Firmness of tomatoes of different ripeness degree**

Investigations showed strong negative correlation (r = 0.83) between lycopene and β-carotene ratio and tomato fruit firmness. Slightly weaker negative correlations were found between lycopene content and fruits firmness, and between β-carotene content and fruits firmness, 0.81 and 0.73 respectively. Bertin and others also investigated tomato quality changes during ripening process and reported negative correlations between β-carotene and lycopene contents and fruit firmness (Bertin *et al.*, 2001).

Conclusions

1. The highest content of lycopene and β-carotene was found in red-ripe fruits of cv. 'Rutuliai', 10.44 and 1.22 mg 100 g^{-1} respectively.
2. Strong positive correlation (r = 0.86) was found between lycopene content and a*****/b***** ratio of colour coordinates.
3. Fruits of hybrid 'Kassa' F_1 of all ripeness degrees were the firmest.

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