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Dear participants,

On behalf of Faculty of Food Technology of Latvia University of Agriculture it’s my pleasure to welcome you to 6th Baltic Conference on Food Science and Technology FOODBAL-2011 “Innovations for Food Science and Production”.

The theme – Innovations for food science and production – ensures that food researchers, Master and Doctor level students and visitors have opportunity to examine and to discuss relevant and current topics. This Conference gives us all the opportunity to share ideas and to continue to improve the way we do the research in food science.

The Conference Organising Committee has created a programme including 4 key note speeches, 27 oral presentations during 6 sessions and poster sessions. We trust you will find the programme informative, stimulating and an opportunity to create new ideas.

I would like to express my very sincere and personal thanks to the Organising Committee for their hard work and administrative support so necessary to make 6th Baltic Conference on Food Science and Technology a great success. In addition I would like to thank all of my colleagues who were involved in the review of papers, again without whom, the review process would have been an absolute impossible. I also wish to thank the ESF project “Formation of the Research Group in Food Science” for financial support of Conference organisation.

I welcome you to Conference and I hope that everything will meet or exceed your expectations and you will enjoy your stay at Jelgava.

Inga Ciprovica
Dean of Faculty of Food Technology
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INFLUENCE OF JERUSALEM ARTICHOKE POWDER ON DOUGH RHEOLOGICAL PROPERTIES

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Abstract
Wheat flour products are the most common and traditional foods around the world. Fresh backed products all day long and a wide variety of pastry products with new flavors, shapes, and sizes are some of the consumers’ demands (Rossell, 2010). The perennial vegetable plant Helianthus tuberosus L. (Jerusalem artichoke) is an interesting plant regarding functional food constituents because it is a rich source for fructooligosaccharides (e.g., inulin), minerals, and vitamins (Kays, Nottingham, 2008). Jerusalem artichoke powder (JAP) is a valuable product which is convenient in use and its addition to bread and pastry product increases their nutritional values.

Quality control equipment Mixolab for flour from French firm Chopin Technologies is a new generation device which allows the complete characterization of the flours in terms of proteins’ quality by determining their water absorption, stability, elasticity, and weakening properties; starch behaviour during gelatinization and retrogradation; consistency modification when adding additives and enzymatic activity of the proteases, amylases (Mixolab, 2010). Mixolab could play a key role in ensuring flour performance matches customers’ expectation in finished product (Partos, 2009). The aim of this study was to evaluate how the addition of JAP in different concentration levels influences thermo-mechanical properties of the wheat flours and dough.

Partial replacement of wheat flour with JAP at different levels (10, 20, 30, 40, and 50%) significantly changed the qualitative and quantitative thermo-mechanical properties of enriched dough. Results indicated relationships between the terms - water absorption capacity, mixing time, dough stability, dough resistance to kneading - and JAP concentration.

Key words: Jerusalem artichoke, wheat flour, rheological properties, Mixolab

Introduction
Commercial bakeries have today understood the changes in consumer lifestyles and have shifted their production processes, products, and even distribution channels to meet the new requirements. Consumers demands and needs become more important and bakeries are facing new challenges for satisfying them. Fresh backed products all day long and a wide variety of pastry products with new flavors, shapes, and sizes are some of the consumers’ demands (Rossell, 2010).

Jerusalem artichoke is a plant with distinctive chemical properties. Inulin is stored as a reserve carbohydrate in the tubers, whereas starch is the storage form of carbon in most plants. Together with a low–fat and mineral–rich profile, inulin gives Jerusalem artichoke tubers their unique value in the human diet (Kays, Nottingham, 2008). Jerusalem artichoke processed in powder could be well applied in bakery products to increase their nutritional value. Also, despite the large amount of information available on the nutritional and physiological properties of fructans, very little information is available on their effects on dough and bakery products (Partos, 2009).

Flour is the primary and important raw material in the preparation of bakery products, their quality is dependent of flour properties and quality (Kunkulberga, Seglins, 2010).

The Mixolab of Chopin Technologies is an instrument that obtains in a single test comprehensive data on the behaviour of all flour components. It is a new generation device which allows the complete characterization of the flours in terms of proteins’ quality by determining their water absorption, stability, elasticity, and weakening properties; starch behaviour during gelatinization and retrogradation. Mixolab could play a key role in ensuring flour performance matches customers’ expectation in finished product.

The aim of this study was to evaluate how the addition of JAP in different concentration levels influences thermo-mechanical properties of the flours and dough.
Materials and Methods

Experiments were carried out in the Laboratory of Food Analysis and Laboratory of Packing Material Investigations at the Department of Food Technology in the Latvia University of Agriculture.

Materials used in the study were: commercial high quality wheat flour „Ekstra” (type 405) – produced in joint-stock company „Dobeles Dzirnavnieks” (Latvia); JAP produced in local Ltd. “Herbe” (Latvia).

The investigated samples were obtained by mixing the wheat flour with JAP in concentrations 10, 20, 30, 40, and 50% of total flour amount, as well as was studied wheat flour (control sample) and JAP.

Content of moisture (%) was carried out using oven–dried PRECISA XM 120 at 0.01 g / 0.01%. Method was based on samples (3 grams) drying at temperature 140±1 °C and measuring the moisture of the wheat flour, wheat flour and JAP mixes, and JAP.

The preparation and characterization of the dough’s was according to the standard method (ICC-Standard Method No. 173, 2006; AACC 54-60.01; adapted ISO 5530–1:1997). Dough rheological investigations were performed by Mixolab (Chopin Technologies, Villeneuve la Garenne, France) which simultaneously determines dough characteristics during the process of mixing at constant temperature, as well as during the period of constant heating and cooling (Mixolab, 2010).

All the measurements were performed using the Mixolab “Chopin+” protocol. The Mixolab analyser measures in real time the torque (expressed in Nm) by passage of the dough between the two kneading arms thus allows the study of the physically-chemical behaviour of the dough. The wheat flour, flour’s mixes, and JAP was placed into the Mixolab bowl, mixed at 80 rpm for sample homogenization and heated up to 30 °C. At this moment, the apparatus adds the distilled water to achieve pre-fixed hydration. Special attention was paid to the determination of the water absorption to ensure the complete hydration of all the components. Required amount of flour for analysis was calculated by Mixolab software according to input values of flour mixtures moisture as well as water absorption. The total mass of flour and distilled water placed into bowl was 75g. After dough mixing stage samples temperature increase with the speed 4°C min⁻¹ until the mixture reached 90 °C; at this point, there was a holding period for 8 min at 90 °C, followed by a temperature decrease with the speed 4°C min⁻¹ until the mixture reached 50 °C. The mixing speed during the entire assay was 80 rpm. Total analysis time was 45 min.

The following parameters were recorded: water absorption (%) or the percentage of water required for the dough to produce a torque of 1.1±0.07 Nm, and this allows for comparison of different samples at the same optimal consistency; dough development time (min) or the time to reach the maximum torque at 30 °C; stability (min) or the elapsed time at which the torque produced is kept at 1.1 Nm; mechanical weakening (Nm) or the torque difference between the maximum torque at 30 °C and the torque at the end of the holding time at 30 °C; ripening stability (Nm), which is calculated as the ratio of the torque after the holding time at 90 °C and the maximum torque during the heating period; and setback (Nm), which is defined as the difference between the torque produced after cooling at 50 °C and the torque after the heating period.

The experiment was realized in four reiterations for each flour type.

The Figure 1 shows a typical Mixolab Standard curve. The explanation of parameters that were obtained from the recorded curve was following: parameter C1 placed in tolerance levels in all samples and used to determine absorption; C2 measures the weakening of the protein based on the mechanical work and the temperature, C3 measures starch gelatinization, C4 measures the stability of the hot-formed gel and amylase activity, C5 measures starch
retrogradation during the cooling period. It was also measured the stability and curve between points C3 and C2 and also between points C3 and C4 (Figure 1.).

![Figure 1. Typical Mixolab Standard curves (Mixolab, 2010)](image)

In a typical Mixolab curve can be distinguished five different stages: 1st dough mixing, 2nd gluten strength, 3rd dough viscosity, 4th amylase activity, 5th starch retrogradation.

**Results and Discussion**
Mixolab Standard offered a complete and detailed characterisation of raw materials - wheat flour, wheat flour and JAP mixes, and JAP, including the behaviour of the warm dough, and all the results are represented graphically in Figure 2.

The first stage lasts 8 minutes at temperature 30 °C that includes the initial mixing wherein the hydration of the flour compounds occurs together with the stretching and alignment of the proteins, leading to the formation of the viscoelastic structure. During this stage an increase in the torque was observed until a maximum was reached and resisted the deformation. The studies showed that dough formation time increases in the concentrations 30, 40, 50, and 100% of JAP of total flour content significantly in comparison with 100% wheat flour (p≤0.05). The flour mixtures with 10% and 20% of JAP, and 100% wheat flour dough formation time is similar and almost two times shorter than flour mixtures with 30, 40, 50% of JAP and more than four times as 100% of JAP (Figure 2). Addition of JAP in concentration greater than 20% of total flour content was bringing prolonged time of dough development that is connected with increasing of dietary fiber. The parameters that were obtained from the recorded curve describe Table 1.

<table>
<thead>
<tr>
<th>Samples</th>
<th>C1 (Nm)</th>
<th>C2 (Nm)</th>
<th>C3 (Nm)</th>
<th>C4 (Nm)</th>
<th>C5 (Nm)</th>
<th>Amplitude (Nm)</th>
<th>Dough stability (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat flour</td>
<td>1.04</td>
<td>0.37</td>
<td>1.66</td>
<td>1.11</td>
<td>1.51</td>
<td>0.07</td>
<td>7.80</td>
</tr>
<tr>
<td>JAP 10%</td>
<td>1.04</td>
<td>0.35</td>
<td>1.50</td>
<td>1.39</td>
<td>1.71</td>
<td>0.08</td>
<td><strong>11.02</strong></td>
</tr>
<tr>
<td>JAP 20%</td>
<td>1.06</td>
<td>0.28</td>
<td>0.78</td>
<td>0.84</td>
<td>0.95</td>
<td>0.11</td>
<td>7.00</td>
</tr>
<tr>
<td>JAP 30%</td>
<td>1.10</td>
<td>0.26</td>
<td>0.27</td>
<td>0.33</td>
<td>0.55</td>
<td>0.08</td>
<td>7.46</td>
</tr>
<tr>
<td>JAP 40%</td>
<td>1.12</td>
<td>0.23</td>
<td>0.25</td>
<td>0.24</td>
<td>0.40</td>
<td>0.07</td>
<td>5.47</td>
</tr>
<tr>
<td>JAP 50%</td>
<td>1.04</td>
<td>0.00</td>
<td>0.23</td>
<td>0.18</td>
<td>0.00</td>
<td>0.06</td>
<td>8.34</td>
</tr>
<tr>
<td>JAP</td>
<td>1.01</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.04</td>
<td>6.84</td>
</tr>
</tbody>
</table>
Water absorption is 54% of wheat flour used in the experiments. The partly substitution of wheat flour by JAP is changed water absorption in flour’s mixtures. The greater part of the wheat flour was replaced with JAP, the more flour mixture decreased absorption (Figure 4) that go with previous other authors studies. Water absorption of fibre–enriched dough decreased because JAP contains low molecular weight sugars and oligosaccharides (Rouille et al., 2005). If 10% wheat flour replace with JAP, then flour mixture water absorption is 47%, which is 13% lower than wheat flour. By contrast, a mixture of flour made from 50% wheat flour and 50% JAP have water absorption 33% that is about 38% less than wheat flour (100%). Comparison between wheat flour (100%) and Jerusalem artichoke flour, water absorption of JAP is almost twice smaller and is only about 31%. Water absorption decreasing in samples with JAP is connected with inulin which content in JAP is about 50% on dry matter. Inulin forms a barrier around the starch grains and thus limits the possibility of water fixation (Karolinska et al., 2007).

The wheat flour, wheat flour and JAP mixes, and JAP between have different moisture content too (Figure 3). Linear correlation coefficient are observed a strong linear correlation (r=0.87) between moisture content and water absorption in flours, this means that rising flour’s moisture content, increases the water absorption of flour. Addition of JAP decreases wheat flour and JAP mixes water absorption.

Dough stability are substantially (p≤0.05) reduced during mechanical kneading if amount of JAP is 20% and more it could be connected with dietary fiber content increasing and inability to keep the dough structure. Dough resistance to kneading was excellent, when wheat flour was substitute by JAP in concentration 10% that may be related to increasing of pentose, which enhances the flour technological properties and improves dough structure formation and dough stability. The values of parameter Amplitude shows that the flour stability increases with JAP addition in concentration up to 40% (Table 1).

The gluten protein’s large molecular size and low charge density appear to allow them to interact by both hydrogen and hydrophobic bonds (Hoseney, Rogers, 1990). JAP is made from vegetable which does not have gluten at all. Wheat flour substitution with JAP changed dough elasticity significantly during mixing and heating (p≤0.05). Addition of JAP reduces the gluten content and, therefore, the resistance to kneading goes down. The thermal weakening of protein was take time more in samples with JAP, and the higher amount of JAP,
the more thermal weakening of protein lasted, and it is can explain with dietary fiber content to high level. If JAP content is 50% and more, gluten content is deficiency, and in result them a formation of homogeneous dough is not possible, because the protein-chain under the mechanical-pressure force has brocket down. Dough from 50% wheat flour and 50% JAP loosed the protein-chain structure at all after approximately 30 minutes, but dough from JAP - after just approximately 13 minutes (Figure 2). According before observed data it is possible explain mechanical weakening values decreasing gradually with JAP concentration increasing.

The temperature increases and the third stage starts with the gelatinisation of starch, the granules absorb the water available in the medium and they swell, so the viscosity increases. Wheat flour sample formed viscoelastic dough and torque used for dough mixing was 1.69 Nm. Addition of JAP starch gelatinising process altered radical. Viscosity of dough with 10% JAP only was satisfactory, but in dough’s, which comprise JAP more than 10%, viscosity doesn’t forms. This situation is related with starch amount decreasing and pentoses content increasing, as well as inulin content increase. Pentosans are swelling and form a gel, so the dough is more sticky and moist (Kunkulberga, Seglins, 2010). Because pentosans bind large quantities of water, so influence of the JAP dough is formed heavier and denser then dough from wheat flour. In addition heating the inulin at 60 °C for 30 minutes slightly changed the gel structure; however, heating at 80 °C caused drastic change in the structure of the inulin gels (Glibowski, Wasko, 2008).

The amylase activity and the physical breakdown of the starch granules are associated with a reduction in the viscosity in the fourth stage. The highest stability of the hot – formed gel was observed in samples with 10% of JAP that is thanks to the inulin which stabilizing dough structure at this concentration of JAP (Karolini-Skaradzinska et al., 2007). In constant heating rate go on enzymatic activity which is the highest in dough from wheat flour, but with addition of JAP the starch’s ability to withstand amylolysis decrease at least twice as much.

A decrease of the temperature resulted in an increase of the torque, which is referred to setback and corresponds to the gelatinising process. This last stage is related to the retrogradation. The dough with 10% of JAP is the strongest during starch retrogration time in the cooling period (Figure 2). According literature the Mixolab’s results allow imagining the appearance of the finished product (Partos, 2009). Mixolab’s results of this experiment shows that addition of JAP in concentration 10% of total amount of flour in pastry products would be the most desirable from several aspects – dough formation time is the shorter, dough resistance to kneading is the higher, dough retain viscosity, and gives a thick structure of
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dough, also dough is the strongest during starch retrogradation time in the cooling period. From experiment measured flours was backed cakes and among them the cake with 10% JAP really looks the best and that volume is the largest in comparing with other samples.

Conclusions
1. The addition of JAP was inducing a decrease in water absorbability of flour.
2. Correlation between water absorption and flour moisture of all experimental samples exist a strong linear correlation: rising flour's moisture content, increases the water absorption of flour.
3. Addition of JAP in concentration greater than 20% of total flour amount was bringing prolonged time of dough development.
4. JAP influenced positively the rheological properties of dough, bringing about its strengthening in concentration up to 10% of total amount of flour.
5. According to Mixolab’s results is possible the appearance of the finished products quality predict.
6. The samples with JAP in concentration 50% and 100% was observed disintegration of the protein–chain under the mechanical-pressure force (respectively, after 30 minutes, and 13 minutes).

Acknowledgement
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References
Abstract
Private consumption is shaped by an array of complex and interrelated factors, including demographics; income and prices; trade, as well as social and psychological factors such as habits, culture and taste. Reducing the environmental impacts related to the consumption of food is a major challenge that requires efforts at all phases of the food value chain. The majority of environmental impacts related to consumption of food are from agricultural activities, including in particular cattle farming; therefore the main focus of the study is directed to meat consumption.

The objective of this paper is to analyze the main influencing factors of consumer behavior and their impact on sustainable food choices in Europe.

The research is based on Principle Component Analysis (PCA) and Robust correlation analyses. To study the problem elements also are used methods of analysis, synthesis and logical construction.

The research results show, that one of the strongest factors influencing meat consumption is income; however there are found other latent factors. Excessive meat consumption is unsustainable also in terms of health and in some countries has a correlation with obesity.

Key words: sustainable food consumption, consumer behavior, environment.

Introduction
Consumer behaviour is key to the impact that society has on the environment. The actions that people take and choices they make – to consume certain products and services or to live in certain ways rather than others – all have direct and indirect impacts on the environment, as well as on personal (and collective) well-being (Jackson, 2005).

Sustainability covers economic, ecologic and social aspects and therefore, sustainable food refers to fair trade food, organic food, local food and seasonal food (Avermaete, Mathijs, 2008).

Food and drink cause 20–30% of the various environmental impacts of private consumption. Meat and meat products, in different degrees of processing, are the most important sources of impact, followed by dairy products (CSP, 2008).

According to a study by the European Commission's Joint Research Centre, meat consumption contributes 24% of the overall environmental impacts caused by total consumption in the EU-27, but accounts for only 6% of total expenditure. The same study shows that in the EU-27 meat and dairy products contribute about 30–40% of aquatic and terrestrial eutrophication, 14% of Glasshouse gas (GHG) emissions, and 35% of nature occupation caused by total European consumption.

The impacts on environment include impacts from: energy, water use and waste generation in agriculture and the processing industry; the use of fertilizers and pesticides; emissions from livestock; land use and transport; and biodiversity loss from clearance of ecosystems to make way for food and feed cultivation, and pollution of water courses. (EEA, 2010).

Unsustainable consumption patterns are characterized by too much fat, overdoses of sugar and a lack of fruits and vegetables. Unhealthy dietary habits influence the development of metabolic syndrome, type II diabetes, cardiovascular diseases, osteoporosis, and postural deformities like scoliosis, effects related in part to excessive weight gain (Ahrens et al, 2006).

Changing behaviours – and in particular motivating more sustainable behaviours – is far from straightforward. Individual behaviours are deeply embedded in social and institutional contexts. We are guided as much by what others around us say and do, and by the ‘rules of the
game’ as we are by personal choice. We often find ourselves ‘locked in’ to unsustainable behaviours in spite of our own best intentions (Jackson, 2005). The aim of this paper is to analyze the main influencing factors of consumer behavior and their impact on sustainable food choices in Europe.

To attain the aim the following tasks are set:
1. to describe the challenge of behavioural change towards sustainable food consumption;
2. to analyze the influencing factors of consumers food choice and define the barriers for sustainable food consumption;
3. to explore general trends in consumer food consumption;
4. the main focus of the study is directed to meat and vegetable consumption. Because of lack of statistic data for all EU member states, there are analyzed 24 European countries.

Materials and Methods
For assessing the European countries’ attitudes towards the issue of sustainable consumption, some classification maps were used which are based on the robust correlation matrix (MCD) and robust principal components analysis (ROBPCA) (Hubert et al, 2005). Principal components analysis (PCA) is a common technique for finding patterns in data of high dimension. This techniques includes covariance, eigenvectors, eigenvalues and standard deviation but outliers can have deleterious effects on covariance matrix and standard deviation. Thus, it is essential to use robust alternative of PCA which is called as ROBPCA. Outliers can be caused by Economical Crisis, inflation and other economical and social processes; outliers appear to deviate from the rest of the data and they may cause inaccurate interpretations, therefore robust techniques are proposed in academic literature.

Results and Discussion
The terminology and the context of sustainable consumption are relatively recent. But the debate about sustainable consumption can only really be understood or evaluated in the context of much older and deeper debates about consumption, consumer behaviour and consumerism itself (Jackson, Michaelis, 2003). “Sustainable consumption is not about consuming less, it is about consuming differently, consuming efficiently, and having an improved quality of life” is stated by United Nations Environmental Program. Shifting consumption patterns towards more sustainable behaviours relies on a robust understanding not just of what motivates consumers, but also on how behavioural change occurs, and how (if at all) it can be influenced by public sector interventions (Jackson, 2005). Food and drink is a regularly purchased group of products which consumers are familiar with and form a central and essential part of people’s lives. The physiology and psychology of the consumption of food lends itself to particular consumer behavior (PSI, 2006). The consumption of food is highly normative and is increasingly motivated by factors beyond necessity. Food relates to everything from our health, skin and life expectancy, to our personality, lifestyle and family. So much so that buying and eating food is no longer just an issue of sustenance, but one of status, personal self-modelling and identity: opening a refrigerator in front of strangers ‘is like baring the soul’ (Lonneker et al, 2008). In addition, attitudes towards food vary across countries, with some countries (for example, Italy) putting a strong emphasis on the role of food within the family, while in other countries food behaviour has been strongly affected by increasingly fragmented modern lifestyles, whereby food consumption patterns are characterized by heterogeneity. Together, both these cultural differences and the psychological influences on behaviour relating to food present a challenge to efforts to encourage the consumption of environmentally-preferable food (such as organic produce) (PSI, 2006).
Unlike other behaviours, food behaviour is much less likely to change (so long as people do not experience physical nutritional deficiencies or experience an adverse reaction to the food such as food poisoning) (Capaldi, 2006).

There are four main barriers for sustainable consumption.
1. Lack of an unsatisfied need with respect to sustainability leads to habitual purchase behaviour, which excludes new products such as sustainable products.
2. A negative attitude towards sustainable products will never lead to sustainable behaviour.
3. The lack of clear information about food products in general and specifically sustainable products could have a negative impact on the decision-making process due to uncertainty and social influences.
4. Availability of sustainable products is determining for the consumer’s ability to purchase sustainable products (Kirwan et al, 2002).

Some general trends in European food consumption are:
Replacement of beef and lamb in diets by pork and, particularly, poultry across the EU as a whole (FAO, 2010), although beef consumption is growing in newer Member States. This is due to a combination of factors including price differences, a general trend towards healthier food and the ease by which poultry can be combined with pre-prepared foods (Danish EPA, 2004; Omann et al., 2007). This trend accelerated in 2008 due to increases in food prices, and total meat consumption even dropped by 2.2% in the EU-27 compared to 2007 (EEA, 2010). Increasing consumption of fruit by 11% in the EU-15 in 1990–2005 (FAO, 2010). This may be due to greater availability and reduced prices of (imported) fruit. Increasing expenditure and frequency of eating take-away food and in restaurants (Omann et al., 2007; Danish EPA, 2004). A dramatic increase in quantities of imported food. Meat imports to the EU-15 increased by 120% between 1990 and 2007. Cereal imports increased by 83%, frozen vegetables by 174%, and bananas by 92% over the same period (FAO, 2010).

Demand for local, sustainable and organic food production is increasing. Organic farming has become one of the fastest growing segments of agriculture in many parts of the world with 82 per cent growth between 2006 and 2008 (Willer et al., 2007).

These trends have differing consequences for the environmental impacts of eating and drinking. (EEA, 2010).

Consumers food choice is influenced by food products’ prices and quality and consumers’ income. But what consumers regard as ‘quality’ has undergone considerable change during the past decades. Grunert approximate today’s consumer food quality perception by distinguishing four groups of quality attributes for food products: sensory attributes, health attributes, process attributes, and convenience attributes. Health has been of increasing importance for consumer food choice for the last 50 years or so, and today analyses of consumer food quality perception many times indicate that health and sensory considerations have about equal weight (Grunert, 2003).

In order to see the impact of income on the meat consumption, the correlation analyses between meat consumption and Gross Domestic Product (GDP) in time period from 1995 to 2007 has been done. GDP is considered an indicator of living standard and national income. The countries for their correlation values can be classified in 3 groups:

- **Positively correlated ones**: United Kingdom (0.9712), Finland (0.9515), Sweden (0.9379), Portugal (0.8967), Lithuania (0.8776), Romania (0.8512), Poland (0.8424), Italy (0.7428), Latvia (0.7309), Ireland (0.6807), Czech Republic (0.4687), Spain (0.4087). Strong correlation is showing that meat consumption in these countries is dependent on income and could be also explained with different consumption cultures, where the meat is important, but in case of lower income can be easily replaced with other products.
Negatively correlated ones: Bulgaria (-0.8922); Netherlands (-0.7702); France (-0.6454); Austria (-0.5465), Belgium (-0.4788), Slovakia (-0.3926). Negative correlation means that meat consumption decreases even the total income increases. And there could be found two explanations. In case of Bulgaria the strong correlation could be explained with high inflation and decrease of real income, so the price of product is having the impact on meat consumption. In case of other countries is presumed that the role of meat choice is playing the awareness of health issues, therefore total meat consumption decreases even the wealth of people is in increasing.

Weakly correlated ones: Cyprus (0.3318), Hungary (0.3001), Estonia (0.2620), Germany (0.1627), Denmark (-0.0613), Greece (-0.0531), where two last ones are uncorrelated. On these countries income has almost no impact and meat consumption is either a part of eating custom and considered as first necessity product, which is not elastic or there are other stronger indexes, which are shaping consumption behavior in these countries.

With the use of Principal Component Analysis (PCA) the map of meat consumption of 24 European countries is created. In the map outlying observations are labeled. In figure 1, Latvia is the most outlying country, it means, in Latvia more than in other countries meat consumption has increased in particular time period (time effect) (1995–2007). Also Bulgaria’s meat consumption is affected by time, though it is showing the same strong, but decrease of consumption amount.

Figure 1. Outlying countries in ROBPCA map according to the meat consumption

Here, X axis indicates ‘score distance’ means – score distance is the ratio of weighted eigenvectors to eigenvalues. 2 LV means number of chosen principal components. In this analysis was decided to use two principal components according to the scree plot-decision rule and number of eigenvalues greater than 1. Y axis indicates ‘orthogonal distance’ means – it is the distance between an observation and its projection in the k-dimensional subspace.

In order to explore unsustainable nature of meat consumption in terms of health, correlation analyses has been done. Below, correlation values represent the relation between obesity and meat consumption. Since the obesity ratios of 24 countries for all years were not attainable, only 6 countries and Europe in average were chosen for correlation analysis.

In average in Europe the correlation value is negative and not strong, what shows, that meat consumption alone is not the cause of increasing obesity ratio in Europe. Even though in some countries as Latvia this value is positive and strong, but in France negative and strong, what can be explained with different eating patterns and diets, which has different (opposite) influence on health and weight gain. Since most literature asserts that increased consumption of vegetables is sustainable and has a positive impact on health, robust correlation value between the obesity ratio and vegetable consumption was obtained for the same countries.
Table 1

<table>
<thead>
<tr>
<th>Country</th>
<th>Latvia</th>
<th>Lithuania</th>
<th>Estonia</th>
<th>Germany</th>
<th>Spain</th>
<th>France</th>
<th>Finland</th>
<th>Europe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation</td>
<td>0.7299</td>
<td>0.3823</td>
<td>0.0617</td>
<td>-0.3517</td>
<td>-0.0031</td>
<td>-0.7315</td>
<td>0.1847</td>
<td>-0.2211</td>
</tr>
</tbody>
</table>

In average in Europe the correlation value is not significant, what shows no direct impact of vegetable consumption on decrease of obesity level, however in Southern European countries as Spain and France, where vegetable consumption is a part of everyday eating culture, the correlation value is significant and negative, what means as more vegetables are consumed as lower obesity ratio and vice versa. Latvia has especially strong and positive correlation value, what could be considered as antagonist and not trustable outcome, if not to see the structure of consumed vegetables. Statistics show, that potatoes are the main vegetables in Latvian diet (at least 10 times more than any other vegetables). The dish of meat and potatoes by most of dieticians is not considered as a part of healthy diet. The same consumption patterns are sharing also neighbor countries Lithuania and Estonia with relevant and positive correlation.

Table 2

<table>
<thead>
<tr>
<th>Country</th>
<th>Latvia</th>
<th>Lithuania</th>
<th>Estonia</th>
<th>Germany</th>
<th>Spain</th>
<th>France</th>
<th>Finland</th>
<th>Europe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation</td>
<td>0.7070</td>
<td>0.4411</td>
<td>0.4501</td>
<td>0.0861</td>
<td>-0.4347</td>
<td>-0.5067</td>
<td>0.1459</td>
<td>0.0259</td>
</tr>
</tbody>
</table>

In order to see the groups of countries with similar correlation patterns between vegetable consumption and obesity a tolerance ellipse, which is based on MCD (robust covariance matrix), has been drawn. Firstly, for 24 European countries the median values of yearly obesity ratio and yearly vegetable consumption (1995–2007) per capita have been obtained. In figure 2, Southern European countries are outliers and take place outside of the ellipse, what can be explained with similar food consumption patterns (the amount of vegetable consumption is higher as in other European countries). These countries also have conjunctive geographical placement.

![Tolerance ellipse (97.5%)](image_url)

**Figure 2. Outlying countries in MCD tolerance ellipse plot according to vegetable consumption.**

**Conclusions**
1. To change consumer behavior towards sustainable food choices is slow and life long process, what is only achievable with strong involvement of government, civil society organizations and consumers themselves.
2. The growth of demand of organic products, decrease of meat consumption in old Member states are heralds of increased consumer awareness and education about positive impacts of sustainable food consumption on health and environment.

3. Meat consumption in Europe is positively or negatively correlated with GDP, it means the income and indirectly also the price of the product have an effect on meat consumption.

4. Meat consumption in average in Europe has weak correlation with obesity. Latvia is an outlier in terms of meat and vegetable consumption and its positive correlation with obesity. It can be explained with national dietary patterns – high consumption of meat and potatoes in the same time. Latvia’s meat consumption is also affected by income and has one of the highest increase of total meat consumption in time period from 1995–2007.

5. Southern European countries in average are consuming more vegetables, but it is not a factor, which is reducing obesity rate.

References
EVALUATION OF SOUR CHERRY CULTIVARS GROWN IN LATVIA FOR PRODUCTION OF CANDIED FRUITS

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Abstract

Different sour cherry cultivars are grown in Latvia suitable both for fresh market and for processing. Fresh local sour cherry fruits are available for consumers only in July. Sour cherries are favourite fruits because of pleasant taste, juicy flesh and attractive dark red color. Fruits are rich in natural antioxidants – vitamins and polyphenols, but are low in calories. One of processing methods preserving valuable nutrients in products in significant quantities is drying. Dried cherry fruits or candied fruits can be used as energetic delicacy and healthy goody. It is a great alternative for people liking sweets and caring for their health. A priority of dried fruits is their long keeping time, as well as their use in preparing other dishes. The aim of the study was to evaluate suitability of sour cherry cultivars grown in Latvia for production of candied fruits. Five sour cherry cultivars were chosen: ‘Bulatnikovskaya’, ‘Orlica’, ‘Shokoladnica’, ‘Tamaris’ and ‘Zentenes’. To evaluate suitability of sour cherry cultivars for drying, candied fruits and syrup, sensory estimation establishing the level of liking was performed and biochemical parameters: content of soluble solids, total acids and phenolic compounds were determined. As a result of the study it was found that higher level of liking had candied fruits and syrup made from the cultivar ‘Shokoladnica’, regardless of their higher acidity. On the other side, the highest content of polyphenols was ascertained in candied fruits of the cultivar ‘Orlica’ and their by-product, while the highest content of soluble solids was found in candied fruits and syrup made from the cultivar ‘Bulatnikovskaya’.

Key words: sour cherries, drying, candied fruits

Introduction

Orchards in Latvia are not imaginable without sour cherries. Horticulturists enjoy flowering cherries in spring and the attractive ruby fruits in summer. Consumers like cherries because of their appearance as well as their pleasant and fresh taste. Due to high cherry quality they are sometimes called “diamond fruits” (Ruisa, 2008).


Recent research has proven that fresh and candied cherries are rich in antioxidants influencing health favourably as they neutralize harmful radicals and reduce the risk of several diseases. The content of β-carotene in cherries is 19 times higher than that in strawberries and high-bush blueberries. Cherry fruits contain vitamins C, B₁ and PP, folic acid, organic acids, glucose, fructose, pectin, several minerals like K, Fe, Cu. Melatonin, a natural regulator of sleep which helps to fall asleep easy as well as prevent aging processes, is also found in cherries (Russel, 1996). Sour cherry fruits are used mainly for processing therefore it is essential to keep them fresh for a longer time. Different methods are employed to preserve fruits, of which the most common are freezing and drying. According to data of the World Trade Organization, the USA is the largest producer of frozen sour cherries; however, the most important country of export is Poland. Nearly 92% of the cherries exported from Poland go into the market of EU Member States. Dried cherry fruits can be used both as a delicacy and a healthy goody with high nutritive value. It is recommended by researchers to eat dried cherries 100 g per day, as they work as effective prophylactic means against heart and coronary disease - the leading reason of mortality in Europe and in Latvia (Kask, 1998).

In the process of making dried cherries up to 50% of initial water is removed from the product by osmotic pressure (Shi, 2008), and as a result cherry syrup is obtained. It can be used as a natural remedy to relieve pain, especially for reducing muscular pain (Audriņa, 2008). Reduced amount of water shortens the drying time, improving the sensory qualities of the product and preventing nutrient losses in the further drying process.
In this study the suitability of sour cherries grown in Latvia for production of candied fruits is evaluated.

Materials and Methods
The research was performed at the Fruit and Berry Experimental Processing Department of the Latvia State Institute of Fruit-Growing. Five sour cherry cultivars grown in Latvia were included in the study: ‘Bulatnikovskaya’, ‘Orlica’, ‘Shokoladnica’, ‘Tamaris’ and ‘Zentenes’. These cultivars are suitable for commercial growing in our country and are characterized by good winter-hardiness of trees, good productivity and dark red fruit colour.

Technology of obtaining candied fruits. Stones were removed, 40% of sugar from fruit mass was added, then put into temperature of +4±1 °C for 48 hours. After then the syrup was poured off and fruits were dried in a drying chamber with forced air circulation at the temperature 45 to 50 °C, until the humidity of the product decreased to 40–43%. Fruits were mixed periodically during drying.

Biochemical analyses. Physical and chemical parameters of fruits and berries alter after processing (Gūtmanis, 1961). To clear up which cultivars are more suitable for the chosen way of processing, as able to preserve the physical and chemical parameters to a maximal extent, the following analyses were carried out: the content of soluble solids (Brix%), accordingly to standard LVS EN 12147: 2001; the content of total acids (%), accordingly to standard LVS EN 12147: 2001; the total content of phenols (mg 100 g⁻¹) by the method of spectrometry (Singlotion, 1990).

Sensory evaluation. The hedonic evaluation method was used based on ISO 4121:2003. Taste and the liking degree were evaluated for the samples (Cliff, 1999).

Statistical analysis. The results obtained in the experiments were summarized and analyzed by MS Excel program. Data were ranged, groups were analyzed by the descriptive statistical method Description statistic, the significance was determined for validity of 95%. The obtained groups of data were compared using the mean (arithmetical) value and the mean validity range (the mean Sx; the mean +Sx). Standard declination was used for the characterization of dispersion of the obtained data. Correlation analysis was used for the evaluation of results to establish the closeness of relationships between chemical parameters and sensory evaluation.

Results and Discussion
Sensory evaluation of candied cherry fruits. Sensory aspect is one of the determinative parameters in consumer choice of product and its further purchasing. Candied fruits of the cultivar ‘Shokoladnica’ received the highest rating: 7.30 points from 9.0 (Figure 1). As found by experts, these fruits had mild sweet-and-sour taste and good structure. American researchers also have found that at taste panels cultivars of this type are preferred (Turner, 2004). When eating candied fruits consumers are sometimes bothered by the astringent taste caused by tannins. Research has demonstrated that with increase of astringency the product gets lower rating in sensory evaluation (Guyer, 1993). Candies made from the cultivar ‘Orlica’ were rated negatively because of smallish fruits and a relatively high content of tannins. Their rating was 7.03 points, and they were also marked as something over-sweet, yet the texture of candies was accepted as good and the overall taste as pleasant. Fruits of cultivars ‘Tamaris’ and ‘Zentenes’ are characterized by a relatively tight skin (Ruisa, 2008). So, their total structure after drying was made up mainly by skin, while the flesh was least tangible. Candied cherries of these cultivars were rated as too dry and firm. Candies made from the cultivar ‘Zentenes’ received 6.8 points and were accepted as more suitable for glazing. Candied cherries made from ‘Tamaris’ had the lowest rating and received 5 points because of the strongly tangible tannins. Candies of the cultivar ‘Bulatnikovskaya’ had good texture; however, their rating reduced because the consumers found them as too sweet.
Figure 1. Results of sensory evaluation of candied cherry fruits

Biochemical content of candied fruits and its influence on sensory parameters. Candied fruits containing higher content of total acids received higher sensory evaluation, and a close positive correlation was found in this aspect ($r=0.42$). Candied fruits of the cultivar ‘Shokoladnica’ had the highest content of total acids: 2.53% (Table 1). This cultivar was recognized as the most suitable for producing candied fruits. The lowest content of total acids was observed for the cultivar ‘Zentenes’: 2.16%. Yet no statistically significant differences were found among cultivars in the content of total acids. This is also demonstrated by the fact that the ratio of soluble solids and total acids was equal for all cultivars: 0.02, except ‘Shokoladnica’ with 0.03. However, the last difference also was not statistically significant. The highest content of total phenols was ascertained for candied cherries of the cultivar ‘Orlica’: 657.17 mg 100g$^{-1}$, and no significant difference was observed for the cultivar ‘Tamaris’— 653.78. Tannins are included in the content of total phenols, so, the higher that content, the lower are the sensory parameters as it was found by Bernalte et al. (Bernalte, 2009).

Table 1

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Content of total acids, %</th>
<th>Content of total phenols, mg·100g$^{-1}$</th>
<th>Content of soluble solids Brix %</th>
<th>Ratio of soluble solids and total acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shokoladnica</td>
<td>2.53</td>
<td>257.29</td>
<td>96.23</td>
<td>0.03</td>
</tr>
<tr>
<td>Tamaris</td>
<td>2.32</td>
<td>653.78</td>
<td>100.67</td>
<td>0.02</td>
</tr>
<tr>
<td>Bulatniovskay</td>
<td>2.45</td>
<td>609.60</td>
<td>104.69</td>
<td>0.02</td>
</tr>
<tr>
<td>Zentenes</td>
<td>2.16</td>
<td>515.23</td>
<td>98.31</td>
<td>0.02</td>
</tr>
<tr>
<td>Orlica</td>
<td>2.51</td>
<td>657.15</td>
<td>97.41</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Negative interrelationships were ascertained between the content of total phenols and sensory evaluation ($r=-0.32$), as well as between the content of soluble solids and sensory evaluation ($r=-0.39$). It can be explained by the high sensory rating of ‘Shokoladnica’ candied fruits which also had the lowest content of total phenols: 257.29 mg 100g$^{-1}$ and soluble solids: 96.23 Brix %. The highest content of soluble solids was observed for candies from ‘Bulatniovskay’ and ‘Tamaris’; difference between these cultivars was not significant: 104.69 Brix % and 100.67 Brix % respectively.

A medium close positive correlation was established between the content of total phenols and the content of total acids. In the dry mass of candied cherries increased content of total acids...
was observed if the content of total phenols was higher ($r=0.69$). Positive correlation ($r=0.53$) was ascertained between the content of soluble solids and total phenols. This correlation is not close, but a tendency was observed that with increasing content of total phenols and soluble solids sensory evaluation decreases.

Statistical evaluation of the data showed that there are no significant differences among the candied cherries from different cultivars ($p=0.98$).

Estimating the biochemical and sensory parameters of cherry syrup, it was found that they are similar to the parameters of candied cherries.

Conclusions
1. ‘Shokoladnica’ was the most suitable cultivar for candied fruit production because of its mild sweet-and-sour taste and good structure.
2. There was a tendency that the highest sensory evaluation had candied cherries containing the highest amount of total acids.
3. Negative interrelationships were ascertained between the content of total phenols and sensory evaluation ($r=0.32$), as well as between the content of soluble solids and sensory evaluation ($r=0.39$).
4. Significant differences among the candied cherries from different cultivars were not established.

References
THE INFLUENCE OF METEOROLOGICAL CONDITIONS AND NITROGEN FERTILIZER ON WHEAT GRAIN YIELD AND QUALITY

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Abstract
Technological quality and protein composition of the wheat grain are influenced significantly by the system of growing, by variety, locality, year conditions and growing technology. A field experiment of four winter wheat (Triticum aestivum L.) varieties was designed to study the influence of meteorological conditions and the effect of additional top dressing of nitrogen fertiliser rates to different varieties grain yield and bread quality data. Field experiment was carried out on sod calcareous medium loam soil of the Research and Training farm “Peterlauki” of Latvia University of Agriculture. The varieties, which were studied, according to the duration of the vegetation period can be relatively divided in three groups: early varieties, mid-early and mid-late varieties. The meteorological conditions in three year period were different compared to average long – term observations and this difference influenced plant development and yield. Obtained data show that fertilizer influence on winter wheat grain yield was significant. The application of nitrogen increased grain yield of late varieties by 10% as compared to early varieties. Grain crude protein content was affected by fertilizer application and by differences of meteorological conditions - from 93 to 172 g kg\(^{-1}\). Significant positive correlation was found among protein content and gluten content (r=0.93) and protein content among Zeleny index (r=0.82). The yield and baking quality parameters are depends on meteorological conditions in the investigated years and individually of varieties.

Key words: wheat, nitrogen, meteorological conditions, grain quality

Introduction
Relevant problem for wheat growers, grain handlers, millers and bakers is obtaining wheat quality accordingly food requirements. Winter wheat (Triticum aestivum L.) yield is highly and significantly influenced by annual dynamics and cultivar – specific differences (Muchova, 2003). Wheat grain yield and quality depends on many factors – agrometeorological conditions and soil quality, but nitrogen fertilization is one of the important factors influencing quality parameters of winter wheat, especially additional nitrogen fertilizer in spring time from the spring upon resumption of vegetative growth (BBCH 22-24) till stem elongation stage (BBCH 31-32). Therefore many researchers studied influence of nitrogen fertilizer on grain yield and quality changes, especially in the regions abounds in precipitation (Cox et al., 1985; Terman et al., 1979; Skudra and Ruza, 2008). Grain protein content significantly varied depending on the differences among cultivars (Mašauskiene, Cesevičiene, 2006) usually from 7–20%. Protein content shows grain suitability for processing. Protein content from 12–13% are suitable for bread making, but grain with higher protein content are used for lower quality grain improver. The aim of research was to investigate the influence of meteorological conditions and the effect of additional top dressing of nitrogen fertilizer rates to different varieties grain yield and bread quality data.

Materials and Methods
The effect of nitrogen fertilizer on winter wheat grain yield and quality was studied on the Training and Research farm „Peterlauki” of the Faculty of Agriculture, Latvia University of Agriculture. The experiments were carried out on sod-pseudogley sandy clay loam soil (Stagnic Luvisols according to FAO classification) from 1998 to 2000. The soil agrochemical properties were: organic matter (Tyrin’s method) – 17–23 g kg\(^{-1}\); pH\(_{KCl}\) – 6.6–7.0; high plant available phosphorus and potassium level. Before sowing, winter wheat was placement – fertilised with complex fertiliser N\(_{12}\)P\(_{52}\)K\(_{60}\). There were four winter wheat varieties with different nitrogen applications: early season variety ‘Donskaja polukarlikovaja’ with one nitrogen regime N-60+60 absolute matter, kg ha\(^{-1}\) (further in text: D-120), mid early variety...
‘Sirvintas-1’ with two fertiliser regimes N-0, and N-60+60 (further in text: S-0, S-120 according nitrogen regimes); late varieties ‘Moda’ with three nitrogen regimes: N-0, N-60+60, and N-60+70+40 (further in text: M-0, M-120, M-170 according nitrogen regimes), and ‘Bussard’ with one nitrogen regime N-60+70+40 (further in text: B-170). Split nitrogen dressing was applied in the following way: at an early period of vegetation (BBCH 15-20) for the first time; at an end of shooting into stalks (BBCH 29-31) for the second time; at an end of shooting into ears (BBCH 57-59) for the third time. The experiment treatments were arranged in four replicates. An intensive plant protection was used.

The meteorological conditions in 1998-2000 were different compared to average long-term observations and this difference influenced plant development and yield. In 1998 the start of the vegetation period was favorable; rainfall in May and July was more than 288% and 160% of the norm, respectively. Finally sowings are logged and was some grain yield loses. The year 1999 was characterized by wet and warm – from spring till autumn air temperature was approximately 2–3°C below long-term observation temperature, but rainfall approximately 50–60% from norm. In 1999, spring was early. The plants suffered from deficient moisture. In 2000 spring was early, but with atypical frosts. In summer was congenial weather for wheat developing: wet, cold with low disease dissemination. But in grain harvesting time was rainy and grain quality was not so good.

The plots were combine-harvested at grain ripeness. The cleaned grain was milled and following qualitative indices were determined: protein content was calculated by multiplying nitrogen content determined by Kjeldahl method by coefficient 5.7, g kg$^{-1}$ (ISO 712:1998), falling number and 1000 kernel weight by Hagberg–Perten method (ISO 3093:1982), flour sedimentation by Zeleny method (ICC 116/1:1994), wet gluten content having washed dough according to Perten, having calculated data for grain of 14 % moisture (ICC 155:1994).

Results and Discussion
The highest grain yields were obtained varieties ‘Moda’ (M-120 – 7.57 t ha$^{-1}$) and ‘Bussard’ (7.14 t ha$^{-1}$) from late varieties (Figure 1).

![Figure 1. Grain yield of winter wheat variants depending on nitrogen fertilizer, average 3 years](image)

LSD$_{0.05}$ (separate difference) = 0.20, A factor (variants) = 0.08, B factor (years) = 0.12

Nitrogen fertilizer increasing did not give grain yield increasing for variety ‘Moda’, but gives crude protein content increasing (Figure 2).
There were obtained comparatively high yields in none nitrogen fertilizing variants – higher than 5 t ha\(^{-1}\), because trial was arranged at high soil potential productiveness.

The results of dispersion analysis showed that winter wheat varieties under diverse fertilizer management essentially effected meteorological conditions by year as Fischer’s criterion \(F_{\text{fact}}=84.93 > F_{0.05}=2.25\). Wheat varieties with different length of vegetation period have their own yield potential, growth and development as well as yield formation process.

Grain crude protein content was affected by fertilizer application and by differences of variety – from 93 to 172 g kg\(^{-1}\) (Figure 2). Depending on fertilizer rate gluten content varied from 143 to 351 g kg\(^{-1}\), but falling number – from 123 to 416 s, and 1000 kernel weight – from 36.6 to 54.9 g. The higher Zeleny number was obtain of early varieties (maximum 57), but lower Zeleny number (16.1–22.5) occurred in treatments receiving no additional nitrogen fertilizer. Grain in those variants characterized by very low baking quality indices and did not achieve food grain requirements. Obtained grain quality parameters in nitrogen fertilized variants were useful for direct baking or mixing up comparatively weak flour.

**Figure 2. Grain quality depending on nitrogen fertilizer, average 3 years**

<table>
<thead>
<tr>
<th>Variants</th>
<th>Protein content, g kg(^{-1})</th>
<th>Gluten content, g kg(^{-1})</th>
<th>Falling number, s</th>
<th>Zeleny index</th>
<th>1000 kernel weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-120</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>S-0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-120</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M-0</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>M-120</td>
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<td>M-170</td>
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<td></td>
</tr>
<tr>
<td>B-170</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 3. Correlation among grain yield and quality**

- Correlation coefficient
- Crude protein content in grain
- Wet gluten content
- Falling number
- Zeleny sedimentation value
- 1000 kernel weight

\[ 1998 \quad 1999 \quad 2000 \]
The significant influence of year was confirmed for all of the evaluated quality parameters (Figure 3.), but it is weak. Mašauskiene et al. (2006) reported that the cause of this fact is that the amount of the wheat grain protein complex depends on environmental factors and not so much on the genotype. Only the positive significant correlation with grain yield there was found in 2000 among falling number \( (r_{0.05}=0.84) \) and negative significant correlation \( (r_{0.05}=-0.87) \) among 1000 kernel weight.

**Conclusions**

1. On evaluation data of 4 winter wheat varieties with different nitrogen fertilizer in 1998-2000 the yield potential was on the level 5–8 t ha\(^{-1}\), but there was a great influence on grain yield and quality traits of year.
2. The results of dispersion analysis showed that winter wheat varieties under diverse fertilizer management essentially effected meteorological conditions by year as Fischer’s criteria \( F_{\text{fact}}=84.93 > F_{0.05}=2.25 \).
3. Grain crude protein content was affected by fertilizer application and by differences of varieties – from 93 to 172 g kg\(^{-1}\). Depending on fertilizer rate gluten content varied from 143 to 351 g kg\(^{-1}\), but falling number – from 123 to 416 s, and 1000 kernel weight – from 36.6 to 54.9 g.
4. The positive significant correlation with grain yield there was found in 2000 among falling number \( (r_{0.05}=0.84) \) and negative significant correlation \( (r_{0.05}=-0.87) \) among 1000 kernel weight.
5. The yield and baking quality parameters are depended on meteorological conditions of years and individually of fertilized varieties.

**References**

ENZYME ACTIVITY OF DIFFERENT CEREALS GROWN USING ORGANIC AND CONVENTIONAL AGRICULTURAL PRACTICES

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Abstract
Enzymes play an important role in cereal processing not only because in many instances they have an impact on processability, but also they add to final product quality. High activities of different hydrolytic enzymes could cause the losses of grain quality and lead to processing problems and unsatisfactory end-products. However, the information on the impact of various cultural practices and conditions on the variation of enzymes activity levels in cereals is rather limited. The present study is therefore aimed to compare the activity levels of most important hydrolytic enzymes (α-amylase, endoxylanase and protease) in wheat, barely, rye and oats grown by organic and conventional agricultural practices. To address this issue, different registered cultivars and up-and-coming lines of winter wheat (4 varieties), winter rye (3 varieties), spring barley (6 varieties), and oats (3 varieties) grown during 2009 harvest year were involved in the test: The α-amylase activity in organically and conventionally grown cereals varied from 224 till 1335 U (units) g⁻¹ and from 814 till 1546 U g⁻¹, endoxylanase activity – from 0.13 till 0.65 U g⁻¹ and from 0.06 till 0.15 U g⁻¹, protease activity – from 4.89 till 4.95 U g⁻¹ and from 4.87 till 4.95 U g⁻¹, respectively. The data demonstrated that organic wheat, rye, and oats had lower α-amylase activity in compare with conventional counterparts. Also organic rye, barley and oats distinguished much higher endoxylanase activity than conventional ones. Contrary tendency was found during investigation of α-amylase activity in barley and endoxylanase activity in wheat. Comparing protease activity, significant differences have not been found between various agricultural practices. These results warrant further studies investigating links between specific agricultural practices and enzyme activities in important food cereals.

Key words: cereals, enzyme activity, organic farming, conventional farming.

Introduction
Cereals worldwide are extremely important for both human and animal nutrition, hence the great economic importance of their processing into a wide range of products. In the last decade, enzymes have become increasingly important in cereal processing not only because in many instances they have an impact on processability, but also they add to final product quality (Kruger, Lineback, 1987). High activities of different hydrolytic enzymes could cause the losses of grain quality and lead to processing problems and unsatisfactory end-products. These enzymes are mainly responsible for mobilising the insoluble storage reserves in the endosperm: amylases degrade starch into dextrins and glucose, xylanolytic enzymes catalyse the breakdown of non-starch polysaccharides, proteases hydrolyse proteins and produce amino acids.

Factors such as resource availability, soil quality, climate, and insect and animal herbivory pressures are known to affect levels of nutrients in cereals (Brandt, Mølgaard, 2001). However, the information on the impact of various cultural practices and conditions on the variation of enzymes activity levels in cereals is rather limited. Given that increasing evidence indicates a role for hydrolytic enzymes in cereal processing, efforts need to be directed in understanding relationships between cultural practices and activities of different enzymes in cereals.

Conventional, organic, and sustainable agriculture are the primary cultural practices used in the production of foods. The goal of each of these practices differs greatly with respect to crop yield, land and pesticide use, and environmental impact. Conventional agricultural practices utilize high-yield crop cultivars, chemical fertilizers and pesticides, irrigation, and mechanization. Although conventional practices result in reliable high-yield crops, there is concern regarding the negative biological and environmental consequences and long-term
sustainability associated with these practices (Robertson et al., 2000). Organic crops cannot be genetically engineered, irradiated, or fertilized with sewage sludge. Additionally, farmland used to grow organic crops is prohibited from being treated with synthetic pesticides and herbicides for at least 3 years prior to harvest.

Most of existing studies have compared the nutritional quality of organically and conventionally grown plants in terms of macronutrients, vitamins, and minerals. Woese and others (1997) reported on the quality of foods grown under different production methods and examined between 1926 and 1994. The authors concluded that no major differences in nutrient levels were observed between the different production methods in some cases while in other cases contradictory findings did not permit definitive conclusions about the influence of production methods on nutrient levels. Worthington (2001) reviewed a number of studies that compared crops produced with organic fertilizer or by organic farming systems to crops produced using conventional farming systems. It was reported that organic crops contained more vitamin C, iron, magnesium and phosphorus than did conventional crops. Bourn and Prescott (2002) also compared the effect of inorganic and organic fertilizers on the nutritional value of crops. They concluded that the study results were too variable to provide any definitive conclusions concerning the effect of fertilizer type on mineral and vitamin content of crops. Davis and others (2004) compared USDA nutrient content data for 43 garden crops between 1950 and 1999. Statistically reliable declines were noted for 6 nutrients (protein, calcium, potassium, iron, riboflavin, and ascorbic acid), with declines ranging from 6% for protein to 38% for riboflavin. However, they attributed the decreases in nutrient content to changes in the cultivars (plant varieties) used. All these data demonstrate that the results of the nutritional quality of conventionally and organically are difficult to interpret because cultivar selection and growing conditions varied widely and different methods of sampling and analysis were used in the investigations. Additionally, these studies did not address levels of enzyme activities in conventionally and organically grown cereals, although the enzyme activities could be differ between these two practices.

The present study is therefore aimed to compare the activity levels of most important hydrolytic enzymes (α-amylase, endoxylanase and protease) in wheat, barely, rye and oats grown by organic and conventional agricultural practices.

**Materials and Methods**

The organic and conventional field trials were conducted during 2009 harvest year in Central Lithuania (Dotnuva, 55°24’N, 23°50’E) at the experimental fields of the Institute of Agriculture. The soil of the experimental site is Endocalcari - Epipogleyic Cambisol (CMg-n-w-can) close to neutral acidity, moderately supplied with available phosphorus and potassium, organic matter (humus) in conventional and organic fields respectively 2.6 and 2.4%. Pre-crop – black fallow. The meteorological conditions of cereal growing and harvesting period in 2009 year are shown in Table 1.

**Table 1**

| Meteorological conditions of wheat growing and harvesting during 2009 |
|-----------------------------|-----|-----|-----|-----|-----|
| **Parameter**               | April | May | June | July | August |
| **Average temperature 2009 (ºC)** | 8.4  | 12.2| 14.7| 18.2 | 16.7  |
| **Average temperature 1924-2006 (ºC)** | 5.4  | 11.9| 15.4| 16.7 | 16.2  |
| ± deviation over previous period | +3.0 | +0.3| -0.7| +1.5 | +0.5  |
| **Average rainfall 2009 (mm)** | 8    | 37  | 96  | 105 | 75    |
| **Average rainfall 1924-2006 (mm)** | 42   | 52  | 68  | 79  | 76    |
| ± deviation over previous period | -34  | -15 | +28 | +26 | -1    |
| **Days with raining ≥ 1 mm** | 1-3  | 6-12| 6-17| 13-18| 8-11  |
Eleven registered cereal cultivars and six up-and-coming lines developed at the Plant Breeding Department of the Institute of Agriculture were involved in the test: winter wheat (*Triticum aestivum* L.) cultivars 'Ada', 'Alma', 'Tauras' and 'Sirvinta 1'; winter rye (*Secale cereale* L.) cultivar 'Joniai' and lines LZI 424, LZI 512; spring barley (*Hordeum vulgare* L.) cultivars 'Simba', 'Luoke', 'Aura DS' and lines 8056-2, 8056-6, 8611; oat (*Avena sativa* L.) cultivars 'Miglia', 'Ivory' and line 1551-3. The varieties were grown in 3 replications with a plot size of 5.0 × 1.7 m². The crop was sown in a well prepared seedbed with a Hege 80 at a rate of 4.5 million seed ha⁻¹. All fertilities in conventional field trials were applied annually. Winter wheat and rye have got N₁20P₅₀K₅₀, spring barley – N₉₀P₆₀K₆₀ and oat – N₆₀P₆₀K₆₀ respectively. Phosphorus and potassium fertilisers were applied in autumn, for winter cereals – pre-sowing. Nitrogen was applied in spring – pre-sowing spring crop or after resumption of vegetative growth (BBCH 23-24) of winter crop. Conventional farming field crops as sprayed with herbicides – at the end of tillering - beginning of booting (BBCH 28–30), taking into account prevalent weed species; other pesticides and additional fertilisation were not applied. The organic field was certified for organic agriculture; no agrochemicals and fertilizers were used. Harvest time – fully ripe grain maturity (BBCH 89). The plots were harvested with a Wintersteiger harvester. Combine-harvested grains from each plot were dried and sampled for analyses.

For analytical tests, grains were milled in Laboratory Mill 120 (Perten Instruments AB, Sweden) at a particle size of 0.8 mm and stored by -20 °C. Frozen samples were defrosted and analyzed for α-amylase, endoxylanase and protease activities. For enzyme activity measurements 5 g of milled grains were extracted with 50 ml of particular buffer for 1 hour and centrifuged at 10000×g for 20 minutes by 4 °C.

α-amylase activity was determined using an ICC Standard method No. 108 (ICC, 1998). Wheat extract was prepared in the calcium chloride solution (pH 6.0). Soluble starch (1%) was used as substrate. One unit (U) of amylase activity was defined as the amount of amylase which is able to catalyze 1 g soluble starch hydrolysis to dextrins under assay conditions.

Endoxylanase activity was determined by the dinitrosalicylic acid assay (Miller, 1959). Wheat extract was prepared in 10 mM sodium acetate buffer (pH 4.5). One unit (U) of endoxylanase activity was defined as the amount of enzyme required to releases 1 µmol of xylose equivalents per min from the birchwood xylan (5 mg ml⁻¹) under the assay conditions used (pH 4.5; 40 °C). The xylose solution (2.5 mM) was used to prepare xylose standards (0–0.45 µmol ml⁻¹) and construct the calibration curve.

Protease activity was determined by Sigma’s enzymatic assay of protease using tyrosine as a standard (Sigma Quality Control Test SSCASE01.001, 1999). Wheat extract was prepared in 10 mM sodium acetate buffer (pH 7.5) with 5 mM calcium acetate. Casein solution (0.65%) was used as substrate. One unit (U) of protease activity was defined as the amount of enzyme required to liberate 1 µmol of tyrosine per min per ml under the assay conditions. The tyrosine solution (0.2 mg ml⁻¹) was used to prepare tyrosine standards (0–0.08 mg ml⁻¹) and construct the calibration curve.

All analyses were performed in triplicate. The calculation of the mean values and standard deviations were performed using a Microsoft Excel 2000 program and a statistical program Analyse-it. The means were compared by one-way analysis of variance (ANOVA). The p-values of <0.05 were considered significant.

**Results and Discussion**

α-amylase, endoxylanase and protease activity levels determined in different winter wheat, winter rye, spring barley and oats cultivars are presented in Table 2.
Table 2

Enzyme activities in conventionally and organically grown cereals

<table>
<thead>
<tr>
<th>Cereals</th>
<th>Agricultural practice</th>
<th>Enzyme activity (U g(^{-1}))</th>
<th>α-amylase</th>
<th>endoxylanase</th>
<th>protease</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Wheat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ada</td>
<td>Conventional</td>
<td>1064±103</td>
<td>0.24±0.04</td>
<td>4.95±0.00</td>
<td></td>
</tr>
<tr>
<td>Alma</td>
<td>Conventional</td>
<td>1105±29</td>
<td>0.14±0.03</td>
<td>4.91±0.02</td>
<td></td>
</tr>
<tr>
<td>Tauras</td>
<td>Conventional</td>
<td>1126±29</td>
<td>0.12±0.03</td>
<td>4.92±0.02</td>
<td></td>
</tr>
<tr>
<td>Sirvinta1</td>
<td>Conventional</td>
<td>1546±7</td>
<td>0.11±0.04</td>
<td>4.91±0.01</td>
<td></td>
</tr>
<tr>
<td>Average value</td>
<td></td>
<td>1210±33</td>
<td>0.15±0.06</td>
<td>4.90±0.01</td>
<td></td>
</tr>
<tr>
<td>Ada</td>
<td>Organic</td>
<td>1026±17</td>
<td>0.10±0.02</td>
<td>4.91±0.00</td>
<td></td>
</tr>
<tr>
<td>Alma</td>
<td>Organic</td>
<td>1081±39</td>
<td>0.12±0.04</td>
<td>4.89±0.03</td>
<td></td>
</tr>
<tr>
<td>Tauras</td>
<td>Organic</td>
<td>1038±33</td>
<td>0.17±0.01</td>
<td>4.90±0.01</td>
<td></td>
</tr>
<tr>
<td>Sirvinta1</td>
<td>Organic</td>
<td>1136±39</td>
<td>0.13±0.01</td>
<td>4.91±0.04</td>
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<tr>
<td>Average value</td>
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<td>0.13±0.03</td>
<td>4.92±0.03</td>
<td></td>
</tr>
<tr>
<td>Joniai</td>
<td>Conventional</td>
<td>1053±59</td>
<td>0.11±0.05</td>
<td>4.89±0.01</td>
<td></td>
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<tr>
<td>LZI 424</td>
<td>Conventional</td>
<td>1131±7</td>
<td>0.12±0.06</td>
<td>4.88±0.00</td>
<td></td>
</tr>
<tr>
<td>LZI 512</td>
<td>Conventional</td>
<td>1012±44</td>
<td>0.15±0.03</td>
<td>4.89±0.00</td>
<td></td>
</tr>
<tr>
<td>Average value</td>
<td></td>
<td>1066±61</td>
<td>0.13±0.02</td>
<td>4.89±0.01</td>
<td></td>
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<tr>
<td>Joniai</td>
<td>Organic</td>
<td>999±22</td>
<td>0.80±0.09</td>
<td>4.90±0.01</td>
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<tr>
<td>LZI 424</td>
<td>Organic</td>
<td>904±44</td>
<td>0.70±0.10</td>
<td>4.95±0.09</td>
<td></td>
</tr>
<tr>
<td>LZI 512</td>
<td>Organic</td>
<td>697±74</td>
<td>0.46±0.09</td>
<td>4.90±0.01</td>
<td></td>
</tr>
<tr>
<td>Average value</td>
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<td>867±154</td>
<td>0.65±0.18</td>
<td>4.90±0.03</td>
<td></td>
</tr>
<tr>
<td>Simba</td>
<td>Conventional</td>
<td>913±51</td>
<td>0.18±0.01</td>
<td>4.88±0.01</td>
<td></td>
</tr>
<tr>
<td>Luoke</td>
<td>Conventional</td>
<td>986±7</td>
<td>0.03±0.01</td>
<td>4.90±0.00</td>
<td></td>
</tr>
<tr>
<td>Aura DS</td>
<td>Conventional</td>
<td>1100±22</td>
<td>0.14±0.02</td>
<td>4.91±0.02</td>
<td></td>
</tr>
<tr>
<td>8056-2</td>
<td>Conventional</td>
<td>830±7</td>
<td>0.18±0.01</td>
<td>4.93±0.02</td>
<td></td>
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<tr>
<td>8056-6</td>
<td>Conventional</td>
<td>732±73</td>
<td>0.14±0.02</td>
<td>4.91±0.02</td>
<td></td>
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<tr>
<td>8611</td>
<td>Conventional</td>
<td>1160±72</td>
<td>0.10±0.01</td>
<td>4.93±0.05</td>
<td></td>
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<tr>
<td>Average value</td>
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<td>954±162</td>
<td>0.13±0.06</td>
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<td></td>
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<tr>
<td>Simba</td>
<td>Organic</td>
<td>1335±55</td>
<td>0.30±0.00</td>
<td>4.88±0.01</td>
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<tr>
<td>Luoke</td>
<td>Organic</td>
<td>1057±18</td>
<td>0.16±0.02</td>
<td>4.91±0.03</td>
<td></td>
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<tr>
<td>Aura DS</td>
<td>Organic</td>
<td>901±55</td>
<td>0.21±0.04</td>
<td>4.90±0.01</td>
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<tr>
<td>8056-2</td>
<td>Organic</td>
<td>1096±12</td>
<td>0.30±0.04</td>
<td>4.93±0.02</td>
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<tr>
<td>8056-6</td>
<td>Organic</td>
<td>875±18</td>
<td>0.16±0.01</td>
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<tr>
<td>8611</td>
<td>Organic</td>
<td>758±12</td>
<td>0.19±0.06</td>
<td>4.88±0.00</td>
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<tr>
<td>Average value</td>
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<td>1004±204</td>
<td>0.22±0.07</td>
<td>4.89±0.02</td>
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<tr>
<td>Migla</td>
<td>Conventional</td>
<td>834±11</td>
<td>0.06±0.00</td>
<td>4.89±0.00</td>
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<tr>
<td>Ivory</td>
<td>Conventional</td>
<td>885±50</td>
<td>0.06±0.01</td>
<td>4.87±0.00</td>
<td></td>
</tr>
<tr>
<td>1551-3</td>
<td>Conventional</td>
<td>814±61</td>
<td>0.06±0.05</td>
<td>4.87±0.01</td>
<td></td>
</tr>
<tr>
<td>Average value</td>
<td></td>
<td>844±37</td>
<td>0.06±0.01</td>
<td>4.88±0.01</td>
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</tr>
<tr>
<td>Migla</td>
<td>Organic</td>
<td>810±37</td>
<td>0.10±0.02</td>
<td>4.95±0.04</td>
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<tr>
<td>Ivory</td>
<td>Organic</td>
<td>697±61</td>
<td>0.09±0.00</td>
<td>4.89±0.01</td>
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<tr>
<td>1551-3</td>
<td>Organic</td>
<td>224±43</td>
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<td>4.91±0.02</td>
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<tr>
<td>Average value</td>
<td></td>
<td>577±311</td>
<td>0.09±0.01</td>
<td>4.91±0.03</td>
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</table>
The results showed that organically grown wheat, rye, and oats had lower α-amylase activity in compare with conventionally grown cereals. The average of α-amylase activity in organically grown wheat, rye, oats was found 13, 23 and 46% lower than that measured in conventionally grown samples, respectively. Differences in the levels of α-amylase activity between the organic and conventional barley were not significant. Contrary tendency was found during investigation of endoxylanase activity. In this case, organically grown rye, barley and oats distinguished for higher (5.0, 1.7 and 1.5 times, respectively) average endoxylanase activity than conventionally grown samples. The levels of endoxylanase activity in organic and conventional barley samples had not significant difference. Comparing protease activity, significant differences have not been found between various agricultural practices. The protease activity of organically and conventionally grown cereals was in the range between 4.88 and 4.92 U g⁻¹.

The activities of hydrolytic enzymes, especially amylase, are important factors that may limit the utilization of cereals. α-amylase is unique in modifying starch and its functional properties. In breadmaking, some α-amylase is needed to sustain the production of sugars required for proper fermentation and consequent gas production. However excess α-amylase can have disastrous effects on bread quality (Buchanan, Nicholas, 1980). Endogenous xylanases are involved in re-modeling and expansion of cereal cell walls during normal cell growth and development and in more drastic cell wall degradation occurring during seed germination (Dornez et al., 2009). Xylanases hydrolyse the backbone of cereal cell wall arabinoxylans and have a significant impact on bread-making, brewing, animal feed efficiency, pasta production, etc. Proteases hydrolyse the peptide linkage, releasing protein fragments or free amino acids and play important role in biscuit manufacture, where the low-protein soft wheat and plastic properties of dough are required. The data presented showed that the results of the impact of organical and conventional cultural practices on the variation of enzymes activity levels in cereals were difficult to interpret because there were inconsistent differences in the enzyme activities of tested grains. Therefore we can conclude that organic farming system produce cereals of the same quality as conventional ones, but using far fewer external inputs in the form of fertilisers and plant protection agents, thereby safeguarding natural resources. These results are in line with recent studies on organic wheat farming, in which wheat nutritional value (protein content, amino acid composition and mineral and trace element contents) and baking quality were found not to be affected by the farming systems: organically or conventionally (Mader et al., 2007). Thus these findings suggest that organic farming can contribute substantially to solving problems related to high-external-input agriculture and to producers and consumers confidence in organic foods.

Conclusions

1. The results of our study showed the inconsistent variation of enzyme activity levels in tested cereals grown by organic and conventional agricultural practices. The α-amylase activity in organically and conventionally grown cereals varied from 224 till 1335 U g⁻¹ and from 814 till 1546 U g⁻¹, endoxylanase activity – from 0.13 till 0.65 U g⁻¹ and from 0.06 till 0.15 U g⁻¹, protease activity – from 4.89 till 4.95 U g⁻¹ and from 4.87 till 4.95 U g⁻¹, respectively. The data demonstrated that organic wheat, rye, and oats had lower α-amylase activity in compare with conventional counterparts. Also organic rye, barley and oats distinguished much higher endoxylanase activity than conventional ones. Contrary tendency was found during investigation of α-amylase activity in barley and endoxylanase activity in wheat. Comparing protease activity, significant differences have not been found between various agricultural practices. These findings suggest that organic farming system could produce cereals of the same quality as conventional ones, but using far fewer external inputs in the form of fertilisers and plant protection agents.
2. On the other hand, data regarding the levels of enzyme activities in organic food are too limited to allow any conclusion. We feel that these results warrant further studies investigating links between specific agricultural practices and enzyme activities in important food cereals.

References
ASSESSMENT OF THE RHEOLOGICAL PROPERTIES OF FLOUR USING THE MIXOLAB

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Abstract
The rheological properties of dough are important basis for the process of production of quality products (Létang et al., 1999; Moreira et al., 2010). The main techniques used for measuring flour dough’s properties are empirical or fundamental. Empirical measurements can be conducted by means of farinograph, Falling Number apparatus, rapid visco analyser, etc., and multifunctional new apparatus like Mixolab (Jansen et al., 1996; Moreira et al., 2010). The aim of research was to evaluate the rheological properties of dough made from different cereal flours and flour blends using Mixolab.

Whole grain flour of triticale, rye, hull-less barley, rice, maize and flour blends were used in this research. Flour blends were made from triticale in a combination with other flour in various proportions. Wheat flour was used as a control. Rheological properties of mixed flour dough were studied using Mixolab (Chopin Technologies), which was capable of evaluating rheological properties and enzymatic activity of flour and flour blends.

In the Mixolab test triticale flour demonstrated equivalent dough properties to wheat flour: formation time, protein weakening and starch gelatinization peak value. But values of amylase activity and starch retrogradation in triticale flour were lower than in wheat flour. Evaluation of the Mixolab test results demonstrated that decrease of triticale flour proportion in flour blend resulted in increase of the dough stability, but did not change substantially dough properties.

Key words: triticale, wheat, hull-less barley, flour blend, Mixolab.

Introduction
The cereals or grain crops are the most important sources of food for people. The type of cereal mainly used in food depends on specificity of the region, in the Far East it is rice, in America – maize, in Central Asia, North America and Northern and Eastern Europe – rye and wheat. There are various grindings of rye and wheat flour mainly used in preparation of bread and pastry production in Latvia.

In order to extend the product assortment and improve their nutritional value, there can be used triticale, hull-less barley, buckwheat, hull-less oat, and other grain flour that are used elsewhere in the world and various scientific studies demonstrate their value (Taketa et al., 2004). Triticale (Tritiseocale Wittmack) is a hybrid crop developed by crossing wheat (Triticum) and rye (Secale). The nutritional value of triticale is close to that of wheat and rye (Salmon et al., 2002). In Baltic countries in 1997 Estonians adapted Western triticale cultivars to Northern growing conditions and they have done serious investigations on triticale for bread making (Tohver et al., 2005).

For expanding the range of bakery and pastry production in the world there are being developed various recipes for product enriching with fibre, especially β-glucan, proteins, vitamins and other nutrients for a healthier diet. It can be done making a flour blend from whole grain triticale, rye, hull-less barley, rice and maize flour (Straumite et al., 2010). The Mixolab allows the characterization of the physicochemical behavior of dough when submitted to dual mixing and temperature constraints. Therefore, it is possible to record the mechanical changes due to mixing and heating simulate the mechanical work as well as the heat conditions that might be expected during the baking process (Rosell et al., 2007).

The rheological properties of dough are important basis for the process of production of quality products (Collar and Armero, 1996; Létang et al., 1999; Rosell et al., 2007; Moreira et al., 2010). During the baking process, flour compounds are subjected to mechanical work and heat treatment that promote changes in their rheological properties (Bollain and Collar, 2004). Many investigations into the cross-linking of wheat protein have demonstrated that the enzyme catalysis reaction not only affects the biochemical
characteristics of the dough, but also the rheological properties (Köksel et al., 2001; Autio et al., 2005). The main techniques used for measuring flour dough’s properties are empirical or fundamental. Empirical measurements can be conducted by means of farinograph, Falling Number apparatus, rapid visco analyser, etc., and multifunctional new apparatus like Mixolab (Jansen et al., 1996; Moreira et al., 2010). The aim of research was to evaluate the rheological properties of dough made from different cereal flours and flour blends using Mixolab.

Materials and Methods
Triticale, rye and hull-less barley crops of 2010 cultivated at the Priekuli Plant Breeding Institute (Latvia) were used in the current study. For this research 4 samples of flour blends were made too (Table 1). Triticale, rye and hull-less barley used for study were ground in a laboratory mill Hawos (Hawos Kornmühlen GmbH, Germany) obtaining whole grain fine flour. Rice and maize flour was purchased from Joint Stock Company Ustuniu Malunas (Lithuania).

Table 1

<table>
<thead>
<tr>
<th>Sample composition per 100 g of flour blend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flour type</strong></td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td>Whole grain triticale, g</td>
</tr>
<tr>
<td>Whole grain rye, g</td>
</tr>
<tr>
<td>Whole grain hull-less barley, g</td>
</tr>
<tr>
<td>Rice, g</td>
</tr>
<tr>
<td>Maize, g</td>
</tr>
</tbody>
</table>

Moisture content in raw materials and flour blends was determined using an express oven-dry method, where 2.00±0.06 g of sample was placed for drying in a moisture scale Precisa XM120 (Precisa Instruments AG, Switzerland) till a constant weight (when 4 subsequent measurements did not change more than 0.01 g) at temperature +110±1 °C.

Mixing and pasting behaviour of dough made from whole grain flour of triticale, rye, hull-less barley, rice, maize and flour blends (Table 2) was studied using the Mixolab analyser (Chopin, Tripette et Renaud, Paris, France). All measurements were performed using the Mixolab standard Chopin+ protocol (ICC No. 173). Mixolab is a sensor that allows measurement of the rheological behaviour of dough that is subjected to both kneading and heating. It measures, in real time, the torque (Nm) produced by the dough between two kneading arms. The test is based on preparing a constant hydrated dough mass so as to obtain a target consistency during the first test phase.

Table 2

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameters</th>
<th>Wheat, rye, hull-less barley, and flour blends</th>
<th>Rice, maize</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dough mass</td>
<td>75 g</td>
<td>90 g</td>
</tr>
<tr>
<td>2</td>
<td>Kneading speed</td>
<td>80 rpm</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Target torque (C1)</td>
<td>1.10 Nm</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Tank temperature</td>
<td>30 °C</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Temperature, 1st level</td>
<td>30 °C</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Duration, 1st level</td>
<td>8 min</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Temperature, 2nd level</td>
<td>90 °C</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1st temperature gradient</td>
<td>4 °C min⁻¹</td>
<td></td>
</tr>
</tbody>
</table>
The dough weight for rice and maize testing was changed from 75 g to 90 g due to the specific nature of the system (Table 2). The whole test can be divided into five different stages (Figure 1): dough development (1), protein denaturation (2), starch gelatinization (3), amylase activity (4) and starch retrogradation (5).

![Figure 1. Typical curve from Mixolab analysis of wheat dough](image)

The first stage C1 at constant temperature 30 °C determines the water absorption capacity of the flour and measures the characteristics of dough during mixing (stability, elasticity, and absorbed power), shows the maximum torque at 30 °C. The parameters that were obtained from the recorded curve were water absorption (%) or the percentage of water required for the dough to produce a torque of 1.1±0.03 Nm.

Means and standard deviations of the means were calculated using Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA).

**Results and Discussion**

Moisture content of flour used in the research was from 10.3% (rice flour) to 11.9% (whole grain rye flour), but in flour blend samples – from 8.3% to 9.5%. Moisture content in the flour blend samples increased, with increasing proportions of other flours used in combination with triticale flour (Table 3).

![Moisture content in flours and flour blend samples](image)
It has been already established that rheological tests on dough can predict its behaviour in a bakery, although only if the rates and the extent of the deformation are in the same range as those during dough processing (Bloksma, 1990; Dobraszczyk and Roberts, 1992). The data of peak viscosity, pasting temperature and setback, can be useful predictors of bread firming behaviour during storage (Collar, 2003). As it is well known wheat flour possesses the unique bread making properties due to the ability of wheat storage protein to form viscoelastic dough when wetted and kneaded (Cauvain and Young, 2007). Therefore, Mixolab curve obtained for the wheat flour system was used as a standard curve.

The early stages (C1 and C2) mainly represent the properties of the protein in wheat, whole grain triticale, whole grain rye, whole grain hull-less barley, rice, maize flour and flour blend samples, but the latter stages (C3, C4 and C5) show the properties of the flour starch. The Mixolab test data showed optimal water absorption 76.30±1.00% (14% basis) for whole grain hull-less barley flour with moisture content 10.8%. Whole grain hull-less barley flour did not reach optimum torque of 1.1 Nm at the mentioned water absorption. At lower water absorption for hull-less barley flour, it was not possible to analyse the properties of starch in Mixolab analyser, because the dough stuck around the kneading arms.

### Mixolab parameters of flours and flour blends samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Water absorption (%)</th>
<th>Dough stability (min)</th>
<th>C1 (Nm)</th>
<th>C2 (Nm)</th>
<th>C3 (Nm)</th>
<th>C4 (Nm)</th>
<th>C5 (Nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flour:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat (control)</td>
<td>57.03</td>
<td>10.21</td>
<td>1.10</td>
<td>0.48</td>
<td>1.65</td>
<td>1.27</td>
<td>1.82</td>
</tr>
<tr>
<td>Whole grain triticale</td>
<td>62.40</td>
<td><strong>3.66</strong></td>
<td>1.12</td>
<td>0.45</td>
<td>1.62</td>
<td>0.39</td>
<td>0.60</td>
</tr>
<tr>
<td>Whole grain rye</td>
<td>65.00</td>
<td>9.34</td>
<td>1.08</td>
<td>0.68</td>
<td>1.98</td>
<td>0.51</td>
<td>0.93</td>
</tr>
<tr>
<td>Whole grain hull-less barley</td>
<td>76.30</td>
<td>8.81</td>
<td>0.66</td>
<td>0.28</td>
<td>1.21</td>
<td>0.30</td>
<td>0.52</td>
</tr>
<tr>
<td>Rice</td>
<td>90.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.31</td>
<td>1.26</td>
<td>1.61</td>
</tr>
<tr>
<td>Maize</td>
<td>90.00</td>
<td>0.29</td>
<td>0.21</td>
<td>0.04</td>
<td>1.22</td>
<td>0.87</td>
<td>1.20</td>
</tr>
<tr>
<td><strong>Flour blends:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>59.67</td>
<td><strong>6.03</strong></td>
<td>1.04</td>
<td>0.46</td>
<td>1.53</td>
<td>0.32</td>
<td>0.50</td>
</tr>
<tr>
<td>B</td>
<td>59.30</td>
<td><strong>6.23</strong></td>
<td>1.06</td>
<td>0.47</td>
<td>1.51</td>
<td>0.31</td>
<td>0.48</td>
</tr>
<tr>
<td>C</td>
<td>60.00</td>
<td><strong>6.87</strong></td>
<td>1.08</td>
<td>0.50</td>
<td>1.51</td>
<td>0.31</td>
<td>0.48</td>
</tr>
<tr>
<td>D</td>
<td>59.87</td>
<td><strong>7.19</strong></td>
<td>1.08</td>
<td>0.50</td>
<td>1.48</td>
<td>0.33</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Values represent the means; n = 3.

Water absorption of whole grain triticale flour was 62.40±0.35%, but rye flour 65.00±0.07% (Table 4), while rice and maize flour had the highest water absorption 90% (by standard method modification). For rice flour the optimal value of water absorption to characterize starch gelatinization, amylose activity and starch retrogradation was 90%, but reducing the water absorption Mixolab analyser showed only data on dough development and protein denaturation. Water absorption for all samples of flour blends was from 59.30±0.10% to 60.00±0.10%.

When dough temperature increased, dough viscosity decreased and the intensity of this decrease depended on protein quality. Flour protein content is not only an indicator of nutritional value, but it has also an important effect on dough rheological properties. Maize flour showed the lowest peak value 0.04 Nm what characterise protein weakening due to mechanical and thermal constraints. It can be assumed that rice and maize flour has lower protein quality. Rye flour showed the highest peak value 0.68±0.01 Nm, what means better
flour protein quality or the highest gluten strength, comparing with other studied flour samples. But, value of C2 was equivalent for wheat, triticale flour, and flour blend samples (Table 4 and Figure 2). It means they have the same gluten strength.

Also, the second part of the curve (C3, C4, C5) involves heat treatment and therefore its parameters are related to starch behaviour (Köksel et al., 2009), it can be observed that wheat flour has the highest curve comparing with other flour curves. As it can be seen in Table 4 and Figure 2, triticale and flour blends, according to Mixolab parameters, did not reach the optimal properties during the heating and cooling period contrary to wheat flour. The C3 value of whole grain rye flour is the highest among the studied flours, what means high dough viscosity during heating. It depends on amylase activity and starch quality.

Comparing (Table 2) wheat flour with triticale flour and flour blend samples peak values of C4 and C5 decreased. It showed that the triticale flour and flour blends had low amylase activity when heating and low starch retrogradation during the cooling period. The low starch retrogradation value corresponds to a long shelf life of the end product.

Comparing dough stability of triticale (3.66 min) with dough stability of flour blend samples (sample A–6.03 min; sample B–6.23 min; sample C–6.87 min and sample D–7.19 min) it was found that the stability of triticale dough increased in mixing time at temperature 30 °C when proportion of other flour increased in a flour blend. Dough stability shows dough resistance to kneading: the longer time it takes the “stronger” dough gets. Sample D shows the highest dough stability in mixing comparing to other flour blend samples. In the research the highest dough stability showed wheat flour – 10.21 min.

Conclusions
1. Moisture content in the studied flour was from 10.3% (rice flour) to 11.9% (whole grain rye flour), but in flour blend samples from 8.3% to 9.5%.
2. Rice and maize flour had the highest water absorption (90%) necessary for measurement of dough rheological properties; change of the dough weight from 75 to 90 g is required in the standard method.
3. Triticale dough stability increased in mixing time at temperature 30 °C when proportion of other flour was increased in a flour blend.
4. Triticale flour and flour blends had low amylase activity when heating and low starch retrogradation during the cooling period.
5. Maize and rice flour showed high starch retrogradation peak value, comparing to whole grain triticale, rye and hull-less barley flour, what means shorter shelf life of the end product.
Acknowledgment
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References
THE EFFECT OF SODIUM SELENITE AND SELENATE ON THE QUALITY OF LETTUCE

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Abstract

Selenium is essential microelement for humans, animals and some species of microorganisms. In human and animal cells Se incorporates in antioxidative system, but it is toxic at high dietary intake. Selenium enters the food chain through the plants which take it up from soil. Se concentration in plants depends on the chemical form of Se, its concentration and bioavailability in soil and soil microorganisms. The aim of the study was detect the effect of sodium selenite and selenate on yield quality of two lettuce varieties. Two varieties of lettuce plants (Lactuca sativa): iceberg lettuce ‘Tarzan’ and lettuce ‘Riga’ were grown in 1L pots with peat substratum. Plants during growth season were once treated with 50 mg m⁻², 100 mg m⁻² or 200 mg m⁻² of sodium selenite or selenate. Control – without treatment. Fresh and dry weight of plants, pigment content in plant leaves, ascorbic acid content and antiradical activity were tested three times during vegetation period. Plants treated with selenium had higher leaves pigment content in comparison with untreated ones. No correlation between selenium concentration and antiradical activity was observed. Ascorbic acid content depended on lettuce variety and selenium preparation. No effect of selenium was observed on plant weight. Accumulation of selenium depended on plant and its variety. Selenium concentration in vegetables correlated with Se dose given to plants. Variants were sodium selenate was used accumulated more Se in comparison with selenite ones.

Key words: Lettuce, sodium selenite, sodium selenate, pigments, ascorbic acid, antiradical activity

Introduction

Selenium is a comparatively rare element, and until the 1950s, this element was considered by most scientists to be only very toxic. It was believes to be responsible for considerable losses of farm animals in parts of US (Reilly, 1998). The microelement selenium is needed for normal functioning of human body because it is part of some enzymes and hormones, interacts with vitamins, participate in oxidizing processes, metabolism of proteins, carbohydrates, and fats. Selenium is part of enzyme glutathione peroxidase, the main part of antioxidative defence system in living cells. Therefore selenium and its compounds have notable antioxidative properties.

The selenium content in foodstuffs depends mainly on its content in plant and animal raw materials, but this, in its turn, is affected by the content of selenium in the soil. The content of selenium in soil is found within a range in the world from 0.1 to 4 mg kg⁻¹ (England, Scotland) or from 5 to 1200 mg kg⁻¹ (Colombia, Venezuela, China's central districts), that further determines the Se content in the food chain (Combs, 2001; FAO, WHO, 2001; Tan et al., 1991). In the years 1960, it was already found that Latvia belongs to countries with a low selenium level in the soil. In addition, a large part of Latvian soil is characterized by a high acidity and high content of iron. Thus selenium may form insoluble compounds resulting in a reduced selenium containing ion mobility and bioavailability to plants.

Although Se is not considered to be required by higher plants, there are indications that it shows positive effects on plants. It is known that selenium antioxidant properties can stimulate plant growth (Hartikainen et al., 2000), delay plant senescence (Djanaguiraman et al., 2005), protect plants against fungal infection and from herbivory (Hanson et al., 2003) and protect plants against different types of abiotic stress (Hartikainen, Xue, 1999). Hartikainen et al. (2000, 2001) demonstrated that depending on the dosage, Se has a dual effect on ryegrass and lettuce – at low concentrations, it acts as an antioxidant and can stimulate the plant growth, whereas at higher concentrations it acts as a pro-oxidant reducing the yields. At the higher Se level a significant increase in total chlorophylls also is possible (Xue et al., 2001).
Materials and Methods

Experiments were carried out in spring 2010 at the greenhouse of the Institute of Soil and Plant Sciences, Latvia University of Agriculture for investigation of the effect of sodium selenite and selenate effect on biochemical parameters of lettuce, garden cress and spinach. Two varieties of lettuce plants: iceberg (crisphead) lettuce (*Lactuca sativa* L. var. *capitata* L.) cv ‘Tarzan’ and leaf lettuce *Lactuca sativa* L. var. *secalina* Alef. cv ‘Riga’ were grown.

Each lettuce at the phase of 1st true leaf was placed in 1 L vegetation pot with peat substrate “Biolan for Professional”, pH KCl 6.5, N 70 mg L⁻¹, P 60 mg L⁻¹, K 300 mg L⁻¹ with microelements (Fe, Mn, Zn, Cu and B). Lettuce plants at the stage of 3rd true leave were treated with selenium. Sodium selenite or selenate was dissolved in tap water and calculated dose (table 1) was added to substrate. Experiments were done in 10 replicas.

<table>
<thead>
<tr>
<th>Dose mg m⁻²</th>
<th>Calculated selenium dose, µg m⁻²</th>
<th>Sodium selenite mg per pot</th>
<th>Sodium selenate mg per pot</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>170</td>
<td>0.57</td>
<td>0.406</td>
</tr>
<tr>
<td>100</td>
<td>340</td>
<td>1.13</td>
<td>0.812</td>
</tr>
<tr>
<td>200</td>
<td>680</td>
<td>2.26</td>
<td>1.624</td>
</tr>
</tbody>
</table>

Content of chlorophylls and carotenoides in plant leaves was determined spectrofotometrically in ethanol extract (Гавриленко et al., 2003), antiradical activity with DPPH (1,1-difenyl-2-picrylhydrazyl) radical (Sroka, 2006), ascorbic acid with 2,6-dichlorophenolindophenol (Ермаков, 1972). The content of selenium was determined by a standard method AOAC 996.16, based on the wet digestion with nitric and perchloric acids, reaction with 2,3-diaminonaphthalene (DAN) reagent and fluorimetrical determination at excitation wavelength of fluorometer at 375 nm and emission at 525 nm. Plants were tested three times during vegetation period once in decade. (1st, 2nd and 3rd decade of May). Obtained data was analyzed with Anova and correlation analyses.

Results and Discussion

Obtained results showed that selenium content in lettuce depended on used selenium preparation, variety of lettuce and sampling time.

Both varieties more intensively accumulated selenium in the form of selenate (Fig. 1 and 2). In average cv ’Riga’ accumulated selenate 2.7 times more intensively than selenite, but cv ‘Tarzan’ 1.7 times. Particularly differences between selenium forms were observed when the largest dose (200 mg m⁻²) was used. ’Riga’ accumulated 5.8 times more selenium, but ’Tarzan’ accordingly 2.5 times. Data analyses showed significant differences between selenium preparations. Both varieties varied significantly in their ability to accumulate Se. Lettuce variety ’Riga’ accumulated higher amounts of selenium in comparison with ’Tarzan’.

In average selenium content in leaf lettuce exceeded iceberg lettuce 2.2 times. (Fig. 1 and 2). Strong correlation between selenium dose and Se accumulation in lettuce leaves was observed (coefficient of correlation for selenate >0.95, but for selenite ≥0.9).

Rate of selenium accumulation depended of variety. Cultivar ’Riga’ accumulated both forms of selenium sharply, therefore Se concentration in the lettuce leaves during all time of vegetation was relatively stable. iceberg lettuce ’Tarzan’ accumulated selenium gently. All treatments promoted increase of selenium concentration in lettuce leaves from 1st till 2nd decade of plant growth after Se application. Higher doses stimulated accumulation during 3rd decade as well. (Fig. 2).
In average selenite promoted even accumulation of Se during vegetation in comparison with selenate which uptake was sharper.

Content of chlorophylls depends on lettuce variety, sampling time and selenium dose and preparation (Table 2). In average cv ’Tarzan’ contained 15.4% more chlorophylls as cv ’Riga’. ’Riga’ was less sensitive to selenium treatment. No significant effect of sodium selenite on chlorophyll content was observed. Sodium selenate increased chlorophyll content at early stages of plant development. The significant enlargement of chlorophyll content was observed at 1st and 2nd decade of research as result of sodium selenate dose 50 mg m$^{-2}$.
Effect of selenium treatment on chlorophylls and carotenoides content in lettuce leaves, mg g⁻¹

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Se dose</th>
<th>Chlorophylls, mg g⁻¹</th>
<th>Carotenoides, mg g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>'Riga' selenite</td>
<td>'Tarzan' selenite</td>
</tr>
<tr>
<td>1st decade</td>
<td>0</td>
<td>0.466</td>
<td>0.466</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.499</td>
<td>0.549a</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.474</td>
<td>0.589a</td>
</tr>
<tr>
<td>2nd decade</td>
<td>0</td>
<td>0.499</td>
<td>0.504</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.523</td>
<td>0.606a</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.543</td>
<td>0.499</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.512</td>
<td>0.492</td>
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<tr>
<td>3rd decade</td>
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<td>0.668</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.592</td>
<td>0.636</td>
</tr>
<tr>
<td></td>
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<td>0.571</td>
<td>0.605</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.618</td>
<td>0.722</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td></td>
<td>0.077</td>
<td>0.077</td>
</tr>
</tbody>
</table>

Table 2

a- Number is significantly higher than control
b- Number is significantly lower than control

Total chlorophyll content decreased during vegetation in the leaves of lettuce cv 'Tarzan'. In average selenium applications delayed that decrease and the most effective was higher doses of Se (100 and 200 mg m⁻²). Similar results was obtained also with soya (Djanaguiraman, M., et al., 2005) and lettuce (Xue et al., 2001), where effect was described as senescence prevention. At the first decade after lettuces’ treatment sodium selenite significantly increased chlorophylls content in the cv 'Tarzan' leaves (Table 2).

Similarly as chlorophylls, no effect on carotenoides content was observed in cv 'Riga' under selenite treatment, but sodium selenate promote carotenoides accumulation in all sampling times as result of 50 and 200 mg m⁻² Se doses (Table 2). 'Tarzan' is less sensitive and only largest dose (200 mg m⁻²) significantly increased carotenoides content in lettuce.

Antiradical activity of lettuce leaves depended on sampling time, preparation and its dose and plant variety. In average cv. 'Riga' showed larger activity in comparison with ‘Tarzan’. The highest antiradical activity was observed in cv. 'Riga’ leaves at the 1st decade of research under selenate treatment and at the 3rd decade under selenium dose 200 mg m⁻². No correlation between antiradical activity and selenium dose or selenium content in lettuce leaves was observed (Fig. 3)

Ascorbic acid content in lettuce leaves depended on plant cultivar and selenium application. In average leaves of lettuce cv 'Tarzan' contained 19% higher concentration of ascorbic acid as 'Riga’ ones. The elevation of ascorbic acid as the result of selenium treatment was observed for cv. ‘Tarzan’. Sodium selenate increased ascorbic acid concentration more efficient in comparison with selenite. (Fig. 4)
The growth-promoting response to Se was not observed for both selenium preparation in opposite to literature mentioned (Hartikainen et al., 1999). For improvement of lettuce quality sodium selenate is recommended. For leaf lettuce it can be used in concentrations 50–100 mg m\(^{-2}\) and earlier (1\(^{st}\) decade after treatment) utilization can be suggested. Iceberg lettuce can be treated with larger doses of selenate or selenite and extended utilized.

Conclusions
1. Selenium concentration in vegetables correlated with Se dose given to plants. In average lettuce variety ’Riga’ accumulated selenate 2.7 times more intensively than selenite, but lettuce variety ‘Tarzan’ 1.7 times. Therefore for improvement of lettuce quality sodium selenate is recommended and it can be used in concentrations 50–100 mg m\(^{-2}\).
2. Plants treated with selenium had higher leaves pigment content in comparison with untreated ones. In average variety 'Tarzan' contained 15.4% more chlorophylls as variety 'Riga'.

3. Sodium selenate promotes carotenoides accumulation in all sampling times as result of 50 and 200 mg m$^{-2}$ Se doses. 'Tarzan' is less sensitive and only largest dose (200 mg m$^{-2}$) significantly increased carotenoides content in lettuce.

4. Sodium selenate increased ascorbic acid concentration more efficient in comparison with selenite. The elevation of ascorbic acid as the result of selenium treatment was observed for lettuce variety 'Tarzan'.

5. No correlation between selenium concentration and antiradical activity was observed.

Acknowledgements
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References
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EVALUATION OF COLOSTRUM QUALITY AND NEW POSSIBILITIES FOR ITS APPLICATION

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Abstract

Colostrum is the secretion from the mammary gland during the first 24 h after calving and is an important source of nutritional, growth, and antimicrobial factors for a newborn calf. Despite the importance of nutrients in colostrum, published data describing bovine colostrum composition in Latvia is extremely limited. Most researches, investigating colostrum, focuses narrowly on the total concentration of immunoglobulins (Ig) and ignores other nutrients, and separate Ig concentrations; therefore the aim of the present study was to evaluate quality of colostrum. A total 29 samples of colostrum were collected from the conventional farm located in Auce. The experiments were carried out in the Latvia University of Agriculture. The content of protein and fat, and pH detected according to the standard methods. Concentrations of IgA, IgG, IgM were determined by turbodynamic method. The data were processed by using the SPSS software package SPSS 11.0. and MS EXCEL. Research results show, that the concentration of separate nutrients is significantly different compared with data from literature. Due high nutritional value, particularly increased concentration of immunoglobulins, colostrum may find beneficial application in new functional food development.

Key words: colostrum, chemical composition, immunoglobulins.

Introduction

Colostrum is the secretion from the mammary gland during the first 24 h after calving (Jaster, 2005) and is an important source of nutritional, growth, and antimicrobial factors for a newborn calf. It is well documented that colostrum of good quality (i.e., containing high levels of Ig) fed as soon as possible after birth is a necessity to decrease disease susceptibility and neonatal mortality (Wittum and Perino, 1995). Colostrum contains high levels of immunoglobulins, which play an important role in establishing passive immunity in the young calf, and play an important role at the localized intestinal level (Jaster, 2004). Colostrum contains 3 types of Ig, IgG, IgM, and IgA, where IgG accounts for more than 75% of the total (Korhonen et al., 2000). There are 3 types of Ig in colostrum of dairy cattle: IgG, IgM, and IgA, which typically account for about 85 to 90, 5, and 7%, respectively, of total Ig in colostrum (Roy, 1990). To be classified as colostrum of satisfactory quality, international recommendations set a minimum concentration of 50 g of Ig g l⁻¹, based on studies showing significantly higher rates of low serum Ig concentrations in calves receiving colostrum with an IgG content below this limit (Besser et al., 1991).

Colostrum quality varies distinctly among different factors: as individual features, breeds, parity, health status of the cow (Dardillat et al., 1978, Gulliksen et al., 2008). Management and feeding of high-quality colostrum can reduce calf mortality, strengthen immunity, and increase animal life span (Quigley and Drewry, 1998). Delaying the intake of colostrum reduces passive transfer of Ig and postpones provision of essential nutrients that supplement the meager reserves in the bovine neonate. The composition of colostrum is important in satisfying the nutritional requirements of neonatal dairy calves, particularly for nutrients that only minimally cross the placenta (Kehoe et al., 2007). Calves also require fat and protein for energy and muscle development in the first days of life, as well as growth factors and many other nutrients that are concentrated in the first lacteal secretions of the dam postcalving (Roy, 1990; Quigley and Drewry, 1998).

At the same time increasing antibiotic resistance among pathogens gives emphasis to the need to develop new means to prevent diseases by nutritional intervention. Modulation of the gastrointestinal flora has turned out to be an integral part of health promotion. It is suggested
that combining bovine milk or colostral Igs with probiotic lactic acid bacteria could provide considerable prospects for health promotion in the future (Mehra et al., 2006).

Despite the importance of nutrients in colostrum, published data describing bovine colostrum composition in Latvia are extremely limited. Most researches, investigating colostrum, focus narrowly on the total concentration of immunoglobulins (Ig) and ignore other nutrients, and separate Ig concentrations; therefore the aim of the present study was to evaluate quality of colostrum.

Materials and Methods

A total 29 samples of colostrum were collected from the conventional farm “Ligotnes” located in Auce. The experiments were carried out in the Latvia University of Agriculture. The content of lactose, protein and fat, density, and pH were detected according to the standard methods (see Table 1). Immunoglobulins (IgA, IgG, IgM) concentrations were determined by turbidimetric method (Грант, 1973).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number of samples</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>The content of lactose</td>
<td>29</td>
<td>LVS ISO 5765–1:2003</td>
</tr>
<tr>
<td>The content of protein</td>
<td></td>
<td>LVS EN ISO 8968–5:2002</td>
</tr>
<tr>
<td>The content of fat</td>
<td></td>
<td>LVS EN ISO 8968–5:2002</td>
</tr>
<tr>
<td>Density</td>
<td></td>
<td>LVS 186:1999</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>LVS EN ISO 6092: 2003</td>
</tr>
</tbody>
</table>

Colostrum from the first milking was collected and freeze immediately after calving. The samples were taken from a healthy quarter. The individual ID number of each cow and the calving date was registered for each colostrum sample. The aim of the study was not to evaluate the influence of factors as cow’s breed (Latvian Brown 76%, Holsteins Black 10% and Danish Red 14%), lactation number (e.g. 1.–6.), season (summer, winter), and feed (was equal for all cows) on the colostrum composition and quality, therefore it was not taken into consideration in this publication.

Samples were analyzed at least in duplicate. The data was processed by using the SPSS software package SPSS 11.0. and MS EXCEL.

Results and Discussion

As was mentioned previously, calves require fat and protein for energy and muscle development in the first days of life (Roy, 1990, Quigley and Drewry, 1998), therefore the content of this nutrient in colostrum is extremely important. The findings of our study were compared with the concentrations reported by Kehoe et al. (2007), the one of the last published review of colostrum composition. The compositional analysis of colostrum content is presented in Table 2.
Mean content of fat in colostrum was 6.48±2.90%, the results of the research relate to the data from previous studies, where fat content was slightly higher – 6.70%, (Kehoe et al., 2007), but significant difference was not established. The content of fat in colostrum is less likely to be affected by keeping and feeding conditions: high quality feed, well balanced and rich in cellulose, not chopped, in sufficient amount was available promote higher content of fat in colostrum. The content of protein in colostrum was in very wide range from 8.48 to 22.68%. Mean protein content was 16.30±3.89% and it was similar to the data from other report (Kehoe et al., 2007). The content of lactose in colostrum was in very wide range from 2.51 to 5.80%. Mean content of lactose was 3.67%, it was significantly higher comparing with other author report (p<0.05) – 2.49% (Kehoe et al., 2007). Higher concentration of lactose should be evaluated negatively, because this fact does not coincide with the physiology of the neonate calf, in which lactase is found in low concentrations at birth (Zabielski et al., 1999), it means, that in this case digestion of lactose is quite difficult for calf.

Immunity is provided to neonatal calves by passive immunity derived from colostral Ig ingested and absorbed during the first 24 h of life (Stott et al., 1979). Therefore the most significant factor affecting colostrum’s quality is concentration of Ig. Concentration of Ig in colostrum ranged from 8.01 to 35.69 g l⁻¹. Mean concentration of Ig was 19.40 g l⁻¹, it was significantly lower than those in the previous research – 34.96 g l⁻¹ (Kehoe et al., 2007). To be classified as colostrums of satisfactory quality, international recommendations set a minimum concentration of 50g of IgG l⁻¹ (Besser et al., 1991), it means, that there were not colostrum samples analysed in current research, which could be classified as colostrum of satisfactory quality.

The concentrations of individual classes of immunoglobulin are shown in Fig. 1 and Fig. 2.

![Figure 1. Concentration of IgG in the first milking colostrum](image)

The concentration of IgG in colostrum ranged between 2.55 to 31.03 g l⁻¹, mean concentration of IgG was 13.65±7.32 g l⁻¹, which was significantly lower than the data reported by Pritchett et al. (1991) found an average of 48.2 g l⁻¹ for IgG1 and Kehoe et al. (2007) – 40.96 g l⁻¹ for IgG.
The volume of colostrum produced, parity, dry period length, vaccination, and many other factors have been reviewed and have been reported to affect the IgG content in colostrums (Kehoe et al., 2007). Any of these factors may have played a role in accounting for the low IgG content in colostrum in the current research, but for stricter conclusion about factor significantly decreasing IgG concentration and about possibility to increase the concentration of IgG in colostrum in the future, the research in this area should be done.

Conversely results were obtained after evaluation mean concentrations of IgA. Concentrations of IgA in the current study were significantly higher than the report by Kehoe et al. (2007) – 1.66 g l⁻¹. In the current study, mean concentration of IgA was 2.67±1.08 g l⁻¹. The higher concentration of IgA could be explained with significant cow’s immunity fortification influence.

Mean concentrations of IgM was 3.03±1.08 g l⁻¹, it was lower than the report by Kehoe et al. (2007) – 4.32, but it fall within the range of literature values because of the high variation of IgM concentrations, ranged from 3 to 12 g l⁻¹.

<table>
<thead>
<tr>
<th>Immunoglobulin concentration in the first milking colostrum and milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
</tr>
<tr>
<td>13.65±7.32</td>
</tr>
<tr>
<td>IgA</td>
</tr>
<tr>
<td>IgM</td>
</tr>
<tr>
<td>Ig total</td>
</tr>
</tbody>
</table>

The concentration of Ig, independent from Ig class, was significantly higher in colostrum comparing with milk (see Table 3). This increased pool of immunoglobulins can be enriched further through concentration techniques, leading to production of Ig products containing high antibody concentration. Such preparation may find beneficial application as in human healthcare and wellbeing by preventing infection and controlling diseases, as in new functional food development.
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Conclusions
1. The mean concentration of main nutrients (fat – 6.48±2.90% and protein – 16.30±3.89%) in colostrum was according to the data from the literature. Since these components are necessary for energy and muscle development in the first days of life the high concentration of them are extremely important and significant.
2. Significantly higher mean content of lactose in colostrum (3.67%), comparing with data from the literature, evaluated negatively – the digestion of lactose for calf is more difficult.
3. Mean concentration of IgG was 19.40 g l⁻¹, it was significantly lower to be classified as colostrum of satisfactory quality.
4. Due high nutritional value, particularly increased concentration of immunoglobulins, colostrum may find beneficial application in new functional food development.

Acknowledgment
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References:
POTENTIAL TO INCREASE THE STABILITY OF MILK RIBOFLAVIN AGAINST PHOTO-OXIDATIVE DEGRADATION

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²Faculty of Food Technology, Latvia University of Agriculture, Liela 2, LV-3001, Jelgava, Latvia

Abstract
Milk and other dairy foods are excellent natural sources of riboflavin (vitamin B₂). Exposure of milk to light as one of the most serious dangers of riboflavin degradation, can take place at several stages from milking to the consumers, initiating the sensibilization of riboflavin, and resulting in the oxidation of milk, losses of nutritional value, and strong off-flavours that can make the oxidized milk unacceptable to consumers. The stability of milk components is dependent of a delicate balance between the anti- and pro-oxidative factors. Carotenoids are known as one of the strongest natural antioxidants. The aim of this study was to compare the influence of cow feed carotenoids on the milk riboflavin stability against photo-oxidative degradation. Milk was obtained from three experimental and one control cow groups. Experimental group’s feed was supplied with carotenoid sources: carrots, red palm oil and red palm oil concentrate. Milk samples were collected after 6 week long feed supplementation and exposed to direct sunlight for 1.5 and 3 hours. The losses of riboflavin measured by the fluorometric method was significantly lower (p<0.05) in all experimental groups milk when cow diet was enriched with carotenoids from carrots, red palm oil, and red palm oil carotenoid concentrate, compared to the control group (respectively, 10.03, 10.82, 11.13, and 21.22% after 1.5 h storage, and 15.66%, 18.95%, 18.64%, and 23.48% after 3 h storage). A strong positive correlation between milk yellow colour intensity and its riboflavin stability was also observed.

Key words: dairy products, vitamin B₂, cow feeding, antioxidants, carotenoids.

Introduction
Milk and other dairy foods are excellent natural sources of vitamin B₂ (riboflavin), and other water-soluble vitamins: B₁ (thiamine), B₆ (pyridoxine), B₁₂ (cianocobalamin), niacin (nicotinic acid), and pantothenic acid. Light is one of the most serious dangers of riboflavin degradation that is extremely sensitive to wavelengths of 420–560 nm (Eitenmiller et al., 2008, Dairy Science and Technology Handbook, 1993). Exposure of milk to light can take place at several stages from milking to the consumers and initiate the sensibilization of riboflavin, and result in the oxidation of milk. The oxidation processes cause the deterioration of nutritional quality of dairy products, and give rise to strong off-flavours, making them unacceptable to consumers (Havemose et al., 2004). The water-soluble vitamin riboflavin present in milk acts as a potent photosensitizer (the ability of riboflavin to generate singlet oxygen in milk in its capacity as a photosensitizer has been confirmed earlier) and has been implicated in the photooxidation of milk fat (MacGibbon and Taylor, 2006). Light-activated riboflavin is an agent in the development of sunlight flavor in milk via methionine oxidation to methional. Other amino acids, besides methionine, may be affected by the presence of light and riboflavin. It also catalyzes the photodegradation of ascorbic acid (Dairy Science and Technology Handbook, 1993, MacGibbon and Taylor, 2006). The extent of off-flavour development is a function of the wavelength involved, and the intensity and duration of exposure. Light has been shown to penetrate milk to an appreciable depth (MacGibbon and Taylor, 2006). The high sensitivity of flavins to light degradation is a significant factor influencing food packaging. The light induced loss of riboflavin in fluid milk packaged in glass containers and subjected to sunlight was one of the first nutrient losses in food products documented by scientific study (Eitenmiller et. al. 2008). However, glass and other transparent material bottles or other packages are often used as packing of milk and dairy products still nowadays, perhaps due to preference of consumers. The stability of milk components are dependent of a delicate balance between the anti- and pro-oxidative processes in milk influenced by different factors. One of these factors is the presence of antioxidants. Carotenoids and vitamin E act as fat-soluble antioxidants in, for example, the milk fat globule membrane which is regarded as a major site of...
auto-oxidation. β-carotene (BC) is also particularly involved in prevention of photo-oxidation, as it absorbs light in a concentration-dependent manner that would otherwise be absorbed by riboflavin, thereby inducing quality changes (Noziere et al., 2006). Factors influencing the potential for oxidized flavor development can be manipulated by changing the cow’s diet. Numerous studies have shown that antioxidants, as tocopherols and carotenoids, can be transferred from the feed to milk, and thereby improve the oxidative stability of milk (Havemose et al., 2004). However the potential of feed carotenoids, as defence mechanism against photo-oxidative degradation of milk components such as riboflavin, as well as against following oxidation of milk lipids, that was mentioned earlier, is not as clearly shown by previous investigations. Furthermore – carotenoid content in the cow feed often is not sufficiently estimated, especially in winter and spring months when cow feed is poorer in it. Amongst the richest sources of carotenoids crude palm oil (0.05 to 0.2%) (Stołyhvo, 2007) and carrots (0.006 to 0.055%) (Kotecha et al., 1998) are considered, containing mainly α- and β-carotenes. The aim of this study was to compare the influence of supplementation of cow feed by different carotenoid sources on the milk riboflavin stability against photo-oxidative degradation and colour.

**Materials and Methods**

*Experimental design.* For this experiment 20 cows were selected in a conventional type dairy farm “Strautni”, and divided into 4 groups – control group (G1) and 3 experimental groups (G2, G3, and G4) by 5 cows in each. The stage of lactation (1st – 3rd month), cow breed (Holstein, Latvian Brown and crossed) and lactation number (i.e. 1–5) were as similar as possible in all groups. The basic feed was equal in all groups, i.e. silage was fed to ad libitum and rapeseed animal feed – 2 kg per cow per day. In the G2 diet additionally 7 kg carrots per cow per day were included. In the G3 diet additionally red palm oil NVRSO – 100 g per cow per day was included. In the G4 diet additionally red palm oil carotenoid concentrate 5 g per cow per day was included. Groups 1, 2, and 4 also received rapeseed oil 100 g per cow per day, mixed in dry forage.

*Milk sample collection and storage.* Individual cow milk samples were obtained in day 42 of feed supplementation from afternoon milking. Equal amounts of each group’s cow’s milk were pooled together getting 1 pooled sample of each cow group. Pooled samples were immediately cooled to the temperature of 4–8 °C, and transported to the laboratory next morning. A total 4 pooled milk samples were divided into smaller subsamples and stored at 4–6 °C temperature until analyses of raw milk colour. Other subsamples for analysis of milk riboflavin were stored in direct sunlight for 1.5, and 3 h at room temperature or in dark at 4–6 °C temperature for 3h, and sub sequentially frozen at temperature of –18 °C until analysis not longer than 1 month.

*Total carotenes in feed* were determined in accordance with ГОСТ 13496.17–95 method, by measuring the concentration on the photometer FEK-56 M by the wave length 450 nm. The extraction of total lipids from feed for analyses of α- and β-carotene concentration was performed by the method of Hara and Radin, 1978. Concentration of α- and β-carotene in feed was determined by HPLC using the technique consisting of a Waters Alliance 2695 HPLC with photodiode array detector, monitoring between 280 and 600 nm, using a 150 x 4.6 mm, RP C18 column and Empower Pro software. The flow rate was 2 ml min⁻¹ and the mobile phase consisted of acetonitrile, methanol-acetate ammonium 50 mM, dichloromethane and water (70:15:10:5). Concentration of carotenoids was calculated by using external standards. The above mentioned feed and milk analyses were carried out in the Scientific Laboratory of Biochemistry and Microbiology of the Research Institute of Biotechnology and Veterinary Medicine ‘Sigra’ of the LLU.

*Milk riboflavin content* was determined based on a method described by Havemose et al., 2004. Milk samples (5 ml) were mixed with 0.5 ml of 2 M sodium acetate (Polskie Odczynniki
Chemiczne) and 1.5 ml of 2 M acetic acid (Chemipur, Poland). The samples were slowly agitated for 5 min. before centrifugation at 1500 x g for 10 min. The supernatant was filtered through a filter (Filtra acido hidrohlorico extracta 90) and the fluorescence was read using a TD-700 Fluorometer (Turner Designs, Sunnyvale, CA), emission 520 nm. All analytical procedures were conducted using glassware wrapped in aluminum foil to avoid light exposure resulting in additional riboflavin degradation during sample preparation.

*Milk colour analysis.* The colour of milk samples was measured using a ColorTec – PCM colour meter, USA (CIE 1976 L*a*b* colour model), which has been calibrated with a standard. The milk riboflavin and colour analyses were carried out in the Research Laboratory of Food Packing Materials and in the Scientific Laboratory of Microbiology of the Faculty of Food Technology of the LLU.

*Milk fat content* was determined by automated infrared analysis using Milcoscan equipment (method ISO 9622-1999) in the laboratory of Milk Quality Control of the Sigulda CMAS. All analytical reagents used in analysis were of analytical or higher purity. Samples were analyzed at least in duplicate. The results were calculated, analyzed, and graphs were made using MS Office program Excel.

**Results and Discussion**

*Milk riboflavin content and stability in relation to cow feeding.*

The content of total carotenes in cow group’s G1, G2, G3 and G4 feed was measured, and it was 225, 1325, 275, and 320 mg per cow per day, respectively.

![Riboflavin content in milk](image)

**Figure 1. Decrease of riboflavin content in milk during storage in direct sunlight**

The riboflavin content in raw milk was from 0.58 till 0.80 mg l\(^{-1}\) what is below the quantities showed in literature: 0.8–1.2 mg kg\(^{-1}\) (Рогожин, 2006), 1.0–2.8 mg kg\(^{-1}\) (Горбатова, 2004); the average riboflavin content of fluid whole milk according to Miller et al., 2007, is about 1.8 mg kg\(^{-1}\), but in the study of Zagorska, 2007, the average riboflavin content in Latvia raw milk, obtained in conventional agriculture was 2.65±0.10 mg l\(^{-1}\). Such of our experiment can be explained by the very high sensitivity of riboflavin to different implications of mechanical, light and other influence from milking and sample collection to laboratory analysis. Comparing the riboflavin content in all group milk, the significantly lowest (p<0.05) riboflavin content was in G3 milk (0.567 mg l\(^{-1}\)). The riboflavin content in other group’s raw milk before storage in direct sunlight was rather similar (0.77–0.80 mg l\(^{-1}\)). During the storage in direct sunlight the degradation occurred in all groups milk (Fig. 1).

Already after 1.5 h storage in direct sunlight, the losses of riboflavin were considerable (10.03–21.22%), and, moreover, by approximately 10% higher in G1 milk than in the three
The experimental group’s milk (Table 1). The losses of riboflavin measured 3h after milk storage in direct sunlight continued to increase, and in G1 milk still were the most expressed – by approximately 5–8% higher, as compared to G2, G3, and G4. So, it can be assumed that feed supplementation with carotenoids helps to prevent the photo-oxidative degradation of riboflavin. The most pronounced positive effect was observed when feed carotenoid content was the highest, and cow feed was supplemented by carrots 7 kg per cow per day.

### The influence of milk storage in direct sunlight to the riboflavin degradation.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Losses of riboflavin, %, from the initial amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Storage time 1.5 h</td>
</tr>
<tr>
<td>G1 (control)</td>
<td>21.22</td>
</tr>
<tr>
<td>G2</td>
<td>10.03</td>
</tr>
<tr>
<td>G3</td>
<td>10.82</td>
</tr>
<tr>
<td>G4</td>
<td>11.13</td>
</tr>
</tbody>
</table>

**Milk riboflavin stability in relation to milk colour.**

According to Noziere, Graulet et al., 2006, carotenoids are responsible for the yellow coloration of cattle milks derived from breeds or diet regimens that have a high carotenoid concentration. The yellow colour intensity (the b* component value) that represents the entire carotenoid pigment amount in milk was significantly lowest in G1 milk among all groups (6.42±0.188). The b*components of all the three experimental groups milk were significantly higher (p<0.05) and rather close, respectively, 7.82±0.095 for G2, 8.10±0.057 for G3, and 8.47±0.095 for G4. This can be explained by the significantly higher carotenoid concentration in all the three experimental cow groups feed. The correlation between yellow colour intensity and losses of milk riboflavin was calculated, results showed in Figure 2. Correlation was stronger (r=- 0.93) after the first 1.5 h of storage in the sunlight, and decreased later – after 3 h of storage in sunlight (r=- 0.74).

![Figure 2. Correlation between losses of milk riboflavin and the intensity of its yellow colour](image)

The fat content was measured, and it was 3.81, 4.02, 3.91, and 4.58 % in G1, G2, G3, and G4 milk, respectively. Recalculating the colour intensity in all groups to average 4.08% fat content, the correlation between yellow colour intensity and losses of milk riboflavin was smaller, but also rather tight: R=-0.83 and -0.69 after 1.5 h and 3 h storage in sunlight, respectively.
However, it should be necessary to collect more data to appreciate the feed and milk carotenoid influence on milk riboflavin stability.

Conclusions
1. Milk riboflavin photo-oxidative stability strongly depends on cow’s diet, i.e. – its carotenoid content. The degradation of vitamin B2 in direct sunlight was significantly lower (P<0.05) in all the three experimental cow groups milk when cow diet was enriched with carotenoids from carrots, red palm oil, and red palm oil carotenoid concentrate, compared to the control group (respectively, 10.03, 10.82, 11.13, and 21.22% after 1.5 h storage, and 15.66%, 18.95%, 18.64%, and 23.48% after 3 h storage).
2. A strong positive correlation between milk yellow colour intensity and its riboflavin stability was observed. The correlation between milk colour intensity and losses of milk riboflavin after 1.5 h and 3 h storage in direct sunlight was r= 0.93, and - 0.74, respectively.

Acknowledgements
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DAIRY CONSUMPTION AND PRODUCTION TRENDS

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Abstract
According to the NACE classification, the dairy industry belongs to the processing industry, production of food products, and is one of the most important industries in Latvia’s national economy. The research aim is to identify the newest trends in processing dairy products and in consumer behaviours. To achieve the aim, the following research task is set forth: to analyse the output, import, export, and consumption of dairy products in Latvia in 2005–2010. The following methods are applied in the research: general research methods: analysis and synthesis, documentary analysis, monographic and graphic methods. Statistical analysis was applied for social and economic studies. Materials used in the research: data of the Latvian Central Statistical Bureau, the Rural Support Service of the Ministry of Agriculture, and the Latvian Central Union of Dairy Farmers. The main conclusions: The dairy industry of Latvia meets the consumption needs of its population. The export of dairy products exceeds their import. More and more dairy products of low value added are exported or the proportion of raw milk increases in the total export of dairy products.

Key words: dairy, production, consumption, Latvia

Introduction
According to the NACE classification, the dairy industry belongs to the processing industry, production of food products, and is one of the most important industries in Latvia’s national economy. The production of dairy products in 2010 compared to 2009 had positive trends – there was a 10% increase in output and an 8% increase in sales, whereas an opposite trend was observed for food products in total, as a slight decrease was still observed for the entire food industry. Yet the proportion of the food industry in the processing industry increased over the recent years. In 2008 it was approximately one fourth (23%), but in 2010 it reached one third (32%) if measured by output. The research aim is to identify the newest trends in the production and consumption of dairy products. To achieve the aim, it is necessary to analyse the output, import, export, and consumption of dairy products in Latvia in 2005–2010; the household consumption of dairy products is analysed for the period 2004–2009.

Materials and Methods
The following methods are applied in the research: general research methods: analysis and synthesis, documentary analysis, monographic and graphic methods. Statistical analysis was applied for social and economic studies. Materials used in the research: data of the Latvian Central Statistical Bureau, the Rural Support Service of the Ministry of Agriculture, and the Latvian Central Union of Dairy Farmers.

Results and Discussion

Output quantities of dairy products
The year 2005 is chosen as the base year, and in general there are the following trends in the production of dairy products in Latvia: in 2005, the output of milk for consumption was 100.4 thousand tons, and there was an almost 4% increase in 2006 and 2007, but since 2008 its output decreased. An especially sharp decrease by approximately 29% was in 2009 when the output of milk for consumption amounted to only 71.6 thousand tons. A stabilising trend was observed in the year 2010. If the output of milk is measured per capita in litres, its quantity decreased from 46 l per capita in 2007 to 32 l per capita in 2009 and 2010, taking into account the real decrease in the number of residents.

Over 10 months of 2010, compared to the same period a year ago, the output of cream fell by 15%, the output of sour cream declined by almost a third (31%), while the monthly output of yogurt fluctuated over the respective months of 2010, reaching the same quantity as a year
ago. Yet over the 10 months of 2010 compared to the previous year, the outputs of ice-cream and dry whole milk fell respectively by 4% and 31%, whereas the output of dry skimmed milk almost doubled (172%); the production of condensed skimmed milk was almost stopped in 2010; the output of butter increased by 14%, but the output of butter mixtures fell by 27%; a 22% decrease in the output of whole milk curd was observed, yet the outputs of curd products and skimmed milk curd increased by 29%. In total, the outputs of curd and curd products increased by 7%, but the output of cheese rose by 60%. The total output of cheese and curd rose by 37%.

There is no stable trend regarding the changes in the output of all types of cheese (including curd). Their output fluctuated from 38.1 thousand t in 2007 to the smallest quantity of 28.8 thousand t in 2009 (see Fig.1). The cheese exports were greater than its imports in the period 2005-2009, but the situation changed in 2009 and the cheese imports exceeded its exports by 2.1 thousand t.

Over the recent five years in Latvia, the output of cheese was greater than its consumption, except the year 2009 when Latvia’s population consumed 32.2 thousand t, which is 3.4 thousand t more than the quantity produced (28.8 thousand t). Producers of cheese explained that by the massive imports of cheap cheese (1.6-1.9 LVL/kg) from Germany in 2009 when local producers were not able to compete and then it was not profitable to produce cheese in Latvia.

The consumption of cheese can be better forecasted than its output quantities. The changes in the recent five years can be expressed by a medium strong polynomial correlation

\[ y = -0.368x^2 + 3.674x + 24.17 \]

\[ R^2 = 0.792 \]

Not sharply, but the total cheese consumption tends to decrease. It is very possible that it can be related to the decrease in the number of Latvia’s population.

Figure 1. Output, import, export, and consumption of all types of cheese in Latvia in 2005–2010 (thsnd.t)

*provisional data
Figure 2. Assortment of cheeses produced in Latvia in 2010 (tons)

Hard, semi-hard, soft, sour-milk, melted, and fresh types of cheese are produced in Latvia. After analysing the assortment of cheese, one has to say that more than half (56%) of the output of cheese consists of semi-hard sorts of cheese. Approximately a third is composed of hard sorts of cheese, but a tenth is made up of melted cheeses.

Exports of milk and dairy products
All groups of dairy products are exported by Latvia. However, the sharp change in economic conditions in 2008 and 2009 significantly impacted the export structure of dairy products. The changes in exports of cheese and curd in 2004–2009 were not as substantial as it was observed for butter, milk, cream, and other dairy products. The highest prices were in 2008, but the largest export quantities were in 2007. There was a sharp decrease in the exports of cheese and curd and also in the prices in 2009, yet their exports were greater than in 2004. Over the recent three years (2008-2010), in terms of value (LVL), the largest exports are: milk and cream, uncondensed and free of sugar or other sweeteners (41–44%); cheese and curd (27-36%); milk and cream, condensed and with sugar or other sweeteners (9–16%).

The most important export markets for milk and dairy products in nine months of 2010 were Lithuania (41%), Germany and Russia, followed by Estonia, Italy, the Netherlands, and Azerbaijan. Other countries compose only 9%.

Imports of milk and dairy products
Latvia imports all types of dairy products. Approximately half of the imported dairy products, in terms of value, consist of cheese and curd (45–51% in 2008–2010). The second place in 2010 was taken by milk and cream, uncondensed and free of sugar or other sweeteners. Cheese and curd are imported by Latvia from Austria, Belgium, Bulgaria, the Czech Republic, Germany, Denmark, Estonia, Spain, Finland, France, Italy, Lithuania, the Netherlands, Poland, and Sweden. In terms of value, the largest imports of cheese are from Lithuania (41%), the second place belongs to the Netherlands (17%), followed by Germany, Estonia, and Poland with 9% each. The proportions of the other countries in the imports are insignificant.

Export-import ratio
The exports of dairy products exceed their imports during both the recent year and the recent six years.
In terms of volume (measured in tons), the exports of milk and dairy products exceed their imports according to a linear progression. It is a very positive trend on the one hand, but a different trend is observed if exports are measured in terms of value. It is a positive fact that the exports exceed the imports. Therefore, the dairy industry contributes to the country’s government budget. Yet a sharp decrease in the export-import gap, in terms of value, is
observed. It indicates that dairy products of low value added are more and more exported or the export proportion of raw milk increases. It can be explained by a decrease in the purchase price of raw milk and by Latvia’s inability to compete regarding raw milk purchase with neighbouring countries, first of all, Lithuania.

**Per-capita consumption of dairy products**

The Central Statistical Bureau’s Household Budget Study Department regularly and continuously collects data on consumption behaviours of residents. The latest data are available for the year 2009. Household studies are conducted since 1952. In the period 2008–2009, data were collected on the following types of dairy products: whole milk (l), lower fat milk (l), yogurt (kg), cheese (kg), homemade cheese, curd (kg), and cream (kg). Consumption calculations are done per one household member a year.

<table>
<thead>
<tr>
<th>Dairy products</th>
<th>All households</th>
<th>Towns</th>
<th>Rural areas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2008</td>
<td>2009</td>
<td>2008</td>
</tr>
<tr>
<td>Whole milk, L</td>
<td>43.95</td>
<td>39.84</td>
<td>35.22</td>
</tr>
<tr>
<td>Lower fat milk, L</td>
<td>7.54</td>
<td>10.78</td>
<td>9.27</td>
</tr>
<tr>
<td>Yogurt, kg</td>
<td>7.50</td>
<td>6.83</td>
<td>8.94</td>
</tr>
<tr>
<td>Cheese, kg</td>
<td>6.08</td>
<td>6.32</td>
<td>6.57</td>
</tr>
<tr>
<td>Homemade cheese, curd, kg</td>
<td>7.85</td>
<td>8.05</td>
<td>8.11</td>
</tr>
<tr>
<td>Cream, kg</td>
<td>12.52</td>
<td>12.06</td>
<td>11.98</td>
</tr>
</tbody>
</table>

In 2009, every Latvian resident on average consumed 39.84 l of whole milk, 10.78 l of lower fat milk, 6.83 kg of yogurt, 6.32 kg of cheese, 8.05 kg of curd or homemade cheese, and 12.06 kg of cream. Whole milk was more popular among rural residents, whereas urban residents mostly consumed skimmed milk. Rural residents consume more cream than urban residents.

Over the recent five years, the consumption of milk per capita tended to decline, yet the year 2009 can be regarded as the year of change in the food consumption behaviour of Latvia’s population.

The total consumption of milk (whole milk + lower fat milk) decreased by almost 12 l per capita a year during the period 2004-2009. Every Latvian resident consumes on average 1 l of milk less a month than six years ago. An especially substantial decrease in the consumption of whole milk – by 24 l – took place in Latvia’s rural areas. Yet an increase in the consumption of skimmed milk by 2 l per rural resident does not compensate for the amount of calcium absorbed from milk.

![Figure 3. Consumption of whole milk and lower fat milk (kg per capita a year)](image-url)
Urban residents consumed skimmed milk almost twice as much as rural residents in 2004, it means that this difference decreased. In 2009, an urban resident consumed 12.46 l, while a rural resident consumed 7.24 l of lower fat milk. A very surprising trend was observed for the consumption of cheese and yogurt over the recent years. During the recent five or six years, the consumption of cheese increased by a whole 1 kg/year per capita, thus exceeding the level of 6 kg per capita. The consumption of yogurt reached its highest level (7.5 kg) in 2008. It was followed by a decrease of almost 700 g in 2009. It has to be noted that urban residents consume yogurt twice as much as rural residents.

An increase in the consumption of cheese in 2009 occurred owing to urban residents. On average, urban residents consume 7.0 kg of cheese a year, while rural residents consume only 4.88 kg year\(^{-1}\). Rural residents consume homemade cheese and curd also less than urban residents. Every urban resident consumes also 8.46 kg of homemade cheese and curd a year, while a rural resident consumes only 7.18 kg of these products. The consumption of curd by rural residents decreased in 2009 compared to 2008, whereas urban residents consumed more of it.

The food industry became even more significant over the recent three years, as its share in the processing industry increased. The dairy industry is of great importance in developing Latvia’s industry. The dairy industry was and still is one of the most important export industries. Over the recent years, the exports of raw milk increased, yet the dairy products of higher value added increase their proportion in the export-import balance in 2010.

During the recent 5-6 years, the output of dairy products reached its highest level in 2007 in both absolute and relative figures. According to the data for 9–10 months of 2010, the dairy industry’s output reached its lowest level in 2009 during the period of five recent years. Changes took place in the assortment of dairy products in 2010. The output of dry skimmed milk significantly increased. The production of condensed skimmed milk stopped. The outputs of cheese (almost by 2/3), curd, curd products, skimmed milk curd, and dry skimmed milk increased. A decrease was observed in the outputs of sour cream (-31%), dry whole milk (-31%), butter mixtures (-27%), whole milk curd (-22%), cream (-15%), and ice-cream (-4%).

The quantity of cheese produced in Latvia completely meets the domestic demand for it, except the year 2009. According to the Ministry’s of Agriculture forecast, the quantity of cheese produced in the country in 2010 has to exceed the domestic demand for it. Latvia’s cheese is exported to 14 countries, while cheese is imported from 15 countries. The total exports of cheese and curd exceeded their imports in 9 months of 2010. This year, the most significant export markets for cheese and curd are in Germany, Russia, Italy, and
Estonia. Almost half of the imported cheese and curd come from Lithuania, which can be explained by the invasion of a Lithuanian supermarket chain in Latvia.
In the group of milk and dairy products, the consumption of whole milk decreased, while that of lower fat milk increased in 2009 compared to 2008.

Conclusions
1. Latvia’s dairy industry meets the domestic demand for dairy products.
2. In terms of volume, the exports of raw milk and dairy products exceed their imports, thus the dairy industry provides revenues for the country’s government budget. Yet a sharp decrease in the export-import gap, in terms of value, is observed.
3. It indicates that dairy products of low value added are more and more exported or the export proportion of raw milk increases.
4. Rural residents, compared to urban residents, more consume cheaper and relatively fatter dairy products.
5. The changes in the economic situation affected the choices of individuals and their food consumption behaviours as well.

Acknowledgment
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FORMATION OF 5-HYDROXYMETHYLFURFURALDEHYDE IN LATVIAN WHOLE MEAL RYE BREAD DURING BAKING

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Abstract

During the baking heat exchange process, various chemical transformations take place and form new chemical compounds, and the final result is bread. Furans are some of these volatile aromatic compounds. They are formed in the heat exchange processes of food production—baking, heating, and roasting. One of these compounds is 5-hydroxymethylfurfuraldehyde (HMF), which is widely distributed in food products such as bread, coffee, honey, milk products, meat, fish, beer and others. Aromatic volatile compounds endow rye bread with its characteristic aroma and taste. The formation of furan and its derivatives in food products is not clearly understood. In 2004, the Europe Food Safety Authority started to collect data from EU member states of HMF content in food products. There is no such information available on Latvian foodstuffs. Specifically, in Latvia, there have been no investigations on HMF formation in rye bread during bake processing to date. The aim of this study was to investigate the dynamic of HMF formation in whole meal rye bread crumb and crust, as influenced by baking time. The bread was produced in a Latvian commercial bakery. The content of HMF in whole meal rye bread was determined by using liquid chromatography. The study showed that Latvian whole meal rye bread baked at the optimal time of 60 minutes contains 320.6 mg kg\(^{-1}\) HMF. Its content in rye bread crumb reached 22 mg kg\(^{-1}\), which is substantially different from the HMF content of bread crust. During the prolonged baking time, the rate of HMF formation is no significant.

Key words: furan, HMF, rye bread, baking

Introduction

The Maillard reaction, first described by French scientist Louis-Camille Maillard in 1912, is comparable to a revolution in the food industry. For nearly 100 years food scientists have studied expressions of this reaction in food during thermal treatment, which leads to its characteristic colour, smell, taste, and structure. Also during the Maillard reaction (MR), aromatic compounds are formed which either enhance food flavours or reduce nutritional value, as happens during overly long-term food storage.

During the rye bread baking process, the Maillard reaction takes place, characteristically occurring in systems with intermediate moisture content, a temperature above 50 °C and a pH of 4–7, and caramelization also takes place during this process at temperatures above 100 °C (Ramirez-Jimenez et al., 2001).

5-hydroxymethylfurfuraldehyde (HMF) is one furan derivative, formed during the rye bread baking process as a result of the MR. HMF in food is a concern because, based on animal tests, it is considered possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC, 1995). A hot debate arose around HMF a few decades ago and the compound’s toxicity, mutagenicity, and carcinogenicity are still controversial. The information contained in the scientific world is unequivocal. The threshold LD50 of HMF is 3.1 g kg\(^{-1}\) body weight in rats and dogs. There are studies at doses of 75 mg kg\(^{-1}\) which confirm HMF effects on animals, as well as researches that confound the assumption about the hazard of HMF (Michail et al., 2007).

The European Food Safety Authority (EFSA) founded a group to evaluate food safety, and during 2004–2009 collected data from EU Member States for furan and HMF content in food (EFSA, 2004). The scientific report lists 2908 furans containing samples from 20 different food groups (EFSA, 2009). There is no such information on Latvian foodstuffs. The EFSA working group, in summarizing findings obtained about the extent of HMF consumer exposure, origin, formation and toxicity, concluded, that the information does not represent a true picture of HMF in foodstuffs. Currently available data on food safety are insufficient to make evaluations or recommendations concerning potential changes in dietary regime. Further studies and data are required. Based on industry-provided data, the EU has
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recommneded a modified theoretical daily consumption (mTAMDI) of HMF, which is 1600 µg/person/day (EFSA, 2005).

Most Latvian consumers consider that the bread is the most important food in their diet after meat, dairy products and vegetables. Latvia is exceptionally rich in unique and consummate rye bread traditions. In Latvia rye bread is traditionally baked on the clay floor of a wood-fired oven. Such rye bread has a distinctive aroma, springy texture, and a slightly moist crumb with a fantastic taste. To date there have been no studies in Latvia on furan compounds and HMF formation during the baking of traditional rye bread. The Latvian Food Centre database has no information on the HMF content of any bread that consumers could access.

The aim of this study was to determine the formation of HMF in Latvian traditional whole grain rye bread during baking.

Material and Methods

The samples used in this experiment were obtained from rye whole grain flour (stock company ‘Jelgavas Dzīrnavas’, Latvia). The dough was made with scald, natural starter, sugar, salt, malt and cumin.

The whole meal rye bread was baked in a Latvian commercial bakery under several conditions. Four 1kg loaves were formed and placed in the prebaking chamber for 1 minute at a temperature of 400 °C, and then the loaves were placed for baking in a chamber oven at a temperature of 250 °C. One loaf was used for measuring temperature during baking of loaf crust surface and crumb temperature inside the loaf by using thermo-couple. The start temperature of crumb was 37 °C and crust start temperature was 87 °C. Characteristics of the samples are given in Table 1.

Table 1: Characteristics of analyzed samples

<table>
<thead>
<tr>
<th>Rye bread loaf</th>
<th>Baking time, min</th>
<th>Temperature of crumb, °C</th>
<th>Temperature of crust, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nr. 1</td>
<td>30</td>
<td>94</td>
<td>134</td>
</tr>
<tr>
<td>Nr. 2</td>
<td>45</td>
<td>99</td>
<td>141</td>
</tr>
<tr>
<td>Nr. 3</td>
<td>60</td>
<td>100</td>
<td>149</td>
</tr>
<tr>
<td>Nr. 4</td>
<td>75</td>
<td>100</td>
<td>156</td>
</tr>
</tbody>
</table>

After baking the loaves were left to cool at room temperature for 12 hours. The loaves were cut into 1 cm thick slices. For analysis the crust was very carefully separated from the crumb by using a conventional knife. The crumb and the crust ratio of the samples was identified. The study was conducted in the Scientific Laboratory of Natural Chemical Substances at the Department of Chemistry of the Latvia University of Agriculture in 2011.

For the HMF analysis, 5g of the homogenized sample was extracted in 50 ml of distilled water. The ten-minute extraction was carried out in an ultrasonic bath. After extraction the samples were centrifuged for 10 minutes at 6000 rpm (HERMLE Z 206A, Germany). The liquid phase of the sample was filtered through a 0.45 µm nylon filter, and was further used for HMF analysis by high performance liquid chromatography (HPLC), (CHIMADZU, Japan). The content of HMF in the samples was determined by the calibration curve method. Standard solutions of 20; 30; 40 mg l⁻¹ were prepared of HMF (Sigma-Aldrich, Germany).

The filtrate of the sample (10µl) was injected into the HPLC, and analyzed by using (10/90) acetonitrile (CH₃CN/H₂O) by flow rate of 1.3 ml min⁻¹ as a mobile phase, and a Alltech C 18 column (4.6x250mm) connected to a UV/VIS detector (SPD-20A) set at 280 nm. Data were acquired and processed using Shimadzu LabSolutions software (Lesolution Version 1.21 SP1).
The colour of the samples was measured by Tristimulus colorimeter Color Tec/PCM (Accuracy Microsensors, Inc), reading the colour spectrum of the three coordinates, where L* is darkness, a* is redness, b* is yellowness. Statistical evaluation was performed using SPSS 14.0 for Windows. Significance was defined at p ≤ 0.05 level.

Results and Discussion
A variety of structural, physiochemical, sensory changes and transformations take place during rye bread baking. The MR and caramelisation explain most of these changes, although it is difficult to characterize all the complexity of the compounds formed during the baking process. The HMF is one of the compounds formed during rye bread baking. The data obtained on HMF content in the rye bread crumb and crust at different baking times is shown in the Figure 1.

Figure 1. The HMF content in rye bread crumb and crust at different baking times

The section of bread loaf – crumb or crust – and baking time, are significant factors (p=0.001) which affect the formation of the HMF in bread. The HMF content of the rye bread crumb at the beginning of the baking process is 0.1 mg kg\(^{-1}\). At optimal baking time – 60 min, – it increases to 2.2 mg kg\(^{-1}\). Continuing baking for another 15 min, it reached 2.5 mg kg\(^{-1}\). The temperature in the crumb increases from 94 ºC at 30 min of baking to 100 ºC at 75 min of baking. The HMF content of bread crust is 57.2 mg·kg\(^{-1}\) at an early stage of baking, and increase to 271.5 mg·kg\(^{-1}\) at the end of baking. The HMF content of the samples at the beginning of baking differs significantly from the content at the end of baking, which can be explained by temperature effects.

Figure 2. The HMF growth rate in the crust at different (30-45; 45-60, and 60-75 min) stages of baking
During baking, the water content on the surface of the loaf becomes less than in the middle and this, combined with the high temperature, is one of the factors that makes the crust different from the crumb. In these circumstances the MR takes place in the crumb, where HMF is a by-product, but in the crust, at a temperature of 134–156 ºC, sugar caramelisation takes place (Ramirez-Jimenez et al., 2001). Throughout the baking time the HMF content in bread crumb increases smoothly, but the amount is negligible compared to the amount in the crust. The HMF in the crust increases, but its growth rate at various stages of baking is different (Fig. 2.).

At an early stage of baking from 30 to 45 min the HMF growth rate was 3.9 mg min⁻¹. The highest growth rate of HMF in the crust was observed from 45 to 60 min, where it reached 6.1 mg min⁻¹, but after 60 min it fell to 4.3 mg min⁻¹. A similar kinetic was observed in a study on the cake baking process (Ait Ameur et al., 2008), where at the final stage of baking the growth of HMF decreased.

The colour L* a* b* measurements of loaf crumb and crust are summarized in Table 2.

<table>
<thead>
<tr>
<th>Baking time</th>
<th>Crumb</th>
<th>Crust</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 min</td>
<td>L*: 45.65±1.293, a*: 4.06±0.190, b*: 23.61±1.195</td>
<td>L*: 39.30±1.293, a*: 5.41±0.190, b*: 19.65±1.195</td>
</tr>
<tr>
<td>45 min</td>
<td>L*: 45.39±1.293, a*: 3.75±0.190, b*: 23.14±1.195</td>
<td>L*: 35.05±1.293, a*: 5.23±0.190, b*: 12.44±1.195</td>
</tr>
<tr>
<td>60 min</td>
<td>L*: 44.49±1.293, a*: 3.19±0.190, b*: 21.50±1.195</td>
<td>L*: 31.12±1.293, a*: 4.17±0.190, b*: 11.80±1.195</td>
</tr>
<tr>
<td>75 min</td>
<td>L*: 43.80±1.293, a*: 2.74±0.190, b*: 20.08±1.195</td>
<td>L*: 29.29±1.293, a*: 3.40±0.190, b*: 7.28±1.195</td>
</tr>
</tbody>
</table>

HMF, colour factors and temperature correlation were determined by correlation analysis. HMF is in a close negative correlation with the colour factor L*(r=-0.978), and factor b*(r=-0.955), and a close positive correlation with temperature (r=0.928). A close positive correlation between HMF and colour factor 100-L* has been observed in studies with biscuits and bread (Ramirez-Jimenez et al., 2001). The regression analysis showed that the HMF content can be predicted from L* factor and the temperature changes (HMF=1296.775 – 24.13 L* – 2.2 · temperature). This regression model is significant (p=0.001), there is close correlation (r=0.986, R²=0.971).

During rye bread baking structural changes take place in the loaf, increasing the thickness of the crust. The ratio of the crust and crumb was determined for evaluation of HMF in bread (Fig. 3.).

**Figure 3. Crumb and crust ratio of bread at different time of baking**
At the beginning of baking, the thickness of the crust is thinnest, and gradually becomes thicker with increased baking time while the crumb volume reduces. At the optimum baking time of 60 min bread crust is 14.6% and crumb is 85.4%. The total content of HMF in the bread was calculated based on the HMF content in the crust and crumb as well as on crust and crumb ratio at various baking times (Fig. 4).

![Figure 4. HMF content in the bread at various baking time](image)

After studies and estimates the HMF content of 1 kg rye whole meal bread loaf at the optimal baking time (60 min) is 32.1 mg kg\(^{-1}\). According to the literature, the content of the HMF in whole meal rye bread is reported to vary from 12.2 to 211.02 mg kg\(^{-1}\) (Bobere et al., 2009; Hiller B. et al., 2011). Latvian nutritionists recommended consuming 150–200 g of rye bread per day. In this case the exposure of HMF would be 4.82–6.42 mg per day. Spanish researchers have calculated HMF exposure of 9.7 mg per day, of which 4.9 mg per day is ingested with coffee (Arribas-Lorenzo and Morales, 2010). Norwegian scientists have concluded, that HMF is more actively assimilated into the human metabolism when consumed in coffee, dried fruits, and alcohol. Monosaccharides, disaccharides, and starch are not essential to the body’s metabolic process (Husoy et al., 2008). From this perspective, bread products are not a source of HMF potentially risky to human health. The issue of HMF in food is still not sufficiently investigated, but it is necessary to look for ways to optimize HMF content in bread without losing its sensory properties. The FDA recommends that consumers eat a balanced diet, choosing a variety of foods that are low in trans fat and saturated fat, and rich in high-fiber grains, fruits, and vegetables.

**Conclusions**

1. The HMF content does not exceed 2.2 mg kg\(^{-1}\) in wholemeal rye bread crumb, but in the crust it was 57.2 mg kg\(^{-1}\) at the early stage of baking, and increased to 271.5 mg kg\(^{-1}\) at 75 min of baking.
2. Baking time and temperature have significant (p=0.001) impact on the HMF content in bread.
3. Wholemeal rye bread contains 32.1 mg kg\(^{-1}\) of HMF; prolonging baking time by 15 min, increases the content of HMF by 1.7 times.
4. The HMF is in close correlation (r=0.986) with the darkest shades of colors characterized by the factors L*. The greatest increase of HMF in the bread crust was found at 45 and 60 min of baking.
Acknowledgements
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References
VOLARIZATION OF SAFFRON INDUSTRY BY-PRODUCTS: BIOACTIVE COMPOUNDS FROM LEAVES

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Abstract
Interest in the development of bioprocesses for the production or extraction of bioactive compounds from natural sources has increased in recent years due to the potential applications of these compounds in food, chemical, and pharmaceutical industries. Obtention of 1kg of spice from saffron stigmas generates 150 000 blooms and 1 500 kg of leaves, which are presently both considered as waste. But due to the biologically valuable compounds like flavonoids, antioxidants which could be found in blossom and leaves of crocus species and could potentially used as functional components for food products and diet supplement. Therefore, the main objective of analysis was proper evaluation of C. sativus leaves usability for further valorization based on flavonoids and antioxidants composition and titration. Analysis were performed on dried leaves of C. Sativus collected in different parts of the world, regions and provinces in the framework of the European Program CROCUSBANK. Samples of C. Sativus were extracted with a in-house developed micro-extraction technique and extracts analyzed by HPLC-UV and by HPLC-MS and spectrophotometer. 8 flavonoids were identified and titrated in C. sativus leaves from which 2 (kaempferol-8-C-gluco-6,3-O-diglucoside and kaempferol-8-C-gluco-6-O-glucose) were reported for the first time in saffron. Comparison of flavonoids and antioxidants from samples harvested in different countries states that flavonoid concentrations vary independently of origin, while different cultivation conditions or different picking periods seemed to greatly influence.

Key words: Crocus sativus, bioactive compounds, HPLC-MS

Introduction
Bioactive compounds are extra nutritional constituents that naturally occur in small quantities in plant and food products (Kris-Etherton et al., 2002). Most common bioactive compounds include secondary metabolites such as antibiotics, mycotoxins, alkaloids, food grade pigments, plant growth factors, and phenolic compounds (Hölker et al., 2004). Phenolic compounds comprise flavonoids, phenolic acids, and tannins, among others. Flavonoids constitute the largest group of plant phenolics, accounting for over half of the eight thousand naturally occurring phenolic compounds. Variations in substitution patterns to ring C in the structure of these compounds result in the major flavonoid classes, i.e., flavonols, flavones, flavanones, flavanols, isoflavones, and anthocyanidins. Similarly to the flavonoids, phenolic acids constitute also an important class of phenolic compounds with bioactive functions, usually found in plant and food products. Phenolic acids can be divided in two subgroups according to their structure: the hydroxybenzoic and the hydroxycinnamic acids. The most commonly found hydroxybenzoic acids include gallic, hydroxybenzoic, protocatechuic, vanillic and syringic acids, while among the hydroxycinnamates, caffeic, ferulic, p-coumaric and sinapic acids can be pointed out (Bravo, 1998). In the last few years, great attention has been paid to the bioactive compounds due to their ability to promote benefits for human health, such as the reduction in the incidence of some degenerative diseases like cancer and diabetes (Conforti et al., 2009), reduction in risk factors of cardiovascular diseases antioxidant, anti-mutagenic, anti-allergenic, anti-inflammatory, and anti-microbial effects (Balasundram et al., 2006), among others. Due to these countless beneficial characteristics for human health, researches have been intensified aiming to find fruits, vegetables, plants, agricultural and agro-industrial residues as sources of bioactive phenolic compounds. Saffron with its unique aroma, color, and flavor can by no means be considered a new introduction to 21st century cuisine and medicine. In fact, the history of saffron usage dates back nearly 3000 years,
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spanning many continents, civilizations, and cultures (Deo, 2003). Saffron, the highly desirable golden spice, is the dried elongated stigmas and styles of the blue-purple saffron flower (*Crocus sativus*, L.), a member of the Iridaceae (iris) family with origins in the Middle East. At nearly $40–50 per gram, it is the world's most expensive spice. It is estimated that it takes approximately 75,000 crocus blossoms and 1 500 kg of leaves, which are presently considered as waste. But due to the biologically valuable compounds like flavonoids, antioxidants which could be found in blossom and leaves of crocus species and could potentially used as functional components for food and health areas. Therefore, the main objective of analysis was proper evaluation of *C. sativus* leaves usability for further valorization based on flavonoids, antioxidants composition and titration.

Materials and Methods

*Plant and extracts.* Leaves of *Crocus sativus* were collected from different countries, regions, provinces and localities in Spain, Castilla-La Mancha University of Agriculture. Raw leaves were dried at temperature of 30 °C for 18 hours. Drying the leaves lost about 50% of their weight. *Crocus sativus* leaves precise locations shown in Table 1. The aqueous-methanolic extract of the leaves was prepared as follows: 5 g of chopped, dried leaves of the plant were extracted with 100 ml of methanol 80% by maceration maceration method. The solvent was then removed under reduced pressure. The plant ingredient concentration in the final extract was adjusted to be 10 g% by adding methanol to the dried extract.

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HPLC and LC-MS analysis. The analysis was performed using a Dionex ASI-100 auto injector, P680 HPLC pump and UV Helwett Packard (1100 series) detector (Dionex, CA, USA) with Varian ChromSep reversed-phase column (column HS C18, 100x3 OMM) (Varian, USA). Detection is performed at 350 nm wave length. Injected 20 ml of the extract. Flow rate – 0.65 ml·min\(^{-1}\). Elution – gradient. Eluent A - acetonitrile (ACN), eluent B - water with 0.1% TFA (v / v) using the gradient change: 0 min − 3% A (97%. B), 30 min − 20% A (80% B) 35 min 3% A (97%. B), 45 min 3% A (97%. B). Analysis was done at room temperature. Flavonoids identified according the analytes retention times and mass spectrum using a Thermo-Finnigan LCQ DECA Fisher XP MAX ion trap mass spectrometer with electro spray ionization (ECI) and the source of the negative ion mode. Mass Spectrometer pump Thermo-Fisher Spectra System (TFSP, San Jose, CA, USA) P1000XR, auto injector TFSP 6000LP Photodiode Array Detector and a TFSP AS 3000. Used column Varian Pursuit XRS 5 C18 (250x4.6 mm ID, 5 mm). Flow rate – 1 ml·min\(^{-1}\). Injected 10 ml of the sample into the mass spectrometer. Elution used is the same as describe before.

Antioxidant analysis. For the determination of total polyphenolics the Folin-Ciocalteau reagent was used. The 0.1% concentration of extracts dissolved in methanol. 1ml prepared extract mixed with 5 ml of Folin-Ciocalteu reagent and 4 ml of 7.5% Na\(_2\)CO\(_3\) solution. Absorbance was measured spectrophotometrically after 30 minutes at wavelength \(\lambda = 765\) nm. Absorption of the zero point measured using distilled water. The results were expressed as gallic acid equivalents (GAE) in mg g\(^{-1}\). Average results were obtained from three parallel determinations.

Results and Discussion
Qualitative Analysis of Crocus sativus leaves. The chemical composition of crocus leaves samples from 50 different sources was determined using reverse phase LC-MS. The chromatographic conditions employed allowed identification of 8 major components in each sample and a well-resolved baseline separation was obtained. Each component was identified by comparison of its retention time as well as by LC-MS-MS analysis through the detection (m/z) of its corresponding pseudomolecular ion (M – H\(^{+}\)). Figure 1 shows one representative
chromatogram, one with the highest concentration of kaempferol-8-C-glyc-6,3-O-diglycoside (26.92 RT). This diglycoside is one of the new compound observed in crocus leaves. The peak identification at 350 nm is as follows: kaempferol-8-C-Glyc-6-O-diglycoside (25.80 RT); kvercetin-8-C-glycoside (26.70 RT); kamferol-8-C-Glyc-6,3-O-diglycoside (29.70 RT); kvercetin-3-O-maltotrioside (27.23 RT); orientin (luteolin-8-C-glycoside) (31.42 RT); kaempferol-3-O-sopfobioside (32.12 RT); vitexsin (apigenin-8-C-glycoside)(34.44 RT). According to our analysis different crocus leaves samples did not differ in their chemical composition, but did differ in the concentration of each component.

Figure 1. LC-MS/MS crocus leaves chromatogram

Figure 2 describes structural formulas of two new glucosydes which were observed first time in Crocus leaves. Currently, in order to clarify the existence of new flavonoids *Crocus sativus* L. conduct further investigations.

Kaempferol-8-C-glyc-6,3-O-diglycoside

Kaempferol-8-C-gluco-6-O-glucose

Figure 2. Glucosydes

Quantitative analysis of *Crocus sativus* leaves. Figure 3 shows the concentration of kaempferol-8-C-glyc-6,3-O-diglycoside compound detected in the 50 tested samples. The results indicate that the differences might be due to the origin of the sample, to the dissimilar
drying processes possibly involving varied time periods, as well as to storage conditions. Spain, Aragon region crocus leaves had the highest total concentration of diglycoside (45.99%) followed in order by the Azerbaijan, Marocco and Spain Castilla la Mancha region. As well as the Spain, Castilla la Mancha region (9.73%) showed the lowest. Spanish leaves had low and high concentration of components in comparison to the sample analysed from La Mancha, Spain. This variation could be the result of different drying processes used, or the time and conditions under which the plant product was packed and stored in each country, all of which could affect the concentration of kamferlo diglycoside as they are thermally labile and photochemically sensitive components.

Figure 3. Kaempferol-8-C-glyc-6.3-O-diglycoside content in *Crocus sativus* leaves

*Total polyphenolic content.* The analysis and comparison of 50 extracts, observed different amounts of total phenolic compounds. In all extracts shown in Figure 4. The highest content of phenolic compounds was characterized in crocus leaves from Spain, Castilla-La Mancha region, in Albacete province of San Pedro locality (17%).

Figure 4. Total phynenolic content in *Crocus sativus* leaves
The lowest amount of phenolic compounds in *Crocus sativus* leaves were in extract from Spain, Castilla-La Mancha region, in Albacete province, Zulema locality (4.48%). Total phenolic compounds in all extracts from different sources were average between 8.55%–9.49%. Influence of phenolic compounds content in saffron, grown in the same country, but in another province or locality may have different growing conditions, plant material preparation time and harvest time.

**Conclusions**

1. Plants produce a wide variety of bioactive compounds with significant applications in the health and food areas (Sarikaya and Ladisch, 1999). Such compounds include a variety of flavonoids, phenolic acids, lignans, salicylates, stanols, sterols, glucosinolates, among others (Hooper and Cassidy, 2006).

2. In fact, plants are considered to be excellent sources of phenolic compounds with very interesting nutritional and therapeutic applications (Li et al., 2008). Among these compounds, a strong correlation between antioxidant activity and the total phenolic content in the plants has been observed, suggesting that phenolic compounds could be the major contributor of their antioxidant capacity (Li et al., 2008).

3. Phenolic compounds are widely distributed in plants, usually being found in higher concentrations in leaves and green steams (Bennett and Wallsgrove, 1994). These compounds are considered natural defense substances, and their concentration in each plant may be influenced by several factors including physiological variations, environmental conditions, geographic variation, genetic factors and evolution (Figueiredo et al., 2008).

4. The large biodiversity of plants existent provides a great exploration field for researches on bioactive phenolic compounds and their biological properties (Shetty and McCue, 2003).

**References**


ACTIVITY OF NATURAL ANTIOXIDANTS EXTRACTED FROM GREATER CALAMINT, SWEET CICELY AND COLTSFOOT CULTIVATED IN LITHUANIA AND IN FRANCE

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2Universite de Toulouse, INP-ENSIACET, Laboratoire de Chimie Agro-industrielle, Toulouse, 31030, France

Abstract
The residues obtained after hydrodistillation of three aromatic plants (Calamintha grandiflora, Myrrhis odorata and Tussilago farfara) were separated into solid and liquid fractions. The liquid fraction was dried while the solid fraction was extracted with acetone, methanol or ethanol. Two main types of antioxidant activity tests were employed: i) assays to evaluate oxidation of fats, oils and other fat containing foods (Oxipress); ii) assays to evaluate radical scavenging activity in model systems (DPPH, FRAP). The antioxidant activities were expressed as gallic acid equivalents (GAE) with the aim of standardizing these methods and that allow data comparisons. The three antioxidant assay methods give different antioxidant activity trends. An initial screening of ethanol extracts from the three aromatic plants for antioxidant activity in oil reported them as the best. Despite hydrodistillation residues presented strong antioxidant activities, the total phenolics content in acetone, methanol, ethanol leaves extracts for Tussilago farfara was about twice lower than others plants extracts.

Key words: Aromatic plants, antioxidant activity, by-products valorization

Introduction
Agricultural and industrial residues/by-products obtained during processing of raw plants represent potential natural sources for antioxidants that are rarely exploited (wastes could be represent up to 99.5% of the raw material). Taking into account this situation our aims are analysing and evaluating such by-products as possible sources for food antioxidants. Chemical and biological diversity of aromatic and medicinal plants depending on such factors, as cultivation area, climatic conditions, genetic modifications and others is an important impetus to study flora present in different growing sites, countries and geographical zones. Aromatic and medicinal plants are used since ancient times for different purposes, e.g. for flavouring of foods, for preservation and for disguising unpleasant odours. Mostly this is linked with the discovery of synthetic drugs, which usually are more effective. Nevertheless a big part of the world population still widely relies on traditional herbal medicine. Many medicinal plants contain large amounts of antioxidants such as polyphenols, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases (Anderson et al. 2001). Herbs are most often defined as any part of a plant that is used in the diet for its aromatic properties (Haeskaylo, 1996; Davidson and Jaime, 2006; Smith and Winder, 1996). Recently, however, herbs have also been identified as sources of various phytochemicals, many of which possess important antioxidant activity (Velioglu et al., 1998; Larson, 1988; Kähkönen et al., 1999). Two main types of antioxidant activity tests were employed assays to evaluate oxidation of fats and assays to evaluate radical scavenging activity in model systems. This work is aimed at a preliminary screening for radical scavenging activity of extracts from aromatic plants namely Calamintha grandiflora, Myrrhis odorata and Tussilago farfara has been performed using DPPH, FRAP radical scavenging assays as well as the total phenolic content estimated by the Folin-Ciocalteu assay. Residue extracts from these plants were added to sunflower oil, and oxidative deterioration was measured by the Oxipres method.

Materials and Methods
Plant Material. The following plants greater calamint, sweet cicely and coltsfoot (Calamintha grandiflora, Myrrhis odorata and Tussilago farfara) were harvested from the collection of
medicinal plants at Kaunas Botanical Garden (Vytautas Magnus University, Lithuania) and in Midi-Pyrénées (south west of France), during April-August, 2009.

Sample Preparation. Preparation of Extracts. The residues (by-product) of hydrodistillation were separated into solid and liquid fractions. The solid residue was dried at 30 °C and extracted with acetone, methanol and ethanol while the liquid fraction (water extract) was divided in two parts, one of which was spray-dried and the other freeze-dried. The yield of extracts varied from 8.0 to 17.1%. The extractions were performed in triplicate.

Evaluation of Antioxidant Activity. DPPH Radical Scavenging Assay. The RSC of aromatic plants extracts against stable 2,2-diphenyl-2-picrylhydrazyl hydrate radical DPPH (Sigma-Aldrich, Germany) was determined by a slightly modified spectrophotometric method of (Brand-Williams et al. 1995). A 2 ml aliquot of DPPH• solution was mixed with a 50 µl of extract solution in 1 cm path length disposable microcuvette. The decreasing absorbance was read during 30 min reaction time at 1 min intervals until the absorbance curve reached the plateau phase. The amount of extract required to decrease the initial DPPH• concentration in the reaction mixture by 50% is referred as an effective concentration, IC_{50}. FRAP assay. The ferric reducing power (FRAP) assay was performed in a Biotek EL808 microplate reader (Vermont, USA). The working FRAP reagent was prepared by mixing the acetate buffer (300 mM pH 3.6): 10 mM TPTZ (2.4.6-tripyridyl-s-triazine) (Sigma-Aldrich, Germany) in 40 mM HCl: 20 mM FeCl3.6H2O in the ratio 10:1:1 (Benzie and Strain, 1996). Firstly, 300 µl of freshly prepared FRAP reagent was heated to 37 °C and an absorbance (A0) of a blank reagent was read at 593 nm. Then 10 µl of 0.1% sample solution in water and 30 µl H2O were added (final dilution of samples in the reaction mixture was 1:34) and the absorbance (A) were recorded every 1 min during the whole monitoring period which lasted up to 30 min The change in the absorbance (ΔA593nm) between the final reading and A0 was calculated for each sample and related to the ΔA593nm of a FeII reference solution which was measured simultaneously. Total Amount of Phenolic Compounds. The concentration of phenolic compounds in extracts was determined by Folin–Ciocalteu method (Folin & Ciocalteu 1927). For the preparation of a calibration curve 1 ml reference gallic acid solutions in ethanol (aliquots of 0.025, 0.075, 0.100, 0.175, and 0.350 mg ml⁻¹) were mixed with 5 ml of standard Folin-Ciocalteu reagent and diluted with distilled water (1:10) and 4 ml of 7.5% sodium carbonate solution in distilled water. The absorption was read after 30 min at 765 nm. The concentration of phenolics compounds (C) was expressed in milligrams of gallic acid equivalents (GAE) per gram of plant extract.

Method of OXIPRES. Infused oil were prepared by mixing sunflower oil with different concentrations of extracts (0.05%, 0.1%, 0.2%) 5 g of infused oil was weighted (nearest 0.001 g) into a reactor tube which then placed thermostated (110 °C) and under oxygen atmosphere (5 bars). Pressure changes were recorded by using OXIPRESS apparatus (Mikrolab Aarhus). The protection factor (PF) value of sunflower oil in case of using plants extracts and their antioxidant activities (AA) were calculated by the following formulas:

\[
PF = \frac{IP_X}{IP_K};
\]

\[
AA = \frac{IP_X - IP_K}{IP_{BHT} - IP_K};
\]

where: IP_X – induction period of sample with additive, h;
IP_K - induction period of sample without additive, h;
IP_{BHT} – induction period of sample with BHT, h.
Results and Discussion

The antioxidant activity of the extracts was determined using a DPPH and FRAP scavenging assay. DPPH assay is known to give reliable information concerning the antioxidant ability of the tested compounds (Huang et al., 2005). The principle of the assay is based on the color change of the DPPH solution from purple to yellow as the radical is quenched by the antioxidant (Parthasarathy et al., 2009). The results of DPPH scavenging assay by different plant extracts are summarized in the Table 1. The acetone extracts were the most effective DPPH radical scavengers for Calamintha grandiflora. IC\textsubscript{50} values 0.112 mg ml\textsuperscript{-1}. The IC\textsubscript{50} values of Myrhis odorata ranged between 0.828 and 0.982 mg ml\textsuperscript{-1}. The lowest IC\textsubscript{50} value (highest antioxidant activity) of 0.049 mg ml\textsuperscript{-1} was obtained for Tussilago farfara Leaves-AE (France). The free radical scavenging activity of spray dried water extract (Calamintha grandiflora-SD) exhibited slightly lower IC\textsubscript{50} value against DPPH radicals (0.158 mg ml\textsuperscript{-1}). The DPPH radical seems to be more reactive with leaves acetone extract for Tussilago farfara (France). The roots extracts for Tussilago farfara had very low antiradical activity comparing with other extracts of blossom and leaves. Methanol and ethanol extracts were more active against the DPPH radical than acetone for Myrhis odorata.

Based on the result obtained from the analyses DPPH it is important to notice that, there is not significant similitude between the same plants of different climates and country (Lithuania and France), the only important difference is obtained by the use of different solvent extraction. On the other hand, the results obtained from the FRAP analyses showed in the figure 1 above, we can find that the plants located in Lithuania has more antioxidant activity that the plants located in the Midi- Pyrénées zone. The FRAP is versatile and can be readily applied to both aqueous, alcohol and acetone extracts of different plants. In this assay, the antioxidant activity is determined on the basis of the ability to reduce ferric (III) iron to ferrous (II) iron. The results

<table>
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<tr>
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</tr>
<tr>
<td>Calamintha grandiflora-AE</td>
<td>–</td>
</tr>
<tr>
<td>Calamintha grandiflora-MeOH</td>
<td>–</td>
</tr>
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<td>Calamintha grandiflora-EtOH</td>
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<tr>
<td>Calamintha grandiflora-SD</td>
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</tr>
<tr>
<td>Myrrhis odorata -AE</td>
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<td>Myrrhis odorata -MeOH</td>
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<td>Tussilago farfara Roots-EtOH</td>
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were expressed as mg ferrous iron equivalents per ml of sample. The ferric reducing power (FRAP) assay of the alcohol extracts was in the range of 0.147–0.986 mg Fe (II)/ml (Figure 1).

![Figure 1. Ferric Reducing Capacity assay of Calamintha gradiflora, Myrhis odorata and Tussilago farfara](image)

For the aqueous extracts, the antioxidant activity ranged from 0.014 to 0.334 mg Fe (II) ml\(^{-1}\). Ethanol, methanol, acetone extracts for plants exhibited highest antioxidant capacity, than the spray dried and freeze dried water extracts.

**Table 2**

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<td>Extract</td>
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<td><em>Tussilago farfara</em> Blossom (France)</td>
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<td><em>Tussilago farfara</em> Leaves (France)</td>
<td>151.36±1.87</td>
</tr>
<tr>
<td><em>Tussilago farfara</em> Roots (France)</td>
<td>13.58±0.01</td>
</tr>
<tr>
<td><em>Tussilago farfara</em> Blossom (Lithuania)</td>
<td>94.79±0.98</td>
</tr>
<tr>
<td><em>Tussilago farfara</em> Leaves (Lithuania)</td>
<td>102.00±0.01</td>
</tr>
<tr>
<td><em>Tussilago farfara</em> Roots (Lithuania)</td>
<td>6.05±0.01</td>
</tr>
</tbody>
</table>
The amounts of total phenolic compounds in the herbs are presented in Table 2. The leaves extracts of *Tussilago farfara* possessed approximately twice-higher amounts of phenolics (102–204.57 GAE) than the other plant extracts. However, the total phenolics content in *Calamintha grandiflora* acetone extract was lower than in methanol, ethanol extracts, and lower than in some other herbs having lower antioxidant activities.

Table 3

Antioxidant activity (AA) the extracts of *Myrrhis odorata* and *Calamintha grandiflora* as compared with the effect of BHT and their effect on the stability of sunflower oil, expressed in protection factor values (PF)

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Conc %</th>
<th>IP Lithuania</th>
<th>France</th>
<th>PF Lithuania</th>
<th>France</th>
<th>AA Lithuania</th>
<th>France</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Myrrhis odorata</em></td>
<td></td>
<td></td>
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<tr>
<td>-AE</td>
<td>0.20</td>
<td>2.17</td>
<td>2.19</td>
<td>1.09</td>
<td>1.10</td>
<td>0.08</td>
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<tr>
<td></td>
<td>0.10</td>
<td>2.09</td>
<td>2.11</td>
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<td>0.04</td>
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<td>1.04</td>
<td>0.02</td>
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<tr>
<td>-MeOH</td>
<td>0.20</td>
<td>2.19</td>
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<td>0.09</td>
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<tr>
<td></td>
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<td>1.01</td>
<td>1.04</td>
<td>0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>-FD</td>
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<td>2.36</td>
<td>2.42</td>
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<td>1.21</td>
<td>0.17</td>
<td>0.2</td>
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<tr>
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<td>0.10</td>
<td>2.21</td>
<td>2.34</td>
<td>1.11</td>
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<td></td>
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<td>2.29</td>
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<td>1.15</td>
<td>0.09</td>
<td>0.14</td>
</tr>
<tr>
<td>-SD</td>
<td>0.20</td>
<td>2.51</td>
<td>2.21</td>
<td>1.26</td>
<td>1.11</td>
<td>0.25</td>
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</tr>
<tr>
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<td>2.34</td>
<td>2.02</td>
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<td>1.01</td>
<td>0.16</td>
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<tr>
<td></td>
<td>0.05</td>
<td>2.26</td>
<td>2.01</td>
<td>1.13</td>
<td>1.01</td>
<td>0.13</td>
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<tr>
<td><em>Calamintha grandiflora</em></td>
<td>0.20</td>
<td>–</td>
<td>2.48</td>
<td>–</td>
<td>1.24</td>
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<td>0.35</td>
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<tr>
<td>-AE</td>
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<td>2.72</td>
<td>–</td>
<td>1.36</td>
<td>–</td>
<td>0.33</td>
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<tr>
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<td>–</td>
<td>2.68</td>
<td>–</td>
<td>1.34</td>
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<td>0.23</td>
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<tr>
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<td>0.10</td>
<td>–</td>
<td>2.71</td>
<td>–</td>
<td>1.36</td>
<td>–</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>–</td>
<td>2.58</td>
<td>–</td>
<td>1.29</td>
<td>–</td>
<td>0.28</td>
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<tr>
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<td>1.49</td>
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<tr>
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<td>–</td>
<td>2.78</td>
<td>–</td>
<td>1.39</td>
<td>–</td>
<td>0.38</td>
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<tr>
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<tr>
<td></td>
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<td>–</td>
<td>2.54</td>
<td>–</td>
<td>1.27</td>
<td>–</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>–</td>
<td>2.30</td>
<td>–</td>
<td>1.15</td>
<td>–</td>
<td>0.14</td>
</tr>
<tr>
<td>-SD</td>
<td>0.20</td>
<td>–</td>
<td>2.78</td>
<td>–</td>
<td>1.39</td>
<td>–</td>
<td>0.38</td>
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<tr>
<td></td>
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<td>–</td>
<td>2.51</td>
<td>–</td>
<td>1.26</td>
<td>–</td>
<td>0.25</td>
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<tr>
<td></td>
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<td>–</td>
<td>2.37</td>
<td>–</td>
<td>1.19</td>
<td>–</td>
<td>0.18</td>
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<td>BHT</td>
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<td>1.00</td>
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<tr>
<td>Sunflower oil</td>
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</tr>
</tbody>
</table>

This finding showed that the content of total phenolics in herbs is not a reliable indicator of their antioxidant activity. The structures of the individual constituents need to be elucidated and assessed in order to obtain more precise results and information. The oxipres method, which was used to evaluate the antioxidant activity (AA) of extracts, is a very convenient procedure performed without using any chemicals. The change of pressure at the end of the induction
period can be rather precisely measured. In case of adding plants extracts to sunflower oil, the pressure drop was delayed for some period of time, indicating the antioxidative effect of the added substance. The effects of the applied extracts at 0.05, 0.1 and 0.2% concentrations are summarized in Table 3. The extracts obtained from *Calamintha gradiflora* and *Myrhis odorata* were found to be the most effective natural antioxidants. The effect of extracts on the stability of Sunflower oil during accelerated oxidation was comparable with the effect of butylated hydroxytoluene (BHT) at the same concentration. The extracts of *Calamintha grandiflora* was the most effective natural antioxidant as compared with extracts of *Myrhis odorata*. It has also should be emphasized that no positive correlation was found between the total amount of phenolic compounds and AA. For instance, methanol extract of *Myrhis odorata* which were rich in the total phenolics possessed the lowest AA.

**Conclusions**

The test result in the DPPH analyses obtained in preliminary investigations show that the type of compounds that have a different antioxidant activity differ depending on the solvent used and do not depend in the zone of origin. This study suggests that the *Myrhis odorata, Calamintha gradiflora* and *Tussilago farfara* extracts contain valuable antioxidant active components, which might be helpful in preventing or slowing the progress of various oxidative stresses. We can find a practical application of these plants in different areas, particularly in formulation and production of food additives. It also can be concluded that methanol and ethanol extraction are a suitable way to prepare extracts with antioxidant activity in oil, this conclusion was based in the observations obtained by the analyses. Continues with this investigation a further analyses on the isolation and identification of antioxidants components of the plants mentioned previously will be realised.

**References**

ANTIOXIDANT ACTIVITIES AND PHENOLIC COMPOSITION OF EXTRACTS FROM NEPETA PLANT SPECIES

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2 Kaunas Botanical Garden of Vytautas Magnus University, Z. E. Zilibero g. 6, LT-46324, Kaunas, Lithuania

Abstract

The aim of this study was to assess antioxidant properties of four in Lithuanian growing Nepeta varieties, namely N. cataria, N. cataria var. citriodora, N. transcaucasica and N. bulgaricum. The plants were extracted using different polarity solvents, namely acetone, methanol, ethanol and water. The antioxidant properties were assessed by determining total content of phenolics, using free DPPH• radical scavenging assay, accelerated oil stability test and peroxide value measurement. Antioxidant activities of the analysed herb extracts were greatly dependent on the extraction solvent. Methanolic extract of N. cataria exhibited significantly higher antioxidant capacity comparing to other extracts. The sub-fractions isolated from the plant material were analysed by HPLC-MS and the concentration of some phenolic acids was determined. Rosmarinic acid was the major component in all extracts; luteolin, and caffeic acid were other identified constituents, however present in considerably lower amounts.

Key words: Antioxidant activity / DPPH• / Rosmarinic acid / polyphenolics/Nepeta spp.

Introduction

Catnips are perennial flowering plants, which have been used as aromatic herbs and for some other purposes. They belong to the genus Nepeta, a member of the Mint family (Lamiaceae), comprising about 250 species, which are native in Asia, North Africa and Europe; however it is most abundant in the Mediterranean region. Catnip leaves possess pleasant minty or lemony scent which depends on the plant species and variety. Some common species of Nepeta genus are N. cataria (field balm, true, catnip, and catmint), N. cataria var. citriodora (lemon catnip), N. transcaucasica and N. bulgaricum. The plant is also interesting because of its behavioral effects on cats; in addition its essential oil is an insect repellent (Kokdil et al., 1999). Some positive effects of plant preparations on human health were reported as well (Giamperi et al., 2009). Zenasni et al., (2008) indicated that the major component in N. atlantica, N. tuberosa and N. cataria oils was the stereoisomer 4α-α, 7-α, 7α-β-nepetalactone constituting up to 70% of oil, they also suggested that nepetalactone plays an important role in antibacterial activity against E.coli and S. aureus strains. Tittel et al., (1982) reported that the main constituents of N. cataria var. citriodora were citronellol (15.6%), elemol (11.9%), geraniol (9.5%), β-elemene (7.5%), β-caryophyllene oxide (4.5 %), α-cadinol (5.0%), nerol (3.7%), isopulegol (3.3%), cadinol (2.6%), citronellol (2.6%), hexahydrofarnesyl acetone (2.2%), linalool (1.8%), and neral (1.5%). Lee et al., (2010) determined that rosmarinic acid was one of the main antioxidant of N. cataria. Miceli et al., (2005) evaluated the anti-inflammatory activities of different species of Nepeta genus. In one of previous studies instrumental and sensory assessment of catnip essential oil was performed using different isolation techniques (Baranauskiene et al., 2003); however the data on antioxidant properties of various plant species and varieties are rather scarce. The aim of this study was to evaluate antioxidative properties and composition of extracts isolated from four in Lithuania grown Nepeta varieties by different polarity solvents, such as methanol, acetone and water.

Materials and Methods

Materials. Leaves of Nepeta cataria (field balm, catnip or catmint), Nepeta cataria var. citriodora (lemon catnip), Nepeta transcaucasica and Nepeta bulgaricum were collected from Kaunas Botanical Garden at Vytautas Magnus University, Lithuania. The plants were harvested during flowering stage, dried at room temperature and stored in glass containers in the dark. Before extraction dried material was ground in a laboratory mill (1095 Knifetec, Canada).
Extraction procedures. The extracts were isolated in a solid-liquid extractor (IKA Werke, Staufen, Germany) operating on the fluidized bed extraction principle. Extraction was completed in 3 hours; heating temperatures were 90 and 70 °C for methanol and acetone, respectively. The products are further referred as methanol (ME) and acetone (AE) extracts. Water extracts (WE) were prepared from the liquid herbal residues obtained after hydrodistilling the essential oil in a Clevenger-type apparatus. The solid residues were dried and extracted with acetone in a solid-liquid extractor to obtain deodorized acetone extract (DAE).

Evaluation of antioxidant properties. The oxidative stability of extracts was determined using an Oxipres apparatus (Mikrolab Aarhus, Denmark), which measures oxygen pressure changes in the vessel containing rapeseed oil with or without plant additives (Trojakova et al., 1999). The Shaal oven test was used to evaluate antioxidant activity by measuring weight gain during oil storage (Trojakova et al., 2001). Antioxidant power of Nepeta ssp. extracts in rapeseed oil were expressed as protection factors (PF). Radical scavenging capacity of extracts was measured using a method described by Brand-Williams et al., (1995). The assay was carried out by mixing 0.05 ml methanol solution of each extract with 2.0 ml of $6 \times 10^{-5}$ M methanol DPPH$^-$ solution. The absorbance was recorded at 515 nm using a spectrophotometer (Spectronic Genesys 8, JAV).

Determination of total phenolic compounds (TPC). The amount of TPC in crude extracts was determined by Folin Ciocalteu method as described elsewhere (Taga et al., 1984). Gallic acid solutions in ethanol (0.045, 0.090, 0.135 and 0.180 mg ml$^{-1}$) were prepared for calibration curve. The concentration of TPC was expressed in gallic acid equivalents (GAE) in 1 g of extract.

Analysis of extract composition by HPLC. Composition of plant ME, AE, DAE and WE extracts was analysed by HPLC (Skalicka-Wozniak et al., 2008) with UV detection at 210–400 nm. Quantitative determination was performed at 254 nm by measuring two replicate samples. Rosmarinic acid, caffeic acid and luteolin were used as reference compounds for identification and quantitative assessment. Calibration curve was plotted by using rosmarinic acid, caffeic acid and luteolin reference compounds at four different concentrations, 0.8, 0.4, 0.2 and 0.1 mg in 10 ml. Quantification was performed by comparing the chromatographic peak areas of compounds present in extracts with those of external standards. The linear dependence was between peak area and concentration was determined for all reference compounds($R^2=0.9991$).

Statistical data assessment. The measurements of radical scavenging capacity and the amount of TPC were performed in triplicate and the results were expressed as a mean ± standard deviation (SD). Other experiments were carried out in duplicate.

Results and Discussion
A screening of several Nepeta plants species was carried out in order to evaluate plant extracts for their antioxidative power using in situ assays, radical scavenging assay and determination of TPC. Three different solvents, acetone, methanol and water were used; their choice was based on polarity differences: dielectric constant (k) of water is 80.1; methanol 32.6 and acetone 20.7 (Demirel et al., 2010).

Two methods were selected to assess the influence of extracts on the PF of rapeseed oil oxidation at different storage conditions. In Oxipress apparatus the oil was heated at 110 °C and in general the effect of extracts on oil stability was insignificant; PF was almost equal for all analysed samples (Table 1). However, oil stability assay using weight gain measurement, (due to binding oxygen in the course of oxidation) performed at lower temperature (50 °C) revealed quite remarkable antioxidative effect of catnip extracts. In this case AEs were stronger antioxidants than other added to oil extracts; the highest antioxidative activity was found for N. cataria var. citriodora AE and DAE extracts, which increased oil PF up to two times.
Comparison of Antioxidant Power (Expressed as Protection Factors) of 0.2% *Nepeta* ssp. Extracts in Rapeseed Oil

<table>
<thead>
<tr>
<th>Additives</th>
<th>Oxipres method at 110 °C</th>
<th>Weight gain method at 50 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. cataria</em> var. <em>citriodora</em> (AE)</td>
<td>1.3</td>
<td>2.0</td>
</tr>
<tr>
<td><em>N. cataria</em> var. <em>citriodora</em> (ME)</td>
<td>1.3</td>
<td>1.7</td>
</tr>
<tr>
<td><em>N. cataria</em> var. <em>citriodora</em> (DAE)</td>
<td>1.2</td>
<td>1.9</td>
</tr>
<tr>
<td><em>N. cataria</em> var. <em>citriodora</em> (WE)</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td><em>N. cataria</em> (AE)</td>
<td>1.2</td>
<td>2.0</td>
</tr>
<tr>
<td><em>N. cataria</em> (ME)</td>
<td>1.3</td>
<td>1.2</td>
</tr>
<tr>
<td><em>N. cataria</em> (DAE)</td>
<td>1.3</td>
<td>1.7</td>
</tr>
<tr>
<td><em>N. cataria</em> (WE)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td><em>N. bulgaricum</em> (AE)</td>
<td>1.3</td>
<td>1.7</td>
</tr>
<tr>
<td><em>N. bulgaricum</em> (ME)</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td><em>N. bulgaricum</em> (DAE)</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td><em>N. bulgaricum</em> (WE)</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td><em>N. transcaucasica</em> (AE)</td>
<td>1.2</td>
<td>1.9</td>
</tr>
<tr>
<td><em>N. transcaucasica</em> (ME)</td>
<td>1.2</td>
<td>1.1</td>
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<td><em>N. transcaucasica</em> (DAE)</td>
<td>1.3</td>
<td>1.9</td>
</tr>
<tr>
<td><em>N. transcaucasica</em> (WE)</td>
<td>1.3</td>
<td>1.0</td>
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</table>

It is well known that antioxidative activity of plant extracts depends on plant species and extraction solvents. Radical scavenging capacity was assessed by using DPPH• assay, which is one of the most frequently used methods for this purpose. The method is based on the ability of constituents present in plant extract to donate an electron to a free DPPH• radical; the reaction may be followed by the colour changes. The results of the antioxidant activity measurements are presented in Table 2. It was found that MEs were the most effective radical scavengers except for *N. cataria* var. *citriodora*. Thus, *N. transcaucasica*, *N. cataria* and *N. bulgaricum* MEs inhibited approximately 80% in the reaction present DPPH• radicals, while *N. cataria* var. *citriodora* ME only 44%. Tepe et al., (2007) also reported that ME of *Nepeta flavida* possessed strong antioxidative effect. The radical scavenging percentage of other extracts was from 19 to 53%. The DAEs were the weakest radical scavengers for all *Nepeta* samples. Most likely, the majority of antioxidatively active constituents as polar compounds are dissolved in water during hydrodistillation; WEs obtained from the liquid hydrodistillation fraction were remarkably stronger antioxidants than DAEs. AEs isolated from the whole plant material were stronger radical scavengers than DAEs. It may be explained by two possible reasons: first, part of components dissolving in water during hydrodistillation at 100 °C may be also extracted by acetone from the whole material; second, AE may contain essential oil components which are removed during hydrodistillation and which may possess some antioxidant activity. Such activity was reported for *Nepeta* species essential oils (Giamperi et al., 2009; Saleh et al., 2010; Dapkevicius et al., 1998). It is interesting noting, that WEs were stronger radical scavengers than AEs, again except for *N. cataria* var. *citriodora*. 
Table 2
Radical Scavenging Capacity and Phenolic Compound Composition of Nepeta Extracts

<table>
<thead>
<tr>
<th>Additives</th>
<th>DPPH• radical scavenging, %</th>
<th>Content (mg 100 g⁻¹ extract)</th>
<th>Total phenolics mg GAE g⁻¹ extract</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Luteolin</td>
<td>Rosmarinic acid</td>
</tr>
<tr>
<td><strong>N. transcaucasica</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AE</td>
<td>33.0±2.4</td>
<td>tr.</td>
<td>60.6</td>
</tr>
<tr>
<td>DAE</td>
<td>21.1±2.8</td>
<td>5.3</td>
<td>59.7</td>
</tr>
<tr>
<td>ME</td>
<td>79.9±1.5</td>
<td>3.5</td>
<td>1082.8</td>
</tr>
<tr>
<td>WE</td>
<td>45.3±0.9</td>
<td>3.7</td>
<td>184.9</td>
</tr>
<tr>
<td><strong>N. bulgaricum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AE</td>
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<td>3.5</td>
<td>387.3</td>
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<tr>
<td>DAE</td>
<td>22.2±0.8</td>
<td>16.5</td>
<td>72.2</td>
</tr>
<tr>
<td>ME</td>
<td>81.1±1.6</td>
<td>3.9</td>
<td>1404.7</td>
</tr>
<tr>
<td>WE</td>
<td>52.6±1.1</td>
<td>6.9</td>
<td>587.3</td>
</tr>
<tr>
<td><strong>N. cataria var. citriodora</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AE</td>
<td>48.1±2.1</td>
<td>2.0</td>
<td>377.9</td>
</tr>
<tr>
<td>DAE</td>
<td>18.5±1.8</td>
<td>7.2</td>
<td>42.2</td>
</tr>
<tr>
<td>ME</td>
<td>44.7±0.5</td>
<td>5.7</td>
<td>476.5</td>
</tr>
<tr>
<td>WE</td>
<td>29.4±0.6</td>
<td>3.9</td>
<td>166.7</td>
</tr>
<tr>
<td><strong>N. cataria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AE</td>
<td>48.1±2.5</td>
<td>2.2</td>
<td>487.8</td>
</tr>
<tr>
<td>DAE</td>
<td>22.9±2.0</td>
<td>9.7</td>
<td>41.1</td>
</tr>
<tr>
<td>ME</td>
<td>80.9±1.4</td>
<td>4.4</td>
<td>1743.8</td>
</tr>
<tr>
<td>WE</td>
<td>52.7±0.9</td>
<td>7.8</td>
<td>193.3</td>
</tr>
</tbody>
</table>

tr. – trace amount

The content of TPC in the analysed extracts varied from 2.1 (WE of N. cataria var. citriodora) to 23.1 mg GAE g⁻¹ (ME of N. cataria). Methanol extracted the biggest amount of phenolic compounds from all studied Nepeta varieties, except for N. cataria var. citriodora, when TPC was slightly higher in AE (Table 2). In general, the content of TPC was in correlation with radical scavenging capacity ($R^2=0.7$), however there were some exceptions. For instance, higher amount of TPC was found in N. cataria AE, than in its WE, while WE possessed higher radical scavenging capacity than AE. It may be explained by the differences in the composition and antioxidant capacity of individual constituents present in the extracts.

Antioxidative properties of Nepeta plants was mainly related to the presence of phenolic acids, particularly rosmarinic and caffeic acids (Lee et al., 2010). According to our results, rosmarinic acid was dominating in all extrtacts, except for WEs of N. transcaucasica N. cataria var. citriodora and N. cataria, when the concentration of caffeic acid was higher than that of rosmarinic acid (Table 2). Flavonoid luteolin was identified in all extracts, however its concentration in most cases was lower comparing to phenolic acids.

**Conclusions**
1. It was shown that Nepeta plants analysed in this study contain the compounds possessing antioxidant activity.
2. In general, methanol extracts demonstrated superior antioxidant properties comparing to less polar, acetone extracts.
3. The extracts isolated from deodorized plant material possessed lower antioxidant power that the extracts isolated from the whole plants.
4. The major component in all extracts was strong antioxidant rosmarinic acid, while caffeic acid and luteolin were present in lower amounts.
5. The extracts of *Nepeta cataria* demonstrated higher radical scavenging capacity than other tested plant varieties. In general it may be concluded that extracts of *Nepeta* species possessed medium antioxidant power in rapeseed oil.

References
DETERMINATION OF VOLATILES, TOCOPHEROLS AND COLOUR CHANGES IN AROMATISED OILS WITH MARJORAM (ORIGANUM MAJORANA L.)

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Abstract
Grape seed oil was aromatised by mixing it with dried and ground marjoram herb. Headspace volatiles of marjoram and aromatised oil were assessed by solid phase microextraction – gas chromatography – mass spectrometry (SPME-GC-MS). γ-Terpinene (14.6%), 4-terpineol (11.7%), α-terpinene (11.5%), β-phellandrene (11.1%), sabinene (11.0%) and α-thujene (7.0%) were major constituents among 22 compounds identified in dry marjoram headspace. However only 10 hydrocarbon terpenes were detected in aromatised oil headspace; their concentrations increased when higher amount of herb (6% compared to 3%) was added and kept longer time (4 weeks compared to 48 hours and 1 week). Percentage composition of volatiles was almost similar for all oil samples. Two tocopherols isomers, α- and γ- were found in oil samples, the major being α-tocopherol (241.8–274.3 mg kg⁻¹). The concentration of tocopherols significantly increased in the oils aromatised with marjoram. Colour of oils was evaluated using CIELAB method; maceration of oil with herb additives resulted in the changes of a* and b* colour characteristics of oil. Most likely, these changes were due to the migration of chlorophyll and carotene type pigments from herb to the oil.

Key words: Marjoram, headspace volatiles, tocopherols, grape seed oil

Introduction
Lipid rich foods, fats and oils deteriorate during long term storage and/or heating. Deterioration process proceeds through several degradation reactions. Using of antioxidants is one of the ways to inhibit oxidation. Antioxidants in terms of their origin can be classified in two main groups, synthetic and natural. The most popular antioxidants in food production are synthetic phenols, such as BHT, BHA, TBHQ and PG. However, there is some uncertainty regarding health risks associated with the consumption of synthetic additives. Therefore, the interest in natural additives has been increasing during last decades (Yanishlieva et al, 2006). Since the ancient times various parts of plants and their extracts have been used for food flavouring and preservation (Hinneburg et al., 2006; Pokorny 2001; Nakatani, 1997). Lamiaceae family plants containing phenolic and volatile compounds were extensively studied as a source of natural antioxidants (Fecka and Turek, 2008; Politeo et al., 2006; Triantaphyllou et al., 2001).

The composition of marjoram volatile compounds was previously studied and 4-terpinenol, γ-terpinene, α-terpinene, sabinene, β-phellandrene and p-cymene were reported as the most important volatiles in marjoram essential oil. However, it was shown that chemical composition of marjoram is rather variable and depends on geographical origin, plant vegetation phase and handling procedures of harvested herb (Baranauskienė et al., 2006; Politeo et al., 2006).

Seed oils are good sources of tocopherols. The level of individual tocopherols in seed oil also depends on many factors. For instance, it was reported that tocopherol content in grape seed oil was dependant on the production region, oil quality and some other factors (Beveridge et al., 2005; Crews et al., 2006).

The aim of this work was to evaluate the changes of volatile compounds, tocopherols and colour during aromatization process of grape seed oil with marjoram herb.
Materials and Methods

Materials and preparation of aromatised oil

Refined grape seed oil was obtained from a specialized retailer in Belgium and stored in dark glass bottles at 4 °C. Dried marjoram herb (Origanum majorana L.) was purchased in a local market in Lithuania and stored in dark and dry place before analysis. Aromatised oils were prepared by adding 3% and 6% (w/w) of marjoram herb to 100 ml grape seed oil and keeping the samples 48 hours, 1 and 4 weeks at 18±2 °C temperature. After aromatization the samples were filtered and kept in cool dark place before further analysis.

Analysis of volatile compounds. One g of marjoram, 2.5 g of grape seed oil and aromatised oils were weighed in vials and closed with a septum (Gerstel, Mulheim-an-der-Ruhr, Germany). Solid phase microextraction (SPME) and sample injection into a chromatograph was performed by using Gerstel MPS-2 autosampler. The samples were preheated for 2 min at 30 °C and headspace volatiles were extracted on DVB/CAR/PDMS fiber (Supelco, Inc., Bellefonte, PA, USA) during 60 min, at 30 °C followed by desorption for 5 min at 250 °C. GC-MS analysis was performed on a Hewlett-Packard 6890 GC Plus coupled with a HP 5973 MSD and equipped with a CIS-4PTV injector and HP5-MS capillary column (30 m length, 0.25 mm i.d., 0.25 µm film thickness). Injector operating in splitless mode was heated at 250 °C; GC oven temperature was programmed from 40 °C (2 min hold) to 150 °C at 5 °C min\(^{-1}\) and from 150 to 250 °C at 10 °C min\(^{-1}\) (2 min hold). Temperature of transfer line to MSD was 260°C. Helium was used as a carrier gas at 1 ml min\(^{-1}\) velocity. MSD parameters were as follows: ionization EI 70 eV; acquisition parameters in full scan mode: scanned m/z 40–200 (0–20 min), 40–400 (>20 min). The analytes were identified by comparison of their mass spectra with mass spectral libraries (Nist'98 and Wiley 6th) and by comparing calculated GC peak retention index (IR) with data present in literature (Adams, 2007).

Analysis of tocopherols. Tocopherols were determined by HPLC equipped with UV diode array detector which was set at 292 nm wavelength (AOCS, 1999). Two g of oil were dissolved in 25 ml of LC-MS grade hexane and directly injected into a normal phase Altima Silica column (250 mm length 4.6 mm id, particle size 5 µm). Tocopherols were eluted in 20 min with hexane/isopropanol (99.5:0.5) at a flow rate 1.5 ml min\(^{-1}\). The concentrations of tocopherols were determined from a calibration curve which was plotted using data obtained by measurements of reference compounds.

Colourimetric measurements. The colour was evaluated on a CIELAB instrument under CIE Illuminant D65 by using 10 g of aromatised oil in a plastic Petri dish; each sample was measured ten times with spectrophotometer (CM-2600d/2500d Konica Minolta) connected to a computer.

Statistical analysis. Statistical analysis was made using software Microsoft Excel 2003. The results were compared by t-test and p-value.

Results and Discussion

Volatile compounds in marjoram and aromatised oils

Twenty-two volatile compounds (Table 1) were identified in dry marjoram herb. The major compounds were γ-terpinene (14.6%), 4-terpineol (11.7%), α-terpinene (11.5%), β-phellandrene (11.1%), sabine (11.0%) and α-thujene (7.0%). Aromatisation of grape seed oil proceeds due to a migration of herb constituents into oil. The process depends on various parameters, such as temperature, surface area of plant material, contact time and the properties of constituents present in herb. Moreover, the release of dissolved in oil constituents from oil, their concentration in headspace and consequently possible impact on flavour of aromatised oil will depend on the solubility of individual constituents and their vapour pressure.
The number of compounds detected in headspace of aromatised oils was from 8 to 12. For instance, 8 compounds were detected when oil was kept 48 hours with 3% of herb, while this number increased to 9 and 12 when the time was increased to 1 and 4 weeks, respectively. When higher amount of herb (6%) was added to the oil, 12 compounds were detected independently on the maceration time. The headspace profiles of aromatised oils were different from those of the marjoram herb. All identified in the headspace of aromatised oils compounds were hydrocarbon monoterpenes, sabinene and $\gamma$-terpinene being the major quantitatively constituents. Other detected compounds were $\alpha$-thujene, $\alpha$-terpinene, $\alpha$-pinene, myrcene, $\alpha$-phellandrene, $p$-cymene, $\beta$-phellandrene and terpinolene. The percentage of monoterpenes in headspace was quite similar in all oil samples: GC peak area of sabinene was 19.7–25.5%, $\gamma$-terpinene 13.9–15.0%, $\alpha$-thujene 11.4–13.4%, $\alpha$-terpinene 8.7–12.6%. The same hydrocarbon monoterpenes were major constituents in herb headspace, however, important oxygenated compounds, 4-terpineol and cis-sabinene hydrate (Table 1) were not detected among oil headspace volatiles. Most likely, oxygenated constituents as possessing polar hydroxyl groups were less soluble in grape seed oil. It is in agreement with Adams et al. (2011) who recently reported that hydrocarbon monoterpenes where dominating in headspace of aromatised with herbal material oil.

Although percentage composition of aromatised oil headspace volatiles was quite similar, the concentration of detected compounds, as it can be judged from their peak area, increased with increasing the amount of added to oil herb and maceration time (Figure 1). It indicates that

---

### Table 1

Volatile compounds in marjoram

<table>
<thead>
<tr>
<th>Compound</th>
<th>RI calculated</th>
<th>RI literature $^a$</th>
<th>GC peak area $\times 10^7$</th>
<th>% of PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$-thujene</td>
<td>926</td>
<td>931</td>
<td>15.6</td>
<td>7.0</td>
</tr>
<tr>
<td>$\alpha$-pinene</td>
<td>931</td>
<td>939</td>
<td>3.1</td>
<td>1.4</td>
</tr>
<tr>
<td>sabinene</td>
<td>973</td>
<td>976</td>
<td>24.4</td>
<td>11.0</td>
</tr>
<tr>
<td>myrcene</td>
<td>993</td>
<td>991</td>
<td>9.2</td>
<td>4.2</td>
</tr>
<tr>
<td>$\alpha$-phellandrene</td>
<td>1004</td>
<td>1005</td>
<td>7.3</td>
<td>3.3</td>
</tr>
<tr>
<td>$\alpha$-terpinene</td>
<td>1017</td>
<td>1018</td>
<td>25.2</td>
<td>11.5</td>
</tr>
<tr>
<td>$p$-cymene</td>
<td>1024</td>
<td>1026</td>
<td>10.1</td>
<td>4.6</td>
</tr>
<tr>
<td>$\beta$-phellandrene</td>
<td>1029</td>
<td>1031</td>
<td>25.1</td>
<td>11.1</td>
</tr>
<tr>
<td>$E$-$\beta$-ocimene</td>
<td>1049</td>
<td>1050</td>
<td>1.2</td>
<td>0.5</td>
</tr>
<tr>
<td>$\gamma$-terpinene</td>
<td>1060</td>
<td>1062</td>
<td>32.0</td>
<td>14.6</td>
</tr>
<tr>
<td>cis-sabinene hydrate</td>
<td>1069</td>
<td>1065</td>
<td>13.8</td>
<td>6.2</td>
</tr>
<tr>
<td>terpinolene</td>
<td>1087</td>
<td>1088</td>
<td>10.9</td>
<td>4.9</td>
</tr>
<tr>
<td>camphor</td>
<td>1143</td>
<td>1143</td>
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</tr>
<tr>
<td>4-terpineol</td>
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<td>1179</td>
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</tr>
<tr>
<td>$E$-dihydrocarvone</td>
<td>1198</td>
<td>1200</td>
<td>3.8</td>
<td>1.7</td>
</tr>
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<td>carvone</td>
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<td>1242</td>
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<td>0.3</td>
</tr>
<tr>
<td>anethole</td>
<td>1285</td>
<td>1283</td>
<td>1.6</td>
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</tr>
<tr>
<td>copaene</td>
<td>1379</td>
<td>1376</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>$\beta$-caryophyllene</td>
<td>1421</td>
<td>1418</td>
<td>6.8</td>
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</tr>
<tr>
<td>$\alpha$-humulene</td>
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<td>1454</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>curcumene</td>
<td>1486</td>
<td>1482</td>
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<td>0.6</td>
</tr>
<tr>
<td>$\beta$-bisabolene</td>
<td>1511</td>
<td>1510</td>
<td>tr</td>
<td>tr</td>
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</table>

--Adams. (2007) Identification of essential oil components by gas chromatography/ mass spectrometry. tr= traces, <0.05
diffusion of marjoram volatile components to oil is a long process. For instance, the total amount of headspace volatiles in oil kept with 3% of marjoram during 4 weeks was higher 4.5 times than that in oil macerated 48 hours.

![Figure 1. Total content of headspace volatile compounds (total GC peak area) above grape seed oil aromatised with marjoram](image)

**Effect of aromatisation on the concentration of tocopherols**

Tocopherols are important oil soluble antioxidants naturally present in oil bearing seeds, which are also present in other plant materials including aromatic herbs. Gómez-Coronado et al. (2004) reported that 100 g of fresh marjoram leaves contain 32.3 mg α-tocopherol and 0.42 mg γ-tocopherol. In our study α and γ-tocopherols were found in analysed grape seed oils (Table 2). The concentration of tocopherols increased in aromatised oils, which indicates migration of tocopherols from herb to oil during maceration. It may be observed that this process continues during all time of maceration; e.g. significant increase (p>0.05) in γ-tocopherol concentration was observed after 4 weeks as compared to 48 hours and 1 week. The concentration of tocopherols was also dependant on the added herb amount.

**Concentration of α- and γ-tocopherol, mg/kg in pure (GSO) and aromatised with marjoram (GSOM) grape seed oil**

<table>
<thead>
<tr>
<th>Oil sample</th>
<th>α–tocopherol</th>
<th>γ–tocopherol</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSO</td>
<td>232.6±1.1\textsuperscript{a}</td>
<td>30.6±0.9\textsuperscript{a}</td>
</tr>
<tr>
<td>GSOM (3%) 48h</td>
<td>241.8±6.5\textsuperscript{b}</td>
<td>33.9±1.7\textsuperscript{b}</td>
</tr>
<tr>
<td>GSOM (3%) 1w</td>
<td>248.1±8.5\textsuperscript{b}</td>
<td>37.2±5.2\textsuperscript{b}</td>
</tr>
<tr>
<td>GSOM (3%) 4w</td>
<td>257.6±6.1\textsuperscript{c}</td>
<td>40.0±3.9\textsuperscript{c}</td>
</tr>
<tr>
<td>GSOM (6%) 48</td>
<td>256.0±11.7\textsuperscript{c}</td>
<td>38.3±11.0\textsuperscript{c}</td>
</tr>
<tr>
<td>GSOM (6%) 1w</td>
<td>262.0±6.3\textsuperscript{c}</td>
<td>45.9±9.6\textsuperscript{c}</td>
</tr>
<tr>
<td>GSOM (6%) 4w</td>
<td>274.3±1.3\textsuperscript{d}</td>
<td>59.0±9.9\textsuperscript{c}</td>
</tr>
</tbody>
</table>

**Effect of aromatisation on oil colour**

Colour is important quality characteristic influencing food acceptability and depending on the presence of pigments (Escolar et al., 2007). Therefore, the measurements of spectrophotometric characteristics were performed in order to assess the impact of aromatisation on oil colour.
An $L^*$ value reflects relative lightness of oil. It may be observed that aromatisation did not have any effect on this colour characteristic; it was in the range of 60.9–61.9 for all samples. The negative $a^*$ value is related to the greenish cast, which is related with chlorophyll content (Giacomelli et al., 2006). Obtained results show that $a^*$ value during aromatisation significantly changed ($p<0.05$) and became more negative, in the course of maceration. It also depended on the amount of added to oil herb (Figure 2). The positive $b^*$ value is related with the carotene content (Giacomelli et al, 2006). The $b^*$ value of grape seed oil also significantly changed ($p<0.05$) during aromatisation and became more positive. These changes may be explained by the migration of marjoram pigments to oil.

Conclusions
The results obtained in this study demonstrate that marjoram constituents, particularly volatile aroma compounds, tocopherols and pigments dissolve in grape seed oil during herb maceration (extraction). It seems, that this process proceeds rather slowly during the whole period of maceration and depends on the amount of added to oil herb. The main volatile compounds identified in the headspace of aromatised with marjoram oils were hydrocarbon terpenes, sabinene and $\gamma$-terpinene, while abundant in dried herb terpene alcohols 4-terpineol and sabinene hydrate were not detected in oil. The concentration of $\alpha$-tocopherols steadily increased in oils in the course of aromatisation. Such colour characteristics as $a^*$ and $b^*$ values were also influenced by marjoram herb; the changes of these characteristics were maceration time and herb concentration dependant.

References


LEAF VOLATILE OIL CONSTITUANTS OF SCHINUS TEREBINTHIFOLIUS AND SCHINUS MOLLE FROM TUNISIA

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Abstract

Members of the genus Schinus mainly S. terebinthifolius (Brazilian pepper tree) and S. molle (Peruvian pepper tree) have long been recognized as a consolidate sources of functional ingredients namely essential oils and phenols. Despite the substantial data on the volatile oil constituents of their fruits, the leaf volatile fraction has not received much interest. Consequently, the aim of the present study is to analyse the chemical composition of the essential oils of the aforementioned species.

The Results show that the hydrodistillation of air dried leaves yielded a pale yellowish oil with pungent and pepper like aroma in 0.75 and 1.06% (w/w dw) for S. terebinthifolius and S. molle, respectively. In both oil samples, monoterpenes hydrocarbons were the most represented class of volatile. Amongst their derivatives, α-phellandrene (46.64–22.16%), β-phellandrene (28.53–6.49%), α-pinene (4.94–5.20%), and β-myrcene (5.04–0.84%) were the main components. Even if the oil samples showed the same main constituents, there was a considerable qualitative and quantitative difference.

In summary, both species could be considered as potential sources of volatile oil because of their higher essential oil yields. Such feature appears to be characteristic of the genus Schinus. Moreover, the occurrence of some volatile compounds (e.g. α-phellandrene, α-pinene, β-myrcene and β-caryophyllene) with documented biological activities could justify the traditional uses of these species.

Key words: Schinus molle - Schinus terebinthifolius - Leaves- essential oil composition- GC/MS.

Introduction

Schinus molle and Schinus terebinthifolius (Anacardiaceae) are evergreen trees native to South America, but they have been introduced and naturalized in many countries of the world (Taylor, 2005). In Tunisia, both species have been introduced as ornamental species at the end of the 1900s by the French colonizers. Their successful introduction in a non-native range is attributed to their high drought and heat tolerance, great potential to compete for nutritive resources and light, high growth rate and prolific seed production, as well as, their phytotoxic activities (Iponga et al., 2008; Zahed et al., 2010).

Theses species are highly aromatic and as consequence, numerous investigations of their volatiles oils have been undertaken. The basic constituents of the oils were α-phellandrene, β-phellandrene, myrcene and α-pinene (Marongui et al., 2004; El Hayouni et al., 2008; Zahed et al., 2010), to which are attributed the antibacterial, anti-fungal, anti-inflammatory, cytotoxic, insecticidal and allelopathic activities (Yequin et al., 2003, Abdel-Sattar et al., 2010, Zahed et al., 2010). However, most previous studies concerned with the chemical composition of the essential oil of these species were focused on fruits, and little is known about the leaf oil constituents of these species. Therefore, the present study was intended at identifying the chemical composition of the essential oil of the leaves of S. molle and S. terebinthifolius from Tunisian origin.

Materials and methods

Leaves from S. molle and S. terebinthifolius were randomly collected from plants growing in El Ghazala (Northern Tunisia), air-dried at room temperature (20±2 °C), ground, sifted through 0.5 mm mesh screen to obtain a uniform size and then submitted to hydrodistillation for 2 h by using a simple laboratory Quickfit apparatus that consists of 1000 mL distillation flask, a condenser and a receiving vessel. The obtained oils were recovered, dried over
anhydrous sulphate sodium and subsequently analyzed by gas chromatography mass spectrometry (GC-MS). GC-MS analyses were performed with an Agilent 6890 N gas chromatograph coupled to an Agilent 5975 B and an Agilent Chemstation software. Separation of oil constituents was performed on DB-5 capillary column (30 m × 0.25 mm, film thickness 0.25 µm). The temperature program was as follow: isotherm at 50 °C for 2 min, then ramped at 3 °C min⁻¹ to 300 °C and finally held at this temperature for 10 min. Helium was used as carrier gas at a flow of 1 mL min⁻¹. One microliter of diluted oil samples in hexane was injected in the splitless mode. Identification of individual compounds was performed by matching their mass spectral fragmentation patterns with corresponding data (NIST 05 library).

Results and Discussion
The hydrodistillation of the dried leaves yielded 0.75 and 1.06% (w/w on dry weight basis) for *S. molle* and *S. terebinthifolius*, respectively, of pale yellowish oils with a pungent and pepper-like aroma. These values were nearly similar to those obtained by Zahed et al. (2010) for *S. molle* leaves but greatly differed from those reported by Barbosa et al. (2007) who found 0.44% (w/w on fresh weight basis) for *S. terebinthifolius* leaves. The relative amount percentage of the main compounds identified in both oil samples are listed in Table 1.in order of their elution in the DB-5 column. As can be seen, both qualitative and quantitative differences were observed between the analyzed oils. Monoterpenes hydrocarbons were found to be the main chemical classes in both species accounting for 38.59 and 86.64% in *S. terebinthifolius* and *S. molle*, respectively. Whatever the species, this fraction was dominated by α-phellandrene, β-phellandrene and α-pinene. However, the leaf oils of *S. terebinthifolius* were distinguishable from those of *S. molle* by the abundance of sesquiterpenes (hydrocarbons and oxygenated sesquiterpenes). The main sesquiterpenes identified were γ-elemene, aromadendrene, allo-aromadendrene, β-cubebene, (E)-bisabolene, spathulenol, globulol and γ-gurjinene. At this point, the latter compounds in addition to some other monoterpenes namely (+)-4-carene, γ-terpinene and cis-oicimene could be considered as marker compounds since both species were cultivated, processed and under the same conditions and hence the observed differences were considered to be genetically determined. Studies in this way are currently in progress.

From biochemical stand point, the present composition displayed great qualitative and quantitative differences with previous investigations. Even if the main components identified herein were reported in other studies, there were striking differences in term of quality and quantities. In fact, in previous phytochemical investigation on the essential oil from *S. molle* leaves collected from Mograne (Northeastern Tunisia), we have reported that α-phellandrene, limonene+β-phellandrene, myrcene and α-pinene were the main components (Zahed et al., 2010). Seven years earlier, Wannaz et al. (2003) studied the chemical composition of the leaf essential oil of *S. areira* and found that α-phellandrene, limonene+β-phellandrene, myrcene and α-pinene were the basic constituents. The same constituents in addition to elemol were reported as the main compounds in leaf oil constituents of Italian *S. molle* (Maffei and Chialva, 1990). Alpha pinene, limonene and β-pinene were found to be characteristic of the leaf oil constituents of Brazilian *S. terebentifolius* while sabinene and limonene were found to be the main constituents of *S. molle* leaves (Atti dos Santos et al., 2009). More recently, Abdel-Sattar et al. (2010) reported p-cymene, α-terpinene and β-pinene as the main volatile compounds of *S. molle* leaves from Saudi Arabia.

Overall, the chemical composition of the leaf essential oil from *S. molle* and *S. terinthifolius* varied considerably depending on the genetic background, origin of cultivation, season, plant parts analyzed and analytical methods.
### Table 1

Chemical composition of the essential oils from *S. terebinthifolius* and *S. molle* leaves

<table>
<thead>
<tr>
<th>Compounds</th>
<th>RI</th>
<th>Percentage (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>S. molle</em></td>
<td><em>S. terebinthifolius</em></td>
<td></td>
</tr>
<tr>
<td>α-Pinene</td>
<td>936</td>
<td>4.94</td>
<td>5.20</td>
<td></td>
</tr>
<tr>
<td>(+)-4-Carene</td>
<td>1011</td>
<td>–</td>
<td>1.77</td>
<td></td>
</tr>
<tr>
<td>o-Cymene</td>
<td>1025</td>
<td>2.92</td>
<td>2.30</td>
<td></td>
</tr>
<tr>
<td>α-Phellandrene</td>
<td>1032</td>
<td>46.64</td>
<td>22.16</td>
<td></td>
</tr>
<tr>
<td>cis-o-cimene</td>
<td>1048</td>
<td>–</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>β-Phellandrene</td>
<td>1050</td>
<td>28.53</td>
<td>6.49</td>
<td></td>
</tr>
<tr>
<td>γ-Terpinene</td>
<td>1057</td>
<td>–</td>
<td>1.23</td>
<td></td>
</tr>
<tr>
<td>β-Cubebene</td>
<td>1390</td>
<td>–</td>
<td>10.06</td>
<td></td>
</tr>
<tr>
<td>γ-elemene</td>
<td>1428</td>
<td>–</td>
<td>3.52</td>
<td></td>
</tr>
<tr>
<td>γ-Gurjunene</td>
<td>1432</td>
<td>–</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>(+)-Aromadendrene</td>
<td>1440</td>
<td>–</td>
<td>1.18</td>
<td></td>
</tr>
<tr>
<td>allo-aromadendrene</td>
<td>1462</td>
<td>–</td>
<td>1.20</td>
<td></td>
</tr>
<tr>
<td>bicyclogermacrene</td>
<td>1496</td>
<td>6.42</td>
<td>27.11</td>
<td></td>
</tr>
<tr>
<td>elemol</td>
<td>1540</td>
<td>2.38</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>δ-cadinene</td>
<td>1578</td>
<td>1.64</td>
<td>2.04</td>
<td></td>
</tr>
<tr>
<td>Camphene</td>
<td>955</td>
<td>1.49</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>β-caryophyllene</td>
<td>1420</td>
<td>–</td>
<td>3.19</td>
<td></td>
</tr>
<tr>
<td>β-myrcene</td>
<td>992</td>
<td>5.04</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>γ-E-Bisabolene</td>
<td>1509</td>
<td>–</td>
<td>3.89</td>
<td></td>
</tr>
<tr>
<td>(-)-Globulol</td>
<td>–</td>
<td>–</td>
<td>2.24</td>
<td></td>
</tr>
<tr>
<td>α- Menth-8-ene</td>
<td>–</td>
<td>–</td>
<td>1.74</td>
<td></td>
</tr>
<tr>
<td>Spathulenol</td>
<td>1578</td>
<td>–</td>
<td>1.85</td>
<td></td>
</tr>
</tbody>
</table>

*RI: Retention index relative to n-alkanes (C<sub>7</sub>–C<sub>20</sub>) on DB-5 column

### References

VALIDATION OF MONOMERIC ANTHOCYANIN DETERMINATION METHOD FOR BILBERRY JUICE AND MARC EXTRACTS

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Abstract
The solid waste generated in industrial berry juice production was considered as a low cost raw material for the extraction of natural antioxidants. Berries contain phenolic compounds with high antioxidant potential, including anthocyanin. Quantitative determination method for monomeric anthocyanins in bilberry juice and marc was validated. An official method from Association of Analytical Communities was used to determine anthocyanins in juice and marc extracts by measuring light absorption for solutions with pH values 1.0 and 4.5 at 520 nm and 700 nm. Results were expressed as cyanidin-3-glucoside equivalent, as it is the most common anthocyanin pigment. Calibration curve was obtained, linearity was checked, working interval and accuracy was determined and samples were tested. Mentioned method was evaluated as appropriate for quantitative anthocyanin analysis in bilberry juice and marc. Necessity of calibration curve was approved using extinction coefficient of cyanidin-3-glucoside instead. Method assures adequate precision and accuracy as well.

Key words: natural antioxidants, monomeric anthocyanins, bilberry juice, bilberry marc

Introduction
Anthocyanin pigments are responsible for the red, purple and blue colours of many fruits, vegetables, cereal grains, and flowers. Food scientists study these compounds because of their obvious importance to the colour quality of fresh and processed fruits and vegetables. Interest in anthocyanin pigments has intensified because of their possible health benefits as dietary antioxidants. Over 300 structurally distinct anthocyanins have been identified in nature (Wrolstad, 2001). Anthocyanins are one class of flavonoid compounds, which are widely distributed plant polyphenols. Flavonols, flavan-3-ols, flavones, flavanones, and flavanonols are additional classes of flavonoids that differ in their oxidation state from the anthocyanins. Anthocyanin is a glycoside composed of an aglycon named anthocyanidin and a sugar residue. The six most widespread anthocyanidins are cyanidin, delphinidin, peonidin, pelargonidin, petunidin and malvidin in decreasing order of occurrence. Anthocyanidins occur most commonly as O-glycosides. When anthocyanidins are coupled to sugars, anthocyanins are formed. The possible sugar residues are D-glucose, D-galactose, L-rhamnose, L-arabinose and D-xylose. These five sugars are involved in the formation of monoglycosides, in which a sugar residue almost always is located at the hydroxyl group of C-3. Diglycosides may be formed when another sugar is linked to monoglycoside at C-3 or to another hydroxyl group at C-5. More rare triglycosides may be formed similarly from diglicosides. Three linked sugars in a side chain may form linear or branched structures. Acylated anthocyanins are typically found in some plant foods such as blueberry, red onion and potato. Acylation by phenolic acid appears to be related to the stabilization of anthocyanins in the acid environment of the cell sap (Riihinen, 2005). As sugars can be coupled at different places and many different sugars are present in plants, it is clear that a very large range of anthocyanins can be formed (Food-info, 2011). While there are six common anthocyanidins, more than 540 anthocyanin pigments have been identified in nature (Anderson, Francis, 2004), with most of the structural variation coming from glycosidic substitution at the 3 and 5 positions and possible acylation of sugar residues with organic acids. Anthocyanins lend themselves to systematic identification as the component anthocyanidins, sugars and acylating acids can be liberated by acid hydrolysis and subsequently identified by chromatographic procedures. Saponification of acylated anthocyanins will produce the anthocyanin glycosides and acylating acids for subsequent identification. These methods are described in several publications (Durst, Wrolstad, 2001; Hong, Wrolstad, 1990; Wrolstad et al., 2002). Electrospray (ES-MS), tandem (MS/MS), and liquid chromatography mass spectroscopy (LC-MS) are powerful
techniques for identifying anthocyanins from their discrete mass units and fragment ions (Giusti et al., 1999; Wang et al., 2003). For more complete identification, NMR can be used for sugar identification and determining the position of sugar attachment and angle of the glycosidic linkages (Giusti et al., 1998; Anderson, Fossen, 2003). Anthocyanins reversibly change colour with pH, which limits their effective use as food colourants for many applications, but also provides an easy and convenient method for measuring total pigment concentration (Giusti, Wrolstad, 2001). The described method is a modification of methods originally described by Fuleki and Francis (1968). Samples are diluted with aqueous pH 1.0 and 4.5 buffers and absorption measurements are taken at the wavelength of maximum absorption of the pH 1.0 solution. The difference in absorption between the two buffer solutions is due to the monomeric anthocyanin pigments. Polymerized anthocyanin pigments and nonenzymic browning pigments do not exhibit reversible behavior with pH, and are thus excluded from the absorption calculation. It is customary to calculate total anthocyanins using the molecular weight and molar extinction coefficient of the major anthocyanin in the sample matrix. The number of anthocyanins for which molecular extinction coefficients have been determined is limited, however (Giusti, Wrolstad, 2001). When using this procedure, extinction coefficients that have been determined in aqueous solutions should be used rather than those determined in acidic ethanol or methanol because of solvent effects (Lee et al., 2005). There is a need for a standardized method for determining total anthocyanins in commerce, since products are being marketed on the basis of their pigment content (Wrolstad et al., 2005). Association of Analytical Communities is published this pH differential method as official anthocyanin identification method called by Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colourants and Wines. The principle is that monomeric anthocyanin pigments reversibly change colour with a change in pH; the coloured oxonium form exists at pH 1.0, and the colourless hemiketal form predominates at pH 4.5. The difference in the absorption of the pigments at 520 nm is proportional to the pigment concentration. Results are expressed on a cyanidin-3-glucoside basis. Degraded anthocyanins in the polymeric form are resistant to colour change regardless of pH and are not included in the measurements because they absorb at pH 4.5 as well as pH 1.0 (AOAC, 2005).

### Table 1

<table>
<thead>
<tr>
<th>Subclass</th>
<th>Flavonoid</th>
<th>mg 100g⁻¹ edible portion</th>
<th>Min</th>
<th>Max</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthocyanidins</strong></td>
<td>Cyanidin</td>
<td>15.02</td>
<td>4.79</td>
<td>28.72</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Delphinidin</td>
<td>29.54</td>
<td>20.82</td>
<td>47.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Malvidin</td>
<td>49.21</td>
<td>32.95</td>
<td>69.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Peonidin</td>
<td>7.05</td>
<td>1.01</td>
<td>19.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Petunidin</td>
<td>11.73</td>
<td>7.19</td>
<td>18.25</td>
<td></td>
</tr>
<tr>
<td><strong>Flavan-3-ols</strong></td>
<td>(-)-Epicatechin</td>
<td>1.11</td>
<td>1.11</td>
<td>1.11</td>
<td>4</td>
</tr>
<tr>
<td><strong>Flavonols</strong></td>
<td>Myricetin</td>
<td>0.82</td>
<td>0</td>
<td>2.60</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Quercetin</td>
<td>3.11</td>
<td>1.70</td>
<td>7.30</td>
<td>7</td>
</tr>
</tbody>
</table>
Bilberries are one of the raw materials for dietary supplement production in medical company Silvanols Ltd. therefore it is necessary to define quality requirements for bilberry juice and marc. Juice is one of the components for dietary supplement production. For now berry marc is a waste with usage potential in the nearest future. During the storage and recycling processes anthocyanins decrease, so it is necessary to determine anthocyanin content in both – juice and marc, but not in the berries before juice squeezing.

There is Table 1 given below in order to approximately comprehend anthocyanin content in bilberry juice and marc. The USDA Database for Flavonoids was created as a response to the interest of scientific community in types of flavonoid compounds and their varied biological properties. According to their data bilberries contain anthocyanidins in significant quantities.

Materials and Methods

The validation of monomeric anthocyanin determination method was carried out in Silvanols Ltd. company in Riga, Latvia, 2010. The object of the validation was bilberry (Vaccinium myrtillus) juice and marc. Juice was obtained from previously frozen and thawed berries picked in the woods of Preili region in 2010. After the juice squeezing process berry marc was dried in hot air dryer at 70 °C until marc moisture was <8% and grounded for 25–30 seconds in blender grinder. Then marc extracts in ethanol/water solution were prepared. Extraction kinetics study was made with 6 samples prepared from 0.1–0.12 g bilberry marc, 10 ml water and 20 ml ethanol. The samples were then extracted for 15, 30, 60, 90, 120 and 180 min on magnetic stirrer. After extraction samples were filtered with water-jet vacuum pump, residues were washed twice with purified water; filtrate was quantitatively transferred to 100 ml flask and refilled with purified water to the mark. For further experiments 4 ml extract was taken and transferred to 25 ml flask which was refilled with buffer solutions – with potassium chloride buffer to obtain pH 1.0, but sodium acetate buffer to obtain pH 4.5.

To determine other parameters in validation process – linearity, working range, accuracy, precision, repeatability and robustness – samples were prepared by the same method, but the extraction time was 2 hours constantly. There was Cyanidin-3-glucoside chloride 99.58% used as a reference material to obtain cyanidin-3-glucoside spectrum and calibration curve and also in accuracy test.

The pH differential method – AOAC Official Method 2005.02 (mentioned above) which is applicable to the determination of monomeric anthocyanins in fruit juices, beverages, natural colorants, and wines within the range of 20–3000 mg L⁻¹ as cyanidin-3-glucoside equivalents was the selected method for monomeric anthocyanin determination.

All absorption measurements were done by UV/VIS spectrophotometer Lambda 25 by Perkin Elmer, wavelength precision ±0.1 nm, bandwidth 1 nm. All experiments were made in triplicate and mean values with standard deviations are reported. A linear correlation analysis was performed with the software SPSS 14.00 for Windows in order to evaluate cyanidin-3-O-glucoside calibration curve.

Results and Discussion

Cyanidin-3-O-glucoside chloride solution at pH 1.0 has orange-red colour with absorption maximum at 520 nm, but at pH 4.5 this solution is colourless with no absorption.

Bilberry juice and marc extract solutions are orange-red at pH 1.0 with absorption maximum at 520 nm, but at pH 4.5 these solutions are in light blue colour with absorption maximum at 500–550 nm. Obtained cyanidin-3-O-glucoside calibration curve is showed in Figure 1. Using four point calibrations the correlation coefficient is 0.9999.

Monomeric anthocyanin extraction kinetics indicated in Figure 2. As we see monomeric anthocyanin extraction from bilberry marc is complete in 90 minutes, however, the recommended extraction time is 2 h.
Figure 1. Cyanidin-3-O-glucoside calibration curve

Figure 2. Monomeric anthocyanin extraction kinetics from bilberry marc

Determined working and linear ranges approved that there is no necessary to obtain calibration curve to detect monomeric anthocyanins in bilberry marc and also in the juice. Calculations instead can be done by using cyanidin-3-O-glucoside molar extinction coefficient, because there is no difference in results as represented in Table 2. Recommended bilberry marc volume for analysis is 0.1–0.12 g, but juice volume – 0.5 ml according to selected method.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>From calibration curve</th>
<th>With molar extinction coefficient</th>
<th>From calibration curve</th>
<th>With molar extinction coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bilberry juice</td>
<td>Bilberry marc</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>513.31 mg l⁻¹</td>
<td>512.98 mg l⁻¹</td>
<td>50.59 mg g⁻¹</td>
<td>50.39 mg g⁻¹</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.4823 mg l⁻¹</td>
<td>0.3801 mg l⁻¹</td>
<td>0.6912 mg g⁻¹</td>
<td>0.6848 mg g⁻¹</td>
</tr>
<tr>
<td>Relative standard deviation</td>
<td>0.09</td>
<td>0.07</td>
<td>1.37</td>
<td>1.36</td>
</tr>
</tbody>
</table>

Experimental data represent very high correlation between results: juice samples from 0.2–0.8 ml indicate correlation coefficient R=0.9999, but marc samples from 0.6–0.14 g – R=0.9998. Experimental data also represent a very high accuracy percentage for both analytes – juice and marc, in both cases it was close to 100%.

Precision and repeatability also was determined. Precision was characterized by maximal relative standard deviation, which was 0.30% for juice sample measurements and 1.88% for
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marc extract measurements, but repeatability was also expressed with standard deviation, that was 0.27% for juice sample measurements and 1.80% for marc extract measurements. Method robustness measurements indicated that the method precision was not exceeded in both cases.

Conclusions
1. Validation process approved that the method is suitable for monomeric anthocyanin determination in bilberry juice and marc extracts.
2. It was approved that it is not necessary to obtain calibration curve to calculate test results as it is enough to use cyanidin-3-O-glucoside molar extinction coefficient for calculations in both cases – juice and marc extracts.
3. Selected method provided adequate precision for monomeric anthocyanin content determination. Maximum relative standard deviation does not exceed 1.88% for marc extract and 0.30% for juice measurements.

References
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EXTRACTS OF JAPANESE QUINCE SEEDS – POTENTIAL SOURCE OF ANTIOXIDANTS

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Abstract

Japanese quince (Chaenomeles japonica) is a minor fruit crop in Latvia and Lithuania; it is used for production of juice, aroma and fruit fibers. The seeds are by-products of food processing that could be used further for different purposes. The seeds of Japanese quince contain about 10 to 20% of oil. The composition of this oil is quite unique: nearly 90% of it is formed by two fatty acids - linoleic (52.4%) and oleic (35.6%). We have also found out that the extracts of Japanese quince seeds can be used to improve stability of vegetable oils – 10 wt-% additive of ground seeds to hempseed oil and 5 wt-% additive to rapeseed oil can increase the oxidative stability of these oils about 2.0 and 1.6 times, respectively. Unfortunately, the seeds of Japanese quince contain also amygdalin – toxic cianogenic glycoside. Due to this compound the usage of seeds of Japanese quince are very limited, especially in case of their hydrophilic extracts. Our research was focused on hydrophilic extracts of seeds in order to find out both the best method to prepare polyphenols rich extracts, as well as to determine the amount of toxic amygdalin in the ethanol/water extracts of seeds and in the extracted seeds. We have found out that the largest amount of total polyphenols can be obtained when whole seeds are extracted with the mixture of ethanol and water under reflux.

Key words: Japanese quince, seeds, antioxidants, polyphenols, amygdalin

Introduction

Japanese quince (Chaenomeles japonica) is wide spread in Latvia. The chemical composition of the fruit has been studied. A few studies are devoted to the composition of carbohydrates (Barcelo, 2000), pectines (Thomas, 2003), polyphenols (Wojdyło, 2008), organic acids (Ruisa, 1996) and volatile compounds (Jordán, 2003).

Only a few studies are dedicated to the composition of the seeds of Japanese quince. Most of these studies describe fatty acid composition (Gora, 1979; Ruisa, 1996; Granados, 2003; Дейнека, 2005; Mierina, 2009). The compositions of triglycerides (Deineka, 2004), phospholipids and other compounds containing phosphorus (Gora, 1979; Mukhamedova, 1979), as well as phytosterols and α-tocopherol (Gora, 1979) have been analyzed. There are some authors, who detected cyanogenic hydrogen (Ruisa, 1996) and benzaldehyde (Granados, 2003) – degradation products of amygdaline – in the seeds of Japanese quince. Amygdalin itself has been identified in seeds of Japanese quince by us (Moskaluka, 2010). This paper is focussed on the characterization of total polyphenol content in seeds of Japanese quince and detection of the amount of cyanogenic compounds in the extracts and extracted seeds.

Materials and Methods

Japanese quince seeds

Fruits of Japanese quince were cut and seeds and pulp were separated. In order to remove damaged seeds, they were washed with water; after that seeds were air-dried at 40±2 °C with forced air circulation (oven Orakas). The water content of the seeds was 5.74%. The seeds were packed under vacuum for 2 kg in bags made of polypropylene; they were stored at 18±2 °C in dark until further experiments.

Preparation of the extracts of Japanese quince seeds

The Japanese quince seed oil was obtained (see table 1) from seeds (cut in half or finely ground in coffee grinder and sieved by particle size d<0.069 mm) by extraction with petroleum ether or mixture of CHCl₃:MeOH (2:1) under reflux or by mixing at room
temperature (RT). Solvent was removed by vacuum filtration and the extract concentrated by rotary evaporation. Deoiled seed meal was air dried for further analysis. Hydrophilic extracts of seeds (whole or ground (d<0.069 mm)) were prepared by extraction with ethanol, water or water:ethanol mixture (1:1:v:v) under reflux (method A, B or C, respectively) or at room temperature (temperature about 16 °C) (method D, E or F, respectively). The ratio “seed:solvent” was 1:5 (g:ml). Duration of extraction was varied from 0.5 to 24 hrs. Mixtures were drained; extracts and air dried seed residues were used for further analysis.

Determination of polyphenol content
Total amount of polyphenols (TAP) was determined according to modified method (Singleton, 1999); amount of polyphenols was expressed as GAE (mg 100g⁻¹) – mg of gallic acid equivalents per 100 g of Japanese quince seeds.

Determination of total amount of cyanogenic compounds
Total amount of cyanogenic compounds was determined by argentometry (Fend, 2003). In order to determine amount of cyanogenic compounds in extracts, 20 ml of extract was refluxed, the vapour was collected in a flask containing 20 ml 2.5% NaOH solution; 8 ml 6 M NH₄OH solution and 2 ml of 5% KI solution was added to the distillate, followed by titration with 0.02 M AgNO₃ solution.

In order to determine the amount of cyanogenic compounds in deoiled seed meal or seed residue, 2 g of seed material was macerated in 20 ml H₂O for 2 hrs at room temperature. The obtained extract was further used as described previously. The amount of cyanogenic compounds was expressed as HCNeq (mg kg⁻¹) – mg of HCN equivalents per 1 kg of Japanese quince seeds.

Chromatographic analyses
HPLC analysis was carried out with Agilent Technologies 1200 Series chromatograph, equipped with UV detector (wave length 210 and 260 nm). Merck Spherisorb SB C18 reverse phase column (4×150 mm, 5 µm) was used as a stationary phase for HPLC analysis. The column was eluted with mixture of 0.01 M KH₂PO₄ and 6% MeOH (1:15, v/v), flow rate 1 ml min⁻¹.

Fatty acid composition was analyzed with Agilent Technologies 6890 N gas chromatograph (Stránský, 2005).

Results and Discussion
The area where Japanese quince is grown in Latvia is nearly 200 ha; the yield is around 1.5 to 2 t ha⁻¹ and approximately 50–70 t of fruits of Japanese quince have been sold in year 2009. According to Granados (Granados, 2003), fruits of Japanese quince contain about 5 to 9% of seeds – so, at least 5–7 t of seeds are produced as a by-product of food manufacturing in Latvia every year. These seeds would be a valuable product. Japanese quince seeds contain about 6 to even 20% of oil (Granados, 2003; Mierina, 2009). The fatty acid composition of the seed oil is unique - nearly 90% of it is formed by two fatty acids – linoleic (52.4%) and oleic (35.6%); the minor fatty acids detected in the oil are palmitic (9.90%), stearic (0.92%), arachidic (0.55%) and linolenic (0.63%) acid. The amount of extracted oil strongly depends on conditions of extraction. We compared the impact of solvent, temperature and duration of extraction on the yield of extracted oil (see table 1). As the yield of oil reached even 20.4% (when finely ground seeds were extracted with petroleum ether at reflux), we tried to obtain oil with oil press (Taby press type 20), too. This so called cold-press method (temperature about 60 °C) gave just a few drops of oil (from 50 g of seeds). In order to improve the result, seeds were preheated before the pressing, but the yield of oil did not exceed 2%.
Table 1

<table>
<thead>
<tr>
<th>Method of extraction</th>
<th>Yield of the oil, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Petroleum ether</td>
</tr>
<tr>
<td>No. Size of seeds</td>
<td>Temperature</td>
</tr>
<tr>
<td>1 cut in half</td>
<td>Reflux</td>
</tr>
<tr>
<td>2 d&lt;0.069 mm</td>
<td>Reflux</td>
</tr>
<tr>
<td>3 d&lt;0.069 mm</td>
<td>RT</td>
</tr>
<tr>
<td>4 d&lt;0.069 mm</td>
<td>RT</td>
</tr>
</tbody>
</table>

Our previous studies show that it is possible to increase oxidative stability of vegetable (rapeseed or hempseed) oil with lipophilic (oil) extracts of Japanese quince seeds. The oxidative stability of vegetable oils was studied (Mierina, 2009) at different conditions with two methods: a) the samples were kept at accelerated oxidation conditions and the oxidative stability was monitored by peroxide values, b) Rancimat method. We used cold pressed rapeseed and hempseed oil to evaluate the antioxidant potential of the seeds of Japanese quince. Variable extracts were prepared from 1 to 20 g of seeds and 100 g oil. In order to find out the optimal concentration that provides highest oxidative stability of vegetable oil, the experiments were carried out into 3 stages; the concentration was scaled down in each next stage. We found out that the highest antioxidant activity can be reached when 5 g or 10 g of seeds were extracted with 100 g of rapeseed or hempseed oil, correspondingly; the antioxidant activity (1.13 and 2.47) of these extracts were detected according to Bandoniene (Bandoniene, 2000).

We established that seed oil (obtained with petroleum ether according method No. 2, table 1) contains 238 mg of polyphenols per 100 g of oil; this amount is equal to 35 GAE. We found out that less than 0.5% additive of Japanese quince seed oil can improve the oxidative stability of rapeseed oil.

We apprrobated different conditions of extraction in order to establish the best method both to prepare extracts rich in polyphenols and to determine the total amount of cyanogenic compounds in extracts and extracted seed material. The whole and finely ground seeds were used for extraction. We have compared six variable extraction conditions: the seeds were extracted with ethanol, water or mixture of water and ethanol (1:1) under reflux (method A, B or C, respectively) and at room temperature (method D, E or F, respectively). In order to optimize the extraction procedure, the impact of the duration of extraction on the total amount of polyphenols and cyanogenic compounds was studied.

We found out that depending from the extraction conditions the TAP varied from 1 to more than 200 GAE (fig. 1 and 2). The lowest result was obtained when whole seeds were extracted with the solvent at room temperature (method D, E and F) even within 24 hrs. When whole seeds were extracted with water, mixture of water and ethanol or ethanol, TAP was only 7 or 1 GAE; due to this whole seeds were not studied further. When ground seeds were extracted at room temperature, the content of polyphenolic compounds was not high – TAP varied from 20 to 50 GAE. The prolongation of extraction from 0.5 to 24 h did not lead to significant increase of TAP. The extraction at room temperature provided the highest amount of polyphenols and cyanogenic compounds was studied.

When the extraction of seeds was carried out under reflux, the TAP increased from 50 GAE after 0.5 h to more than 200 GAE after 4 or more hrs. TAP did not change significantly, if extraction time was short (0.5 hrs) and extraction was carried out under reflux or at room temperature; the same situation was observed for all prepared ethanol extracts at any duration of extraction. TAP was remarkably higher, when seeds were extracted with water or mixture of water and ethanol under reflux (in comparison with room temperature). TAP was similar in
case of ethanol and ethanol/water extracts, if extraction was carried out under reflux for less than 5 hrs. Nevertheless, TAP increased nearly twice in case of ethanol/water extracts in comparison with water extracts if the duration of extraction increased up to 6 or 7 h (see fig. 1a and 1b).

![Graph](image1)

**Figure 1. TAP dependence from solvent and duration of extraction of ground seeds**

(a – reflux, b – room temperature)

When whole seeds of Japanese quince were extracted under reflux, TAP strongly depended from the solvent used for extraction. The lowest TAP was determined in ethanol extracts; it varied from 8 to 16 GAE depending from duration of extraction. Notably higher TAP was in case of extracts prepared with water and ethanol/water mixture; TAP was similar for both ethanol and ethanol/water extracts, when the duration of extraction was 4 or less hours. When the duration was increased, the total amount of polyphenols was remarkably higher in the extracts prepared with mixture of ethanol and water.

![Graph](image2)

**Figure 2. TAP dependence from solvent and duration of extraction of whole seeds**

Due to the fact that seeds of Japanese quince contain cyanogenic compounds (Ruisa, 1996, Moskaluka, 2010), we have studied their content (expressed as total amount of HCN) in seeds. We already established (Moskaluka, 2010), that intact seeds contain amygdalin, but extracted seed oil (see Table 1) or mechanically pressed oil do not contain it or its degradation products (benzaldehyde, benzoic acid or mandelonitrile) (according to HPLC analysis). Now we have determined that the total amount of cyanogenic compounds in Japanese quince seeds is 690 mg kg\(^{-1}\). This amount decreased in deoiled seed meal (see Table 2). It appeared, that the seeds extracted with petroleum ether or mechanically pressed contained benzaldehyde and mandelonitrile; benzoic acid was found in the seeds extracted with CH\(_3\)Cl:MeOH.

The aim of this study was both to determine the amount of cyanogenic compounds in the prepared hydrophilic extracts and to elaborate extraction procedure for detoxification of Japanese quince seeds. The highest amount of cyanogenic compounds was determined in water extracts of Japanese quince seeds, followed by extracts prepared with mixture of ethanol and water.
The Amount of Cyanogenic Compounds in the Deoiled Seed Meal

<table>
<thead>
<tr>
<th>Method of extraction</th>
<th>Extractant petroleum ether</th>
<th>Extractant CHCl₃:MeOH (2:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HCNeq</td>
<td>Amygdalin*</td>
</tr>
<tr>
<td>1</td>
<td>590</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>110</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>320</td>
<td>+</td>
</tr>
</tbody>
</table>

* – detected with HPLC

The least amount of cyanogenic compounds was determined in ethanol extracts. The amount of these compounds varied from 270 HCNeq in extract of ground seeds prepared according to the method E (extraction time 0.5 hrs) to 5 HCNeq for the extract prepared from ground seeds according to the method D. The amount of cyanogenic compounds decreased in case of all above mentioned hydrophilic solvents if extraction was carried out under reflux, which is most likely due to enzymatic and/or thermal degradation of amygdalin (Li, 1992). The amount of cyanogenic compounds was 2 to 5 times higher in the extracts prepared at room temperature.

The cyanogenic compounds were determined also in the seed residue obtained by extraction with hydrophilic solvents. The amount of cyanogenic compounds varied from 0 to few hundreds of HCNeq. We have established that in order to detoxify the seeds the best of tested solvents was water. When the whole seeds were extracted for 7 hrs with water or ground seeds were extracted for 4 hrs with water or 6 hrs with ethanol or the mixture of ethanol and water under reflux, the amount of cyanogenic compounds were reduced to 0 HCNeq.

Conclusions
1. An additive of both Japanese quince seeds and their oil can be used to increase the oxidative stability of vegetable oils.
2. The total amount of polyphenols strongly depends on solvent, temperature and the seed particles (degree of grounding), but less - on the extraction time. The highest TAP was determined in extracts of ground seeds when extraction was carried out with ethanol or mixture of ethanol and water under reflux. High TAP was detected in extracts of whole seeds prepared by extraction with water and mixture of water and ethanol under reflux.
3. The highest amount of cyanogenic compounds was found in water extracts of seeds; it decreased with increase of extraction temperature. The prolongation of extraction can reduce the amount of cyanogenic compounds in the extract. The extraction procedure used to prepare extracts rich in polyphenols can be used to detoxify seeds, too; for this purpose the best method is extraction of ground seeds with water under reflux.

Acknowledgement
This research was supported by EUREKA project Nr E!6240 „Developement of new products from plant material for health improvement and cosmetics” and the European Social Fund within the project „Support for the implementation of doctoral studies at Riga Technical University”.

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INFLUENCE OF HARVESTING TIME ON THE YIELD AND CHEMICAL COMPOSITION OF SAGE (Salvia officinalis L.)

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Abstract
Sage (Salvia officinalis L.) was harvested at different periods from May 23 to July 11. The yield of crop depended on plant growing phase and was 3.0–10.0 of fresh and 0.5–2.7 t ha⁻¹ of dried herb. Total essential oil (EO) content increased from May 23 to June 20 and was 0.1–0.2 in fresh and 0.4–1.0 cm³ 100 g⁻¹ in dried herb. Seventy compounds were identified in sage EOs by GC and GC-MS. It is evident that S. officinalis grown in Lithuania depends to α-thujone and camphor chemotype: the content of α-thujone in EO varied in the range of 15.2–39.7%; that of β-thujone 5.3–7.9%. Other important components were 1,8-cineole, camphor, borneol, α-humulene, viridiflorol and manool. In addition, the amounts of dry soluble solids, ascorbic acid, carotenes, nitrates and total sugars were determined in fresh raw material at different growth stages.

Key words: Sage, Salvia officinalis, seasonal variation, essential oil, chemical composition, crop yield.

Introduction
Sage (Salvia officinalis L., Lamiaceae) and its products, such as EOs and oleoresins have been widely used as food flavourings and health promoting agents (Perry et al., 1999; Perry et al., 2003; Farhat et al., 2009). In Lithuania aerial parts of sage are used in herbal teas and in the mixtures of medicinal plants for healing digestion and circulation disturbances, bronchitis, angina, skin and other diseases. Sage is also a natural source of flavonoids and polyphenolic compounds possessing strong antioxidant, radical-scavenging and antibacterial activities (Dapkevičius et al., 1998, Delamare et al., 2007).

The studies of sage EO indicate that its composition may be variable (Farhat et al., 2009; Delamare et al., 2007; Marić, Maksimović, Miloš 2006; Mockutė et al., 2003; Mirjalili et al., 2006). ISO 9909 for medicinal uses regulates the amounts of the following constituents in sage EO: cis-thujone (18.0–43.0%), camphor (4.5–24.5%), 1,8-cineole (5.5–13.0%), trans-thujone (3.0–8.5%), α-humulene (≤12.0%), α-pinene (1.0–6.5%), camphene (1.5–7.0%), limonene (0.5–3.0%), bornyl acetate (≤2.5%) and linalool + linalyl acetate (≤1.0%) (Santos-Gomes, Fernandes-Ferreira, 2001; Bernotienė et al., 2007). EO composition of Salvia species depends on many factors and sometimes does not match the profile defined by the ISO 9909 (Farhat et al., 2009). Therefore, the present study was aimed at evaluating and comparing the biological and chemical properties of S. officinalis grown in Lithuania at different growth stages. The results presented in this study expand our knowledge on sage and may be practically applied in the developments of commercial cultivation of this valuable herb.

Materials and Methods
Sage (Salvia officinalis L.) was cultivated in the experimental fields of the Lithuanian Institute of Horticulture. The plants were harvested at various vegetation phases: May 23 – regrowth; June 7 – intensive vegetative growth; June 20 – butonization (just before flowering, formation of inflorescences); June 27 – full flowering; and July 11 – after flowering (in fruiting – seeds ripening). Harvested herbs were dried at 40 °C in the dark. The yield of EO was determined by hydrodistillation of 100 g herb in a Clevenger-type apparatus during 3 hr. Soluble solids were determined by refractometer method using an Abbe refractometer (AOAC, 1990a). Ascorbic acid was determined by titrimetric method using
2,6-dichloroindophenol (AOAC, 1990b). Sucrose was determined by reducing sugars before and after inversion; sugars (reducing) were determined by inversion method (AOAC, 1990c). Nitrates were determined on a potentiometer pH-150 with an ion selective electrode EM-020604 (Methodology directions, 1990). Carotenes were determined by spectrophotometric method measuring extinction at 450 nm in hexane (Scott, 2001).

The EOs diluted in pentane (10 µl in 1 ml) were analyzed on a Fisons 8000 GC equipped with a flame ionization detector and a DB-5 fused silica capillary column (50 m×0.32 mm i.d.×0.25 µm). GC–MS analyses were performed using a Perkin Elmer Clarus 500 GC coupled to a Perkin Elmer Clarus 500 series mass selective detector in the electron impact ionization mode at 70eV, the mass range was m/z 29–550 using an Elite–5MS capillary column (30 m×0.25 mm i.d. ×0.25 µm) (Baranauskiene, 2007).

All analyses were replicated four times. Data were statistically handled by one-way analysis of variance (ANOVA). Duncan’s multiple-range test was applied for the calculation of the significant differences among the harvesting time treatments of plant biochemical composition and the amounts of individual EO components (P=0.05). The effect of different growing time on the yield of sage was described by regression analysis.

**Results and discussion**

**Sage crop yield and EO content.** The crop and the quality of plant material are the most important characteristics in commercial cultivation of spices and aromatic herbs.

![Crop yield of fresh and dried sage](attachment:crop_yield.png)

**Figure 1. The yield of crop (t ha⁻¹) of sage raw material at different growth phases**

It is obvious that the crop yield of fresh harvested sage continuously increased from May 23 till June 27 from 3.0 to 10.0 t ha⁻¹ (Figure 1). The maximum peak was reached at full flowering stage and afterwards remarkably decreased after flowering at seeds ripening (June 11) to 7.0 t ha⁻¹. The effect of different growing time on the yield of fresh sage herb can be described by the third-order polynomial regression equation y=−0.3417x³+2.2176x²−1.7174x+2.9473 with a determination coefficient R² = 0.985. Sage herb may be used in its dried form or for further processing, e.g. isolation of EO, extraction. Therefore it is important to assess the yield of dried raw materials. After drying, the mass of sage decreased ~3–6 times. The similar tendency at different vegetative phases was obtained with crop yield of dried plant material 0.5→2.7→2.3 t ha⁻¹ compared to that of a fresh one (Figure 1). The output of dry sage may be described by the third-order polynomial regression equation: y=−0.1144x³+0.8857x²–1.3203x+1.0869, R²=0.992.
EO content at different harvesting time was in the range of 0.1–0.3 cm$^3$ 100 g$^{-1}$ (fresh) and 0.4–1.0 cm$^3$ 100 g$^{-1}$ (dried) (Figure 2). In another study the yield of EO (w/w %) based on the dry weight of sage from Iran was in the following order: floral budding (0.9%)>vegetative (0.7%)>flowering (0.5%)>immature fruit (0.4%)>ripen fruit (0.2%) (Mirjalili et.al., 2006). Generally, the amount of EO is at its highest level at the floral budding stage when the oil is intensively biosynthesized, and decreased gradually at the fruiting phase, as observed in other plant species.

![Figure 2. The content of EO (cm$^3$ 100 g$^{-1}$) and the yield of EO (t ha$^{-1}$) in sage raw material at different growing phases](image)

The total productivity of oil in fresh sage varied from 2.7 to 25.8 dm$^3$ ha$^{-1}$. After drying EO content slightly reduced in May 23 – June 7, and was from 2.6 to 26.8 dm$^3$ ha$^{-1}$; that indicates that the losses of volatiles during drying were not significant. The total productivity of oil from dried herb on June 20 was determined in higher amount compared to that of fresh sage and constituted 25.8 and 26.8 dm$^3$ ha$^{-1}$ in fresh and dried herb, respectively (Figure 2).

**EO composition.** The composition of sage components exceeding 1 % in EO is presented in Table 1. The major components were $\alpha$-thujone, camphor, $\alpha$-humulene, manool, 1,8-cineole, viridiflrorl, borneol and $\beta$-thujone, however, their content at different vegetative phases varied in a rather wide range. For instance, the percentage of $\alpha$-thujone from May 23 to June 20 steadily increased from 29.4 to 39.7% (fresh herb). The content of viridiflrorl also increased at the same period, while the changes of camphor, $\alpha$-humulene and manool were not so consistent. For example, from May 23 to June 7 the content of camphor significantly decreased from 12.8 to 5.3% and afterwards on June 20 again increased up to 13.7 (fresh herb). After drying it varied between 8.3% (June 7) and 17.7% (June 20). In contrary, the percentages of $\alpha$-humulene and manool significantly increased from May 23 to June 7 and afterwards on June 20 decreased more than twice. The percentages of 1,8-cineole, borneol, $\beta$-pinene were continously decreasing during the whole vegetative period, while the content of $\beta$-thujone was almost stable (5.8–6.1%). Drying of fresh sage resulted in the changes in the composition of EO, most likely due to losses of the most volatile constituents. For example, the percentage of $\alpha$-thujone significantly decreased after drying and was from 24.7 to 30.1%.

In general, it could be concluded that amounts of toxic thujones and other regulated compounds, such as camphor, 1,8-cineole, $\alpha$-humulene, camphene, $\alpha$-pinene, limonene and bornyl acetate met the requirements of ISO9909 standard.
Table 1

Variation in chemical composition of EOs of sage at different growth stages, GC peak area percentage

<table>
<thead>
<tr>
<th>Compound</th>
<th>Identification</th>
<th>May 23</th>
<th>June 7</th>
<th>June 20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>fresh</td>
<td>dried</td>
<td>fresh</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>KI, MS</td>
<td>1.93b</td>
<td>2.13bc</td>
<td>1.68a</td>
</tr>
<tr>
<td>Camphene</td>
<td>KI, MS</td>
<td>2.51c</td>
<td>1.67b</td>
<td>0.95a</td>
</tr>
<tr>
<td>β-Pinene</td>
<td>KI, MS</td>
<td>5.70d</td>
<td>2.04b</td>
<td>4.24c</td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>KI, MS</td>
<td>8.91e</td>
<td>6.58d</td>
<td>5.66b</td>
</tr>
<tr>
<td>α-Thujone</td>
<td>KI, MS</td>
<td>29.35b</td>
<td>24.67a</td>
<td>30.26b</td>
</tr>
<tr>
<td>β-Thujone</td>
<td>KI, MS</td>
<td>6.05b</td>
<td>6.50c</td>
<td>5.83b</td>
</tr>
<tr>
<td>Camphor</td>
<td>KI, MS</td>
<td>12.81d</td>
<td>9.58c</td>
<td>5.25a</td>
</tr>
<tr>
<td>Borneol</td>
<td>KI, MS</td>
<td>7.45e</td>
<td>5.39d</td>
<td>2.74b</td>
</tr>
<tr>
<td>Isobornyl acetate</td>
<td>KI, MS</td>
<td>0.81c</td>
<td>0.94d</td>
<td>0.55b</td>
</tr>
<tr>
<td>Bornyl acetate</td>
<td>KI, MS</td>
<td>0.09a</td>
<td>1.47d</td>
<td>0.32b</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>KI, MS</td>
<td>tr²a</td>
<td>2.74c</td>
<td>0.52b</td>
</tr>
<tr>
<td>β-Caryophyllene</td>
<td>KI, MS</td>
<td>2.35bc</td>
<td>2.78c</td>
<td>3.70d</td>
</tr>
<tr>
<td>α-Humulene</td>
<td>KI, MS</td>
<td>7.90b</td>
<td>12.93d</td>
<td>13.79e</td>
</tr>
<tr>
<td>Viridiflorol</td>
<td>KI, MS</td>
<td>4.37a</td>
<td>5.74b</td>
<td>7.04c</td>
</tr>
<tr>
<td>Humulene epoxide II</td>
<td>KI, MS</td>
<td>0.25a</td>
<td>1.08d</td>
<td>0.31a</td>
</tr>
<tr>
<td>Manool</td>
<td>KI, MS</td>
<td>3.81a</td>
<td>7.85b</td>
<td>10.39d</td>
</tr>
<tr>
<td>Total identified, %</td>
<td></td>
<td>99.94</td>
<td>99.13</td>
<td>98.95</td>
</tr>
<tr>
<td>RSD⁴, %</td>
<td></td>
<td>8.81</td>
<td>7.48</td>
<td>6.64</td>
</tr>
</tbody>
</table>

¹Average GC peak area percentage of four replicates. ²tr, peak area percent ≤0.04%. ³nd, not determined.
⁴%RSD, average coefficient of variance of individual compounds. a-f, Values within rows followed by the same letter do not differ statistically at p=0.05.

The concentration of the main sage EO components was also expressed in absolute units, mg kg⁻¹ (Figure 3). Significant increase in the concentration of α- and β-thujones was observed during the studied period of vegetation. In general, the amounts of α-thujone, β-thujone, α-pinene, 1,8-cineole, and viridiflorol were increasing with the increase of the total EO content. The highest amounts of α-humulene, β-caryophyllene and manool were observed on June 7 during intensive vegetative growth; while on June 20 it again decreased. After drying some losses of volatiles were observed. For example, the amount of α-thujone was from 264.2 to 1271.0 mg kg⁻¹ in fresh herb; while after drying it decreased 1.3–1.8 times and was 148.0–721.3 mg kg⁻¹. The same changes were observed for β-thujone, exception for herb harvested on June 7, when its amount in dried herb (134.1 mg kg⁻¹) was higher than that in fresh herb (110.7 mg kg⁻¹). Also, the amounts of some other components during butonization (June 20) were higher in EO from dried herb compared to the fresh one (Figure 3).

Biochemical composition of sage. The amounts of dry soluble solids, vitamin C, carotenoids, nitrates and total sugars are presented in Table 2. It was observed that carotenoids were biosynthesized more intensively at the second half of vegetation, while in the period from June 20 to June 27 their concentration was similar (P=0.05). The content of dry soluble substances and total sugars was different at various growth phases (P=0.05) and varied in the range of 6.3 and 11.3 % and 1.46–3.47 %, respectively. The concentration of vitamin C was very low (9.6 mg 100 g⁻¹) at the beginning of plant vegetation, however it increased up to 17.6 mg 100 g⁻¹ during intensive flowering phase (Table 2). The amounts of nitrates were quite high during all plants vegetation period and varied from 720 to 980 mg kg⁻¹; it continuously decreased during plant vegetation from May 23 to July 11.
Figure 3. The concentrations of individual constituents in sage at different harvesting period: F=fresh herb; D=dried herb

Table 2

<table>
<thead>
<tr>
<th>Growing time</th>
<th>Dry soluble solids, %</th>
<th>Vitamin C, mg %</th>
<th>Carotenes, mg %</th>
<th>Nitrates, mg kg⁻¹</th>
<th>Total sugars, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 23</td>
<td>6.3a</td>
<td>9.6a</td>
<td>5.5a</td>
<td>980e</td>
<td>1.46a</td>
</tr>
<tr>
<td>June 7</td>
<td>9.2b</td>
<td>15.0c</td>
<td>9.2c</td>
<td>880d</td>
<td>1.68b</td>
</tr>
<tr>
<td>June 20</td>
<td>9.9c</td>
<td>17.6d</td>
<td>11.2b</td>
<td>830c</td>
<td>2.68c</td>
</tr>
<tr>
<td>June 27</td>
<td>10.5d</td>
<td>17.0b</td>
<td>11.6b</td>
<td>760b</td>
<td>3.06d</td>
</tr>
<tr>
<td>July 11</td>
<td>11.3e</td>
<td>16.8b</td>
<td>12.8d</td>
<td>720a</td>
<td>3.47e</td>
</tr>
</tbody>
</table>

a-e, Values within columns followed by the same letter do not differ statistically at p=0.05.

It could be concluded that biochemical composition of *S. officinalis* considerably depends on plant growth phase. In the beginning of plant vegetation after winter the amounts of dry soluble solids, total sugars, vitamin C and carotenes were low, and continuously increased until the intensive flowering phase or after flowering in seed ripening. The content of nitrates was continuously decreasing during all vegetation period.
References

BIOLOGICALLY ACTIVE COMPOUNDS IN ROASTED COFFEE

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Abstract
Coffee is one of the products rich in biologically active compounds. The aim of this research work was to investigate the content of total phenols, flavonoids and caffeine in coffee available in the local markets as well as its changes depending on the brand. Eighteen coffee brands used in the research were analysed. The content of total phenols and total flavonoids was determined spectrophotometrically. The content of caffeine and polyphenols (gallic acid, catechin, caffeic acid, vanillin, chlorogenic acid, epicatechin and ferulic acid) were determined by high performance liquid chromatography. Total phenols in analysed coffee samples ranged from 1300 to 3700 mg gallic acid equivalents (GAE) 100 g⁻¹ coffee, total flavonoids – from 15 to 103 mg quercetin equivalents (QE) 100 g⁻¹ coffee. The content of polyphenols varied in a wide range too: gallic acid from 2.5 mg to 80 mg 100 g⁻¹, catechin from 30 to 80 mg 100 g⁻¹, caffeic acid from 1200 to 2500 mg 100 g⁻¹, vanillin from 100 to 150 mg 100 g⁻¹, chlorogenic acid from 1.4 to 2.8 g 100 g⁻¹, epicatechin from 11 to 30 mg 100 g⁻¹, and ferulic acid from 23 till 120 mg 100 g⁻¹ coffee. The content of caffeine ranged from 0.7 till 1.5 g 100 g⁻¹ coffee. The highest content of caffeine, catechin, caffeic acid and ferulic acid was detected in coffee samples with a higher proportion of the Robusta coffee variety. Total phenols and total flavonoids content did not vary significantly between coffee varieties.

Key words: Coffee, polyphenols, coffee substitutes

Introduction
Phenols of plant origin are one of the most significant primary antioxidants. Coffee is a product rich in these compounds. Evergreen coffee trees are grown in the equatorial region in more than 50 countries of Asia, Africa, America, and the Caribbean region. From about 70 coffee tree types only two have commercial value. They are Arabica (Coffea arabica) – which accounts for 75% and Robusta (Coffea canephora) accounting for 25% of the world’s consumption. The coffee bean is a pit of a berry; it is a seed from the coffee tree. Coffee which is made from Robusta is more concentrated, (the content of caffeine in Robusta beans reaches 4–5%) characterised by its bitterness and is less aromatic. Coffee from Arabica has a much more sophisticated flavour and intense complex aroma resembling those of flowers, fruit, honey and chocolate. Arabica contains more aromatic oils, but it has two times less caffeine than Robusta variety coffee beans. The world’s best coffees are produced only from 100% Arabica coffee beans and therefore are much more expensive than blends containing Robusta coffee beans (Jansen, 2006).

In order to produce the specific and desirable aroma, the consumer is offered not one pure coffee variety but a blend of different varieties. One of the most popular varieties – Arabica and Robusta, mixed in different proportions, offer a wide range of tastes and aromas of coffee beverages.

Coffee roasting is one of the most important stages in the process of turning the beans into a beverage. The chemical changes that take place during the process of roasting are very complex and they have not yet been sufficiently researched (Eggers et al., 2002). During the roasting process at temperatures of 200–300 ºC the most important heat and chemical reactions take place which create a pleasant flavour and aroma of the roasted coffee – the beans change colour and they increase in volume by 50–80%, simultaneously losing 13–20% from its mass at the expense of moisture. Green unroasted coffee beans contain about 250 different compounds, whereas roasted coffee beans have about 655–800 compounds influencing the flavour. Thus from the same coffee beans it is possible to obtain completely different flavour characteristics during the process of roasting.
The main component in coffee that creates a tonic effect is caffeine (Fig. 1.) – a stimulant of the central nervous system.

![Figure 1. Structural formula for Caffeine](image)

From the compounds present in coffee not only caffeine but also different compounds of phenols have a significant role. Phenolic compounds belong to different classes: phenolic acid and its derivatives, flavonoids (Belitz et al., 2008).

Unprocessed coffee beans contain caffeine, chlorogenic acid, caffeic acid (Fig. 2), proteins, lipids, mineral salts and other substances. The content depends on the coffee variety, harvesting and processing methods and also from climatic conditions.

From acids, chlorogenic acid is most represented, accounting for 4.5–11.1% from the dry weight in unroasted coffee. In the medium roasting process chlorogenic acid content decreases by 30%, but after strong roasting it decreases by 70%. Chlorogenic acid exists in the form of several isomers and forms a hydrophobic complex of a proportion 1:1. Coffee substitutes, however, contain about 1% caffeine and about 65% chlorogenic acid (Jansen, 2006).

![Figure 2. The main acids in coffee](image)

The aim of this research work was to investigate the content of total phenols, flavonoids and caffeine in coffee available in the local markets as well as its changes depending on the brand.

### Materials and Methods

Ten samples of coffee from the beans of known origin roasted in Latvia were selected for this research; the six most popular coffee brands available in retailing (two identical Merild coffee brands), in the processing of which the origin of the beans is unknown, and also two coffee substitutes. The names of the coffee brands used in the research are presented in Table 1.

The total phenolic compounds were determined by using the Folin-Ciocalteu method. Each coffee sample (0.5 g) was extracted in 50 ml distilled water 30 min. and filtered. This solution was then mixed with 0.2 N Folin-Ciocalteu reagent for 5 min and then a solution of sodium carbonate was added. After incubating at room temperature for 2 h, the absorbance of the reaction mixture was measured at 760 nm against a methanol blank. Gallic acid was used as standard to produce a calibration curve. The total phenolic content was expressed in mg of gallic acid equivalents (GAE) 100 g\(^{-1}\) of coffee (Kaškoniene et al., 2009; Meda et al., 2005; Marinova et al., 2005).
Table 1

<table>
<thead>
<tr>
<th>Legends of samples</th>
<th>Coffee brands</th>
<th>Legends of samples</th>
<th>Coffee brands</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Robusta Smile Tiger / Vietnam (Coffea canephora)</td>
<td>10</td>
<td>Exclusive (Coffee arabica) mixed selection</td>
</tr>
<tr>
<td>2</td>
<td>India Monsoon Robusta (Coffea canephora)</td>
<td>11</td>
<td>Merild–1</td>
</tr>
<tr>
<td>3</td>
<td>India Robusta Parchment (Coffea canephora)</td>
<td>12</td>
<td>Merild–2</td>
</tr>
<tr>
<td>4</td>
<td>Brazil Santos (Coffea canephora)</td>
<td>13</td>
<td>Double Coffee</td>
</tr>
<tr>
<td>5</td>
<td>Mexico Altura (Coffea canephora)</td>
<td>14</td>
<td>Tchibo</td>
</tr>
<tr>
<td>6</td>
<td>PNG A/X Plantation / Papua New Guinea (Coffea arabica)</td>
<td>15</td>
<td>Pauling</td>
</tr>
<tr>
<td>7</td>
<td>Nicaragua (Coffea arabica)</td>
<td>16</td>
<td>Jacobs espresso</td>
</tr>
<tr>
<td>8</td>
<td>Ethiopia Sidamo (Coffea arabica)</td>
<td>17</td>
<td>Chicory, barley coffee</td>
</tr>
<tr>
<td>9</td>
<td>Indonesia Sumatra lintong (Coffea arabica)</td>
<td>18</td>
<td>Barley – Chicory – acorn coffee</td>
</tr>
</tbody>
</table>

The total flavonoid content (expressed as quercetine equivalent, mg QE 100 g\(^{-1}\)) was determined using the Dowd method. 2% AlCl\(_3\) solution in methanol was mixed with the coffee extract solution. Absorbance readings at 415 nm were taken after 10 min against a blank sample consisting of a 5 ml coffee solution with 5 ml methanol without AlCl\(_3\). The total flavonoid content was determined using a standard curve with quercetin as a standard. The total flavonoid content was expressed in mg of quercetin equivalents (QE) 100 g\(^{-1}\) of coffee (Kaškonienė et al., 2009; Meda et al., 2005; Marinova et al., 2005).

The caffeine content in coffee was determined by HPLC using Shimadzu LC 20 Prominence chromatographer.

**Determination parameters:**
- Detector: DAD SPD – M20A; Column: Perkin Elmer C18, 4.6x250 mm, 5µm; Temperature of the column and detector: 35 °C; Mobile phase: A – acetonitrile; B – 10 mM KH\(_2\)PO\(_4\) (pH = 3.5). 15:85; Gradient regime; Capacity of the injection sample: 10 µl; Total time of the analysis: up to 10 minutes; Rate of the flow: 1.3 ml·min\(^{-1}\).

For determining chromatographic polyphenols the following determination parameters were used.
- Detector: DAD SPD – M20A; Column: Perkin Elmer C18, 4.6x250 mm, 5µm; Temperature of the column and detector: 30°C; Mobile phase: A – methanol un B – acetic acid (100%) : distilled water (2:98); Gradient regime: A:B (20:80); Capacity of the injection sample: 10 µl; Total time of the analysis: up to 30 minutes; Rate of the flow: 1.3 ml·min\(^{-1}\); Wavelength: 278 nm.

**Results and Discussion**

The total phenols content in the analysed samples is characterised in Figure 3. It should be concluded from the data presented in Figure 3, that from the known coffee brands the coffee brand Brasil Santos has a comparatively lower content of polyphenols. Double Coffee has the highest content of polyphenols. Coffee substitutes (Chicory, barley coffee and Barley – Chicory – acorn coffee) have the lowest content of polyphenols.
To fully characterise total phenols content, the total flavonoids content was determined, which is shown in Figure 4.

Tchibo has the highest content of flavonoids. Similarly to total phenols content the total flavonoids content is the lowest in Chicory, barley coffee and Barley - Chicory - acorn coffee. Brasil Santos coffee brand is an exception. The content of flavonoids is even lower there. The content of several phenolic compounds in different coffee brands is presented in Table 2.

**Table 2**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Gallic acid, mg 100g⁻¹</th>
<th>Catechin, mg 100g⁻¹</th>
<th>Caffeic acid, 10³mg 100g⁻¹</th>
<th>Vaniline, mg 100g⁻¹</th>
<th>Chlorogenic acid, 10³mg 100g⁻¹</th>
<th>Epicatechin, mg 100g⁻¹</th>
<th>Ferulic acid, mg 100g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>78.97</td>
<td>68.15</td>
<td>23.24</td>
<td>146.85</td>
<td>2.73</td>
<td>28.55</td>
<td>120.72</td>
</tr>
<tr>
<td>2</td>
<td>4.68</td>
<td>76.05</td>
<td>23.08</td>
<td>99.03</td>
<td>1.75</td>
<td>21.04</td>
<td>63.42</td>
</tr>
<tr>
<td>3</td>
<td>3.86</td>
<td>83.13</td>
<td>24.90</td>
<td>140.96</td>
<td>2.04</td>
<td>19.42</td>
<td>83.95</td>
</tr>
</tbody>
</table>
As shown in Table 2, the content of different polyphenols in the analysed coffee samples fluctuates within quite a large range. An increased content of gallic acid, epicatechin and ferulic acid is observed in the coffee brand Robusta Smile Tiger. The results of chromatographic analyses show that the coffee brand Brazil Santos has a reduced content of polyphenols catechin and Ferulic acid. In untraditional coffee brands a reduced amount of almost all phenols has been observed, which coincides with the results of the previous analyses.

The results of caffeine determination are shown in Figure 5.

As shown in Table 2, the content of different polyphenols in the analysed coffee samples fluctuates within quite a large range. An increased content of gallic acid, epicatechin and ferulic acid is observed in the coffee brand Robusta Smile Tiger. The results of chromatographic analyses show that the coffee brand Brazil Santos has a reduced content of polyphenols catechin and Ferulic acid. In untraditional coffee brands a reduced amount of almost all phenols has been observed, which coincides with the results of the previous analyses.

The results of caffeine determination are shown in Figure 5.

**Figure 5. Content of caffeine in coffee samples**

Increased content of caffeine has been found in the coffee brands Monsooned and India Robusta Parchment Robusta. In coffee substitute’s chicory – barley and chicory – barley – acorn coffee the content of caffeine is rather low.
Conclusions
1. In analysed coffee brands the content of polyphenols varies over a large range.
2. Double Coffee contains the most of total phenols, whereas Tchibo has the highest content of total flavonoids. Robusta Smile Tiger coffee has the highest content of gallic acid, epicatechin and ferulic acid, while Brazil Santos coffee brand has the lowest content of polyphenols.
3. From Robusta variety coffees the highest content of caffeine was found in the brand India Monsooned, but from Arabica variety coffees the highest content of caffeine was found in Merild–2 and Double Coffee samples.
4. Merild–1 and Merild–2 samples differ more than two times in the content of gallic acid, epicatechin and ferulic acid, which indicates that different raw materials were used in the processing of different Merild coffee batches.
5. Coffee substitutes have both a very low content of caffeine and a lower content of antioxidants compared to regular coffee.

References
BIOACTIVE COMPOUNDS IN LATVIAN WILD EDIBLE MUSHROOM BOLETTUS EDULIS

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Abstract
Considering the interest for mushrooms and the demand to search for natural antioxidants and other sources of bioactive-compounds, the aim of this study was to investigate the content of bioactive compounds of two widely used wild edible mushrooms Boletus edulis f. beticola and Boletus edulis f. pinicola collected at Jelgava and Riga regions in Latvia. Ash amount was determined to characterize the mineral content; protein was determined by Lowry method (325–526 mg g⁻¹ of dried mushrooms). Using HPLC the phenolic compounds like gallic acid, caffeic acid, catechin, epicatechin and rutin were detected and quantified. β-carotene and lycopene were determined. DPPH assay was used to evaluate free radical-scavenging activity. In water extracts titratable acidity (0.22–0.26 mmol of NaOH per g of dry mushroom matter) and formol number (0.74–1.40 mmol NaOH per g of dry mushroom matter) were determined. The total content of phenols (TP) as determined by Folin-Ciocalteu assay was higher in the water extracts (11.2–12.5 mg of gallic acid equivalents GAE per 1 g of dry mushroom matter) than in methanol extracts (7.3–8.0 mg of GAE per 1 g of dry mushroom matter). The total content of flavonoids (TF) was higher in the water extracts (0.33–0.37 mg of quercetin equivalents QE per 1 g of dry mushroom matter) than in methanol extracts (0.13 mg of quercetin equivalents QE per 1 g of dry mushroom matter).

Keywords: wild edible mushrooms, bioactive compounds

Introduction
Mushrooms have been widely known and used as a source of food from ancient time. Many species of mushrooms are used also as medicine (Barros et al., 2007a, Dembtsky et al., 2010). Mushrooms are very appreciated, not only for their texture, flavour, but also for their nutritional properties. Mushrooms have been demonstrated antitumor, antifungal, antibacterial activities (Chirinang et al., 2009) and are useful in preventing diseases such as hypertension, hypercholesterolemia, atherosclerosis and cancer (Ribeiro et al., 2006).
The mushrooms showed antioxidant, free radical-scavenging activity (Vidovic et al., 2010). These functional characteristics are mainly due to their chemical composition (Bernas et al., 2006). Boletus mushrooms are valuable food source, low in calories, lipids and high in vegetable proteins, minerals and vitamins. A study of the amino acid composition has showed Boletus edulis to have the highest total amino acid content of all the mushrooms tested. The major amino acids found were glutamine, alanine, glycine, serine, proline (Ribeiro et al., 2008). The major saturated fatty acid was 16:0, the major unsaturated fatty acid was 18:1(9) (Dembitsky et al., 2010). Boletus edulis presents a qualitative profile composed by oxalic, aconitic, citric, malic, quinic, fumaric acids. The major acids are malic and quinic acids (Ribeiro et al., 2006). Mushrooms are characterized by a high level of well assimilable mineral constituents. Potassium and phosphorus compounds were most abundant. Boletus edulis contains appreciable amount of selenium. Mushrooms are an important source of vitamins. The vitamins of group B are abundant, particularly thiamine, riboflavin, pyridoxine, pantothenic acid, niacin, folic acid, cobalamin, as well as other vitamins, such as phyllochinon, tocopherols, ergosterol (Bernas et al., 2006). Mushrooms have powerful antioxidant properties derived from compounds such as selenium, ergothioneine, phenols (Vidovic et al., 2010).
Considering the interest for mushrooms and the demand to search sources of bioactive-compounds, the aim of this study was to investigate the bioactive compounds content of two widely used Latvian wild edible mushrooms – king boletus Boletus edulis f. beticola and Boletus edulis f. pinicola.
Materials and Methods

Samples of *Boletus edulis* f. *beticola* and *Boletus edulis* f. *pinicola* were collected at Jelgava and Riga regions in Latvia in late summer 2010. After collection, the mushrooms were freeze-dried in order to obtain dry matter (Christ Freeze Dryer Alpha 1-2 LD plus). All dried mushroom samples were grounded in a blender and then stored in air-tight bags at the room temperature. Gravimetric method for the determination of ash amount was used as described by Mortensen et al. (1989). The content of proteins in mushroom dry matter was determined by Lowry procedure.

Mushroom powder sample (1 g) was extracted with 50 ml of methanol at 25 °C for 24 h. Water extract was prepared as follows: 1 g of powdered mushroom was boiled in 50 ml of water for 30 min. The mixture was centrifuged (3000×g, room temperature for 10 min), and supernatant portioned and kept frozen at -23 °C until analysis (Ribeiro et al., 2006, Barros et al., 2007a).

For all spectrophotometrical analysis Jenway 6405 UV/Vis. spectrophotometer were used. The total content of phenol compounds in water and methanol extracts was determined by Folin-Ciocalteu assay. Gallic acid (0-0.75 mg ml⁻¹) was used as a standard to produce the standard curve. The absorbance of the reaction mixture was measured at 765 nm. The total content of phenol compounds was expressed as milligrams of gallic acid equivalents (GAE) per gram of mushroom dry matter (Barros et al., 2007a).

The total content of flavonoids (TF) in water and methanol extracts was determined as described previously (Jia et al., 1999, Barros et al., 2007a, Robaszkiewicz et al., 2010). The absorbance of the supernatant was read at 515 nm against a blank. Quercetin (0-0.4 mg ml⁻¹) was used as a standard. The results were expressed as milligrams of quercetin equivalents QE per gram of mushroom dry matter. To determine polyphenol compounds HPLC analysis (Shimadzu LC-20 prominence) of the extracts was performed as described by Vidovic et al. (2010).

The concentration of the content of β-carotene and lycopene was determined spectrophotometrically. Obtained methanol extract was evaporated and 100 mg of the remaining dry matter were stirred with 10 ml of acetone-hexane mixture and filtered. The absorbance was measured at 453, 505, 645 and 663 nm. The content of β-carotene and lycopene was calculated according to the following equations (Barros et al., 2007b):

\[
\text{Lycopene}(mg \cdot 100ml^{-1}) = -0.0458A_{663} + 0.372A_{505} - 0.0806A_{453} \quad (1)
\]

\[
\beta - \text{carotene}(mg \cdot 100ml^{-1}) = 0.216A_{663} - 0.304A_{505} + 0.452A_{453} \quad (2)
\]

The results were expressed as milligrams of carotenoid per gram of dry matter.

The free radical scavenging activity of mushrooms in water and methanol extracts was determined with 1,1-diphenyl-2-picrylhydrazyl DPPH by measuring absorbance at 517 nm (Vidovic et al., 2010, Wang et al., 2010). Radical scavenging activity (% RSA) was calculated by the following equation (3):

\[
\%\text{RSA} = 100 - \left( \frac{A_{\text{sample}} \cdot 100}{A_{\text{blank}}} \right) \quad (3)
\]

In water extract titratable acidity and formol number was determined as described by Tanner and Brunner (1987).

Results are presented as the mean ± standard deviation of three measurements.
Results and Discussion

The yields of dry matter of Boletus edulis f. beticola and Boletus edulis f. pinicola samples were 10.5±0.4% and 9.2±0.6% accordingly. The highest content of protein was found for Boletus edulis f. pinicola – 526±2 mg per 1 g of mushroom dry matter (Table 1).

The content of protein and ash in Latvian mushroom dry matter

<table>
<thead>
<tr>
<th>Mushroom</th>
<th>Protein, mg g⁻¹ in mushroom dry matter</th>
<th>Ash, % in mushroom dry matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boletus edulis f. beticola</td>
<td>325±2</td>
<td>5.56±0.06</td>
</tr>
<tr>
<td>Boletus edulis f. pinicola</td>
<td>526±2</td>
<td>6.07±0.03</td>
</tr>
</tbody>
</table>

Ash amount was determined to characterize the mineral content. Mineral content for Boletus edulis f. pinicola was higher than for Boletus edulis f. beticola (Table 1).

Titratable acidity (total amount of acid in the solution as determined by the titration using a standard solution of sodium hydroxide) was determined in water extracts of mushroom dry matter and results expressed as mmol of NaOH per 1 g of mushroom dry matter. The total amount of acids for Boletus edulis f. pinicola and Boletus edulis f. beticola was very similar (Table 2). Amino acids present in water extract of mushroom dry matter were determined by formol titration and expressed as a formol number. The results were expressed as mmol of NaOH per 1 g of mushroom dry matter. Formol number for Boletus edulis f. pinicola was about 2 times higher than for Boletus edulis f. beticola (Table 2).

Titratable acidity (TA) and formol number (FN) in water extracts of Latvian mushroom dry matter

<table>
<thead>
<tr>
<th>Mushroom</th>
<th>TA, mmol of NaOH g⁻¹ of mushroom dry matter</th>
<th>FN, mmol of NaOH g⁻¹ of mushroom dry matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boletus edulis f. beticola</td>
<td>0.22±0.01</td>
<td>0.74±0.02</td>
</tr>
<tr>
<td>Boletus edulis f. pinicola</td>
<td>0.26±0.01</td>
<td>1.40±0.02</td>
</tr>
</tbody>
</table>

The content of total phenols in water extracts of mushroom dry matter was higher for Boletus edulis f. pinicola (12.5±0.3 mg of gallic acid equivalents GAE per 1 g of mushroom dry matter) than that for Boletus edulis f. beticola (11.2±0.1 mg of GAE g⁻¹). Such tendency was observed also in methanol extracts of mushroom dry matter: for Boletus edulis f. pinicola (8.0±0.1 mg of GAE per 1 g of mushroom dry matter) and for Boletus edulis f. beticola (7.3±0.1 mg of GAE g⁻¹) (Figure 1).

Figure 1. The content of total phenols (TP) in Latvian mushroom dry matter
In general, the concentration of total phenols was higher in water than in methanol extracts (Figure 1). The content of total flavonoids was identical for *Boletus edulis f. pinicola* and *Boletus edulis f. beticola* (0.13±0.1 mg of quercetin equivalents QE per 1 g of mushroom dry matter) in methanol extracts of mushroom dry matter and the same was observed in water extracts (0.37±0.1 and 0.33±0.1 mg of QE per 1 g of mushroom dry matter respectively). The concentration of total flavonoids was higher in water than in methanol extracts (Figure 2).

Figure 2. The content of total flavonoids (TF) in Latvian mushroom dry matter

Using HPLC the phenol compounds like catechin, epicatechin, gallic acid, caffeic acid and rutin were detected and quantified (Table 3).

<table>
<thead>
<tr>
<th>Phenols</th>
<th><em>Boletus edulis f. beticola</em></th>
<th><em>Boletus edulis f. pinicola</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechin, µg g⁻¹ dry matter</td>
<td>1701.2</td>
<td>765.5</td>
</tr>
<tr>
<td>Epicatechin, µg g⁻¹ dry matter</td>
<td>2.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Gallic acid, µg g⁻¹ dry matter</td>
<td>9.5</td>
<td>7.1</td>
</tr>
<tr>
<td>Caffeic acid, µg g⁻¹ dry matter</td>
<td>15.6</td>
<td>17.3</td>
</tr>
<tr>
<td>Rutin, µg g⁻¹ dry matter</td>
<td>0.0</td>
<td>1129.0</td>
</tr>
</tbody>
</table>

The content of β-carotene was almost 2 times higher than the concentration of lycopene. The highest content of β-carotene and lycopene was found in methanol extracts of *Boletus edulis f. pinicola* (Table 4).

The radical scavenging activity (RSA) of mushroom extracts was tested against 1,1-diphenyl-2-picrylhydrazyl (DPPH). The RSA of water extracts of mushrooms was found to be higher than those of methanol extracts. The RSA was higher for *Boletus edulis f. pinicola* than that for *Boletus edulis f. beticola* (Figure 3).
Figure 3. Radical scavenging activity (%RSA) of Latvian mushroom dry matter

The antioxidant activity of the mushroom extracts highly depends on the concentration of active compounds – phenols, flavonoids, carotenoids etc. Our results are consistent with previous reports (Robaszkiewicz et al., 2010, Vidovic et al., 2010).

Conclusions

Both studied edible boletus mushrooms *Boletus edulis* f. *pinicola* and *Boletus edulis* f. *beticola*, collected in Latvia are important source of protein, minerals, phenols and carotenoids.

References

POLYPHENOLS AND VITAMIN E AS POTENTIAL ANTIOXIDANTS IN BARLEY AND MALT

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Abstract

Barley (Hordeum vulgare L.) is an ancient and important cereal grain crop. Whole grain products are recommended for healthy diets as being recognized sources of dietary fiber and antioxidant substances – such as polyphenols and vitamin E. The aim of current research is to compare the content of total phenolic compounds (TPC) and vitamin E of different barley varieties and malt types. One flaky, malting barley variety ‘Klass’, four hull-less barley lines from Latvia and theirs corresponding malt and for comparison four types of industrial malt – Pilsner, Munich, light, caramel and black malt were used for analyses. The total phenolic content was determined by spectrophotometer according to the Folin-Ciocalteu colorometric method with some modifications. Total phenolics were expressed as gallic acid equivalents (mg GAE g⁻¹ dry weight). During research vitamin E content was detected according to standard method AOAC 971.30. All barley varieties and malt samples exhibited significant content of vitamin E, and contained significant levels of phenolic compounds. The content of vitamin E in all barley samples was similar and average increase during malting was 34% for all varieties. Increase of content of vitamin E during malting can be explained by synthesis of vitamin E in germination process of grains. Content of vitamin E in malt depends on kilning temperature: higher kilning temperature, higher losses of vitamin E. A significant increase in TPC (from 2.017 to 3.406 mg GAE g⁻¹ DW) in all malt samples were observed after malting, i.e. steeping, germinating and kilning samples showing greater effect. It increases during malting probably not only by the modification or release of phenolic compounds, but also by the formation of new antioxidants, such as Maillard reaction products.

Key words: Vitamin E; hull-less barley; malt; total phenolic content

Introduction

Cereals and their derivatives are the most important foods in the human diet mainly because of the energy that they provide, due to their high carbohydrate content. However, in recent years, researchers have also begun to study their antioxidant profiles. Barley is widely consumed cereal, because of its dietary and technological properties. In fact, barley meals and fractions are now gaining renewed interest as ingredients for the production of functional foods, due to its bioactive compounds, such as β-glucans and tocols (Bonoli et al., 2004). Moreover, there are several classes of compounds in barley that have a phenolic structure, such as benzoic and cinnamic acid derivatives, proanthocyanidins, flavonols, flavones and many other phenolic compounds (Bonoli et al., 2004). Phenolic compounds in cereals are either in free or bound form. Plant phenolics, including, flavonoids and phenolic acid and tocopherols, are known to protect plants against tissue injuries, high levels of oxygen, free radicals and reactive oxygen species formed by the byproducts of photosynthesis. These molecules also play an important role in the protection of food against lipid oxidation and in human health by counteracting the risk of cardiovascular diseases, cancer and cataract, among other degenerative diseases of aging (Peyrat-Maillard et al., 2001).

In the food industry, hull-less barley (Hordeum vulgare L.) is acknowledged as more valuable and more economical, compared with flaky barley. The hull-less barley has elevated content of β-glucans. Soluble dietary fibre, mainly β-glucans, provides the formation of viscosity; as a result, cholesterol and fat absorption are decreased (Bhatty, 1999; Belicka and Bleidere, 2005). Selected hull-less barley varieties are able to pass flaky barley criteria: moreover, the amount of extract substances in hull-less barley is higher by 4–5% compared to malting barley (Dabina-Bicka et al., 2010).

Attention needs to be focused on the protection of endogenous antioxidant in beer and its raw materials, that is, barley and hop. About 80% of phenolic compounds present in beer are
derived from barley malt, and the remaining comes from hop (Goupy et al., 1999). They contribute to astringency and colour (Shahidi and Naczk, 1995), serve as browning substrates, participate in chill haze formation and are responsible for overall beer stability. Malt contains various compounds of barley (endogenous phenolic compounds) and from the malting process (Maillard reaction products) which can play significant role in malting and brewing through their antioxidant properties (Goupy et al., 1999) In this area, Maillard reaction products (MRP) which are naturally produced in food during thermal processing and home cooking operations by reducing sugars interacting with available amino groups, modify important food properties such as colour, flavour and stability during processing and storage (Maillart et al., 2007).

Vitamin E (α-tocopherol) is also a monophenolic compound present in barley and malt (Bamforth et al., 1993) which can quench free radicals. Their antioxidant activity is based mainly on the tocopherol-tocopherol quinone redox system (Randhir et al., 2008). The vitamin E content of cereal grains is influenced by plant genetics and is adversely affected by too much rain and humidity during harvest (Ball, 2006).

Antioxidants are generally thought to play a significant role in malting and brewing due to their ability to delay or prevent oxidation reactions and oxygen free radical reactions (Zhao et al., 2008). Antioxidant compounds present in barley extracts are complex, and their activities and mechanisms would largely depend on the composition and conditions of the test systems. Extraction solvent had significant effect on barley total phenolic content (TPC) evaluation, and 80% acetone (v/v) was recommended as antioxidants extraction solvent from malting barley for TPC evaluation (Zhao et al., 2006).

The aim of current research is to compare the content of total phenolic compounds (TPC) and vitamin E of different barley varieties and malt types.

Materials and Methods

Barley and malt samples
The research was carried out on four hull-less barley lines ‘3528’; ‘L-400’; ‘3475’; ‘3537’ (further in text abbreviated: A; C; D; B, respectively) and flaky barley (one line ‘Klass’) grains, which were harvested in Latvia in 2010, with germination capacity above 95%. The following technology was used for malt production from the tested grains: washing and steeping of grains (H2O t=17±2 °C) until moisture content in grains reached 38–40%. Then the grains were placed for germination. The grains were germinated for 4 days at 19±1 °C temperature. An 8 hour kilning of the germinated grains was completed in a laboratory kiln. Grains in a thin layer were spread on sieves in a chamber-type drier with hot air circulation at the temperature from +50 °C to +80 °C till a constant moisture content was achieved in the grains (5±1%).

In this study experimentally produced malt from hull-less barley was compared to commercial sorts of malt. Four kinds of malt, which are produced in “Viking Malt” (Lithuania) – Pilsener, and “Slodownia Strzegom” (Poland) – Munich, Light caramel and Black. Kilning and roasting temperatures were following: Pilsener – 75 °C; Munich – 100 °C; Caramel – 150 °C; Black – 230 °C.

Chemicals
Gallic acid and Folin-Ciocalteus phenol reagent were purchased from Sigma-Aldrich (Switzerland). All other chemicals and solvents were of the highest commercial grade and obtained from BARTA a CIHLAR spol.s.r.o. (Czech Republic) - Na2CO3, ethanol, acetone.

Preparation of extracts from barley and malt
Barley and malt was finely ground in a laboratory mill CIATRONIC KSW 2669. Four grams of ground samples were extracted 10 minutes in ultrasound bath (ULTRASONIS, SELECTA P) with 40 ml of solvent. To reach a compromise between alcoholic and acetone
extractions, a 7/7/6 ethanol/acetone/water (v/v/v) mixture was tested (Bonoli et al. 2004). After centrifugation at 3000 min\(^{-1}\) for 10 min using a centrifuge MEDITRONIC BL-C, the supernatant was removed and the extraction was repeated once more. The supernatant was collected in a 50 ml volumetric flask and refilled by solvent till mark (Jakobsone, 2008).

**Total phenolic content (TPC)**

The TPC of the barley and malt extract was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999) with some modifications. First, 0.25 ml of sample was transferred to a 25.0-ml volumetric flask containing 6 ml of H\(_2\)O, to which was subsequently added 1.25 ml of undiluted Folin-Ciocalteu reagent. After 1 min, 3.75 ml of 20% aqueous Na\(_2\)CO\(_3\) was added, and the volume was made up to 25.0 ml with H\(_2\)O. The control sample contained all the reaction reagents except the extract. After 2 h of incubation at 25 °C, the absorbance was measured at 760 nm using a spectrophotometer JENWAY 6300. Total phenols were expressed as gallic acid equivalents (Damien Dorman et al., 2004).

**Vitamin E**

The analyses of vitamin E content were carried out by the AOAC Official Method 971.30 “α-Tocopherol and α-Tocopheryl Acetate in Foods and Feeds” standard colorimetric method (1971–1972). The term “vitamin E” is the generic descriptor for all tocol and tocotrienol derivatives that exhibit qualitatively the biological activity of α-tocopherol (Ball, 2006).

**Statistical analysis**

The differences in the total phenol and vitamin E content were analyzed using the analysis of variance (ANOVA). Tukey’s test was applied to compare the mean values and p-value at 0.05 was used to determine the significant differences.

**Results and Discussions**

Phenolic compounds were considered as a major group of compounds that contributed to the antioxidant activity of cereal (Zhao et al., 2008). Significant amounts of total phenolics were detected in all barley varieties. The content of TPC of the different barley varieties ranged from 1.96 to 2.43 mg GAE g\(^{-1}\) DW (Fig. 1). The results were similar to those reported by Zhao et al. (2008) but higher than those reported by Maillard et al. (1996). This might be due to the differences of the barley varieties and the extraction methods used in studies. The differences of TPC content between hulled barley “Klass” and hull-less barley lines A, B, C were not significant (P>0.05), but higher amount of TPC in line D was detected. Comparing hull-less barley lines, significantly lower content of TPC in line C was detected.

![Figure 1. Total phenolic contents in barley grains and malt](image-url)
Content of TPC in malt was higher than in unprocessed barley (Fig. 1), and the increase ranged from 28% till 65%. Lower content in hull-less barley line C was detected and also lower increase after barley malting – 28%. Increase can be explained by biochemical reactions that occur during germination process. Overall increase of phenolic compounds in legumes, peas after germination was reported (Lopez-Amoros et al., 2006). Also results could be explained by formation of phenolic compounds during Maillard reaction (Maillard et al., 2007).

Content of vitamin E in barley ranged from 34.96 to 39.2 mg kg\(^{-1}\) DW (Fig. 2) and these results are in compliance to Hall (2001). The current research showed that vitamin E content increased during barley malting by 38% comparing to unprocessed barley. Literature studies showed that germination influence the content of tocopherols. In lupins during germination increase of \(\alpha\)-tocopherol, whereas decrease of \(\gamma\)-tocopherol were observed, but did not affect content of \(\delta\)-tocopherol (Frias et al., 2005).

The thermal stability of the vitamin E in food vary from the heating time, heating methods, and food composition. Content of vitamin E in the commercial malt types (Pilsener, Munich, Caramel, Black) was lower than in experimental malt samples (Table 1).

<table>
<thead>
<tr>
<th>Malt</th>
<th>Total phenolics GAE (mg g(^{-1}) DW)</th>
<th>Vitamin E (mg kg(^{-1}) DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pilsener</td>
<td>3.529±0.072</td>
<td>37.7±0.1</td>
</tr>
<tr>
<td>Munich</td>
<td>4.354±0.184</td>
<td>41.7±0.3</td>
</tr>
<tr>
<td>Caramel</td>
<td>6.773±0.293</td>
<td>40.8±0.5</td>
</tr>
<tr>
<td>Black</td>
<td>6.782±0.369</td>
<td>24.2±0.7</td>
</tr>
</tbody>
</table>

Pilsener malt kilning temperature was the same that for experimental samples, but content of vitamin E was lower. In malt produced from varieties Klass, A, B, C, D content of vitamin E ranged from 45.8 to 54.2 mg kg\(^{-1}\), but in Pilsener malt only 37.7 mg kg\(^{-1}\). Other two types of
commercial malts Munich and Caramel showed higher amount of vitamin E than Pilsener malt, because the killing temperature was above 75-80 °C. In accordance with Palmer (2006) report, kilning and roasting temperatures of different malt types were following: Pilsener – 75; Munich – 100; Caramel – 150; Black - 230°C. Thermal stability of vitamin E in the Black malt was destroyed, because kilning temperature was too high, reaching 230 °C. The results of our experiments proved that more intensive synthesis of TPC was detected in Caramel and Black malts, respectively 6.773 and 6.783 GAE mg g\(^{-1}\) DW. The TPC increased as roasting time increased showing that Maillard browning reaction products were generated and were probably responsible for the TPC, which are important with regard to improving beer stability and as a source of antioxidants in the diets of beer drinkers (Palmer, 2006).

For further understanding of interrelationship between biologically active compounds of barley and malted barley, correlation analyses were performed. Strong correlation (r=0.84) was found between vitamin E in barley and malted barley, whereas weak correlation (r=0.53) between TPC in barley and malted barley. These data indicate that the content of vitamin E is mainly influenced by technological operations, but for TPC formation other factors, as grain structure, starch molecule size and also amino acid, reducing sugars content that are involved in synthesis of new polyphenols from Maillard reaction, are significant.

**Conclusion**

1. The content of total phenolic compounds and the vitamin E content of flaky and hull-less barley varieties increase after steeping, germination and kilning in its corresponding malts.
2. The content of vitamin E in barley significantly increased during malting and was heat stable under kilning temperature.
3. As a result of Maillard reaction at higher malt production temperature provides greater content of phenolics in the products.

**Acknowledgements**

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**References**

Abstract
The research objective is the new technology of the granulated green tea with antioxidant properties and its solubility in boiled water. Object of research is served: fresh shoot of tea, steamed tea shoot (granules received from them). Quality of tea is defined by physical and chemical indicators of infusion: colour, transparency, aroma, taste, body. The technological scheme of granule processing is the following: steamed tea leaf, partial drying of steamed reception of granules and drying. Extraction of cellular juice, tea leaves; to achieve the result we used different sizes of a matrix for reception of granules. After the launching experiments, we established the optimum size of granules as following: matrix thickness (3, 5 and 10 mm) and the diameter of a pore (3, 5 and 7 mm). Four times drawing of granules in boiled water defined the physical and chemical indicators of received samples: definition of extractive substances is – tannin method of Vorontsov and catechins method of Bokuchava. As a results manufacturing method of the granulated green tea are yielded. The product is characterized by fast dissolution after drawing it to boiled water and this feature considerably surpasses usual tea. This product is rich with catechins which possess antioxidant characteristic and P vitamin activity. It is remarkable, that this method is simple enough. Farmers engaged in a small-scale tea business can use it. Our technology satisfies market requirements, and we consider sale possibility in those countries where products of green tea are traditionally popular.

Key words: granular green tea, catechin, antioxidant, tannin.

Introduction
China the homeland of tea offers the wide assortment of production of tea (Kalinin, 2002). In Georgia, manufacturing of black tea, green tea, green brick tea and black tiled tea are can be practiced (Majsuradze, 2010; Lazishvili, 2004).

Materials and Methods
Materials used: gentle shoots of tea, steamed flesh (granules received from them); installation for producing granules: inside of the case are placed rotating screw conveyer and the cutting tool consisting of motionless grids which have apertures of various sizes.
Influences are investigated: humidity of a material in formed profiled weights, the sizes of a main matrix, diameter in limits (3–10 mm) and the solubility feature by 4 times drawing in boiled water. In each extract the total amount of extract substances, phenol mixtures and catekhins were defined.
Solubility of granules obtained by us was compared to the Chinese granulated tea purchased in Batumi trading center.

Results and Discussion
The first debt represents considerable interest to establish at what humidity the profiled products are formed.
Experiences showed that optimal steaming of tea leaf is 5 minutes at temperature 95–100 ºC. The best try was the 20% extraction of tea cellular juice from initial mass of tea leaf. Maximum amount of phenol mixtures and catekhins were retained both in cellular juice and granulated tea. This product is rich with catechins which possess antioxidant characteristic and P vitamin activity (Lazishvili and Kobakhidze, 2003). By our technology we made two products: liquid cellular juice which can be used for ice tea, lemonade (Seidishvili et al., 2004) and syrup production; the other is granulated green tea.
It is important to define at what humidity the profiled mass is formed. Experiments showed that it is reached at humidity of 56.1–60.7%. Diameter of matrix should be 5–10mm and the thickness of 3–5mm. However at smaller diameters, for example 3мм and at raised humidity of 60.7% profiled weight is not formed.
Solubility of tea production is one of the major indicators of quality of production. Tea granules received by our technology have appeared quick-dissolving while keeping in boiled water for 10 minutes. More than 85% of soluble substances pass in tincture after 4 multiple drowning in boiled water. For comparison the granulated Chinese green tea is taken. At different apertures of matrix diameter and thickness ($d_3\cdot h_3$, $d_5\cdot h_5$, $d_{10}\cdot h_5$) doesn’t render essential influence on solubility of granules.

That’s how granules received at $d_3\cdot h_3$, $d_5\cdot h_5$, $d_{10}\cdot h_5$ almost equally dissolved in hot water. It is necessary to notice, that by solubility degree on total of soluble substances phenol connections (tannins), including (catekhins) surpass others. See table 1.

### Table 1

<table>
<thead>
<tr>
<th>Frequency rate of infusion</th>
<th>Granules $d_5\cdot h_5$</th>
<th>The Chinese tea &quot;Elita izumrud&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extract, %</td>
<td>Phenol, %</td>
</tr>
<tr>
<td>I</td>
<td>85.8</td>
<td>74.3</td>
</tr>
<tr>
<td>II</td>
<td>10.5</td>
<td>17.1</td>
</tr>
<tr>
<td>I+II</td>
<td>96.3</td>
<td>91.4</td>
</tr>
<tr>
<td>III</td>
<td>2.3</td>
<td>5.7</td>
</tr>
<tr>
<td>I+II+III</td>
<td>98.6</td>
<td>97.1</td>
</tr>
<tr>
<td>IV</td>
<td>1.4</td>
<td>2.9</td>
</tr>
<tr>
<td>I+II+III+IV</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

In tea there are substances which possess ability to dissolve faster in hot water, for example: caffeine, amino acids, monosugar, etc.

It is remarkable, that granulated tea of a new kind offered by us it is also possible to name the granular or ball figurative tea radically differs from the granulated teas produced nowadays. Difference is both on quality and on technology.

Technologies of reception of small granules of tea directly from a fresh tea leaf as CTC (Chanturia, 2000) are known also.

For this purpose dried tea leaf is crushed to fine-dispersed conditions with the sizes of particles 0.8–1.1 mm and thus granules by a method of balling without preliminary consolidation turn out. These small granules are used for preparation of bags of disposable consumption.

The way developed by us does not demand preliminary crushing to such degree to receive fine-dispersed weight (0.8–2.6 mm), and also there is no necessity of balling in the special device. It is enough to press through the granule in apertures of a grid installation. Reception of granules is provided at the expense of properties of steamed tea leaves which arrive in screw of the chamber; they are exposed to intensive mechanical influence from the screw blade of screw conveyer, gradually are condensed and resulted in dense weight. This weight of tea is forced through apertures of a matrix and pressed in profiled continuous or partially continuous weight. Profiled weight remains even after drying.

There are many theories explaining receptions of the connected body from loose materials (pasta, granul forages, briquette of peat, etc.) for each method substance play a role which promote consolidation process. So, for example in macaroni manufacture is gluten, in peat humic acids and etc.
Tea fleshes maintain considerable quantity of substances possessing colloid properties, such as pectin substances and hemicellulose which can give plasticity and viscosity properties to tea and finally, the connected body is formed.

All these substances in process of granulation are exposed to transformations. Most of all phenol connections, in particular katechines change. In formation of profiled weights of a granule, undoubtedly, pectin substances take part in the presence of sugars and acids.

Finishing article, we should state an estimation of this new production of tea at which it is necessary to consider, that in it all elements of flesh are put in pawn. Starting from the most gentle parts of flesh - the first sheet with a kidney, finishing by the coarsened third sheet. Talking about the success of product; the last words are on customer.

However we should give them organoleptical estimation. Experts have noticed that the presented samples on color of infusion, taste, aroma and on color of boiled leaves are quite comprehensible (Table 2).

### Table 2

**Organoleptical estimation of pre-production models of the granulated green tea**

*(Experts for organoleptical estimation – R.Tsintsabadze, A.Sardzhveladze, G.Malanija)*

<table>
<thead>
<tr>
<th>Manufacturing date</th>
<th>Diameter of granules, mm</th>
<th>Appearance</th>
<th>Color of infusion</th>
<th>Taste aroma in balls</th>
<th>Color boiled leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.05.10</td>
<td>3</td>
<td>Has the form of granules</td>
<td>intensive a reddish shade</td>
<td>- 3.50 3.50</td>
<td>Wholeness of granules remain, color is homogeneous</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Has the form of granules</td>
<td>intensive a reddish shade</td>
<td>- 3.00 4.00</td>
<td>Wholeness of granules remain, color is homogeneous</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Has no form of granules</td>
<td>intensive a reddish shade</td>
<td>- 3.00 4.50</td>
<td>No wholeness of granules remain, color is homogeneous</td>
</tr>
<tr>
<td>20.07.10</td>
<td>3</td>
<td>Has no form of granules</td>
<td>intensive a reddish shade</td>
<td>- 4.00 3.50</td>
<td>Wholeness of granules remain, color is homogeneous</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Has no form of granules</td>
<td>intensive a reddish shade</td>
<td>- 3.75 4.00</td>
<td>Wholeness of granules remain, color is homogeneous</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Has no form of granules</td>
<td>intensive a reddish shade</td>
<td>- 3.75 3.25</td>
<td>No wholeness of granules remain, color is homogeneous</td>
</tr>
</tbody>
</table>
The technology offered by us has advantages before STS – technology. The matter is that the majority of its production of 60–70% so small, that demands packing in bags. It is connected with the big expense tare packing a material. And our production can be packed into cardboard boxes.

**Conclusion**

We consider, that the granulated tea received by us with the simplified technology to enrich assortment of tea production.

**References**

Abstract
Celiac disease is a common complex disease caused by a dietary intolerance to gluten proteins found in all wheat types and closely related cereals such as barley and rye (Heap, van Heel, 2009). The only effective treatment is a strict gluten-free diet throughout life (Gobbetti et al., 2003). Gluten-free breads often have poor crust and crumb characteristics, low quality, exhibit poor mouth feel and flavour (Katina et al., 2005, Gallagher et al., 2003). The aim of this research was to investigate the effects of extruded maize flour addition on quality of gluten-free bread. Four flour types (buckwheat, maize, rice, extruded maize) and six types of gluten-free breads made from buckwheat, maize and rice with or without extruded maize flour in various proportions were studied. The main quality parameters of gluten-free flour and breads were determined using the following methods: hardness with Texture analyser TA.XT. plus, moisture content with Precisa XM 120 at temperature 110±1 °C, microstructure was observed using Zeiss „Axioskop 40” microscope and Axioskop 4.7 software. Results showed that extrusion of maize flour has an effect on the size of starch granules, bread moisture, hardness and pore equivalent diameter. After partial flour replacement with extruded maize flour, it is possible to obtain gluten-free bread with more regular, stable and porous texture.

Key words: Gluten-free bread, extruded flour, quality

Introduction
Cereal foods in various forms are an essential component of the daily diet. Nutritionally, they are an important source of carbohydrates, protein, dietary fibre, many vitamins and non-nutrients (Katina et al., 2005).
There is increasing interest in gluten-free products as the number of the celiac patient grows and society is more informed about gluten-free products. Celiac disease is a common complex disease caused by a dietary intolerance to gluten proteins found in all wheat types and closely related cereals such as barley and rye (Heap, van Heel, 2009). The only effective treatment is a strict gluten-free diet throughout life (Gobbetti et al., 2003).
Gluten is the main structure-forming protein in flour, responsible for the elastic and extensible properties needed to produce good quality bread. To ensure the acceptability of gluten-free bread, the loaves must have quality characteristics similar to those of wheat flour bread (Gobbetti et al., 2003). Gluten-free bread is one of the most challenging issues for food technologists due to the fact that wheat gluten has such a wide variety of tasks in bread making, so a wide range of ingredients is needed to achieve a good quality product without it and since a gluten-free diet is essential for patients having celiac disease. Gluten-free breads often have poor crust and crumb characteristics, low quality, exhibit poor mouth feel and flavour (Katina et al., 2005, Gallagher et al., 2003).
Many researchers have tried improving gluten-free bread quality using gluten-free flour mixtures (Sciarrini et al., 2010, Torbica et al., 2010), additives such as hydrocolloids, gums, enzymes, emulsifiers (Renzetti et al., 2008; Peressini et al. 2011). Researchers from Brazil produced gluten-free bread using extruded rice flour as a gluten replacement. Results showed that the gelatinization of starch by extrusion could make the gluten-free bread production process viable and improve the colour of the crust and texture characteristics, which were similar to those of wheat bread, despite presenting a low specific volume (Clerici et al., 2009). The aim of this research was to investigate the effects of extruded maize flour addition on quality of gluten-free bread.
Materials and Methods

White rice (moisture 9.09%), yellow maize (moisture 10.97%), buckwheat (moisture 9.53%) and extruded maize (moisture 6.69%) flour from Joint Stock Company “Ustukiu Malunas” (Lithuania), eggs, sugar, salt, apple vinegar, dry yeast and vegetable oil from local market were the materials used in study.

The general technological scheme used to make the gluten-free bread is presented in Figure 1. Six samples were prepared – maize bread (MB), maize bread with extruded maize flour (MBE), rice bread (RB), rice bread with extruded maize flour (RBE), buckwheat bread (BB), buckwheat bread with extruded maize flour (BBE).

![Figure 1. The general technological scheme of gluten-free bread](image)

The hardness of bread slices (approximately 60×80×10 mm) samples was objectively measured using a Texture Analyser (TA.XT. plus, Stable Micro Systems) equipped with following compression test parameters: probe = 25 mm diameter aluminium cylinder, test speed = 1 mm s⁻¹, distance = 10 mm (Cauvain, 2004). Moisture content was analysed with Precisa XM 120 at temperature 110±1 °C in two reiterations. Structure of bread samples and pores of bread crumb was analysed under the triocular microscope Axioskop 40. Pictures were taken by digital compact camera Canon PowerShot A620 via 16x40 magnification of the microscope. Size and area of cells and starch granules was measured using software Axioskop 4.7.

Means and standard deviation of the means were calculated using Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA).
Results and Discussion

During fermentation lead to further development of the dough structure and formation of gas cells. Yeast fermentation generates carbon dioxide, and the dough expands due to increasing pressure in the gas cells (Oates, 2001). Extruded maize flour affects gas cells stabilization and retention; it helps to develop regular porosity of gluten-free bread crumb (Figure 2). The rice and buckwheat bread samples had the most significant impact of extruded maize flour on specific volume and porosity. Both samples had typical and regular specific volume and porosity (Figure 2c–f). In its turn the added amount of extruded maize flour did not affect the specific volume and porosity of maize gluten-free bread (Figure 2a–b).

An important parameter of bread quality is moisture, as it possesses the ability to bind and return water during the technological process (dough mixing, fermentation, baking and cooling). Figure 3 presents the moisture content of gluten-free bread samples.

Extruded maize flour has better water absorption capacity; it is associated with changes in starch granules during the extrusion. Consequently, the maize and rice bread samples with extruded flour moisture content were higher for about 3.25% (MBE) and 6.99% (RBE) (Figure 3). At the same time the added amount of extruded maize flour did not affect significant moisture content of buckwheat gluten-free bread (49.48% BB and 49.75% BBE). Unlike maize and rice, buckwheat contains more soluble proteins that affect its dough and crumb structure (Hong, Kim, 2006).

Figure 2. Digital images of gluten-free bread slices

a - maize bread, b - maize bread with extruded maize flour, c - rice bread, d - rice bread with extruded maize flour, e - buckwheat bread, f - buckwheat bread with extruded maize flour
Crumb hardness is closely related to bread moisture content, pore size and wall structure. Large pores with thick walls from stronger and less flexible crumb, but small, homogeneous pore structure develop softer and more flexible crumb (Oates, 2001). Figure 4 shows crumb hardness of the gluten-free bread samples.

The obtained results shows that the gluten-free bread samples with extruded maize flour are 8.4% (BBE), 14.5% (MBE) and 40.9% (RBE) softer than the samples without extruded flour. Chemical composition of buckwheat flour which is characterized by larger soluble protein (Hong, Kim, 2006), partially explains the crumb hardness of the buckwheat gluten-free bread samples. Although rice flour contains small amount of protein, it contains a lot of starch (80%). All starch granules do not gelatinize and made bread crumb harder (Arendt, Nunes, 2010, Oates, 2001).

Figure 3. Moisture content of gluten-free bread samples

Figure 4. Crumb hardness of the gluten-free bread samples
Optimally developed dough is one which the continuous and interconnected gluten matrix surrounds most of the starch granules. Association between starch granules and gluten proteins is affected by the quality of flour. Optimally developed dough contains occluded gas cells. The number and size of gas cells, which during baking expansion into open network of pores and determine crumb structure and volume, is influenced by mixing conditions and flour quality (Oates, 2001). Digital images of the gluten-free bread crumb samples are presented in Figure 5.

Both maize bread samples have a homogeneous pore structure. Pores of maize bread are characterized by thin walls and so close to each other that coalescence in one network (Figure 2a, 5a). Equivalent diameter of MBE pores decreased form 578.9 µm to 434.5 µm (Figure 2a–b, 5a–b). Crumb porosity of the maize bread samples with extruded flour (MBE) are homogeneous, because maize flour baking properties are similar to wheat.

Conclusions

1. Moisture content in maize and rice bread samples with extruded flour was higher by approximately 3.25% (maize bread with extruded maize flour) and 6.99% (rice bread with extruded maize flour) comparing to bread without extruded maize flour.
2. Gluten-free bread samples with extruded maize flour are 8.4% buckwheat bread with extruded maize flour, 14.5% maize bread with extruded maize flour and 40.9% rice bread with extruded maize flour softer than the samples without extruded flour.
3. Porosity of gluten-free bread with extruded maize flour crumb becomes homogeneous, equivalent diameter of pores decreased on average form 639.8 µm to 491.9 µm.
Acknowledgment
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References
Abstract

A lot of research has been carried out to substitute the sugar in food products by other sweeteners since sugar consumption is directly related to diabetes and other illnesses such as obesity. Inulin is more commonly known as a prebiotic, soluble dietary fiber. In literature there is comparatively little information about inulin as a sweetener. Inulin syrup used in the research was obtained from the Jerusalem artichoke (*Helianthus tuberosus* L.) tubers. Therefore the aim of the research work was to evaluate properties of agar-agar gel when sucrose was replaced by inulin syrup. Agar-agar gel samples were prepared with different concentrations of inulin syrup to replace sucrose: 0%, 20%, 40%, 60%, 80% and 100%. Gel samples were prepared by adding inulin syrup at specific temperatures: 65 °C and 105 °C. To analyse the texture of the gel samples the Texture Analyser Model TA.XT Plus was used by slicing samples with a wire cutter (A/BC) while the colour was determined in the system of CIE L* a* b* by using the Colour Tec-PCM, and for pH measurements – a pH-metre Jenway 3510 with a combined glass electrode was used.

The results of texture analysis show that strength (hardness) of the gel is influenced by the temperature as well as the concentration of the inulin syrup added to the gel samples. In the structure analysis the strength of the gel samples ranges from 7.406±0.656 N to 10.001±0.730 N and the strength increases in samples when a higher concentration of inulin syrup is added at a temperature of 65 °C, but an opposite situation is observed in the gel samples when prepared at a temperature of 105 °C. The colour parameter L*, which describes lightness of the sample, ranges from 20.92 (20% of inulin) to 18.11 (100% of inulin) while pH of the gel samples ranges from 3.29 (20% of inulin) to 4.18 (100% of inulin).

Key words: inulin syrup, agar-agar, gels.

Introduction

A valuable and balanced diet, safe and qualitative foodstuffs are the basic principles of people’s health and life quality improvement. The main criteria of qualitative food are its wholeness, variety and moderation.

The problem of cardiovascular diseases and the related nutritional problems like obesity and overweight have become an important issue in Latvia lately. One of the causes of this problem is a high consumption of sweets between the main meals.

The sensory feeling of sweetness is one of the five primary taste qualities, and there are several measures of human perception of sweetness.

Traditionally, sucrose has been the sweetener of choice, though a wide range of sugars have been available for bakers. These sugars are derived from the hydrolysis of cereal starch, such as glucose and fructose syrups. Apart from its sweetening action, sugar performs a variety of functions: formation of crust colour, flavour enhancer, texture modifier, development of structure, and shelf-life improvement.

Over the last two decades, inulin-type fructans have become a topic of interest for both food industry and for researchers (Roberfroid, 2005, Gedrovica et al., 2009). Since sugar consumption is directly related to diabetes and other illnesses such as obesity, there is an increased interest in products without added sugar. Jellies are not basic foods, but they are good complements to a diet if they are eaten in correct amounts (Figuerola et al., 2007). Jellies are high-energy products, meaning that the products are not suitable or desirable for people who have glycemic problems, obesity, diabetes, and cardiovascular diseases. Low sugar, on the other hand, means difficulties in gel formation and problems with product texture, stability, and uniformity.

Replacing sugar in jellies, technological problems arise because of the fact that in order to obtain the desired sweet taste in jellies, sugar is essential to form the product structure using the gels with pectin or other gelling material. Some hydrocolloids can be used in low-sugar
jelly formulations by obtaining different product quality. When the amount of sugar in jelly production is decreased or even eliminated, agar is one of the gelling substances that could be used to compensate for sugar and its properties.

Agar is a hydrophilic colloid extracted from certain marine algae of the class *Rhodophyceae*. Agar has been used for many centuries as a high performance gelling agent (Armisen et al., 2000; Stanley, 2006). Its ability to produce clear, colourless, odourless, and natural gels without the support of other colloids has long been exploited by the food industry not only as a stabilizer and gelling agent but also in the manufacturing of confectionery. Agar jellifies at 40 °C and melts at 80 °C (Barrangou et al., 2006).

Agar is a well known thermo-reversible gelling polysaccharide, which sets at 30 to 40 °C. Inulin is legally classified as food or a food ingredient, and not as additive in all countries where it is used (Roberfroid, 2005). In addition to pure inulin and fructooligosaccharides, the Jerusalem artichoke is processed in a number of different ways for the health food market (Frack, 2002; Kaur et al., 2002). The Jerusalem artichoke flour is used in a wide range of foods, such as pasta for diabetic diets, while extracts are sold in pill form for their health-promoting properties. In the food industry, it is used as a prebiotic ingredient and a low-calorie sweetener (Glibowski et al., 2008). Therefore the aim of the research work was to evaluate the properties of agar-agar gel prepared at two different temperatures (65 °C and 105 °C) when sucrose was replaced by inulin syrup.

**Materials and Methods**

The research was carried out at the Latvia University of Agriculture, Faculty of Food Technology.

In gel production the Jerusalem Artichoke Juice Concentrate produced by Topina, Diät Rohstoff Gmb, (Germany) inulin syrup was used to replace sugar, while glucose syrup was obtained from the confectionary factory “Laima” (Latvia).

Sugar and citric acid were purchased in a local grocery store. In the preparation of gelling substance AgarNordS (E 406) (Estonia) was used. It is a low calorie material (non-digestible by humans).

Ten different gel samples were prepared using various concentrations of inulin syrup with agar-agar, sucrose, glucose syrup. To prepare the control sample the following recipe was used: agar-agar powder (2 g), glucose syrup (62 g) – sucrose (104 g), citric acid (2 g of 50% citric acid solution) water (100 g) (Дубцов, 2001; Сборник основных рецептур, 2000). In the research the replacement of sucrose by inulin syrup was in the following range of ratios: 100/0, 80/20; 60/40; 40/60; 20/80; 0/100 %.

Agar-agar was swollen in cold water, and then the mixture was heated until agar-agar was dissolved in water.

1st option: agar-agar/water solution was boiled for 5 minutes at a temperature of 105 °C. Afterwards, the sucrose or inulin syrup, citric acid and the sucrose syrup were added to the boiled solution and cooled down to 65 °C.

2nd option: agar-agar/water solution was boiled for 5 minutes at a temperature of 105 °C then cooled down to 65 °C. Then sucrose or inulin syrup, citric acid and the glucose syrup were added to the boiled solution and cooled agar-agar solution.

Subsequently, the obtained agar-agar/water with sugars and citric acid solution was hot–filled in polystyrene containers (150 ml), which were sealed with their covers and cooled down to 18 °C.

The analyses of the colour, structure and pH were carried out on the next day after all kinds of gel samples were prepared.
The measurement of pH was determined by – the pH-metre (Jenway 3510) with a combined glass electrode. During the pH analysis, the glass electrode was immersed in the jelly samples. The measurements of pH were repeated three times.

The strength (hardness) of gel was characterised by texture profile analysis. The texture was determined using the Texture Analyser Model TA.XT Plus; Stable Micro Systems. The wire cutter (A/BC) was used to slice the gel samples. The measurement was repeated ten times obtaining precise results of the structure analysis.

The colour of the gels was determined in the system of CIE L* a* b* by using Colour Tec-PCM measure and the obtained values were expressed as L* a* b* values. The data were processed by using Microsoft Excel programme.

**Results and Discussion**

For developing new products that meet the people’s needs for a non-sugar diet, most of the foods are prepared by substituting sugar with sugar replacers – other sweeteners which give lower calories (sugar gives 4 kcal while fibre -2 kcal per g of the product) (Commission Directive 90/496/EU, Commission Directive 2008/100/EU). Jelly is a product manufactured by cooking fruit juice with added sugar, glucose syrup and agar-agar (Figuerola, 2007). Sugar serves as a preserving agent and aids in gelling. Too little sugar prevents gelling and may allow yeast and moulds to grow. For proper structure, jelly products require the correct combination of agar, sugar and glucose syrup (Tabata, 1999; Bayarri, 2004). In order to examine how the strength (hardness) of gel changes, the development of an optimum gel model is in progress without adding any extra taste, smell and colour additives (e.g fruit or berry juice).

In our research the inulin syrup is used as a sugar replacer therefore we are investigating the quality differences between the standard recipe of gel and experimental products where sugar is replaced by inulin syrup. Since oligosaccharides give a lower calorie content (around 2 kcal per g) it is suggested that sugar be replaced by inulin as an oligosaccharide which is important for those consumers who like to consume jelly candies (where sugar is used) and have weight problems.

The pH of gels ensured the storage stability and inhibition of the growth of moulds and yeasts. In terms of agar-agar gels, gelling properties change when pH changes.

The pH of experimental gels was not changed in a wide range. The pH value of the control samples was 2.42. Low pH level of the gel solutions can be explained by adding citric acid (described in the section ‘Materials and Methods’). The pH values of other samples analysed in the research ranged from 3.11 (20%) to 4.01 (100% of inulin syrup) when samples were prepared at 65 °C, while in the samples prepared at 105 °C the pH values ranged from 3.29 (20%) to 4.18 (100 %) (Figure 1).

![Figure 1 Changes of pH in gel samples](image)

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By adding inulin syrup (pH 4.7) in the production of gel solutions, the pH value increased compared to the control samples (without inulin syrup).

Regarding the gel strength (hardness), the data of gel structure in the literature sources are insufficient. Even very minor changes in composition or processing variables can dramatically influence the textural properties of jellies (Kim et al., 2001, Matsuhashi, 1990, Panouille et al., 2009).

The strength of experimental gels was affected significantly by chosen inulin syrup concentration and heating temperature. The changes are shown in Figure 2.

The strength increased by increasing the concentration of inulin syrup which was added at a temperature of 65 °C and it ranged from 7.406±0.656 to 10.001±1.730 N. In the gel sample production, inulin concentration influences both the taste and strength of the gels.

An opposite situation was observed at a temperature of 105 ºC. The strength of gels decreased from 12.419±0.679 to 2.490±0.126N. The decrease in hardness of the gels can be explained by the properties of inulin at a certain temperature. In this case, gel production at a temperature lower than 70 ºC affects its gelling ability, while adding inulin at 105 ºC water binding capacity increases forming a colloidal grid.

We could put forward a hypothesis that such a situation has been caused by the properties of agar-agar and inulin and their different gelling temperatures. If we add inulin syrup to the gel solution at 105 ºC, the concentrations of inulin influence the gel strength. While adding inulin syrup to the gel solution at 65 ºC the gel is already formed by the agar-agar and the concentration of inulin syrup has a very small influence on the strength of the gel.

Agar and inulin as polysaccharides have the ability to form a gel and its strength depends on the properties and concentration of polysaccharides. By replacing sucrose with inulin the strength of the gel increases. The strength of the gels become weaker when inulin syrup is added at a temperature of 65 ºC, and the strength of the gels slightly increases by increasing the concentration of inulin. Adding inulin syrup to the gels at a temperature of 105 ºC, the strength of the gel decreases sharply.

From the obtained results it can be concluded that during gel formation agar-agar needs to have a sufficient amount of free – unbound water available to form a gel with proper strength and structure.

In food perception certain factors play a very important role – eg. structure, smell and colour. When a food product is evaluated for consumption all the senses play an important part. A food product is accepted or rejected. The colour is one of the main characteristics of food
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quality. Most consumers associate gel with a bright and mainly light colour. The results of surface colour measurements of experimental gels are presented in Figure 3 and Figure 4.

**Figure 3 Changes of the lightness “L*” of gel samples**

By increasing the inulin syrup ratio in experimental gels the mixture obtains a darker colour than the standard mixture in both cases, since inulin syrup is darker in colour. From the Fig 3 it can be seen that the lightness L* of gels decreases in all samples prepared at a temperature of 65 °C as well as at 105 °C from 20.92 (20% of inulin syrup) to 18.11 (100% of inulin syrup) and from 20.02 (20%) to 16.98(100%). The gel samples are in a dark colour, and that can influence the consumers’ choice in purchasing these products.

Experimental gels’ yellowness b* increases from 4.85 to 6.38 (temperature 65 °C), and from 4.99 till 6.97 (temperature 100 °C) (Fig. 4).

**Figure 4. Yellowness b* in gel samples**

The values of colour factor a* have been determined, but they have not been described in the research because they do not characterise the colour of the gel samples.

**Conclusions**

1. Inulin syrup can be used as sugar replacer in manufacturing of new gel products.
2. Gel strength increased by adding inulin syrup to the standard solution at a temperature of 65 °C from 7 N to 10 N, but by adding inulin syrup to standard solution at a temperature of 105 °C, the strength decreases.

3. When gel samples were prepared at 65 °C, pH ranged from 3.11 to 4.01 while at a temperature of 105 °C – from 3.29 to 4.18.

4. The added amount of inulin syrup influenced the lightness of gel samples.

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References
Abstract
The ability of *Bifidobacterium lactis* (Bb-12) to hydrolyse lactose, lactulose and inulin was studied during milk fermentation. For this purpose, the content of lactose, lactulose and inulin was determined before and after fermentation of milk samples. Pasteurized milk, freeze-dried culture Bb-12 (Chr. Hansen, Denmark), inulin - RAFTILINE® HP (ORAFI, Belgium) with polymerization degree ≥5 and degree of purity 99.5%, syrup of lactulose (Duphalac®, the Netherlands) were used for experiments. The different concentrations (1; 2; 3; 4 and 5%) of lactulose and inulin were used for studying of *B.lactis* ability to assimilate of milk sugar and prebiotics during fermentation. The fermentation process of milk samples enriched with lactulose or inulin was produced at 37 °C for 16 hours. The content of lactose and lactulose was determined by IDF standard 147B:1998 procedure, the content of inulin by AOAC Official Method 999.03 and by AACC Official Method 32.32.

Results showed that bifidobacteria poorly assimilate lactose at the presence of prebiotics in milk. The lactose assimilation decreases together with increase of added prebiotics concentration in milk. However bifidobacteria are able to hydrolyse up to 50% of lactulose in the product, except sample with 5% of lactulose. There was a decrease of lactulose by 2/3. The changes of lactulose content are significant (p<0.05). Consequently *B.lactis* possesses ability to assimilate lactulose. Inulin assimilation degree in fermented milk samples was low (10–20%), because it depends on the inulin polymerization degree and the degree of purity. The inulin assimilation decreases together with the increase of inulin polymerization degree and the degree of purity. The obtained results confirm that most suitable substrate for growing of bifidobacteria in milk is lactulose at any analyzed concentration.

**Key words:** bifidobacteria, lactose, inulin, lactulose, fermented milk

Introduction
Bifidobacteria are the most popular probiotics, they have been associated with health promoting effects. Many studies suggest that prebiotics such fructo-oligosaccharide, inulin, galacto-oligosaccharide, lactulose, isomalto-oligosaccharide are able to stimulate the growth of probiotic bacteria as well bifidobacteria (Özer et al., 2005; Martinez-Villaluenga et al., 2006) but not all prebiotics are suitable substrate for growing of bifidobacteria in milk. Bifidobacteria ferment various types of carbohydrates, the fermentation ability depends on the species. One of most popular species of *Bifidobacterium* spp., which is used for production of dairy products, is *B.lactis*. *B.lactis* is isolated from animal faeces and adapted in milk (Klein et al., 1998). It possesses a relative oxygen and acid tolerance which is not observed in some *Bifidobacterium* species. Therefore, *B.lactis* is able to grow in milk. Many studies indicate different results about the ability of bifidobacteria to hydrolyze prebiotics. Semjonovs et al. (2004) observed that *B.lactis* is not capable of utilizing inulin, whereas levan can be metabolized in relatively small amounts. Kontula et al. (1999) reported that bifidobacteria are able to utilize lactulose and lactitol, Reyed (2007) indicated that bifidobacteria can ferment lactose and lactulose, too. Therefore, the objective of this study was to investigate the ability of *Bifidobacterium lactis* to hydrolyse lactose, lactulose and inulin.

Materials and Methods
The research was performed at the microbiological laboratory of the Department of Food Technology of Latvia University of Agriculture and at the laboratory of the Department of Microbiology and Biotechnology of the Faculty of Biology of the University of Latvia. Pasteurized milk with fat content 2.5% and the strain of *Bifidobacterium lactis* (Bb-12, Chr.Hansen, Denmark) was used for experiments. During the experiments, the culture was maintained at -18 °C. As prebiotics were used inulin RAFTILINE® HP (ORAFI, Belgium)
with polymerization degree ≥5 and degree of purity 99.5% and syrup of lactulose (Duphalac®, the Netherlands) with following composition (%): lactulose – no less than 67, lactose – less than 6, galactose – less than 10.

Different lactulose and inulin concentrations (1; 2; 3; 4 and 5%) were added individually to 100 g of milk. *Bifidobacterium lactis* was inoculated with 2 ml of milk suspension (10⁶ cfu ml⁻¹) and cultured at 37 °C for 16 hours. The control sample was prepared without the prebiotics for comparing with the obtained results. The content of lactose and lactulose was determined by IDF standard 147B:1998 procedure, the content of inulin by AOAC Official Method 999.03 and by AACC Official Method 32.32.

**Results and Discussion**

Martinez-Villaluenga et al. (2006) have indicated that *B.lactis* is characterized by a pronounced ability to ferment lactulose in concentrations from 0.5% to 2%, Özer et al. (2005), in turn, have stressed that *Bifidobacterium bifidum* BB-02 and *Lactobacillus acidophilus* LA-5 more effectively assimilated lactulose, if compared with inulin. Taking into consideration these authors’ conclusions and many contradictory data in literature, the content of lactose, lactulose and inulin in milk was determined before and after fermentation. The content of lactose in milk before and after fermentation is shown in Figures 1 and 2.

The obtained results confirm the conclusions mentioned in literature that bifidobacteria poorly assimilate lactose (Modler, 1994). As it is seen in Figure 1, during the fermentation bifidobacteria have been able to utilize 0.42 g 100 g⁻¹ of lactose in control sample. In the milk samples with lactulose, in turn, the changes of lactose content depend on the added lactulose concentration. It is reported in literature that lactose assimilation depends on the added bifidobacteria species, and with the higher assimilation ability are *B.bifidum*, *B.breve* and *B.infantis* (Lamoureux et al., 2002). It is possible to facilitate the process by adding prebiotics. In order to evaluate the effect of lactulose concentration on lactose assimilation in the samples, a dispersion analysis was applied. The obtained results show that different lactulose concentrations do not have the significant effect on the ability of bifidobacteria to
assimilate lactose (p>0.05) during fermentation. Evaluating the decrease of lactose content in the analyzed control and samples, and comparing with the initial lactose content in milk, it should be considered as significant (p<0.05). That could be explained by *B. lactis* properties. *B. lactis* is adapted in milk (Klein et al., 1998), consequently *B. lactis* is able to grow in milk and use lactose as a nutrient for the cell energy metabolism. Similar tendencies are could observe to analyze the data in Figure 2.

![Figure 2](image)

**Figure 2. The content of lactose in milk before* and after fermentation depending on the concentration of inulin**

*The changes of the content of lactose in milk samples before fermentation are connected with the composition of inulin. The content of glucose in inulin is calculated into the total content of lactose according to the standard method for determination of lactose.*

In the sample with 5% of inulin, lactose assimilation increases, however, there are not established significant differences between the fermented milk samples with 2%, 3% and 4% of inulin and with control samples (p>0.05).
The lactose content in milk samples with inulin and control before and after fermentation (Figure 2) was established as significant (p<0.05). However, it should be remarked that lactose assimilation depends on the type and concentration of the added prebiotics. In literature can find indications about the ability of bifidobacteria to assimilate lactulose (Özer et al., 2005) and derivatives of raffinose (Martinez-Villaluenga et al., 2006). When evaluating results, it should be taken into consideration that *B. lactis* is adapted in milk and consequently the speed of multiplication in milk is higher than the other bifidobacteria species. It does explain the decrease of lactose content in fermented milk samples.
The lactulose and inulin content is analysed in the research before and after milk fermentation in order to be able to find out regularities among lactose, lactulose or inulin assimilation in milk under the influence of *B. lactis*. The content of lactulose in milk before and after fermentation is reflected in Figure 3.
The obtained results show that bifidobacteria are able to ferment up to 50% of lactulose in the product, except milk sample with 5% of lactulose where there was a decrease of lactulose by 2/3. When the lactulose concentration increases, the lactulose assimilation also increases in milk and resulting in the increase of *B. lactis* in samples that indicates to a mutual interaction. The obtained results confirm conclusions mentioned in literature about the bifidogenic effect.
of lactulose (Palframan et al., 2002; Bouhnik et al., 2004) and bifidobacteria ability to assimilate lactulose (Saarela et al., 2003).

The changes of lactulose content are significant (p<0.05). Consequently, a conclusion can be drawn that \textit{B.lactis} possesses ability to assimilate lactulose. It relates to conclusions found in literature that bifidobacteria better multiply in the presence of lactulose (Rycroft et al., 2001). Kontula et al. (1999), in turn, indicated that several microorganisms of the large intestine, including also bifidobacteria, are able to utilize lactulose and lactitol.

When comparing the changes of lactose and lactulose content in the fermented milk samples, it is apparent that \textit{B.lactis} is able better to assimilate lactulose (the content of assimilated lactulose increases from 47% to 66%) in comparison with lactose (the content of assimilated lactose is from 9% to 31%). Evaluating the obtained data, it could be concluded that \textit{B.lactis} in combination with lactulose is suitable for the development of a synbiotic dairy product.

The content of inulin in milk before and after fermentation is given in Figure 4.
The obtained results confirm that bifidobacteria poorly assimilate inulin (10–20%). It is reported in literature that *B. lactis* cannot assimilate inulin (Semjonovs et al., 2004). Biedrzycka and Bielecka (2004) have indicated that the ability of bifidobacteria to assimilate inulin is depending on the polymerization degree and the degree of purity. The inulin assimilation decreases together with the increase of inulin polymerization degree and the degree of purity. That does explain the results of inulin assimilation degree in fermented milk samples.

**Conclusions**
1. The ability of *B. lactis* hydrolyse lactose depends on the type and concentration of added prebiotics.
2. *B. lactis* is characterized as being able to assimilate lactulose to 66% in comparison with lower assimilation level of lactose (to 37%) and inulin (to 20%).
3. Lactulose should be considered as the most suitable substrate for growing of *B. lactis* in milk.

**References**
THE STUDY OF ATTENUATED STARTERS IN HOLANDES CHEESE RIPENING

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Abstract
Ripening is a slow, and consequently an expensive process. Acceleration of cheese ripening has received considerable attention in the scientific literature (Fox et al., 1996, El Soda et al., 2003, Wilkinson, 1993, Upadhyay and McSweeney, 2003). Certain approaches have been used to accelerate the ripening of cheese, including the use of an elevated ripening temperature, addition of attenuated starters, and use of adjunct cultures. The aim of the paper is to investigate the role of attenuated starter to formation of Holandes cheese sensory properties.

Holandes cheeses (Edam type cheese) from one manufacturer with/without attenuated starter culture were chosen for experiments. Cheese samples were ripened for 60 days at 6 °C and 12 °C. Cheeses were analyzed during ripening (60 days) for chemical (aroma compounds), physical (\(a_w\), pH and elasticity) and microbiological (colony forming units of lactic acid bacteria) composition. Mesophilic lactic acid bacteria were enumerated on MRS agar at 37 °C 72 h. Volatiles were detected using solid phase GC/MS, the extraction of components was carried out using of 75 µm CAR-PDMS fibre. pH, \(a_w\) and elasticity of cheese samples were measured using appropriate standards procedures.

Attenuated starter has impact on the sensory properties of Holandes cheese. The starter intensifies the flavour of cheese and accelerates proteolysis without necessarily reducing of ripening time. Modification of the ripening temperature influences the rate of flavour and structure development in Holandes cheeses. In spite of attenuated starter the slow development of flavour are observed in cheeses ripened at 6 °C.

Attenuated starter accelerates ripening, intensifies flavour and controlsthe growing of non starter lactic acid bacteria in Holandes cheese.

Key words: Holandes cheese, attenuated starter, aroma compounds, ripening conditions

Introduction
Cheese ripening is a slow and expensive process. Acceleration of cheese ripening has received considerable attention in the scientific literature (Fox et al., 1996, El Soda et al., 2003, Wilkinson, 1993, Upadhyay and McSweeney, 2003). Various approaches have been used to accelerate the ripening of cheese, including the use of an elevated ripening temperature, addition of exogenous enzymes or attenuated starters, use of adjunct cultures, use of genetically modified starter bacteria and high-pressure treatments.

The simplest and the most successful approach to accelerate ripening studied to date is an elevated ripening temperature (Folkertsma et al., 1996). Modification of the ripening temperature is used to control the rate of flavour development in hard cheeses, and ripening at an elevated temperature results in the rapid development of flavour, although problems can occur with texture.

Finally, recent advances in the genetics of lactic acid bacteria (LAB) and a greater understanding of the role of specific enzymes in the generation of volatile flavour compounds in cheese during ripening will facilitate the development of starter genetically modified to enhance flavour development.

The aim of this paper is to investigate the role of attenuated starter to formation of Holandes cheese sensory properties.

Materials and Methods
Unripened Holandes cheese (Edam type) samples with and without attenuated starter cultures were chosen from one cheese manufacturer in Latvia. Cheese has 45% of fat content in dry matter and not greater than 44% of moisture content according to the branch standard LPCS 11:2001 “Holandes siers”.

Cheese samples were ripened for 60 days at 6 °C and 12 °C for evaluation of the influence of attenuated starter on cheese ripening during the different temperature regimes. The ripening
temperature at 6 °C was selected according to ordinary practice in Latvian cheesemaking and at 12 °C was intended to achieve the classical sensory properties of Holandes cheese. Cheese samples were analyzed during ripening for chemical, physical and microbiological indices. Mesophilic LAB were enumerated on MRS agar at 37 °C 72 h. Volatile compounds were detected using solid phase GS/MS (Clarus 500 GC/MS, PerkinElmer Inc.), the extraction of components was carried out using of 75 µm CAR/PDMS fibre.

pH was measured using 3520 pH Meter – JENWAY (Barloworld Scientific Ltd., Essex, UK). The compression force for measurement of cheeses elasticity was determined by TA.XT Plus Texture Analyser (Stable Microsystems Ltd., Surrey, UK). A spherical probe (P/1S – Ball Stainless) was used for compression of sample. Test speed, distance and trigger force were 2 mm s⁻¹, 5 mm and 0.0493 N, respectively. Water activity was determined by Meter AquaLab LITE (Decagon Inc, USA). All parameters were determined in triplicate or more according to the method of procedure. Analysis of variance (ANOVA) was performed in order to identify significant differences in pH and elasticity between cheese samples with and without attenuated starter.

Results and Discussion
The quality and intensity of cheese flavour cannot be predicted precisely during ripening. There is an economic incentive for the development of methods for the acceleration of cheese ripening, provided that the flavour and texture can be maintained and characteristic of the variety (Fox et al., 2000). Acceleration of cheese ripening can be achieved by various practices among them could be mentioned elevated ripening temperatures and usage of attenuated starter. The influence of above mentioned factors on ripening Holandes cheese have been analyzed.

Colony forming units of non starter lactic acid bacteria in analysed Holandes cheese samples were presented in Figure 1.

Figure 1. The dynamic of colony forming units of non starter lactic acid bacteria in Holandes cheeses with/without attenuated starter during ripening
The differences in non starter lactic acid bacteria colony forming units were observed in cheeses with and without attenuated starter from the beginning of ripening. The highest value of colony forming units was observed in Holandes cheese samples with attenuated starter (7.45 log CFU g\(^{-1}\)) comparing with Holandes cheese samples without attenuated starter (4.62 log CFU g\(^{-1}\)).

On 30 day of ripening the growth of colony forming units was 7.83 and 7.10 log CFU g\(^{-1}\) in comparison with initial numbers, reaching peak value for cheese ripening at 12 °C. The obtained results showed that the lowest growing rate of non starter lactic acid bacteria was detected in Holandes cheese samples with attenuated starter during ripening. Cheese samples maturated at 6 °C the peak value of colony forming units reached after 45 days of ripening. Ripening at 6 °C reduces lactic acid bacteria growth rate. However, successful ripening at elevated temperatures requires careful control of cheese composition and microflora.

During ripening enzymes such as residual chymosin, residual plasmin and enzymes from the starter bacteria and cheese microflora are still active and metabolise residual lactose, protein and fat till different compounds, also loss of water by evaporation is observed. Salt content, lactate concentration, amino acids, small peptides and calcium phosphate are important for changes of water activity from 0.995 at the beginning till 0.971 at the end of ripening in cheese. Water activity influences the proliferation of microorganisms in cheeses. There are find differences in the growing rate of microorganisms between samples with and without attenuated starter during ripening.

The growth rate and population density of non starter lactic acid bacteria are affected by pH of cheese. The changes of cheese pH during ripening are showed in Figure 2.

![Figure 2. The dynamics of pH in Holandes cheeses with and without attenuated starter during ripening](image)

The ripening temperature influences the rate of proteolysis, lypolysis, cheese microflora, texture and aroma of cheese and as a result observes more rapid elevation of pH curves. Also higher pH was determined in Holandes cheese samples with attenuated starter in the beginning of ripening. It could be explained by origin of alkaline products of proteolysis due to proteolytic and peptidolytic activity of attenuated starter (Cogan et al., 2002). Also, attenuated starters can be defined as lactic acid bacteria which are unable to produce...
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significant levels of acid during cheesemaking, but which provide active starter enzymes that are important for cheese ripening and flavour development. There are observed some similarities in the dynamics of pH between cheese samples with and without attenuated starter. Due to higher initial pH of samples with attenuated starter the increasing of pH continues during ripening, reaching pH 5.74 at ripening temperature of 12 °C and pH 5.66 at ripening temperature of 6 °C. This differs significantly (p<0.05) from cheeses without attenuated starter, respectively, pH 5.62 at 12 °C and pH 5.52 at 6 °C.

The development of volatile compounds in Holandes cheese samples have been studied throughout ripening period. 24 compounds, each with a distinctive aroma character, have been identified in cheese, and these provide the largest contribution to diversity of cheese flavour. Compounds identified in cheeses include fatty acids (acetic, butanoic, hexanoic, octanoic, nonanoic, decanoic and dodecanoic acids), ketones (2-pentanone, 2-undecanone, diacetyl, acetoin, 2-octanone, 2-undecanone, 2-nonanone, 2-heptanone), alcohols (3-methyl butanol, 1-pentanol, 2-methyl propanol), aldehydes (nonanal, octadecanal, 3-methyl butanal, benzaldehyde), lactones (δ-dodecalactone) and D-limonene.

The same volatile compounds were present in each type of cheese. Differences were observed in the levels of 3-methyl butanol, 1-pentanol, 2-pentanone, acetoin, 2-octanone, 2-nonanone, 2-undecanone, nonanal, 3-methyl butanal, benzaldehyde. In cheeses with attenuated starter have the higher levels of mentioned components than in cheeses without attenuated starter. The influence of cheese microflora on volatile compounds production was observed. Moreover, the levels of all compounds increased in cheese at a ripening temperature of 12 °C, compared with 6 °C.

The influence of ripening on Holandes cheese texture properties was showed in Figure 3.

![Figure 3](image)

**Figure 3. The changes of texture in Holandes cheeses with and without attenuated starter during ripening**

The changes of cheese elasticity were observed in cheese samples. Some authors (Bertola et al., 2000) were found that texture development was accelerated by increasing the ripening temperature. According to Lawrence (1987) data, the cheese texture changes...
markedly during the first two weeks of ripening and exactly ripening temperature has significant impact on cheese texture during all ripening. Holandes cheese with attenuated starter was softer (p<0.05) and the tendency was maintained during ripening at 6 and 12 °C. It indicated on higher proteolysis rate in cheeses with attenuated starter due to increase of water soluble nitrogen fraction that contribute to water binding and texture softening (Waagner, Nielsen, 2004). Besides softer texture, differences in the flavour profile of cheese can be observed analyzing the volatiles in cheeses. The flavour of the ripened cheese samples is richer and more complex when attenuated starter is used in cheese production. It could be explained with different volatile compounds, each with a distinctive aroma character, have been identified in cheese sample, and these provide the largest contribution to the diversity of cheese flavour.

Conclusions
1. Attenuated starter accelerates ripening, intensifies flavour and controls non starter lactic acid bacteria in Holandes cheese.
2. Attenuated starter is unable to produce significant levels of lactic acid during cheesemaking.
3. The higher levels of 3-methyl butanol, 1-pentanol, 2-pentanone, acetoin, 2-octanone, 2-nonanone, 2-undecanone, nonanal, 3-methyl butanal and benzaldehyde are established in cheeses with attenuated starter than in cheeses without attenuated starter.
4. In spite of attenuated starter the slow development of flavour are observed in cheeses ripened at 6 °C.

References
COMPOSITION OF VITAMINS AND AMINO ACIDS IN LATVIAN CRANBERRIES

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Abstract

Berries contain a diverse array of nutrients with recognized biological activities that promote or contribute to health. The content and diversity of vitamins is often the basis for promoting increased daily intake of berries. The current research focuses on the evaluation of vitamins and amino acid content in wild and cultivated cranberries grown in Latvia. The research was accomplished on fresh Latvia wild growing (Vaccinium oxycoccus L.) and cultivated cranberries (Vaccinium macrocarpon Ait.) harvested in Kurzeme region: wild cranberries and cranberry cultivars ‘Early Black’, ‘Ben Lear’, ‘Stevens’, ‘Bergman’ and ‘Pilgrim’. The following quality parameters of cranberries were controlled using standard methods: vitamin E (AOAC 971.30), vitamin B1 (AOAC 986.27), vitamin B2 (AOAC 970.65), vitamin C (LVSEN 14130:2003), amino acids except tryptophan (ion-exchange method), tryptophan (spectrophotometric method). The results of current research demonstrate that very similar vitamin C content was detected in cranberry cultivars: ‘Stevens’, ‘Bergman’ and ‘Ben Lear’. The lowest vitamin C content was detected in wild cranberries and cranberry cultivar ‘Early Black’, 46.98±3.42 and 47.48±3.42 mg·100 g−1 in dry matter (DM) respectively. During current research it was found, that the content of vitamin B1 and B2 in cranberries was similar. The higher vitamin E content was found in wild and cranberry cultivars ‘Ben Lear’ and ‘Pilgrim’, ie., 1.56 mg 100g−1, 1.50 mg 100g−1 and 1.58 mg 100g−1 in DM respectively. Higher essential amino acid content was found in wild and cranberry cultivars ‘Bergman’, ‘Pilgrim’ and ‘Early Black’ 1.94 g 100g−1, 2.06 g 100g−1, 1.83 g 100g−1 and 2.23 g 100g−1 in DM respectively.

Key words: amino acids, vitamins, cranberry cultivars

Introduction

Berries contain a diverse array of nutrients with recognized biological activities that promote or contribute to health; different kinds of anti-oxidant compounds, including flavonoids, phenolics, carotenoids and vitamins, which are all considered beneficial to human health, for decreasing the risk of degenerative diseases by reduction of oxidative stress, and for the inhibition of macromolecular oxidation (Heber, 2004; Rangkadilok et al., 2007). Vitamins are a diverse group of compounds, both chemically and analytically, because they comprise a range of biomolecules whose common properties reside solely in the fact that they are essential dietary components. These compounds are needed in relatively small amounts to sustain life and good health (Bates, 1999).

Vitamin C (Figure 1) is the most important vitamin for human nutrition that is supplied by fruits and vegetables. L-Ascorbic acid (AA) is the main biologically active form of vitamin C. AA is reversibly oxidised to form L-dehydroascorbic acid (DHA), which also exhibits biological activity. Since DHA can be easily converted into AA in the human body it is important to measure both AA and DHA in fruits to know vitamin C activity (Davey et al., 2000; Deutsch, 2000). AA is widely distributed in plant cells where plays many crucial roles in growth and metabolism. As a potent antioxidant, AA has the capacity to eliminate several different reactive oxygen species, keeps the membrane-bound antioxidant α-tocopherol in the reduced state, acts as a cofactor maintaining the activity of a number of enzymes (by keeping metal ions in the reduced state), appears to be the substrate for oxalate and tartrate biosynthesis and has role in stress resistance (Arrigoni and De Tullio, 2002; Klein and Kurilich, 2000).

In foodstuffs, the vitamins B1, B2 (Figure 1) and B6 may be present in free (thiamine, riboflavin, pyridoxol, pyridoxal and pyridoxamine) and phosphorylated forms (essentially thiamine pyrophosphate, riboflavin-5’-phosphate (FMN), riboflavin-5’-adenosylidiphosphate (FAD) and pyridoxal phosphate (Ndaw et al., 2000). Thiamin (Figure 1) is one of the least stable of the water-soluble vitamins when the pH of the matrix approaches neutrality. Vitamin is characterized by a pyrimidine ring linked by a
methylen bridge to the 3-nitrogen atom in a substituted thiazole; the vitamin is highly susceptible to losses during thermal processing. Riboflavin (Figure 1) is stable to heat and oxidation if protected from light; thus, most food processing operations have little effect on riboflavin content (Eitenmiller et al., 2008).

![Figure 1. Ascorbic acid (reduced form) (a), free thiamine base (b), riboflavin (c), α-tocopherol (d) (Eitenmiller et al., 2008)](image)

Vitamin E (Figure 1), also known as α-tocopherol, is an important vitamin found largely in plant materials. Its complex biological functions may include anti sterility, an antioxidant role. The beneficial role of vitamin E in preventing degenerative physiological process and ageing is still being investigated (Tütem et al., 1997). Biologically, vitamin E functions as the primary antioxidant and as a perox radical scavenger. It is the primary, lipid-soluble, chain-breaking antioxidant that combines actions with other lipid- and water-soluble antioxidants to provide cells with an efficient defense against free radical damage. Free radicals are chemical species capable of independent existence that contain one or more unpaired electrons (Eitenmiller et al., 2008).

Amino acids are important of these is concerned with assessing the nutritional value of food and drink products. The monitoring of fermentation and correlated flavour trends in the development of foods and drinks, and the assessing of levels of amino acid fortification also require these compounds to be analyzed (Callejón et al., 2010). For example, Lysine (Lys) is an essential amino acid in humans because there is no Lys biosynthetic cellular machinery (Seminotti et al., 2008); the threonine (Thr) dehydrogenise catalyzes the NAD^+−dependent (nicotinamide adenine dinucleotide) oxidation of L-threonine to 2-amino-3-ketobutyrat (or 2-amino-3-oxobutanoate (Chen et al., 1995) and the Methionine (Met) is an essential amino acid with an important role in biological methylation reactions. It constitutes the main supply of sulfur in the diet, preventing disorders in hair, skin or nails. Moreover, it helps to reduce cholesterol levels by increasing the lecithin production in liver, being also a natural chelating agent for heavy metals (Agüii, 2004).

The current research focuses on the evaluation of vitamins and amino acid content in wild and cultivated cranberries grown in Latvia.

**Materials and Methods**

The research was accomplished on fresh Latvia wild growing (*Vaccinium oxycoccus* L.) and cultivated cranberries (*Vaccinium macrocarpon* Ait.) harvested in Kurzeme region in the first part of October 2010: wild cranberries and cranberry cultivars ‘Stevens’, ‘Bergman’, ‘Ben Lear’, ‘Pilgrim’ and ‘Early Black’ (the moisture content of berries is summarized in Table 1).

The following quality parameters of cranberries were controlled using standard methods: vitamin E by AOAC 971.30, vitamin B\textsubscript{1} by AOAC 986.27, vitamin B\textsubscript{2} by AOAC 970.65, vitamin C by LVSEN 14130:2003, amino acids except tryptophan by ion exchange method (Štavíková and Htka, 2008), tryptophan by spectrophotometric method (Ren et al., 2007).
## Results and Discussion

Mainly fresh cranberries contain 13.3 mg 100 g⁻¹ of Vitamin C (Nutritional value..., 2011), it is 103.34 mg 100 g⁻¹ in DM; however, in Latvia grown cranberries contain lower Vitamin C content (Table 2), what mainly could be explained with climatic conditions. In the present research after acquired data mathematical processing it was established, that there is substantial difference in Vitamin C content in analysed wild and cultivated cranberries (p=0.001) grown in Latvia. Similar Vitamin C content, i.e., differences was not substantial (p=0.511), was found in cranberry cultivars ‘Stevens’, ‘Ben Lear’ and ‘Bergman’, it was ~1.2 times or by 21% higher comparing to Vitamin C content in wild cranberries – 46.98±2.83 mg 100 g⁻¹ in DM, what is substantially (p=0.001). The lowest Vitamin C content was detected in wild cranberries (Table 2): it was by 10% less (substantially, p=0.001) than vitamin C content in cranberry cultivars ‘Pilgrim’ and by 1% less (not substantially, p=0.053) than vitamin C content in cranberry cultivar ‘Early Black’ (Table 2). Else, there is found substantially difference (p=0.001) in vitamin C content in cranberry cultivars ‘Pilgrim’ and ‘Early Black’. Vitamin C content in berries mainly depend on variety individuality, weather conditions during growing period, age of plant, ripening level, fertilizers presence and other factors.

### Table 1

<table>
<thead>
<tr>
<th>No.</th>
<th>Cranberry cultivar</th>
<th>Moisture content, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wild</td>
<td>85.91±2.40</td>
</tr>
<tr>
<td>2</td>
<td>‘Stevens’</td>
<td>86.52±3.12</td>
</tr>
<tr>
<td>3</td>
<td>‘Bergman’</td>
<td>88.19±2.85</td>
</tr>
<tr>
<td>4</td>
<td>‘Ben Lear’</td>
<td>87.36±2.91</td>
</tr>
<tr>
<td>5</td>
<td>‘Pilgrim’</td>
<td>87.36±2.12</td>
</tr>
<tr>
<td>6</td>
<td>‘Early Black’</td>
<td>86.31±3.73</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation; for the mathematical data processing *Sheffe* test was used and p-value at 0.05 was used to determine the significant differences. Experiments were carried out in triplicate.

### Vitamin C content in dry matter of fresh cranberries

<table>
<thead>
<tr>
<th>No.</th>
<th>Cranberry cultivar</th>
<th>Content of Vitamin C, mg·100 g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wild</td>
<td>46.98±2.83</td>
</tr>
<tr>
<td>2</td>
<td>‘Stevens’</td>
<td>57.05±3.12</td>
</tr>
<tr>
<td>3</td>
<td>‘Bergman’</td>
<td>56.56±1.45</td>
</tr>
<tr>
<td>4</td>
<td>‘Ben Lear’</td>
<td>57.83±3.22</td>
</tr>
<tr>
<td>5</td>
<td>‘Pilgrim’</td>
<td>51.89±2.11</td>
</tr>
<tr>
<td>6</td>
<td>‘Early Black’</td>
<td>47.48±2.75</td>
</tr>
</tbody>
</table>

The Recommended Dietary Allowances (RDA) of Vitamin C are 75 to 90 mg d⁻¹ for adult women and men, respectively (Eitenmiller et al., 2008). Therefore, it will be necessary to use in nutrition about ~1.0 kg d⁻¹ of fresh cranberries to reach RDA. It is known, that many berries contain concentrations of food folate, B vitamins such as niacin, tocopherols, and vitamin K, which are important for human health. Therefore it was very important to analyse B group and vitamin E content in cranberries. As the literature data demonstrate, the content of Thiamin in fresh cranberries is 0.012 mg 100 g⁻¹, Vitamin E − 1.2 mg 100 g⁻¹ (Nutritional value..., 2011). By Eitenmiller (2008) RDA as set by the Institute of Medicine in the Dietary Reference Intake
(DRI) for thiamin (Vitamin B$_1$) are 1.2 and 1.1 mg d$^{-1}$ for adult men and women, respectively. Our experiments show (Figure 2), that cranberries will be not as a source of thiamin B$_1$ in diet, because 100 g of fresh cranberries contains ~0.03 mg of Vitamin B$_1$, i.e., ~3% of RDA. However, positively is to observe, that Vitamin B$_1$ was detected in cranberries. After mathematical data processing it was found, that there is no relevant differences ($p=0.051$, $p=0.833$, $p=0.057$) between wild, ‘Stevens’ and ‘Bergman’ cranberry cultivars and between ‘Ben Lear’, ‘Pilgrim’ and ‘Early Black’ ($p=0.051$, $p=0.290$, $p=0.292$) in Vitamin B$_1$ content. However, relevant difference in Vitamin B$_1$ content was found between wild, ‘Stevens’, ‘Bergman’ and ‘Ben Lear’, ‘Pilgrim’ and ‘Early black’ cranberry cultivars ($p=0.001$). It was ascertained, that in cranberry cultivars ‘Ben Lear’, ‘Pilgrim’ and ‘Early Black’ Vitamin B$_1$ content was equally by 50% less than Vitamin B$_1$ content in wild, ‘Stevens’ and ‘Bergman’ cranberry cultivars, what mainly could be explained with berries individuality and Vitamin B$_1$ formation intense in berries during growing. The RDA for riboflavin (Vitamin B$_2$) range from 0.5 mg d$^{-1}$ for children (1–3 years) to 1.6 mg d$^{-1}$ for males and females. The RDI used for the nutritional label declaration is 1.7 mg (Eitenmiller et al., 2008). Therefore, 100 g of fresh cranberries contains ~0.04 mg of Vitamin B$_2$, i.e., ~3% of RDA. There is found substantial difference ($p=0.001$) in Vitamin B$_2$ content in analyzed cranberries. The content of Vitamin B$_2$ was significantly higher ($p=0.001$) in ‘Bergman’ and ‘Ben Lear’ cranberry cultivars by 74% and 61% respectively comparing to wild cranberries (Figure 2), what mainly could be explained with variety individuality and fertilizers presence during berries growing. However, there is no found significant difference in analyzed vitamin content in ‘Stevens’ ($p=0.833$), ‘Ben Lear’ ($p=0.864$) and ‘Pilgrim’ ($p=0.052$) cranberry cultivars comparing to wild berries.

![Figure 2. B$_1$, B$_2$ and E vitamin content in dry matter of fresh cranberries](image)

By Eitenmiller (2008) Estimated Average Requirement (EAR) and Reference Daily Intake (RDA) are 12 mg and 15 mg of Vitamin E ($\alpha$-T) per day, respectively. The upper intake level (UL) value includes all forms of $\alpha$-T from supplemental intake of all-$rac$-$\alpha$-T. The UL is 1000 mg d$^{-1}$. Therefore, 100 g of fresh cranberries contains ~0.2 mg of Vitamin E, i.e., ~1.3% of RDA. In present research it was found, that there is no significant difference in vitamin E content between wild and ‘Pilgrim’ ($p=0.911$), wild and ‘Ben Lear’ ($p=0.575$) cranberry cultivar However, the content of Vitamin E is significantly ($p=0.04$ and $p=0.02$) lower in cranberry cultivars ‘Stevens’ and ‘Early Black’ by 14% and 16% respectively comparing to wild cranberries.
Higher total irreplaceable amino acid content was found in wild and cranberry cultivars ‘Bergman’, ‘Pilgrim’ and ‘Early Black’ – 1.94 g 100 g\(^{-1}\), 2.06 g·100 g\(^{-1}\) in DM, 1.83 g 100 g\(^{-1}\) and 2.23 g·100 g\(^{-1}\) in DM respectively, lower – in ‘Stevens’ and ‘Ben Lear’ – 1.46 g 100 g\(^{-1}\) and 1.12 g·100 g\(^{-1}\) in DM respectively. Thereby, significant difference (p=0.001) was found in total essential amino acid content in ‘Stevens’ (by 25% less), ‘Ben Lear’ (by 42% less) and ‘Early Black’ (by 15% higher) cranberry cultivars comparing to wild grown berries.

In the present experiments it was established, that the content of threonine, tryptophan, isoleucine and histidine in berries was low. However, equally the content of leucine is by 45%, lysine by 15%, valine and phenylalanine by 14% was higher comparing to threonine, tryptophan, isoleucine and histidine content in cranberries. By Amino Labs (2011) RDA for essential amino acids range from ~4 g d\(^{-1}\) for females to ~5 g d\(^{-1}\) for males. Therefore 100 g of fresh cranberries contains ~0.1 g of essential amino acids, i.e., ~3% of RDA.

**Conclusions**

1. Similar Vitamin C content was found in cranberry cultivars ‘Stevens’, ‘Ben Lear’ and ‘Bergman’, it was by 21% higher comparing to Vitamin C content in wild cranberries – 46.98±2.83 mg 100 g\(^{-1}\) in dry matter; the lowest Vitamin C content was detected in wild cranberries.
2. There is no relevant differences between wild, ‘Stevens’ and ‘Bergman’ cranberry cultivars and between ‘Ben Lear’, ‘Pilgrim’ and ‘Early Black’ in Vitamin B\(_1\) content. In cranberry cultivars ‘Ben Lear’, ‘Pilgrim’ and ‘Early Black’ Vitamin B\(_1\) content was equally by 50% less than Vitamin B\(_1\) content in wild, ‘Stevens’ and ‘Bergman’ cranberry cultivars.
3. There is found substantial difference in Vitamin B\(_2\) content in analyzed cranberries: the content of Vitamin B\(_2\) was significantly higher in ‘Bergman’ and ‘Ben Lear’ cranberry cultivars by 74% and 61% respectively comparing to wild cranberries.
4. There is no significant difference in vitamin E content between wild and ‘Pilgrim’, wild and ‘Ben Lear’ cranberry cultivars; the content of Vitamin E is significantly lower in cranberry cultivars ‘Stevens’ and ‘Early Black’ by 14% and 16% respectively comparing to wild cranberries.
5. Significant difference was found in total essential amino acid content in ‘Stevens’ (by 25% less), ‘Ben Lear’ (by 42% less) and ‘Early Black’ (by 15% higher) cranberry cultivars comparing to wild grown berries.

6. As results of current research demonstrate fresh cranberries could be source of Vitamin C in diet but not of Vitamins B₁, B₂, E and essential amino acids.

Acknowledgments
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References
CHEMICAL, MICROBIAL AND ANTIOXIDANT PROPERTIES OF SELECTED HONEY VARIETIES FROM SOUTH AFRICA

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Abstract
In this study, the authors measured chemical properties, antioxidant potentials, and microbial composition of eight commercially available varieties of honey from South Africa. Their pH values ranged from 3.61 ±0.03 to 6.65±0.02, while the electrical conductivity varied between 12±3 to 93±0.4 mS m⁻¹. Concentrations of mesophilic bacteria ranged from (1.4±0.4) × 10¹ to (1.55±0.09) × 10³ CFUs g⁻¹ wet weight of honey, and there was between (2.3±0.5) × 10¹ to (3.3±2.3) × 10⁵ CFUs·g⁻¹ wet weight of honey of mesophilic fungi. No threats to human health would result from the consumption of any of the honey varieties studied as the faecal coliform concentrations, and the concentrations of *Salmonella spp.*, were below the detection limit of 1–9 CFUs g⁻¹ wet weight. All samples had antioxidant activity as supported by results of the ATBS and DPPH assays. At the same time, the square-wave voltammetry showed that this activity resulted from two compounds at half-position potentials between 0.207±0.003 and 0.293±0.003 V; and from 0.444±0.001 to 0.524±0.031 V. Based on the Kruskal-Wallis analysis at 5% level of significance, the average values of all but one of the parameters were different among the honeys (all p-values <0.0124). Stratification of mesophilic fungi was observed in all the studied samples which probably resulted in no difference among the fungal concentrations, i.e. p-value was equal to 0.0740. Preliminary identification of fungal species is presented.

Keywords: honey, South Africa, composition

Introduction
Saccharides, such as fructose and glucose, have been shown to account for 85 to 95% of honey by weight (Gomes et al., 2010). In South Africa, honey has long been used as the raw materials for fermentation of mead-like alcoholic beverages (Cambray, 2005) and it is also consumed raw as sweetener. Plants used for honey production include *Aloe greatheadii var. davyana* (Human and Nicolson, 2008), *Leucospermum cordifolium* and *Erica species* (Basson and Grobler, 2008). To date, the research activities have focused on the wound-healing properties of honey (Karpelovsky et al. 2007), antimicrobial activity against *Candida albicans,* *Staphylococcus oralis* and *Staphylococcus aureus* (Basson and Grobler, 2008); and *Helicobacter pylori* (Manyi-Loh et al., 2010). A lot of research has been conducted on the biology and diseases of the honey bee (e.g. Dieteman et al., 2006); and beekeeping in South Africa (Charles, 2005). Quality of honey depends on the sensory, chemical, physical and microbiological properties (EU, 2001). Data of this kind is currently missing from the literature in South Africa and so it is the aim of this article to address this knowledge gap.

Materials and Methods
Eight commercially honeys were purchased from supermarkets and local shops in the following provinces of South Africa: Eastern Cape, Western Cape, and North West. The honey samples were stored at room temperature until the analyses were performed. The following chemicals and consumables were purchased from Merck (Pty.) Ltd. (Johannesburg/Cape Town, South Africa): m-FC agar, sodium chloride, nutrient agar, potato-dextrose agar, XLD agar and tetrathionate broth for *Salmonella enumeration*, ascorbic acid, brilliant green, potassium iodide and iodine. Hanna water testers for measurement of pH and electrical conductivity (EC) were purchased from Sigma-Aldrich (Johannesburg, South
Chemicals for the antioxidant assays in the DPPH and the ABTS methods were also purchased from this supplier. Sterile 40 mL urine jars for microbial sampling and dilutions were purchased from EC Labs (renamed to Spellbound Inc., Port Elizabeth, South Africa). Values of pH and EC were measured using the method of Gomes et al. (2010). For microbiological analyses, 5–30 g wet weight of the particular honey sample (accuracy 0.01 g) was dispensed into a 40 mL sterile urine jar. Nine to 15 millilitres of sterile physiological saline solution was added and the sample was homogenised by hand-shaking. The aim was to ensure complete visible dissolution of the honey in question. Decimal dilutions in physiological saline of the samples were then performed under aseptic conditions. Concentrations of mesophilic bacteria (MB) were enumerated after spread-plating onto nutrient agar, while the mesophilic fungi (MF) were spread-plated onto potato-dextrose agar. Colonies were counted after incubations at 30 °C for 48 hours for bacteria and after 96 hours at 32 °C for fungi. Faecal coliforms were spread-plated onto m-FC agar and counted as blue colonies after growth at 44.5 °C for 24 hours.

Separate samples were prepared for enumeration of *Salmonella* spp. Two millilitres of the diluted sample was mixed with 20 mL of m-FC broth and incubated at 37 °C for 18 hours. After this, the 1 mL was re-inoculated onto the XLD agar and *Salmonella* spp. was quantified as the number of red colonies that grew after further 48 hours at 37 °C. All incubations were done in one of the following incubators: the Labcon incubator Model FSIM B (Labmark, Johannesburg, RSA), the TS 606/3-I incubator (WTW, Weilheim, Germany), the Labcon low temperature incubator LTIE 10 (Labmark, Johannesburg, RSA); and/or the Heraeus Model FT 420 (Heraeus Kulzer GmbH, Dormagen, Germany). All sterilisations were conducted using the Model RAU-53Bd REX MED autoclave (Hirayama Manufacturing, Tokyo, Japan). Microbial concentrations are reported as colony forming units 1 g⁻¹ wet weight of the honey (CFUs·g⁻¹).

Antioxidant activity of the individual honey samples was evaluated using the DPPH and the ABTS methods; as well as using square-wave voltametry. For the DPPH assay, a 72 mg mL⁻¹ DPPH solution was made up in 80% methanol, followed by a 100 µg cm⁻³ ascorbic acid stock solution in MilliQ water (Millipore-Microsep, Port Elizabeth, South Africa). The calibration solutions were prepared by diluting the ascorbic acid stock solution with MilliQ water. In this way, a range of solutions with the following ascorbic acid concentrations was obtained: 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, 45 and 50. Two hundred microlitres of DPPH solution was added to each standard in the microtitre plate and the plate was placed into the Powerwave plate UV/VIS spectrophotometer (BioTek, Winooski, USA). Antioxidant activity was evaluated using Eq. (1) after taking reading every 15 seconds for 5 minutes.

\[
\text{Antioxidant activity of honey} = 100 \times \frac{A_{\text{Standard}} - A_{\text{Honey}}}{A_{\text{Standard}}}
\]

In Eq. (1), \(A_{\text{Standard}}\) is the average absorbance at 515 nm for ascorbic acid (dimensionless), and \(A_{\text{Honey}}\) is the average absorbance at 515 nm for the particular honey sample (dimensionless). Antioxidant activity was measured as ascorbic acid equivalents.

For the ABTS method, 7mM ABTS solution was prepared by weighing out 0.3845 g ABTS and dissolving it in 80 cm³ of MilliQ water. Then 0.0662 g of \(K_2S_2O_8\) was added and the volume was made up to 100 cm³ with MilliQ water. The solution was left to stabilise overnight and the ABTS solution was diluted to obtain a solution with absorbance 0.8–1.0 at 734 nm. A 100 µg cm⁻³ stock solution of TROLOX was made up in 100% ethanol and it was further diluted to obtain the following concentrations (µg cm⁻³): 2, 4, 6, 8, 10, 15, 20, 25, 30, 35 and 40. The rest of the procedure was analogous to that of DPPH and results were evaluated according to Eq. (1). To identify the number of antioxidants in each honey sample,
square-wave voltammetry was performed on the Potentiostat/Galvanostat 30 (PGSTAT 30; Eco Chemie, Netherlands) and antioxidation capacity was quantified using the peak potential at half height (half-position potential) and number of electrochemical peaks detected (Harbertson and Spayd, 2006).

If there were statistically significant differences in the properties of individual honey varieties was examined using the one-way Kruskal-Wallis analysis of variance at 5% level of significance (KW analysis; Past statistical software package version 2.0, Paleontological Museum, Oslo, Norway and Geological Museum, Copenhagen, Denmark).

Results and Discussion

No threats to human health would result from the consumption of any of the honey varieties studied as the faecal coliform concentrations, and the concentrations of *Salmonella* spp., were below the detection limit of 1–9 CFUs·g\(^{-1}\) wet weight. The remaining results are summarised in the text below and mainly in Table 1. The pH values of the samples ranged from 3.61±0.03 to 6.65±0.02, while the EC values varied between 12±3 to 93±0.4 mS m\(^{-1}\). Concentrations of MB ranged from \((1.4±0.4) \times 10^1\) to \((1.55±0.09) \times 10^3\) CFUs g\(^{-1}\) wet weight of honey, and there was between \((2.3±0.5) \times 10^1\) to \((3.3±2.3) \times 10^3\) CFUs g\(^{-1}\) wet weight of honey of MF. Morphological examination of the growth characteristics showed that 8 different species of bacteria and 15 different species of fungi were present in the honey samples. More direct identification is currently underway. Preliminary results indicate that 2 of the bacterial isolates belonged to the genus *Bacillus* spp. while 1 fungal isolate was identified as belonging to the species *Saccharomyces* spp. These species have been reported in honey by Snowdon and Cliver (1996). Additional identification will be conducted using DNA sequencing, but this work will commence once additional funding becomes available.

All samples had antioxidant activity as supported by results of the ATBS and DPPH assays. In both assays, there was no statistical difference in the antioxidant properties of the individual honey varieties (p-values > 0.120 in all cases). On average, the antioxidant activities honeys were 43 and 62% lower than the respective assay standard used in the ATBS and the DPPH assays. The square-wave voltammetry showed that this activity resulted from two compounds at half-position potentials between 0.207±0.003 and 0.293±0.003 V; and from 0.444±0.001 to 0.524±0.031 V. Based on the Kruskal-Wallis analysis at 5% level of significance, the average values of all but one of the parameters were different among the individual honey samples (all p-values <0.0124). Stratification of MF was observed in all the studied samples led to this observation (p-value =0.0740). Maximum values for the other parameters from Table 1 were observed for the following samples: Fynbos for pH and EC, Melior for MB, Blue Gum for MF, Goldcrest for the first peak potential and Champagne for the second square-wave peak.

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>EC mS m(^{-1})</th>
<th>MB CFUs g(^{-1})</th>
<th>MF CFUs g(^{-1})</th>
<th>E(_1) (V)</th>
<th>E(_2) (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goldcrest</td>
<td>3.61±0.03</td>
<td>12±4</td>
<td>44±7</td>
<td>400±361</td>
<td>0.272±0.036</td>
<td>0.484±0.047</td>
</tr>
<tr>
<td>Melior</td>
<td>3.75±0.01</td>
<td>40±2</td>
<td>1600±97</td>
<td>33±32</td>
<td>0.208±0.003</td>
<td>0.498±0.003</td>
</tr>
<tr>
<td>Fynbos</td>
<td>6.65±0.02</td>
<td>93±0</td>
<td>70±18</td>
<td>300±294</td>
<td>0.217±0.011</td>
<td>0.476±0.006</td>
</tr>
<tr>
<td>Champagne</td>
<td>3.70±0.04</td>
<td>33±3</td>
<td>90±21</td>
<td>1000±1040</td>
<td>0.209±0.005</td>
<td>0.524±0.031</td>
</tr>
<tr>
<td>Blue Gum</td>
<td>3.90±0.02</td>
<td>13±1</td>
<td>140±13</td>
<td>330000±232191</td>
<td>0.293±0.003</td>
<td>0.444±0.001</td>
</tr>
<tr>
<td>Ikaros</td>
<td>3.77±0.03</td>
<td>27±0</td>
<td>29±6</td>
<td>4000±3987</td>
<td>0.207±0.003</td>
<td>0.493±0.003</td>
</tr>
<tr>
<td>Cape Coast</td>
<td>4.47±0.01</td>
<td>32±1</td>
<td>14±4</td>
<td>23±5</td>
<td>0.266±0.004</td>
<td>0.511±0.004</td>
</tr>
</tbody>
</table>

Table 1: Chemical, microbial and antioxidant properties of the honey varieties
Concentrations of FC, MB and MF, along with the values of pH, EC, are comparable to the data of Gomes et al. (2010). Likely sources of bacteria include the intestines of the worker bees, pollen, and post-harvest handling by the processing plant staff (Snowdon and Cliver, 1996). Sanitary conditions at the processing plants can be considered within regulatory guidelines, as no faecal coliforms or *Salmonella* spp. cells were detected in either of the honey samples. Highly acidic values of the pH measured in the analysed honey varieties indicate that saccharides present in the samples were probably fermented into organic acids (Gomes et al., 2010). The results of the square-wave analysis indicate that all eight honey samples have strong antioxidation potential, since position potential values are below 0.5 V (Harbertson and Spayd, 2006).

**Conclusions**
1. Eight honeys from South Africa showed to be safe for human consumption, and showed to have strong antioxidant potentials.
2. Further research will focused on the precise identification of the mesophilic bacteria and fungi isolated in this study; and identification of the antioxidant compound.

**Acknowledgements**
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**References**
Abstract
The rowanberries (Sorbus aucuparia) are small orange-red “fruits” of a rowan tree. These berries have been described as an important source of flavonoids, and their antioxidant activity affects reactive oxygen species and lipid peroxidation; therefore they are suitable for production of health-food products. The aim of this experiment was to prepare new product – rowanberries in powder sugar, and to determine chemical and physical properties of samples. The experiments were prepared in the Faculty of Food technology of Latvia University of Agriculture and in the Customs laboratory of National Customs Board of State Revenue Service. The berries of cultivars ‘Moravica’ and ‘Michurinskaya Krasnaya’, and hybrid of rowanberry × hawthorn ‘Granatnaya’ were used for investigations. The rowanberries ‘Granatnaya’ in powder sugar were packed in carton and plastic (biodegradable PLA and conventional PP) boxes and kept three weeks at room temperature. The content of ascorbic acid and total carotenoids, the firmness of experimental products and the weight losses of packed samples were analysed. For determination of the organic acid and ascorbic acid content high performance liquid chromatography was used, and the content of total carotenoids was determined by spectrophotometric method. Texture analyser TA.XT.plus was used for measurement of the firmness of samples. The results showed that sweet rowanberries are good raw material for preparation it’s in powder sugar. The analysis of firmness showed that the rowanberries in powder sugar had a fairly hard texture, influenced by the presence of sugar layer. We observed the weight losses during 18 days storage of packed samples in carton boxes.

Key words: rowanberries in powder sugar, firmness, ascorbic acid, total carotenoids

Introduction
The rowanberries (Sorbus aucuparia L.) are small orange-red “fruits” of a rowan tree (also known as mountain ash), which grow in the northern part of Europe. The rowanberries (Sorbus) belong to the subfamily Maloideae of the family Rosaceae and their berries have been promoted as a health-food or can be a source for health-promoting components. The ripe wild rowanberries are picked in the autumn and they are eatable, but very tart in flavour. Sweeter and less astringent than wild rowanberries are different cultivars of sweet rowanberries and hybrids with other species. The hybrid cultivars were developed by cross-breeding rowan with Malus, Pyrus, Aronia and Mespilus (Hukkanen et al., 2006; Poyrazoglu, 2004).

The berries consist mostly of water, and the main components in the dry matter are carbohydrates, primarily sugars, and non-volatile organic. Organic acids are important intermediate products of metabolism (Viljakainen et al., 2001). According to food composition and nutrition tables, sweet rowanberry S. aucuparia L. var. edulis contains 1600-2420 mg of organic acids per 100 g of edible portion, int.al. 10 mg of parasorbic acid, 98 mg of vitamin C per 100 g and 2.5 mg of total carotenoids per 100 g. (Souci et al., 2008)

Packaging protects goods from damage, allows efficient transportation and distribution, offers convenience and prolongs shelf life. Berries are live organisms and, even after harvest, they continue to respire and transpire. If there is not enough oxygen, fermentation occurs, and small amounts of alcohol, acetaldehyde, and other volatile compounds are produced; therefore, packaging materials for fruits should not create too high a barrier to oxygen. On the other side berries are high-moisture products and loss of moisture under normal storage conditions causes wilting and shrivelling of product. Developments in polymer chemistry have resulted in the production of packaging films such as low-density polyethylene (LDPE), polystyrene (PS), polyvinyl chloride (PVC), polyvinylidene chloride (PVDC), and ethylene vinyl alcohol (EVOH). These films have a range of water vapour and gas barrier, and heat-sealing characteristics that enable them to be used for shelf-life extension of products (Gontard et al., 2010;
Smith et al., 2005). Promising alternatives of polymer packaging are biopolymers, produced from regularly renewable raw materials. There are three main forms of renewable bio packaging: polylactic acid (PLA), polyhydroxyalkanoates (PHA) and thermoplastic starch (TPS) (Weber et al., 2002). Although many biobased packaging materials do not provide a high water vapour barrier, and moisture loss or gain is therefore a critical parameter for foods packaged in such materials. There was reported and expected that PLA will be suitable for fruits packaged in atmospheric air as these products do not require any specific gas barrier (Holm, 2010).

The aim of this experiment was to prepare new product – sweet rowanberries in powder sugar, to determine chemical and physical properties of samples and the possible storage time in different packaging materials.

Materials and Methods

Experimental design

Experiments were carried out at the Faculty of Food technology, Latvia University of Agriculture, and the Customs laboratory of National Customs Board, State Revenue Service. The object of this investigation was berries of two sweet rowanberries cultivars 'Moravica' and 'Michurinskaia Krasnaya', and hybrid of rowanberry × hawthorn 'Granatnaya' (hybrid of S. aucuparia × Crataegus sanguinea) grown in Latvia. The berries were picked in the Pure Horticultural Research centre (HRC) collection of genetic resources in September 2010. Part of samples after harvesting was packed in plastic bags, frozen, and kept at -18±2 °C along 3 months.

The samples of experimental product – sweet rowanberries in powder sugar, were prepared from fresh rowanberries and from stored frozen rowanberries followed by thawing. The frozen berries (1 kg) were thawed overnight in refrigerator at +4°C. All rowanberries were dipped in the solution of starch and then coated with powder sugar layer. The coating process with powder sugar was repeated once. The samples of rowanberries 'Granatnaya' in powder sugar were packed in carton (80±5 g in each box) and plastic (biobased PLA and conventional PP) boxes (60±5 g in each box), and stored 18 days at room temperature. The content of organic acids and ascorbic acid and total carotenoids, the firmness of all experimental products and the weight losses of packed samples were analysed.

Chemical and physical analysis

The determination of the organic acids and vitamin C content was based on methods reported by Romero-Rodriguez et al. (1992) and Vanques-Oderiz et al. (1994) by adding some modifications. Samples of experimental products (100 g) were homogenised by a manual blender (Braun). The portion of samples (5–10 g) was weighed into a volumetric flask (50 ml) and 0.001 M sulphuric acid (~ 30 ml) was added for determination of organic acid content, and 0.1% oxalic acid (~ 30 ml) – and ascorbic acid content was determined. Mixture was stirred mechanically for 15 minutes and the solution filtered through a paper filter (DP 503 125, Albet) in 50 ml volumetric flask and filled to mark with correspondent acid solution. Acid extract was then filtered through a membrane filter with pore size 0.2 µm (Sartorius) prior to injection into the chromatographic system. Calibration curve was acquired after two repeated HPLC runs of 5 standard solutions of reference materials. Quantifications of the organic acids and ascorbic acid content of experimental products were performed in duplicate and were based on peak area measurements.

The extract of experimental products was analysed and content of organic acids and vitamin C (ascorbic acid) determined using HPLC Prominence (Shimadzu, Japan) equipped with Ostion LG-KS H+ column (250x8 mm, particle size 10 µm) and an autosampler SIL-20A. Working conditions: the mobile phase – ultra-pure water acidified to pH 2.2 with sulphuric acid. The flow rate was 0.4 ml min⁻¹, column temperature – 30 °C, detection with a UV/VIS detector SPD-20A (Shimadzu) was at 215 nm for organic acids and at 245 nm for vitamin C, and injection volume of
samples – 10 µl. Data were acquired and processed using Shimadzu LabSolutions software (LCsolution Version 1.21 SP1).

The content of total carotenoids was analyzed by the spectrophotometric method at 440 nm (Ермаков, 1987) with petroleum ether (boiling temperature range 80–110 ºC) and measured with UV-VIS-NIR spectrophotometer UV-3100PC (Shimadzu) in 10 mm cuvettes. One to two grams of homogenized rowanberries in powder sugar were placed in a conic retort (100 ml) and 96% ethanol (20 ml) was added, and then samples were stirred by a magnetic stirrer for 20 min. Then petrol ether (25 ml) and water (1 ml) was added and stirring was continued for one more hour. After 3–4 hours the top (yellow) layer was used for the detection of total carotenoids. The carotene equivalent (KE) was found, using graduating curve with K$_2$Cr$_2$O$_7$.

The firmness of all experimental products was measured by texture analyser TA.XT.plus (Stable Microsystems Ltd., Surrey, England) and software Texture Exponent 32. The texture analyser was equipped with a load cell of 50 kg. Samples of rowanberries in powder sugar were positioned under the needle type P/2. The results were expressed as maximum force in Newtons (N), and the maximum force required for sample compression was calculated as an average of 15–20 measurements.

The weight losses of packed samples were determined by weighing on the analytical balances Sartorius SB-210s with precision ±0.0001 g. The results were analyzed before storage (0 days) and after 4, 6, 8, 10, 12, 14 and 18 storage days, and reported as averages of those three determinations.

Data analysis

All values of parameters were expressed as means and standard deviations and calculated using SPSS for Windows (Version 11.0).

Results and Discussion

In this study 6 samples of sweet rowanberries in powder sugar were prepared consisting of 53±6% of berries and 47±6% of starch-sugar layer.

The content of organic acids and ascorbic acid (vitamin C) is a very important parameter that determines the quality of berry products. The amount of organic acids (citric, malic, succinic, and sorbic) of rowanberries in powder sugar is given in Table 1. Malic acid was the dominant compound compared with other organic acids in all investigated samples. The content of malic acid in samples prepared from frozen rowanberries was on 7.5–8.2% less than of those made from fresh rowanberries. We observed that during frozen storage of rowanberries 'Michurinskaya Krasnaya' the content of succinic and sorbic acid increased.

<table>
<thead>
<tr>
<th>Cultivar of berries</th>
<th>Citric acid</th>
<th>Malic acid</th>
<th>Succinic acid</th>
<th>Sorbic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Moravica' fresh</td>
<td>26±3*</td>
<td>769±17</td>
<td>65±2</td>
<td>7±1</td>
</tr>
<tr>
<td>'Moravica' frozen</td>
<td>25±12</td>
<td>706±28</td>
<td>66±12</td>
<td>6±2</td>
</tr>
<tr>
<td>'Michurinskaya Krasnaya' fresh</td>
<td>91±1</td>
<td>1242±28</td>
<td>202±6</td>
<td>10±1</td>
</tr>
<tr>
<td>'Michurinskaya Krasnaya' frozen</td>
<td>70±2</td>
<td>1150±31</td>
<td>222±5</td>
<td>12±1</td>
</tr>
</tbody>
</table>

* - values expressed as mean and standard deviation

The ascorbic acid content of sweet rowanberries is reported in Figure 1, and the highest content of vitamin C was detected in the samples of experimental products made from fresh berries.
Figure 1. The vitamin C content of rowanberries in powder sugar

The total carotenoid content of sweet rowanberries in powder sugar is reported in Figure 2. The content of total carotenoids was less about of 13 and 16% in samples made from frozen and thawed rowanberries than in rowanberries prepared from fresh berries (cultivars 'Michurinskaya Krasnaya' and 'Moravica', respectively).

Figure 2. The total carotenoid content of rowanberries

The results of textural analyses of experimental products are showed in Figure 3. The results of the experiment showed that the rowanberries in powder sugar had a fairly hard texture, improved by the presence of sugar layer.

Figure 3. Comparison of firmness of rowanberries and rowanberries in powder sugar
The weight losses during storage of rowanberry samples in powder sugar packed in carton boxes are reported in Figure 4. The weight loss of samples packed in plastic boxes during the firsts 4 storage days was 1.6–2.2 % and during 6 days storage – 9.6–11.6%. The weight loss of samples packed in cardboard boxes during the firsts 4 storage days was determined 9.9–11.4 % and during 6 days – 21.3–23.5%, and during 18 days – 29.1–32.5%. The sugar coating of experimental sample’ berries packed in plastic boxes (PLA and PP) after 6 days storage moistened, therefore the storage was not followed up.

![Figure 4. The weight loses of rowanberries 'Granatnaya' in powder sugar](image)

The firmness of experimental products during storage is showed in Figure 5.

![Figure 5. The firmness changes of rowanberries in powder sugar during storage time](image)

After 18 days storage in carton boxes the firmness of experimental products – 'Granatnaya' in powder sugar, were increased from 6.3 up to 10.3–12.1 N (samples from fresh berries) and from 7.6 up to 8.9–9.8 N (samples from frozen and thawing berries).
Conclusions

1. Both fresh and frozen stored rowanberries followed thawing are as good raw material for their processing in powder sugar. The new product contains ascorbic acid, organic acids, and carotenoids. Malic acid is the main component of organic acids – in 'Moravica' in powder sugar – 706–769 mg 100 g$^{-1}$ and in 'Michurinskaya Krasnaya' in powder sugar – 1150–1242 mg 100 g$^{-1}$.

2. The rowanberries in powder sugar had a fairly hard texture, improved by the presence of sugar layer. Plastic boxes (PLA and PP) are not recommendable for storage of berries in powder sugar more than 6 days; whereas in carton boxes the storage time may be 12 days.

Acknowledgements

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AMOUNTS OF HEAVY METALS IN BALTIC COD MEAT

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Work was done at the Klaipeda’s State Veterinary and Food Department Laboratory

Abstract
The aim of the study was to analyze the amount of heavy metals in Baltic cod meat. Three year averages of Pb, Cd and Hg in cod muscle are reported (the study was conducted in 2008–2010) and data compared with the acts of the Republic of Lithuania and the European Union. The information and research material (data on the amount of heavy metals) were received from the National Laboratory of Veterinary. During the monitoring period amount of heavy metals Pb, Cd and Hg in cod meat corresponded to the allowable standards and are close to Lithuanian Hygiene Norms MAL. The results of this work obligate to control the amounts of heavy metals in fish muscles regularly.

Key words: heavy metals, fish, cod, contamination

Introduction
Mankind, without let-up through the land resources and promoting the development of civilization does not always ensure the protection of the natural environment of various chemical or biological contaminants. Some heavy metals – lead (Pb), cadmium (Cd) and mercury (Hg) emissions of its toxic properties within the human body and can cause serious problems to the marine environment and human’s health. The heavy metals content of fishery products need to be well established as fishery products are widely consumed by humans (Volbekas, 1990; Turan et al., 2009; Staniškienė et al., 2007).
Since fish is the last link in the aquatic food chain, the heavy metal concentrations in many fish species have been determined in relation to the metal content of the aquatic environment (Kargin, 1998). Heavy metals like copper, zinc and iron are essential for fish metabolism while some others such as mercury, cadmium and lead have no known role in biological systems. For the normal metabolism of fish, the essential metals must be taken up from water, food or sediment. However, similar to essential metals, non-essential ones are also taken up by fish and accumulated in their tissues (Turan et al., 2009).

Industrialization has improved general technology as well as quality of life but has also resulted in an increase in metal concentrations in water. These metals can be classified as potentially toxic (aluminium, arsenic, cadmium, lead, mercury, etc.), probably essential (nickel, vanadium, cobalt) and essential (copper, zinc, selenium) (Munoz-Olivas and Ca’mara, 2001). Toxic elements can be very harmful even at low concentration when ingested over a long time period. Other elements, which are also present in seafood, are essential for human life at low concentration; however, they can also be toxic at high concentrations (Ray, 1994). In our study we investigated lead (Pb), cadmium (Cd) and mercury (Hg) in Baltic cod meat. Mercury gets into water mainly with industrial effluents and atmospheric precipitation and very quickly passes into the bottom sediments. It accumulates there, usually in sulphite form. Elementary mercury and its organic and inorganic compounds are liable to methylation. The toxic products of this methylation (methyl mercury) enter the food chains and accumulate in the aquatic organisms. In the aquatic medium, lead accumulates mainly in the bottom sediments where its level is usually four orders higher than in the water. Like mercury, lead is able, through the action of some micro-organisms, to produce organic methyl derivatives which accumulate in the aquatic organisms. However, as distinct from mercury, lead was not observed to accumulate in fish. In waters, cadmium is accompanied by zinc; it is also contained in industrial effluents. Waters that wash phosphate fertilizers from farm land are also a significant source of cadmium contamination. Like lead, cadmium was not found to significantly accumulate in aquatic organisms (Svobodová, 1991).
Among Baltic fish, the following species have been studied for heavy metal levels: cod (Gadus morhua), herring (Clupea harengus), sprat (Sprattus sprattus), flounder (Platichthys flesus), sea trout (Salmo trutta) and perch (Perca fluviatilis) (Brzezinska et al., 1984; Kannan and Falandysz, 1997; Szefer and Falandysz, 1985; Vuorinen et al., 1998; Vuorinen et al., 1994). There are reports on heavy metals in cod from Baltic waters (Polak-Juszczak, 2009); however no data are available for cod heavy metal content reported from the coastal waters of the western Baltic sea of the Lithuania.

The aim of the paper was to study the distribution of Pb, Cd and Hg in muscle and liver of edible fish, i.e. cod (Gadus morhua), economic zone of the Baltic Sea.

**Materials and Methods**

Data of heavy metals concentrations in Baltic cod was taken from the Lithuanian State Food and Veterinary Service of the National Veterinary Laboratory annual reports (2008–2010). Fish samples were collected from fishing cutters operating in the Lithuanian coastal zone of the Baltic. Cruises were undertaken in the winter-spring (February–March), summer (August), and fall (October–November) seasons. Fish at the fishing vessels were sealed in plastic bags, frozen on board the vessels, and then transferred to the freezer at the laboratory (-18 °C).

Heavy metal content in tissues was assessed by atomic absorption spectrometry. Cold vapour atomic fluorescence spectroscopy was used for Hg assessment and graphite furnace atomic absorption – for Cd and Pb.

Statistical analyses of results are performed using SPSS statistical software.

**Results and Discussion**

Restricted levels of heavy metals in the fish are determined not only by the World Health Organization guidelines, but also by the actual situation of the country, so in different countries it is different. For example, the Lithuanian Hygiene Norm 54: 2001 indicates maximum concentration of lead in fish flesh from 0.2 to 0.4 mg kg⁻¹ and the European Union — 0.2 mg kg⁻¹ (EC, 2000). The evaluation of the average annual content of the various elements based on the data from several years confirmed that the dominating element in all the fish was Hg (Table 1).

**Table 1**

<table>
<thead>
<tr>
<th>Year</th>
<th>n</th>
<th><em>Cd</em></th>
<th>Pb</th>
<th>*Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>24</td>
<td>0.0023±0.0023</td>
<td>0.0420±0.0350</td>
<td>0.0920±0.0510</td>
</tr>
<tr>
<td>2009</td>
<td>23</td>
<td>0.0021±0.0022</td>
<td>0.0250±0.0300</td>
<td>0.0370±0.0400</td>
</tr>
<tr>
<td>2010</td>
<td>25</td>
<td>0.0022±0.2500</td>
<td>0.0320±0.3200</td>
<td>0.0470±0.4300</td>
</tr>
</tbody>
</table>

The toxic elements of Cd and Pb occurred at low levels in the fish studied. In cod, these elements were often at the levels of detection (Cd below 0.002 mg kg⁻¹, Pb below 0.013 mg kg⁻¹).

The concentration of Hg in 2010 was approximately two times lower than in 2008. The differences in average concentrations of Cd and Pb in 2008 and 2010 were much less pronounced; however, this stable level was not noted throughout the studied period. Differences in average concentrations of Hg in cod meat were much more dispersed. This agrees with previously done studies (Polak-Juszczak, 2009). There Polak-Juszczak found the concentration of Hg and Pb in 2003 was approximately fourfold lower than in 1994, while concentrations of Cd were more than twofold lower in sprat, herring and cod from Baltic Sea. Casini et al. (2004) reported that many factors impact the accumulation of metals by fish, including diet. If trace
elements associated with plankton are absorbed into the alimentary tract of consumers, this is the predominant route of exposure (Harms, 1996). This nutritional chain can be applied to herring and sprat. These fishes feed mainly on plankton, in contrast to cod which feed mainly on small herring and benthic animals. This means that the respective variations in the concentrations of trace metals in the water and, thus, plankton, are not reflected directly in heavy metals concentrations in cod (HELCOM, 2002).

Conclusions
1. Baltic cod fillets are a good source of many major and essential elements. In addition, the levels of non-essential elements, such as Cd, Pb and Hg are typically low.
2. Accumulating of heavy metals in fish muscle may be considered as an important warning signal for fish health and human consumption.
3. The present study shows that precautions need to be taken in order to prevent future heavy metal pollution. Otherwise, these pollutions can be dangerous for fish and human health.

References
PREVALENCE OF YERSINIA ENTEROCOLITICA IN THE ENVIRONMENT OF SLAUGHTERHOUSE

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Abstract

A total amount of 64 surface swabs from slaughterhouse rooms and equipment, work tools and clothes were collected in three large scale slaughterhouses between January 2006 and January 2009 during pig slaughter. Samples were tested according to ISO 10273 standard requirements, with subsequent cold enrichment for three weeks in peptone mannitol bile salt broth. Isolated cultures were confirmed with API 20E, after that all Y. enterocolitica isolates were biotyped and serotyped. In general, the prevalence of Y. enterocolitica in the slaughterhouses was 37% (24/64), where 34% (22/64) and 3% (2/64) comprised Y. enterocolitica 1A and Y. enterocolitica 4/O:3, accordingly. Y. enterocolitica 1A was recovered in slaughterhouses A, B and C with the prevalence 42% (8/19), 34% (9/26) and 26% (5/19) of positive cases, while Y. enterocolitica 4/O:3 was observed only in slaughterhouse A with the prevalence 11% (2/19). Y. enterocolitica 1A was found on sink (4/4/100%), door (2/4/50%), meat inspection platform (2/4/50%), floor (5/12/42%), work surface (2/5/40%), table for work equipment (1/3/33%), box for cold storage of products (1/4/25%), apron (1/4/25%), gloves (1/4/25%), footwear (1/4/25%), hook (1/5/20%), box for offals (1/7/14%) samples. Y. enterocolitica 4/O:3 was found on work surface (1/5/20%) and floor samples (1/12/8%). No significant differences (p>0.01) were observed in the prevalence of Y. enterocolitica in environmental samples between slaughterhouses A, B and C. The presence of Y. enterocolitica, bioserotype 4/O:3 in environmental samples, indicated that environment of the slaughterhouse can be a cause of contamination of slaughter products with yersiniae, and greater efforts should be made to maintain hygiene in slaughterhouse on acceptable level.

Key words: non-pathogenic yersiniae, pathogenic yersiniae, hygiene, pig

Introduction

Yersiniosis is a foodborne infection, caused by pathogenic Y. enterocolitica bioserovars (Bottone, 1997). Pathogenic Y. enterocolitica bioserovar 4/O:3 has been frequently isolated from pork and pork products at retail level and in clinical cases in Europe (Fredriksson-Ahomaa et al., 2006). During the case control studies, pork was recognized as a most important source of pathogen, thus meat and meat products could be responsible for transmission of Y. enterocolitica 4/O:3 to consumers (Fredriksson-Ahomaa et al., 2006). The presence of non-pathogenic Y. enterocolitica biovar 1A was often reported on meats also, but its clinical significance is still discussible (Logue et al., 1996). Y. enterocolitica 4/O:3 is distributed in clinically healthy pigs and pathogen could be introduced in pork and the environment of slaughterhouse during the slaughter of Y. enterocolitica 4/O:3 positive animals (Nesbakken, 1988). Sites where Y. enterocolitica could be recovered are important to identify in the plant environment, because they represent possibilities for contamination of slaughter products (Sammarco et al., 1997). As presence of Y. enterocolitica 4/O:3 was detected in pig and in slaughter products in Latvia, possible that pathogen could spread to the slaughterhouse environment (Terentjeva, Bērziņš, 2010). The aim of present study was to detect the prevalence of Y. enterocolitica in the slaughterhouse environment.

Materials and Methods

A total amount of 64 environmental samples were collected in three slaughterhouses in Latvia between January 2006 and January 2009 during pig slaughter. The slaughter capacity of the selected slaughterhouses was 50 pigs per hours. The slaughtering process was similar in the selected plants, and consisted from stunning, bleeding, scalding, dehairing, polishing and evisceration steps.

Samples were collected from the following sampling sites: work surfaces (n=5), doors (n=4), tables for work equipment (n=3), floors (n=12), sinks (n=4), boxes for offal (n=7), meat
inspection platforms (n=4), boxes for cold storage of products (n=4), hooks (n=5), knives (n=4), gloves (n=4), aprons (n=4), footwear (n=4).

An area of approximately 20cm$^2$ of selected sampling site was swabbed with sterile gauze tampon (5cm X 5cm), moistured in 0.9 % of NaCl, placed in sterile sample transporting bags and delivered to the laboratory on ice within 2 h after collection. Samples were diluted with 90 ml PMB broth (Peptone-Mannitol-Bile Salt broth) immediately after arrival to the laboratory.

Samples were tested using the direct plating, the selective enrichment and the cold enrichment according to the ISO and NMKL methods (Anonymous, 1996, Anonymous, 2003). Prior to testing, swabs in PMB and were left for one hour at 22 °C for resuscitation. For the direct plating, 10 µl of suspension were streaked on CIN Agar. For the selective enrichment, 0.1 ml of suspension was transferred into ITC (Irgasan Ticarcillin Chlorate) enrichment broth (Fluka, Switzerland) and CIN agar (Cefsulodin-Irgasan-Novobiocin agar, Yersinia selective agar, OXOID, Basingstoke, Hampshire, UK) and incubated at 25 °C for 48 h. For the cold enrichment, samples in PMB broth were plated out onto CIN agar after one, two and three weeks of incubation at 4 °C with alkali treatment with 0.25% KOH in case no positive isolates were obtained during the first or second weeks of cold enrichment.

A quantity of 10 µl of suspension from ITC broth after incubation, and PMB fater cold enrichment was streaked onto CIN agar plates. CIN agar was incubated at 30 °C for 48 h. CIN plates were evaluated after incubation in order to detect bacterial colonies with yersiniae-like appearance. Presumptive colonies with a “bull eye” like appearance – red centre and transparent surrounded margins, from CIN agar were tested for oxidase reaction and urea hydrolysis. Differentiation of species was carried out with API 20E system (BioMérieux, Marcy l’Etoile, France).

Biotyping of \textit{Y. enterocolitica} positive isolates was performed as follows: strains were tested for pyrazinamidase activity, salicin, xylose, trehalose fermentation and lipase hydrolysis as described by Wauters et al., (1987). Indole reaction was obtained from API 20E kit. Serotyping was carried out as described by the manufacturer with \textit{Yersinia enterocolitica} O:3 antisera (Sifin, Berlin, Germany).

The Chi-square tests were used to detect differences between the prevalence of \textit{Y. enterocolitica} in slaughterhouses.

\section*{Results and Discussion}

\textit{Y. enterocolitica} was isolated from the slaughterhouse environment with the prevalence 37\% (24/64) positive cases, where 34\% (22/64) and 3\% (2/64) comprised \textit{Y. enterocolitica} 1A and \textit{Y. enterocolitica} 4/O:3, respectively Table 1.

\begin{table}[h]
\centering
\begin{tabular}{|l|lll|}
\hline
\textbf{Sampling site} & \textbf{No. of samples} & \textbf{No. of positive samples} & \textbf{No. of positive samples (\%)} \\
& & \textbf{\textit{Y. enterocolitica} 1A} & \textbf{\textit{Y. enterocolitica} 4/O:3} \\
\hline
Work surface & 5 & 2 (40) & 1 (25) \\
Door & 4 & 2 (50) & 0 (0) \\
Table for work equipment & 3 & 1 (33) & 0 (0) \\
Floor & 12 & 5 (42) & 1 (8) \\
Sink & 4 & 4 (100) & 0 (0) \\
Box for offals & 7 & 1 (14) & 0 (0) \\
Meat inspection platform & 4 & 2 (50) & 0 (0) \\
Box for cold storage of products & 4 & 1 (25) & 0 (0) \\
\hline
\end{tabular}
\end{table}
Table 1 shows that *Y. enterocolitica* 1A was found on work surfaces, doors, tables for work equipment, floor, sinks, boxes for offal, meat inspection platforms, boxes for cold storage of products, hooks, knives, gloves, aprons and footwear, while *Y. enterocolitica* 4/O:3 on work surface and floor samples. *Y. enterocolitica* 1A was not recovered from knives, but *Y. enterocolitica* 4/O:3 from doors, tables for work equipment, sinks, boxes for offal, meat inspection platforms, boxes for cold storage of products, hooks, knives, gloves, aprons and work footwear samples. *Y. enterocolitica* 1A was observed in slaughterhouse samples due to wide appearance of microorganism in nature. *Y. enterocolitica* 1A could enter the environment of slaughterhouse from outside sources (Harmon et al., 1984, Sammarco et al., 1997). The principal source of *Y. enterocolitica* 4/O:3 are pigs, and the environment of slaughterhouse could become contaminated with pathogen from pig faces and tonsils (Kapperud, 1991, Fredriksson-Ahomaa et al., 2000). The results on the presence of non-pathogenic *Y. enterocolitica* was in agreement with Sammarco et al., 1997, who found work surfaces and floor samples to be contaminated with bacteria, however, no yersiniae-positive samples were revealed in case slaughtering wall, hand wash basin, handles, hooks, knives and abattoir worker clothing were tested. The presence of *Y. enterocolitica* 4/O:3 in the environment of slaughterhouse was also in agreement with Nesbakken, 1988, who found pathogen on the floor of eviscerating area and viscera table. The highest prevalence of *Y. enterocolitica* 1A was found on sink, where 4/4 (100%) samples were positive, while the lowest on box for offal – 1/7 (14%) positive samples. In our mind, high prevalence on sink was observed due to it contamination with yersiniae from highly contaminated material, such as worker clothing and contaminated equipment. In contrast, Sammarco et al., 1997 reported, that the highest prevalence of non-pathogenic *Y. enterocolitica* was observed on slaughterhouse floor samples, where 3 out of 18 samples were positive (17%), but did not find contamination with *Y. enterocolitica* on hand-wash basin. *Y. enterocolitica* 4/O:3 were found in two samples, and the most probably pathogen was introduced on floor sample with blood from slaughtered animals, and on work surface due to direct contact with contaminated material (Nesbakken, 1988). *Y. enterocolitica* was isolated from the slaughterhouse environment samples in slaughterhouses A, B and C, and the prevalence is shown in Table 2.

### Table 1

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>No. of samples</th>
<th>No. of positive samples (%)</th>
<th>Y. enterocolitica 1A</th>
<th>Y. enterocolitica 4/O:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hook</td>
<td>5</td>
<td>1 (20)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Knife</td>
<td>4</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Gloves</td>
<td>4</td>
<td>1 (25)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Apron</td>
<td>4</td>
<td>1 (25)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Footwear</td>
<td>4</td>
<td>1 (25)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>64</strong></td>
<td><strong>22 (34)</strong></td>
<td><strong>2 (3)</strong></td>
<td></td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Slaughterhouse</th>
<th>No. of samples</th>
<th>No. of positive samples (%)</th>
<th>Y. enterocolitica 1A</th>
<th>Y. enterocolitica 4/O:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>19</td>
<td>8 (42)*</td>
<td>2 (11)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>26</td>
<td>9 (34)*</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>19</td>
<td>5 (26)*</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>64</strong></td>
<td><strong>22 (34)</strong></td>
<td><strong>2 (3)</strong></td>
<td></td>
</tr>
</tbody>
</table>

* differences in the prevalence of *Y. enterocolitica* 1A between slaughterhouses A, B and C were not significant (p>0.01).
The highest prevalence of *Y. enterocolitica* 1A was found in slaughterhouse A, while the lowest- in slaughterhouse C, however, without significant differences (p>0.01). Our findings are similar to Sammarco et al., 1997, who found presence of non-pathogenic *Y. enterocolitica* in the environment of two out of 11 slaughterhouses, but without statistical differences. *Y. enterocolitica* 4/O:3 was detected only in slaughterhouse A. Prevalence of *Y. enterocolitica* 1A in the environment of three slaughterhouses is shown in table 3.

### Prevalence of *Y. enterocolitica* 1A in the environment of slaughterhouse

<table>
<thead>
<tr>
<th>Sampling site</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Work surface</td>
<td>3/1 (33%)</td>
<td>2/1 (50%)</td>
<td>0/0 (0)</td>
</tr>
<tr>
<td>Doors</td>
<td>1/0 (0)</td>
<td>2/2 (100)</td>
<td>1/0 (0)</td>
</tr>
<tr>
<td>Table for work equipment</td>
<td>1/0 (0)</td>
<td>1/1 (100)</td>
<td>1/0 (0)</td>
</tr>
<tr>
<td>Floor</td>
<td>2/1 (50%)</td>
<td>7/2 (29%)</td>
<td>3/2 (66%)</td>
</tr>
<tr>
<td>Sink</td>
<td>2/1 (100)</td>
<td>1/1 (100)</td>
<td>1/1 (100)</td>
</tr>
<tr>
<td>Box for offals</td>
<td>1/0 (0)</td>
<td>3/0 (0)</td>
<td>3/1 (33%)</td>
</tr>
<tr>
<td>Meat inspection platform</td>
<td>0/0 (0)</td>
<td>2/1 (50)</td>
<td>2/1 (50)</td>
</tr>
<tr>
<td>Box for cold storage of products</td>
<td>1/1 (100)</td>
<td>2/0 (0)</td>
<td>1/0 (0)</td>
</tr>
<tr>
<td>Hoof</td>
<td>1/1 (100)</td>
<td>2/0 (0)</td>
<td>2/0 (0)</td>
</tr>
<tr>
<td>Knife</td>
<td>2/0 (0)</td>
<td>1/0 (0)</td>
<td>1/0 (0)</td>
</tr>
<tr>
<td>Gloves</td>
<td>1/1 (100)</td>
<td>2/0 (0)</td>
<td>1/0 (0)</td>
</tr>
<tr>
<td>Apron</td>
<td>2/0 (0)</td>
<td>1/1 (100)</td>
<td>1/0 (0)</td>
</tr>
<tr>
<td>Work footwear</td>
<td>2/1 (50)</td>
<td>1/0 (0)</td>
<td>1/0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>19/8 (42)</td>
<td>26/9 (34)</td>
<td>19/5 (26)</td>
</tr>
</tbody>
</table>

Table 3 shows that variations in the prevalence of non-pathogenic *Y. enterocolitica* exist between slaughterhouses. In our mind, these variations in the prevalence of *Y. enterocolitica* between certain sampling sites as work surfaces, doors, table for work equipment, floor, box for offal and meat inspection platform was observed due to the differences between plants in their structural characteristics, the slaughtering practices and the sanitation practices (Sammarco et al., 1997).

### Conclusions

1. The presence of *Y. enterocolitica*, especially of pathogenic biosetype 4/O:3 in environmental samples, indicated that the environment of slaughterhouse can be a cause of contamination of slaughter products with yersiniae, and greater efforts should be made to maintain hygiene in slaughterhouse on acceptable level.
2. Our study revealed sites in the environment of the slaughterhouse where contamination with yersiniae occurs more often, therefore cleaning and sanitation procedures should be performed more carefully.

### References


ETHANOL EFFECT ON THE RADIOLYSIS OF PORK MUSCLE TISSUE
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Abstract
Conventional preservation with the use of ionizing radiation is most modern and highly economical technology of production of safe food. We used pH-metry and microscopy methods to investigate the effect of short-term treatment of full piece sample muscle tissue surface with ethanol on the radiolysis of his restructured samples of muscle tissue and muscle fibers. Autolytic and microbial processes during storage of samples in sealed post-radiation period at +4 °C were differentiated on the timeline display as a result of ethanol treatment. Overall stabilizing effect of ethanol and electron-beam exposure (variable absorbed dose 12.5 – 50.0 kGy) on the acidity of the restructured samples muscle tissue with preservation of their pH level standards within 3 – 4 weeks, depending on the absorbed dose was marked.

Key words: electron-beam irradiation, muscle tissue, the radiolysis

Introduction
Radiation preservation in which it is used ionizing radiation in majority scientist's opinion considered to be the most modern and highly economical technology production of safe food, including meat or meat food as it gives opportunity to store it without freezing (Васильев и др., 2000; Костенко и др., 1992; O’Bryan et al., 2008; Пикаев, 1995; Элиас, Кохен, 1983). Actually, for radiation treatment of food it is used either gamma- and X-ray or electron beams, generated by electron accelerators (Васильев и др., 2000; Мякин и др., 2006; Zhu et al., 2009). In recent times of radiotechnology development the energy of accelerated electrons is used much wider than the one of gamma and X-ray source. It is due to greater radiation safety and larger power of electron accelerator.

With a sufficient dosage of ionizing radiation, having a bactericidal effect, it provides a complete sterilization in a short time (tens of seconds). However the long-term storage of irradiated meat at cool temperatures is opposed by activity of proteases. Radiation dose, sufficient for achieving of practical sterility incompletely inactivate proteolytic enzymes in meat. Usage of different types of protease inhibitors to repress autolytic activity couldn’t become common use. Therefore, practical solution of meat-food sterilization is concerned with the necessity in inactivating of muscle cathepsins. Studies in this field hasn’t given final results yet.

The goal of this study is to investigate the influence of preliminary sterilization of full-piece sample surface of pork muscle tissue by 96% ethanol on radiolysis of samples, exposed to electron-beam treatment after its chopping. The sterilization by ethanol was used to find an opportunity of time-sharing the beginning of microbial and autolysis of muscle tissue processes, usually associating and strengthening each other. The research was conducted with pH-metry and microscopy methods.

Materials and Methods
In this work restructured pork muscle tissue (pH=5.82) and muscle fiber (pH=6.28) were tested and exposed to radiation. After preliminary sterilization of full-piece sample surface of pork sirloin by 96% ethanol for 2.0 minutes, muscle tissue was ground in meat mincing-machine with 2.5 mm holes on the disk. Muscle fiber was obtained from restructured mincing-machine with the help of extraction of water-soluble constituents of sarcoplasm by bidistilled water during 12 hours. Parallely, unsterilized by ethanol intact samples, obtained from muscle tissue were tested. After grinding, samples (2.0–2.5 g) were packed in polyethylene film
(0.03 mm thickness), airproofed and exposed to radiation. In post-radiation period samples were stored at +4 °C during 17−37 days. Measuring of pH of water slurry samples during storage was performed on pH-meter “Expert” with the glass electrode; batch weight of the sample was 0.3 g, liquid phase volume was 20 ml. In post-irradiation period history curve of pH samples were plotted on pH=f(τ) coordinates, where τ is the time measured in days.

Semiquantitative evaluation of bacterial growth was performed by microscopic examination gram-stained (Сидоренко и др., 2002) touch smears, taken from the surfaces of samples using Zeiss Axiosstar plus microscope (x1000). Sample radiation was carried out, using medium energy electron accelerator (RTE-1B). Absorbed dose in series was: 12.5; 25.0; 37.5 and 50.0 kGy.

Results and Discussion

Fig. 1 and 2 shows the changing of acidity curves of samples, which were restructured from pork muscle tissue, exposed to radiation and stored at +4 °C during 17 days. Samples in series 1 were obtained by restructuring of intact muscle tissue. Series 2 is shown by samples of forcemeat prepared from muscle tissue pretreated by 96% ethanol.

![pH vs time graph](image-url)

**Fig.1. Changing of acidity of intact muscle tissue samples plotted against absorbed dose at storage in post-radiation period**

On Fig. 1 it is shown curves of pH change in water suspension, plotted against storage time of samples in series 1. The first thing that stands out here is that treatment of intact sample by ionizing radiation, regardless of absorbed dose, leads to stabilization of variation range of pH of water suspension samples. While the curve 5, describing pH change in water suspension of control sample, as early as 5 days points to decrease of it acidity.

The second thing, appeared from fig. 1 is the periodic relation of pH=f(τ) for irradiated samples (1−4). Periodicity in interchanging of dominant role of proceeding processes shows their selectivity and dependence from acidity of sample within the range pH=6.15−6.35. Synchronism in forming three maximums pH_max. (5, 10 and 15 days) is presented only on
curves obtained for samples 1 and 4 within pH range 6.1–6.5. It can point to repeatability of post-radiation processes in samples, absorbed doses of 12.5 and 50.0 kGy during irradiation. From the whole picture sample 2 is visibly falling out. On the curve of this sample where pH=f(τ) it is seen the broad band (8–17 days). The pH variance is within its range 6.0–6.6. It says about greater variety of processes in samples, absorbed dose 25.0 kGy, total algebraic effect of which is shown at pH increasing. However, with the dose 37.5 kGy this broad band differentiates with formation of two maximums (10 and 15 days), which is accompanied by decreasing of pH. Dose absorbance of 50.0 kGy leads again to appearance of three maximums on the curve 4. Linear dependence of pH\textsubscript{max} in extreme points on time of their appearance in this curve says about densification of substances decreasing pH of system.

![Graph showing pH changes](image)

**Fig. 2. Changing of acidity of muscle tissue samples, treated by ethanol depending on absorbed dose at storage in post-radiation period**

In fig. 2 curves 1-4 show the changing in acidity during the storage procedure of parallel series two of muscle tissue samples, treated by ethanol before grinding and radiation. In dynamics of changing of acidity in samples of this series from absorbed doses of ionizing radiation it is pointed out quite another behavior. But what really stands out here is the absence of synchronism in appearance of first maximums on curves received at a preset time interval. Moreover, for all samples regardless of absorbed doses, it is exhibited the stabilizing effect of ethanol during first 8 days and this effect strengthens each time with increasing of absorbed doses. Stabilization of pH by pH=f(τ) curves point to excess of free radicals in analyzed system, which cant influence on changing pH of water suspension. Only for samples 1 and 2 of current series the first maximum on pH=f(τ) curves forms on tenth day. But for sample 2 it is lower on pH scale. In the nature the dependence of pH=f(τ) for sample 1 is closer to curve 5, where the change of pH of control samples is presented. The first maximums on curves, gained for samples 3 and 4 are displaced on 12 and 17-th days. The forming of first maximums on pH=f(τ) curves is indicative of that the beginning of periodic changes in samples system is the result of the beginning of directed processes. Comparison of curves 5 for control samples of both series (Fig. 1 and 2) shows that short-term treatment of full piece meat with ethanol without following exposure of ionizing radiation on forcemeat leads to more dynamic decrease of its acidity comparably to radiation-exposed
samples in post-radiation period. If full piece meat surface was pretreated by ethanol, than ground to the forcemeat followed by ionizing radiation that manipulations stabilized pH samples noticeably. Moreover, stabilizing effect of ethanol increases with the increasing of absorbed dose.

In Fig. 3 it is presented the curves 1–4 of changing pH in water slurry of pork muscle fiber samples gained from meat sterilized by ethanol and exposed to electron beam treatment forcemeat. Rate of curves for all samples, which were stored from 7 to 37 days regardless of absorbed dose also is declarative of periodicity of their changing of pH in range 6.2–6.6. This fact correspond the process intensification which are slowed down by radiolysis the first week after radiation as in forcemeat samples of series 2. Synchronism in forming of maximums (рНмах) on all obtained dependences of pH=f(τ) and their closeness on pH scale can point to uniformity of type of processes, taking place in muscle fiber in post-radiation period. Moreover pH = f(τ) curves, received for samples 1 and 2 are completely coinside with throughout the whole storage life. The variation in change of pH of sample with increase of absorbed dose becomes noticeable two weeks later after storage.

Conclusions
1. The investigation has shown that ethanol pretreatment of surface of the compact sample pork muscle exhibits in dynamics change of the acidity of restructured samples in post-irradiation period.
2. The ethanol treatment has shown pH stabilization of irradiated tissues. The periodic changes of pH=f(τ) is connected with two directional processes that proceeds in parallel.
References

FOODBALT 2011
MICROBIOLOGICAL QUALITY AND PHYSIOCHEMICAL PARAMETERS OF COLD-SMOKED SAUSAGES DURING RIPENING

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¹Food and Veterinary Service, Dienvidzemgale branch, Latvia, *e-mail: forest.con@apollo.lv
²Agricultural University of Latvia

Abstract
Many recent studies in food safety have investigated non-thermal processing of ready-to-eat food products, but there is little information about the survival of food pathogens in different ripening stages of cold smoked sausages. Therefore, the microbiological quality (total aerobic count – TAC, *Staphylococcus aureus*, *E. coli*, *Salmonella spp.*, and *Listeria spp.*), water activity (a$_w$), and pH were determined in cold-smoked sausages during the ripening time from days 0 to 21. The temperature in smoke camera was 28 °C for the first 3 days of maturation and 15±1 °C during days 4 to 21. As a result the TAC of starter culture bacteria increased from 5.72 lg cfu g$^{-1}$ at the beginning to a maximum of 9.41 lg cfu g$^{-1}$. The count of *S. aureus* increased from 1.38 to 2.68 lg cfu g$^{-1}$ and *E. coli* decreased from 2.47 to 0.85 lg cfu g$^{-1}$. *Salmonella spp.* was not detected at any time. *Listeria monocytogenes* was found in one of 5 sausage series, but measured only during the first 5 days when the count decreased from 3.41 to 2.08 lg cfu g$^{-1}$. The mean value of pH decreased from 5.80 to 4.65 in the first 5 days and stabilized. Water activity (a$_w$) decreased slowly and generally correlated with air humidity in the ripening camera and the mean value changed from 0.963 to 0.817 a$_w$. A significantly different correlation between the bacterial count and a$_w$ values was found. The results indicate that the microbiological safety of cold-smoked sausages depend on the initial contamination level with food pathogens. The analysis was done at the Faculty of Veterinary medicine of the Agricultural University of Latvia and at the sausage manufacturer’s laboratory.

Key words: food pathogens, cold-smoked sausage, water activity, pH

Introduction
Growing consumer interest to foodstuffs of high nutritional value that guarantee health from food pathogens and proper hygienic conditions has prompted interest in ready-to-eat meat products, partly to cold-smoked (fermented) meat products (Bohaychuk et al., 2006), because the processes used in their production, and specification of content inhibit many pathogenic bacteria. However, when fermentation process is not adequate, there is a potential microbiological risk – some food pathogens may survive and proliferate during ripening (fermentation) (Adams and Mitchell, 2002).

Among the most representative cold-smoked sausages, produced in Latvia, are “Jelgavas Jubilejas”, “Kantvurst”, and some others, manufactured at Jelgava meat factory. Due to tradition and consumption habits the ready-to-eat cold-smoked sausages are produced and consumed in large amounts. On the other hand, these products due to specific recipes of production, very long shelf-life, special storage conditions, and sometimes inappropriate management at meat warehouses or shops might be unsafe for consumption.

Theoretically most of relevant food pathogen bacteria can be found in cold-smoked sausages (Bohaychuk et al., 2006). The most common pathogens which are present in fermented sausages and therefore keep a health risk are *Salmonella*, *E. coli*, and *Staphylococcus aureus*. In E. Drosinos et al. (2006) study it was shown that L. monocytogenes can also survive fermentation. For that reason, bacteria mentioned higher, has been examined and enumerated. *Salmonella* spp. is accounted for the most reported food-borne outbreaks in European Union. Eggs, egg-products and meat are the main sources of outbreaks. Positive to salmonella spp. have occasionally been found up to 5% ready-to-eat meat products (EFSA, 2010).

*Listeria monocytogenes* is a significant foodborne pathogen that is readily present in raw meat products used in the manufacture of processed meats (Sheen, 2008). In ready-to-eat food the occurrence of L. monocytogenes in quantities exceeding the Community *Listeria* criteria (100 cfu g$^{-1}$) remained at low levels in 2008 (EFSA, 2010). Post-processing contamination from a plant environment (equipment, personnel, floors, etc) is the most frequently reason for
its presence in meat or its product surface (Gudbjornsdottir et al., 2004). Fermentation process usually destroys \textit{L. monocytogenes}, but at optimal pH and water activity (a$_w$) conditions it is capable of surviving (Drosinos et al., 2006). The infective dose of \textit{L. monocytogenes} to human risk groups – children, elderly, immunocompromised people and pregnant women is not known, but the latter opinion is that listeriosis can be caused by \textit{L. monocytogenes} population of 2.0 - 3.0 lg (cfu g$^{-1}$) (Kendall et al., 2006).

The pathogenic \textit{Escherichia coli}, including the following sub-species, as enteropathogenic, entero-invasive, enterotoxigenic, and entero-haemorrhagic \textit{E. coli} are most significant for food-borne outbreaks. Entero-haemorrhagic \textit{E. coli} has produced vero-toxins or shiga-toxins and is the most common serotype isolated from the reported cases (EFSA, 2010). Heat-resistant staphylococcal enterotoxins have high heat tolerance and cannot be destroyed by a normal heat treatment. 291 food-borne outbreaks caused by \textit{Staphylococcus} spp., which constituted 5.5% of the total number of bacterial toxin outbreaks in the EU and two fatal cases in one possible \textit{Staphylococcus} outbreak was reported in 2008 (EFSA, 2010).

According to literature data, many recent studies in food safety have investigated non-thermal processing of ready-to-eat food products, but there is little information about survival of food pathogens found in different ripening stages of cold smoked sausages when water activity and pH value changes affect bacterial growth. Therefore, the aim of the study was to determine the survival limits of most popular food pathogens in manufactured cold smoked sausages depending on water activity (a$_w$) and pH values.

**Materials and Methods**

The microbiological tests were done in Agricultural University of Latvia Faculty of Veterinary Medicine and sausage manufacturer laboratory of a real company located in Latvia in 2009–2010. The five series of cold smoked sausages were investigated (total 105 samples) on presence and count of planned investigated bacteria, and the mean values of lg (cfu g$^{-1}$) were estimated, in addiction to pH and water activity (a$_w$) changes at ripening time. Ingredients of a 100 kg cold-smoked sausage raw material were: pig meat – 30 kg, beef – 10 kg, bacon 35 kg, structural emulsion – 25 kg. Salt and species summary – 3.25 kg and starter culture ‘Optistart Plus’ – 0.02 kg (control No. L9694599, prepared by Raps GmbH and Co.KG, Germany, sachet composition in lg values: 11.38 \textit{Lactobacillus sake} L 110, 11.38 \textit{Staphylococcus xylosus} M86, and 10.00 \textit{Debaryomyces Hansenii} DH3).


pH was measured on 0, 1$^{st}$, 3$^{rd}$, 5$^{th}$, 7$^{th}$, 14$^{th}$ and 21$^{st}$ days of maturation. Three individual pieces of sausages were measured each time, and then mean pH value was calculated. The pH-meter Testo 205 (Testo AG Germany), with automatic temperature compensation, was applied. Calibration was done by means of 2 point method with pH standard solutions 4.01 and 7.00.
Water activity was measured with PawKit (Decagon) simultaneously with pH measurings. Calibration of devices was done with saturated NaCl (sodium chloride) 6.0 molal standard solution (0.760 \( a_w \) at 20 °C). Samples for water activity measuring were collected in original polyethylene vessels with caps and measured immediately after collecting.

Statistical analysis. All measurements were reiterated three times, and tests were triplicated. The results represent the mean ± standard deviations. Means were compared by Student’s t test. Differences were considered statistically significant when \( p<0.05 \). Statistical analysis was conducted by means of SPSS 17.0 (SPSS, Chicago, Ill., USA). Tables and chart figures were done by means MS Excel 2007 software.

Results and Discussion
It was observed that bacterial counts – total aerobic count (TAC) and \( S. \) aureus increased from 5.72 to 9.41 lg and from 1.38 to 2.68 lg respectively. The count of detected \( E. \) coli decreased approximately by 1.5 logs (from 2.48 to 0.85 lg) during sausage ripening time of 21 days. \( L. \) monocytogenes was detected in one of five sausages series, but tested only during the first 5 days when the count decreased from 3.41 to 2.08 lg cfu g\(^{-1}\), and was not detected on days 7, 14, and 21 of sausage ripening. \( Salmonella \) spp. was not detected at any time. The mean microbiological test results of 5 manufactured cold-smoked sausages series are summarized and shown in Figure 1.

![Figure 1. The count lg (cfu g\(^{-1}\)) changes of detected bacteria spp. in cold-smoked sausages during 21 day ripening time](image)

The samples of cold smoked sausages had a mean initial pH value of 5.80±0.04, which agrees with the results found by V. Paleari et al. (2003). A rapid decrease in pH was observed during the first three days of fermentation. The final pH of the fermented sausages had a mean value of 4.64±0.05; this drop in pH was due to lactic acid production by the starter culture used for fermentation (Vermeiren and Debevere, 2004). Lactobacilli are the major producers of lactic acid responsible for the decrease in pH and the increase in acidity during fermentation (Schillinger et al., 1991). Lactic and acetic acids are often suggested to be major contributors to the acid aromas and tastes and the development of the texture of fermented sausage (Visessanguan et al., 2005).
The mean value of initial water activity was 0.96 which decreased in the product from 0.963±0.004 to 0.817±0.006 in 21 days. Decreasing trends of \( a_w \) and pH are shown in Figure 2.
Figure 2. Decreasing trends of $a_w$ and pH values in cold-smoked sausages during 21 day ripening time (pH ------, $a_w$ ......)

All measured physical and chemical parameters significantly (p<0.01) correlate with the decreased $L.~monocytogenes$, and $E.~coli$ count, at the same time with the increased TAC and $S.~aureus$ count in first 5 days. The correlation coefficient ‘r’ of values is shown in Figure 3.

(TAC = a, $S.~aureus$ = b, $E.~coli$ = c, $L.~monocytogenes$ = d)

Figure 3. Pearson’s correlation coefficient of values between bacteria lg(cfu g$^{-1}$) and physical and chemical parameters – $a_w$ and pH

In prior study (Siliņš and Liepiņš, 2010) showed that decreasing $a_w$ in cold-smoked sausages during ripening reduced $L.~monocytogenes$ count to approximately lg 0.44 day$^{-1}$. In this study the reduction of $L.~monocytogenes$ count was lg 0.27 day$^{-1}$, but for $E.~coli$ count a decrease rate was lg 0.08 day$^{-1}$. Evaluating correlation coefficients in Figure 3, it is seen that $a_w$ as a factor has more force for degradation of bacteria growth than pH. It is also seen in Figure 3, that $a_w$ and pH value changes did not affect the growth of $S.~aureus$ in partly ripening time in 15 days, what is recommended for microbiological tests of raw sausage material for detecting initial level of contamination and possibility to forecast final count and toxins level, when $a_w$ decreased to 0.86 and $S.~aureus$ growing stopped. In this study $S.~aureus$ growth rate was lg 0.10 every day and could be explain on the one hand as less sensibility to $a_w$ decreasing process and on the other hand as support of genus kin from added starter culture. It will be possible starter culture quality could affect the $S.~aureus$ growth rate, and stimulate customers of more attention on starter culture genus structure.
Conclusions
1. A significant Pearson’s correlation (p<0.01) was established in the decreased count of *L. monocytogenes* and *E. coli*, increased count of *S. aureus* and aerobic bacteria between decreased water activity and pH values in cold-smoked sausages during ripening.

2. The continual decreased changes of water activity and partly pH diminished possible initial count of some bacterial species, such as *L. monocytogenes* and *E. coli* that allows considering cold-smoked sausages being relatively safe and healthy meat product, under condition that initial contamination with *Staphylococcus aureus* is minimal.

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References
CONSUMERS KNOWLEDGE AND ATTITUDE TO TRADITIONAL AND ENVIRONMENTALLY FRIENDLY FOOD PACKAGING MATERIALS IN MARKET OF LATVIA

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Abstract
The packaging industry is one of the most important industries in the world. The quantity of packaging materials increases annually. Food packaging accounts for almost two-thirds of total packaging waste by volume and approximately 50% by weight of total packaging sales. The aim of food packaging is to contain the food, to protect food products from external influences and damage, to preserve food safety and minimize the environmental impact. To clear up a situation in the market of Latvia, as well as to study the consumer’s awareness problem and attitude to traditional and biodegradable polymer packaging materials and their implementation in the market year of 2010, questionnaire was polled. 1200 respondents (50% female and 50% male) from different regions of Latvia (Kurzeme, Zemgale, Vidzeme, Latgale and Riga) were in replay to 19 different questions: how well-informed they are for traditional and eco-friendly packaging and how much more they are willing to pay for eco-friendly food packaging (biobased, biodegradable, recyclable) compared with conventional polymers. Most of consumers inquired in Latvia think that there is too much polymer packaging for food products on the market.

Key words: food packaging, biodegradable, consumers, market.

Introduction
Packaging plays a major role when products are purchased. After all, it is the first thing seen before making purchase choices and it is widely regarded that over 50 per cent of purchasing decisions are made at the shelf, or point of purchase. Therefore, packaging which creates differentiation and identity in the relatively homogenous consumer packaged goods industry is therefore highly important. Today, it is widely acknowledged that packaging decisions can have a significant impact on sales. Therefore, without some evidence of consumer acceptance towards different packaging concepts and designs, manufacturers and retailers will restrict their chances of profitable innovation (Aarnio, Hamalainen, 2008; Marsh, Bugusu, 2007). The quantity of packaging materials increases by 8% every year; in proportion, the amount of waste increases as well, at the same time fossil resource – petroleum is decreasing. In recent years, there has been marked an increased interest in biodegradable materials for application in food packaging, agriculture and other areas. In particular, biodegradable polymer materials (known as biocomposites) are of high interest. Polymers materials, and are continually being employed in an expanding range of areas. As a result, many researchers are investing time into modifying traditional materials to make them more user-friendly, and into designing novel polymer composites out of naturally occurring materials. A number of biological materials may be incorporated into biodegradable polymer materials, with the most common being starch and fiber extracted from various types of plants. The belief is that biodegradable polymer materials’ production at a low cost will reduce the need for synthetic polymers (thus reducing environment pollution), thereby generating a positive affect both environmentally and economically (Kolek, 2001; Kolybaba at al., 2003; Robinson, 2010).

This paper presents the enquiry results of Latvian consumers’ attitude to packaging and the importance of the environmental and functional characteristics of packaging for their purchasing decisions. The aim of this study is to evaluate whether and how purchasing behaviour can be influenced in such a way as to limit the environmental problems caused by
packaging. The study deals with consumers’ attitude to packaging in general, and mainly in
details of biodegradable packaging.

**Materials and Methods**

To clarify the situation in the market of Latvia, as well as to study the consumer’s awareness
problem and attitude to traditional and biodegradable polymer packaging materials and
their implementation in the market year of 2010, questionnaire was polled. 1200 respondents
(50% female and 50% male) from different regions of Latvia (Kurzeme, Zemgale, Vidzeme,
Latgale and Riga) were enquired, from which 33% were younger than 20 years, 34% were
20–45 years old, and 33% – older than 45 years. Respondents were asked to answer on
19 different questions: how well-informed they are for traditional and eco-friendly packaging
and how much more they are willing to pay for eco-friendly food packaging (biobased,
biodegradable, recyclable) compared with conventional polymers. The statistics on a
completely randomized design was determined using the General Linear Model (GLM)
procedure SPSS 10.0 (Arhipova, Bāliņa, 2003). The one-way ANOVA ($p \leq 0.05$) test was used
to determine the significance of differences between the means.

**Results and Discussion**

The obtained results are demonstrated in Figures 1 to 10. The first column in each figure
shows a summarized attitude of respondents all over Latvia, and the next columns present
respondents’ answers from 4 regions of Latvia – Kurzeme, Latgale, Vidzeme, and Zemgale
and at the end the fifth column – of capital city Riga.

![Figure 1. Do you as a consumer consider that there is too much packaging
on foods you buy?](image1)

Most of consumers (approximately 56%) inquired in Latvia, with the exception of Zemgale
(47%), showed a very similar tendency ($p>0.05$), considering that sometimes there is too
much polymer packaging on food products (Fig. 1.).

![Figure 2. Do you as a consumer worry about the amount of packaging waste you get
when buying foods?](image2)
On average, 44% of consumers inquired in Latvia (Fig. 2.) worry about the amount of packaging waste when buying foods and beverages; only for small part (14%) of consumers this question was indifferent (p>0.05). The consumers’ opinion regarding too large used packaging amount in all regions of Latvia except Vidzeme (60%) showed a similar tendency (p>0.05). 

A heartening news have been obtained from respondents all over Latvia (70%) and in most individual regions as well (75–88%), that consumers, besides Riga (only 31%), are quite well informed about packaging materials’ different impact on pollution of environment (p>0.05) (Fig. 3.). In Riga as capital city the waist system is totally co-ordinated, therefore consumers in weekdays don’t have to see scrap-heaps, plastic bottles on the banks of a ditches, and flying plastic bags on the top of trees, therefore they don’t worry (40%) or even don’t think at all (22%) about environmental pollution with plastic waste from packaging. 

Figure 3. Are you informed as a consumer about the packaging material different influence on the environment?

Figure 4 shows that 61% of respondents in Latvia (in Latgale 52%) are nearly informed about the existence of eco-friendly biodegradable packaging. The situation in all regions of Latvia except Latgale (where 27% were not at all informed) (p>0.05) shows a similar tendency of knowledge’s on biopolymers (p>0.05). 

Results presented in the Figure 5 inform that most of respondents all around Latvia as more environmentally friendly packaging preferred degradable polymers (valuating by 1 to 2 points) and paper (1 to 2.5 points), following by reusable glass packaging (3 to 5 points) and Tetra Pack laminated milk and juice carton (4 points, excepting Latgale). Metal cans and traditional polymers (validated by approximately 6 points) were considered as the worst. Obviously, Latvian consumers are not at all informed that metal packaging is the most widely recycled waste therefore can be considered as environmentally friendly packaging.
Figure 5. Which of the mentioned packaging materials do you prefer as more environmentally friendly?

Figure 6. Do you as a consumer use your own containers when purchasing unpacked foods?

Approximately 52% of respondents in Latvia sometimes use their own containers when purchasing any unpacked foods on the market. Only about 15% of Latvian respondents confirmed that it is normally always to use their own special containers for foods’ shopping. The consumers’ practice about frequency of owns packaging container application in all regions of Latvia shows a similar tendency (p>0.05).

Figure 7. How much more are you willing to pay for eco-friendly packaged (biodegradable) products compared with traditionally packed ones?

The information summarized in Figure 7 shows that the majority of respondents come up with the following answers: they are willing to pay not more or only 5% or same of them – 10% more compared to conventional packaging. On average, in Latvia 25% of consumers are unwilling at all to pay more, exclusively 39% agree to pay 5% more and 9% agree pay 10% more for eco-friendly (biodegradable) package compared with traditional package. It is interesting fact in the enquiry that average 1% of respondents could agree to pay twice as
much for environmentally friendly packaging. The respondents’ point of view from region Latgale and capital of Latvia – Riga is substantially different (p>0.05) compared with the average consumers’ view in Latvia.

![Graph showing the preference for shopping bag materials.](image)

**Figure 8. What kind of material do you prefer for shopping bags?**

Figure 8 show that Latvian consumers mainly prefer textile material shopping bags. Analysing the situation in all territory of Latvia, we can see that consumers choose paper and compostable material shopping bags more or less similar. Nevertheless, still 10% of respondents prefer traditionally used polymer shopping bags. Exclusively in Riga the consumer’s choose of shopping bags seems more or less in the same kind. Most of respondents support the idea to use new eco-friendly materials for food packaging. Positive evaluations were given by 82% of respondents in Latvia (Fig. 9).

![Graph showing the evaluation of new eco-friendly materials.](image)

**Figure 9. How do you evaluate the proposal to introduce new eco-friendly materials for food packaging?**

![Graph showing the preference for separate waste collection.](image)

**Figure 10. Do you prefer the separate waste collection?**

In general the respondents (21%) participating in separate waste collection are inhabitants of Riga (Fig. 10), ever if the attitude of capital city Riga population disparate (p>0.05) compared
with the average view at all in Latvia (10%), respectively, in all another regions (4–10%) (p>0.05). A large part of consumers (38–48%) does not separately collect the waste or declare that there is no possibility for it.

**Conclusions**

Most of consumers in Latvia consider too much polymer packaging applied for food products. 82% of respondents in Latvia support the idea of new eco-friendly food packaging implementation, nevertheless only small part of consumers agree to pay more for environment protection from used packaging. To optimize the environment protection effects of food packaging, it is essential to educate consumers and to analyse the system that covers its main purpose “food”, the total production chain and the varying demands of the consumer.

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**References**

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INFLUENCE OF ACTIVE PACKAGING ON THE SHELF LIFE OF SOFT CHEESE KLEO
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Abstract
The research was carried out at the Faculty of Food Technology of the Latvia University of Agriculture (LLU). Soft cheese Kleo produced in Latvia was used for experiments. At present the cheese Kleo has been found on the market place in vacuum packaging (VP) and its shelf life is not more than 15 days. For shelf life extension an active packaging in combination with modified atmosphere (MAP) was investigated. Following packaging materials were used: OPP, PE/PA, PE/OPA and Multibarrier 60. An iron based oxygen scavenger sachets of 50 cc obtained from Packaging Solutions OÜ was used. Cheese samples of 100±10 g were packaged in polymer pouches (110 x 120 mm). Modified atmospheres consisting of carbon dioxide CO₂ (E 290) 30% and nitrogen N₂ (E 941) 70% was used, while VP was selected as the control packaging. Pouches were hermetically sealed by MULTIVAC C300 vacuum chamber machine, stored in a Commercial Freezer/Cooler „Elcold” at the temperature of +4.0±0.5 °C up to 32 days’ and analysed before packaging and in the 0, 5th, 11th, 15th, 18th, 22nd, 25th, 29th and 32nd day of storage. Physical and chemical properties: headspace gas composition, pH, acidity, moisture content, and microbial conditions were evaluated. By use of both usual MAP conditions as well as with oxygen scavenger commitment in the pouch the shelf life was extended, good outside appearance and lactic acid aroma was observed.

Key words: soft cheese, modified atmosphere, oxygen scavengers, shelf life.

Introduction
Soft fresh cheeses are those cheeses that are unripe. White cheese is usually made from raw milk without of starter culture (Abdalla et al., 1997), souring the milk, either with lemon juice or vinegar. Creamy curds were formed then strained to produce a simple cheese. These cheeses have high moisture content, are usually mild and have a very soft texture. These cheeses are typically the most perishable. Cheeses in the fresh category include Italian Style Mascarpone, and Ricotta, Chevre, Feta, Cream cheese, Quark and Cottage cheese.

Optimal packaging solutions could prevent or minimize quality changes, resulting in increased shelf life as well as quality maintenance. Different types of cheeses have to be packed in different packaging concepts (Mortensen, Bertelsen et al., 2005). Most fresh cheeses are packed in air atmosphere due to the short shelf life required. Some experiments proved that chemical composition and sensory characteristics, colour and body of white cheese made from pasteurized cow milk during the storage period 45 days in vacuum packaging did not significantly change (Osman, Abdalla et al., 2009). Find out that packaging and cold storage of Sudanese white cheese in metal containers is better than in plastic containers as low total bacterial, coli forms, E.coli and yeast counts were obtained in cheese packed in metal containers (Ahmed, Alhassan, 2010).

An established fact is that primary spoilage organisms in cheeses are moulds and MAP could be used to prevent mould growth. Gases with low residual oxygen and high CO₂ levels may be used to ensure microbial stability (Mortensen, Bertelsen et al., 2005). Sometimes cream cheeses are pasteurized prior packaging (Gonzales-Fandor, Sanz et al., 2000).

The potential use of Modified atmosphere packaging (MAP) and active packaging for extending the shelf life of dairy products, including cheese, has been demonstrated (Floros, Nielsen, and Farkas, 2000). Limited work has been conducted to date on the use of different gas composition MAP for shelf life extension of soft, creamed-style, and whey cheeses (Pintado et al., 2001; Papaioannou, Chouliara et al., 2007; Dermiki, Ntizimani et al., 2008).

In order to optimize product and packaging compatibility, materials with improved barrier properties should be used. Optimization may include new areas such as active packaging
concepts, and nanocomposite technology (Mortensen, Bertelsen et al., 2005). A study conducted by the Department of Food Science at Cornell University concluded that FreshPax™ is effective in extending the shelf-life of oxygen-sensitive foods, and its use decreases mould and mould spoilage of commercial cheese (FreshPax™ Oxygen Absorbing Packets). Different antimicrobial packaging systems were used as active agents to increase the shelf life of Mozzarella cheeses (Conte, Scrocco C., et al., 2007). The objective of this work was to study two different modified atmosphere packaging (MAP) solution effect on the shelf life of soft fresh cheese Kleo – the use of both usual MAP conditions as well as with oxygen scavenger commitment in the pouches made of different polymer materials and compare them with the commercial vacuum packaging (VP) of the cheese.

**Materials and method**

**Cheese Kleo characteristics**
Experiments were carried out at the Department of Food Technology, Latvia University of Agriculture in 2011. The objects of the research: cheese Kleo – a regional white soft fresh cheese manufactured in local cheese making factory from pasteurized (+78–+82 °C) and normalized cow milk – for experiments was bought on the local supermarket in Latvia. The albumin from the milk have been set down by addition of acid cheese whey, after than filled in self compressing vat for moulding during 16 to 18 hours. The pressed unripe cheese peaces have a cylindrical shape with rounded off side edges, before packaging they are rubbed with salt (NaCl), salt content – from 0.3 to 0.8%. The consistency of cheese is mild, and has a homogenous slightly grainy texture, with irregular breaks, the surface slightly wet, the colour from white to slightly yellowy. The moisture content of cheese should be not more than 64%, fat content – 35±2%.

**Packaging and storage of samples**
Cheese Kleo in currently is sold on the market place in a polymer PA/PE pouch vacuum packaging (VP) weight of 0.3–0.8 kg in each and its shelf-life is not more than 15 days at the temperature 0 to +6 °C. Four different polymer films were used: PA/PE, PE/OPA, Multibarrier 60 and OPP. The structure of performed experiments is shown in Fig. 1.

![Figure 1. The structure of performed experiments](image-url)
Packaging materials for experiments were selected with different water vapor transmutation rate and various thicknesses. The manufactured cheese *Kleo* cylindrical pieces were cut into four parts each, packed by 100±10 g in beforehand from roll stocks made polymer pouches size of 110 x 120 mm. For shelf life extension the use of both usual MAP conditions as well as with oxygen scavenger commitment in the pouch was investigated. Modified atmosphere consisting of carbon dioxide CO\(_2\) (E 290) 30% and nitrogen N\(_2\) (E 941) 70% was used, while vacuum packaging (VP) was selected as the control. For reduced oxygen packaging (ROP) creation (O\(_2\)–0%) in pouches an iron based oxygen scavenger sachets of 50 cc obtained from Packaging Solutions OÜ were used. The samples were hermetically sealed by MULTIVAC C300 vacuum chamber machine and stored in a Commercial Freezer/Cooler „Elcold” at the temperature of +4.0±0.5 °C, controlled by MINILog Gresinger electronic. At the storage time up to 32 days’ physical and chemical properties: headspace gas composition, pH and acidity, moisture content, water activity were evaluated. Content of microorganisms – moulds, yeasts, and lactic acid and *Escherichia coli* bacteria was pointed. At each time of measurement, two identical packages for each packaging material were randomly selected on sampling days (day 0) and after 5\(^{th}\), 11\(^{th}\), 15\(^{th}\), 18\(^{th}\), 22\(^{nd}\), 25\(^{th}\), 29\(^{th}\) and 32\(^{nd}\) day of storage; six measurement repetitions of each sample were performed.

**Physical, chemical, and microbial analysis**


**Statistical analysis**

The results were processed by mathematical and statistical methods. Statistics on completely randomized design were determined using the General Linear Model (GLM) procedure SPSS, version 16.00.

**Results and Discussion**

Water vapor permeability of packaging materials is essential for water loss. In our experiments has been established the mass losses from packages during 32 storage days didn’t exceed 0.3%. Dosebry and Hardy (2000) noted that a 2.5–5.0% weight loss of cheese due to insufficient barrier properties is normal. They also found that dehydration of fresh cheese should be avoided, because a dehydrated surface is a major quality defect in those products. Moisture loss was the main factor of weight loss from the cheese during the storage time caused by different water vapor barrier properties of packaging materials. Water activity \(a_w\) of cheese *Kleo* at the beginning of experiment was 0.98, during the storage time it increased till 0.99.

The gas composition in the headspace changed. Carbon dioxide content decreased in all packages by approximately 10 to 15% during first 5 days of storage due to partial dissolution in the cheese mass. Following the storage CO\(_2\) content as a result of growth of lactic acid bacteria significantly increased (till 58%) in MAP, Multibarrier 60 packages, when in the presence of oxygen scavengers its increase was only till 40% (Fig. 2). The oxygen content (O\(_2\)) in Multibarrier packaging increased a little in the packages with oxygen scavengers incorporated, while in the packages made of the same material without scavenger, including vacuum packaging, the O\(_2\) concentration increased in average till 4.5% (Fig. 3). Phenomena...
showed MAP, OPP packaging increasing the O\textsubscript{2} content in packages till 20%, similar like in air conditions, and all samples after 22 storage days spoiled and turn moldy, therefore further experimentally were not analyzed.

Figure 2. The dynamics of carbon dioxide (CO\textsubscript{2}) content in the headspace of package in MAP (30% CO\textsubscript{2}+70% N\textsubscript{2})
1 – vacuum, PA/PE; 2 – MAP, PE/OPA; 3 – MAP, PE/OPA, oxygen scavenger; 4 – MAP, Multibarrier 60, oxygen scavenger; 5 – MAP, Multibarrier 60; 6 – MAP, OPP.

As shown in Fig. 4, initial pH value of cheese Kleo was 5.8 decreasing within 32 storage days up to 5.0 of the control sample (VP) and 4.8 in samples packed under MAP in PE/OPA without oxygen scavengers. Using oxygen scavenger’s pH values decreased till 4.9 in PE/OPA and till 5.0 in Multibarrier 60 film packaging relating with acidity increase approximately till 130 °T (Fig. 5).

Figure 4. pH dynamic in vacuum and in MAP packed Cheese Kleo during the storage time
1 – vacuum, PA/PE; 2 – MAP, PE/OPA; 3 – MAP, PE/OPA, oxygen scavenger; 4 – MAP, Multibarrier 60, oxygen scavenger; 5 – MAP, Multibarrier 60; 6 – MAP, OPP.

The least decrease in pH values showed sample packed in Multibarrier 60 film without oxygen scavenger in conformity with acidity increase from 40 till 100 °T. The higher decrease of cheese pH value was observed in cheese packed in MAP, OPP – already after 22 storage days it was 4.8 corresponding to higher value of acidity 140 °T, therefore all samples in this packaging grow misty, wherewith this packaging material doesn’t ensure the cheese quality during storage. This is expressed and may be related to the higher lactic acid bacteria (LAB) counts to those (Figure 6) different packaging methods. During the growth of LAB, lactic acid produced with a consequent drop in pH.

Figure 3. The dynamics of oxygen (O\textsubscript{2}) content in the headspace of package in MAP (30% CO\textsubscript{2}+70% N\textsubscript{2})

Figure 5. Acidity dynamics in VP and in MAP packed cheese Kleo during the storage time
1 – vacuum, PA/PE; 2 – MAP, PE/OPA; 3 – MAP, PE/OPA, oxygen scavenger; 4 – MAP, Multibarrier 60, oxygen scavenger; 5 – MAP, Multibarrier 60; 6 – MAP, OPP.
Regarding LAB (Fig. 6) all samples showed similar growth rate. All samples after 10 storage days presented the value 7 log cfu g\(^{-1}\), after 32 days of storage – in average 9 log cfu g\(^{-1}\). Similar results were fond by Dermiki, Ntzimani et al. (2008) in experiments with MAP packed whey cheese. Lactic acid bacteria may spoil soft cheeses in modified atmosphere, because the bacteria are facultative anaerobic, which implies that they cannot be controlled by modified atmosphere packaging (Westall, Filtenborg 1998). Not any mould growth in all packaged samples, excepting MAP, OPP was observed. In cheese packed in high moisture barrier property OPP film the samples get spoiled and moulded after 22 days. The yeast grows in all packaging conditions was equal (Fig. 7) – after 32 storage days in average 9 log cfu g\(^{-1}\) were observed. The incorporation of oxygen scavenger into packages didn’t give the desiderate result for quality maintenance and subsequent shelf life extension of soft cheese Kleo. It was recognized the shelf life of cheese Kleo could be acceptable till 32 days.

Conclusions
1. No significant effect of oxygen scavengers’ application for shelf life extension of soft cheese Kleo was observed.
2. An elevated content of CO\(_2\) (till 55%) produced by lactic acid bacteria growth positively affected the cheese quality parameters in MAP, Multibarrier 60 film packaging without oxygen scavenger sachets incorporated in pouches.
3. The addition experiments should be carried out to determine the effect of active packaging on cheese Kleo shelf life.

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References


Abstract
In definite processes, including pathological, regardless of the origin of solid object start from its surface. The processes develop in deep and change the structure and properties of the sample in volume, but in this case the surface itself is appropriately transformed. One of the most effective methods for studying solid surfaces is EDRS (electronic diffuse reflectance spectroscopy), along with IR (infra red) spectroscopy, Raman and ESR (electronic spin resonance) spectroscopy. The EDRS method in the wave length range 200–750 nm was used. In this work we studied the combined effect of ethanol and radiation with fast electrons (absorbed dose ranged from 12.5–50.0 kGy) on the electronic spectrum of surface of restructured muscle tissue of pork and beef.

Key words: optical diffuse reflection, electron-beam radiation, muscle tissue.

Introduction
The number of research methods of solid surface of biological objects which are complex heterogeneous systems is quite limited (IR-spectroscopy, Raman and EDRS). The limited number is caused by difficulty and non-uniqueness in interpreting of received data. EDRS method is used particularly to determine the quality of meat and fatty products by their colour in visible area of spectral range. The quantity of total protein and its degradation level in meat-food is estimated by absorption of two amino-acids – tryptophan and tyrosine in ultraviolet range (260 and 280 nm) of spectrum (Антипова и др., 2001; Василинец, Колодязная, 2004; Демченко, 1981).

Integrated studies that have been conducted using plant (Нечипоренко и др., 2010; Плотный, 2007; Шамова, 2007) and animal (Нечипоренко и др., 2008a; Нечипоренко и др., 2008b; Нечипоренко и др., 2007) tissues for the last five years (in St. Petersburg State University of Low Temperature and Food Technologies) showed that EDRS method can be used more widely and efficiently.

A number of serial chemical treatments (extraction of water-soluble constituents of sarcoplasm, treatment with sodium chloride, alkali, ethanol, ether) showed in general that electronic spectrum of surface of biomaterials is presented by four clearly differentiating areas. It is well-known that in visible range of spectrum (535–640 nm) distinctly appears a doublet of bands – myoglobin and its oxidized forms – pigment proteins of meat. Mucopolysaccharides give broad intensive and stable band in wavelength interval 410–425 nm, which is deleted from spectrum after treatment of samples by 0.6 M NaOH. In the near ultraviolet area (330–365 nm) lipid components is displayed in the form of 2–3 bands of medium intensity. The most complex and saturated one is the middle ultraviolet range (200–320 nm) of spectrum where absorb some of amino-acids (such as tyrosine, tryptophan, phenylalanine and cystein) and also sugars, oligosaccharides and their different hybrids.

The goal of this study is to investigate the influence of ethanol and electron-beam radiation treatments on optical spectrum of pork and beef muscle tissues.

Materials and Methods
In this work minced pork sirloin (pH=5.82) and beef sirloin (pH=5.87) were tested. The muscle tissue was ground in meat mincing-machine with 2.5 mm holes on the disk. Pork muscle fiber was obtained from forcemeat by extraction of water-soluble constituents of sarcoplasm by bidistilled water during 12 hours. In parallel we studied the series of pork forcemeat samples which was taken from compact muscle tissue samples pretreated by 96%
ethanol for 2.0 minutes. Grind up to forcemeat muscle samples (2.0–2.5 g) were packed in polyethylene film (0.03 mm thickness), airproofed and exposed to radiation. Sample radiation was carried out, using medium energy electron accelerator of resonant-transformer type RTE-1B (Saint-Petersburg Scientific Research Institute of Electrophysical Apparatus). Absorbed doses in series were: 12.5; 25.0; 37.5 and 50.0 kGy. Electron absorption spectra of sample surface were received using spectrophotometer “Specord M-200” (AIZ Engineering GmbH, Germany) within wavelength range 200–750 nm on radiation day.

Results and Discussion
In Fig.1 it is shown electron absorption spectra of intact surface of forcemeat of pork muscle tissue sample (1) and pork muscle fiber sample (4). Their comparison shows that removal of sarcoplasm causes sharp decrease of the whole spectrum. However in this case clearly differentiate bands that belong to lipids (340 and 360 nm), proteins (220, 240, 260 and 280 nm) and one of the bands (290 nm) belong to monosaccharides. In spectrum of intact muscle tissue sample are more exhibited bands of carbohydrates –275, 285 и 300 nm (Элиас, Кохен, 1983).

![Figure 1. Electron absorption spectra of sample surface of pork forcemeat and muscle fibers](image)

1 – intact; 2 and 3 – forcemeat from muscle tissue, pretreated by ethanol; 4 – muscle fiber

Treatment of sample (1) by ethanol also has an effect on all muscle tissue components, but it is more seen in UV-range where proteins and carbohydrates are absorbed (curve 2). At this wavelength range spectrum is located much higher than spectrum of intact sample and it is smoothed. It is of some interest the comparison of curves 2 and 3 received at 2 month interval, which illustrate absorption spectra of sample surface of forcemeat of two different species pretreated by ethanol before grinding of compact muscle tissue.

Smoothing character of both spectra and their closeness let us to assume the uniformity of destructive processes caused by ethanol activity on muscle tissue of the same anatomic part of different animals. Moreover destructive processes of ethanol affect mostly proteins and carbohydrates of sarcoplasm. The band with \( \lambda = 310 \) nm we relate to oligosaccharides. In both spectra indicated differentiation of two strips belonging to lipid components.

The effect of electron-beam treatment on intact minced pork muscle tissue illustrates Fig.2. Bring to notice the inconsequence of electron spectra location in dependence with absorbed dose. Sample that absorbed the dose in 12.5 kGy has a spectrum very similar in UV-area to
the ones pretreated by ethanol and unexposed to radiation muscle tissue samples (Fig. 1, curve 2 and 3).

![Image of optical spectrum](image-url)

**Figure 2. The effect of electron-beam treatment on optical spectrum of surface of intact pork muscle tissue samples**

Absorbed dose is: 1 – 12.5; 2 – 25.0; 3 – 37.5; 4 – 50.0 kGy; 5 – is the control

This shows similar nature of action of ethanol and water radiolysis products on the most sensitive water-soluble components of sarcoplasm. Spectrum of sample absorbed 25.0 kGy declines and we turn to entirely other bands than in control. Absorbance lowering in wavelength range 200–280 nm, formation of maximums resulting from protein structures – 220, 240, 260 and 280 nm (qualitatively very similar to muscle tissue) and also bands that can be given by carbohydrates – 270, 290, 310 nm shows significant destruction of all sarcoplasm components.

Further increase of dosage lead to lift of electron spectra. Spectra concordance in interval 230–290 nm of samples 1 and 4, which absorbed 12.5 and 50.0 kGy respectively, suggests that from the dose of 50.0 kGy begins the destruction of muscle fiber. Evidently that 37.5 kGy dose is intermediate between the doses whereby starts disintegration of sarcoplasm components (25 kGy) and disintegration of muscle fiber (50.0 kGy).

![Image of optical spectrum](image-url)

**Figure 3. Influence of ethanol treatment on optic spectrum of radiated samples of pork muscle tissue**

Absorbed dose: 1 – 12.5; 2 – 25.5; 3 – 37.5; 4 – 50 kGy; 5 – control
It is important to notice that obtained data is in a good agreement with pH-metry data received while post-radiation sample storage of given series (Orehova et al., 2011). Electronic spectra show (Fig. 3) that pretreatment of samples by ethanol strengthens action of ionizing irradiation at low doses. It is seen from the position of curves 1 and 2. Absorbed dose 25.0 kGy in studied range present oneself as a threshold. The spectra of intact and radiation treated samples above threshold dose is not enough different from. However collating spectrums of samples 1 and 2 (Fig. 2 and 3) make possible to notice that ethanol not only enforce the action of ionizing radiation but gives certain direction to radiolysis. In distinction from intact samples in spectra ethanol treated samples is absent bands specific for protein structures, but is present bands corresponding carbohydrates. It is possible to suppose that ethanol direct initial process of radiolysis on carbohydrate components.

Figure 4. Electronic spectra of absorbance of sample transversal section surface of intact pork muscle tissue
Absorbed dose: 1 – 12.5; 2 – 25.5; 3 – 37.5; 4 – 50.0 kGy; 5 − control

By analysis of electronic spectra of sample section surface of intact pork muscle tissue (Fig. 4) we have turn to their identity to spectra showed on fig. 2. Just as in example of forcemeat muscle tissue the greatest destructive effect on sarcoplasm components gives 25.0 kGy doses.

Figure 5. Electronic spectra of absorbance of sample transversal section surface of intact beef muscle tissue
Absorbed dose: 1 – 12.5; 2 – 25.0; 3 – 37.5; 4 – 50.0 kGy; 5 − control
Electronic spectra of absorbance show (Fig. 5) that samples transversal section surface of intact beef muscle tissue are more stability for electron-beam treatment than such samples pork muscle tissue, because in this case the process of radiolysis directed on carbohydrate and lipids components muscle tissue.

Conclusions
1. Surface pretreatment of the compact pork muscle sample by ethanol exhibits changes of surface optical properties of the sample after preparing forcemeat.
2. The protein structures are protected by ethanol, which redirect the process of radiolysis to carbohydrate components of muscle tissue.
3. The influence of ethanol on restructured samples is identical to the section samples of muscle tissue, but the beef muscle tissue is more resistant to the ionizing radiation than the pork.

References
Abstract
In order to ensure hygienic end-product realization, it is necessary as soon as possible to the chilling, using efficient methods, such as the blast chilling equipment. Blast chilling equipment provides food chilling, optimum, 90 – 120 minutes to +3°C, reaching an internal temperature of food, especially important to say, the perishable ready-to-eat product groups (first course, main courses and dressings, snacks), including meat dishes, which are used as a reference model in this study. Given the fact that in recent years, technology increasingly refuse from discrete point, switching to the fuzzy temperatures, a study was conducted as a separate meat dishes microbiological indicators, they are not chilling technology to the +3 °C and +7 °C and +11 °C. Research was carried out in Amica Ltd professional kitchen, using blast chilling equipment, and Microbiology Laboratory of Faculty of Food Technology, Latvia University of Agriculture. The study used two types of Amica Ltd catering companies more often used for meat dishes - pork goulash and minced meat sauce. Research results show that, by combining the food safety standards and cost-efficiency, chilling end temperature of blast chilling in the range of +3 – +11 °C, followed by further storage in freezer. It was recommended to stop the blast chilling +7 – +11°C temperature range, depending on the required shelf life for the finished product. It is therefore essential that the limit, to encourage the blast chilling process, reducing the length to be able to optimize the overall process.

Key words: chilling, blast chillers, ready-to-eat products

Introduction
Fast chiller as the method used in the world catering meals for more than 10 years, Latvian, this technique is used in recent years. In Latvia this technology gained popularity in recent years. It is related to the operation of the installation, production, knowledge which is needed to apply the equipment, as well as the Latvian accession to the European Union, which imposes requirements related to the food business involved parties. This is defined by Regulation EC Nr.2073/2005 The microbiological criteria for foodstuffs, the Regulation EC No. 852/2004 on the hygiene and food industry good hygiene practice guidelines for closed-catering, mobile and temporary facilities (General Legislation of ..., 2010).

The aim of research is to create innovative solutions for fast chilling processes in food services industry.

Microbiological hazards are the most common major source of food related disease and therefore the microorganisms in food may not be in quantities that pose risks to human health. (Marčenkova T., 2010; Effect of Bacterial Growth ..., 2010). Fast chilling is a way to reduce microbiological hazards in food. However, to cool the facility dishes are familiar, a chilling method used, because each dish has its own characteristics associated with both the food structure, the moisture content and production technology (Ruciņš M., 2001).

Previously we don’t have research on how the food is safe for the consumer if the food cool for a blast chilling to a temperature of +3 °C and higher, reaching +10 °C and more. There are also no studies on how the chilling temperature affect processing food shelf life. From a business perspective, it is important to minimize costs. Using a method that provides a specific time, as needed cook and cool, can reduce electricity costs.

Hot dishes can be cooled in two ways, through the fast chilling units and traditional to be refrigerated after cooking left to cool a cold water bath, in a container on the work tables, as well as some food companies are positioned on the sill and to which it is cooled at room temperature, placing them in cold rooms.

There are many reasons why chilling with the blast chilling equipment is needed, such as health security, the efficiency of the building.
Enterprise, using fast chiller equipment, organization of work is facilitated, prepare hot meals are chilled and can be stored up to 5 days in refrigerators. (Blast chillers ..., 2010). But good hygiene practice guidelines for closed-end catering companies determine if a company wants to set a longer shelf life, may be a microbiological testing laboratory where samples are removed from the dishes and found the microbiological parameters. Conversely, if the food is used for chilling the traditional method of chilling in a cold water bath or in specialized cells, for good hygiene practice guidelines for public catering enterprises, this must be done within 2 hours after cooking (Good hygiene practice guide..., 2006). This means that each day is to prepare a particular dish, for example, in one day cooks goulash sauce not only for a certain day, but 2 or 3 days, it is cooled and placed in cold chambers. Cooking time will save about 30–50%. (Blast chillers..., 2010). By contrast, when preparing their own meals each day in small quantities, are consumed much more power and resources.

Materials and Methods
This study is based on two frequently used for meat dishes – minced meat souce and pork goulash cooked according to traditional technologies, professional pan Metos Futura. Technological research conducted Amica Ltd professional kitchen; microbiological analysis - Microbiology Laboratory of Faculty of Food Technology, Latvia University of Agriculture. Cooked foods quick blast chiller Fast Friulinox was used for chilling standard mode (hard), chilling the product of preparation (+82 – +84 °C) to a temperature +3 °C, +7 °C, and +11 °C. Product temperature inside the cabinet was detected by the built-in bimetallic thermometer (Irinox Blast chillers..., 2010).

For determination of the aerobic colony count (ACC) standard LVS EN ISO 4833:2003 “Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of microorganisms. Colony– count technique at 30 degrees C” was used. For isolation of the microorganism’s cultures GPA medium was used. The standard method usage is required by Regulation of Eiropean Comission No. 2073/2005 on microbiological criteria for ready-to-eat foods (Regulation (EC) No.2073/2005).

Data processing is done with Microsoft Excel tools – average, standard error and graphics.

Results and Discussion
The study was conducted during the period from September 2010 to January 2011. In experiments using minced meat sauce and pork goulash, prepared according to a given technology. Ready meals placed in various GN containers of 1 kg at 6 and 2.5 cm thickness; fast chilling standard method (hard) with a chilling period of 90 minutes. The samples were chilled rapidly reaching the end temperature of +11 °C, +7 °C and +3 °C and stored at 72 and 96 hours in a low constant temperature (+2 – +6 °C).

Thickness of 2.5 cm
Chilling for 2.5 cm for is started, the food temperature is +7 °C below normal (+70 °C), which are chosen by the thickness of +63 °C. Chilling process, the chamber temperature is -18º C, but after 15 minutes it rises to 0 ºC degrees. This temperature is maintained to 62nd minute, after that the cabinet temperature falls to -5 ºC, until the end of the cycle. At the end of chilling process the food temperature has dropped to +9 ºC, that is not enough, therefore, is the reopening of the chilling cycle. The new chilling cycle is initiated by -5 ºC temperature in the cabinet. This means that the machine automatically sets the appropriate chilling temperature – the intensity depending on the temperature dishes (first chilling cycle was initiated with the -18 ºC). In the second chilling cycle, the cell temperature decreases from -5 to -19 ºC, until food temperature reaches +4 ºC. The total chilling time cycle is 106 min.
Figure 1. Changes of temperatures during food blast chilling process

**Thickness of 6.0 cm**
The temperature entering the chilling food is +81.8 ºC, what is 11.8 ºC higher than the recommended. Chilling process, the initial temperature of the chamber is -20 ºC, what persists to 25\textsuperscript{th} minute, but installed by the end of the cycle method (90 minutes), the temperature in the chamber is gradually increased to 0 ºC, when installed on the expiry of 90 minutes, but after 90 minutes of chilling temperature of the food has to +18 ºC, which is not enough, therefore is repeated the cycle of chilling process. In the second chilling cycle in 11 minutes the cabinet temperature fall from -3 to -12 ºC, and then quickly goes down to -18 – -20 ºC. In this temperature the food is chilled 24 minutes, then it again rises to -11 ºC and chilling has been stopped because the food has reached +4 ºC temperature. The second chilling cycle lasts 39 minutes, but the total chilling time is 129 minutes.

**A comparison of layers**
Comparing the collected data, it can be concluded that the chilling influence of food start chilling temperature, and depth of food chilling container. At layer thickness 6 cm food beginning temperature is +80.3 ºC (the most by such temperature starts chilling, because the food in a blast chiller immediately after preparation), and the food is chilled in the temperature -20 ºC 35 minutes, but when the layer thickness of 2.5 cm and the food beginning temperature is +63 ºC, the food was chilled -18 ºC for only 15 minutes; after the chilling temperature is 0 ºC and only 90 minutes before the end of the cycle it will again fall to -5 ºC. Comparing the food temperature after 90 minutes chilling can be concluded that the layer thickness of 6 cm, whereas the +18 ºC, the layer thickness of 2.5 cm – +9 ºC, that is, two times lower.

The chilling intensity is different between the two studies – a layer of thickness 6 cm intensity is higher and the initial temperature is higher than the layer thickness of 2.5 cm. Continuing the chilling cycle after 90 min in both trials, the cabinet temperature falls to -20 ºC, but the chilling time at this temperature is different. At layer thickness 6 cm dish reaching +4 ºC,
temperature in the chamber once again rise to -11 °C, but the layer thickness of 2.5 cm it is -20 °C.
Chilling expectancy gap for both studies is 31 min. At layer thickness 6 cm dish was cooled in 129 minutes, but the layer thickness of 2.5 cm 106 minutes, both studies reach the same end temperature.
If the food to chill only to +11 °C (as confirmed by microbiological studies), then a layer of thickness 6 cm requires 109 minutes, but at 2.5 cm requires only 82 minutes.

Storage and microbiology
Ready meals at the end of the chilling process (+11 °C, +7 °C, +3 °C) were stored in refrigerators at +2 – +6 °C degrees 72 and 96 hours.

Figure 2. ACC average number of log CFU g\(^{-1}\) in minced meat sauce and pork goulash

The results (n = 15) shows ACC count, the total sample results are less than 10\(^4\) CFU g\(^{-1}\), but the disappointing results of the tests found two samples (\(\geq 10^3\) CFU g\(^{-1}\)).

When stored samples are cooled to a final temperature +30°C, 72 hours, the average number ACC not exceed the limit – 1.34 log CFU g\(^{-1}\) minced meat and 1.47 log CFU g\(^{-1}\) pork goulash, which confirms the blast chilling equipment manufacturer's recommendations. Samples chilled to +7 °C ACC average number is about the same, within acceptable standards – pork goulash 2.44 and 2.45 log CFU g\(^{-1}\) of minced meat sauce. Analysis shows that the food may be chilled by the chosen technology.

Most microorganisms evolved in samples cooled to a final temperature +11 °C - minced meat sauce ACC is 3.66, but the pork goulash 3.78 log CFU g\(^{-1}\). This is related to sensitive margin temperature (+10 – +60 °C) achievement of the development of microorganisms is to create a favourable environment. ACC average number of meals exceeds the limit, so after such a technology chilled foods considered to be microbiologically unsafe.

When stored samples 96 hours after chilling to +3 °C, ACC average number of appropriate (not exceeding 10\(^3\) CFU g\(^{-1}\) - figures are roughly similar to minced meat sauce and pork
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goulash. Samples chilled to +7 °C over ACC is minced meat in a cream sauce – 2.7 log CFU g\(^{-1}\). Minced meat sauce organisms evolved more, as this can be explained by different food pH environment. Minced meat sauce environment is neutral, as for food technologies sauce add cream, but the pork goulash environment is more acidic.

Survey data show that the prepared meat dishes (pork goulash and minced meat sauce), which are prepared by the process, rapidly chilled and stored in a low 72 and 96 hours, the test meets the criteria used for the study, if the chilling end temperature does not exceed +7 °C. Dishes, which are chilled to the final temperature of +11 °C, it is recommended to realize a shorter period of time, up to 48 hours.

Conclusions

1. The study confirms that the company may be stored for goulash, minced meat sauce up to 96 hours in refrigerators and those ready-to-eat meals are microbiologically safe, if the preparation is rapidly chilled end temperature of +7 °C and +3 °C.

2. Investigated food is microbiologically safe for the consumer, where it is chilled only to +11°C current notional location (+3 °C). Dishes, which are chilled to +11 °C is recommended to realize a maximum of 48 hours.

3. Blast chilling process, and finished dishes chilled +11 °C, the chilling time is reduced by an average of 24 minutes (22%) to the finished dish a layer thickness of 2.5 cm and about 19 minutes (15%) at a layer thickness 6 cm, which allows blast chilling to optimize the overall process.

4. Storing ready-to-eat dishes until 4 days to provide the necessary storage conditions: constant temperature (+2 – +6 °C) in a closed container in which the finished dish placed to prevent any contamination.

References


MODEL DEVELOPMENT FOR FRESH BAKED BREAD NATURAL AND FORCED COOLING

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Abstract
Industrial production of bakery products allows consumers to get fresh bread at any time of the day. However, there are many problems connected with storage and cooling of bread before distributing it to the trading network. Microbiological safety requirements and marketing necessitates the packaging of bread into films after baking. However, this is only possible when the baked bread has cooled. Rate of cooling is, therefore, very critical parameter. Low cooling rates can limit production capacity in a bakery, while higher cooling rates can lead to higher moisture evaporation rate and result in greater weight loss of the product. The moisture distribution in bread at the end of cooling can also result in water condensation on the film, which can accelerate fungal growth. The principal objective of this work is to study the effect of cooling conditions on heat and mass transfer in bread, and assess their influence on product quality. Experiments were carried out with white pan bread which was prepared in a laboratory. Cooling was undertaken in ambient environment (26±2 °C, RH 50 %) by placing the hot product on a rack, as well as in a climatic cabinet with controlled temperature, humidity and level of convection. A mathematical model based on Fourier’s second law for heat transfer and the Fick’s second law for the mass transfer was developed to simulate heat and mass transfer processes in the bread during cooling, and validated using experimental data. The model is able to predict mass and volume changes during the „cooling” time.

Key words: Bread cooling, model development, heat and mass transfer.

Introduction
Heat and mass transfer in bakery products production occurs during proofing, baking and post-baking cooling. Several works are available in the literature on the modelling of heat transfer during baking, for example, DeVries et al. developed a quantitative model for heat transport in dough and crumb during baking (deVries, 1988). Modelling of simultaneous heat and water transport in the baking process can be also found in Sablani (1988) work. Zanoni (1993) represented results of thorough study on the baking process modelling (Zanoni, 1994). In 2007 Wagner published an article concerning water transport in bread during baking (Wagner, 2007). In the other words there is a lot of information available in literature, which describes heat and mass transfer in bread during baking, but there is a lack of knowledge in the modelling of the bakery products cooling. In 1993 van Sluis used a finite element computer program designed for the simulation of the cooling of bovines carcasses, and adapted his model to bakery products (van der Sluis, 1993).

Heat and mass transfers are taken into account, and chilling and freezing can be simulated. The heat transfer on the surface takes into account the convection and the radiation, but not the evaporation. N. Hamdami in this paper determined, thermo-physical properties, including apparent and true densities, specific heat, enthalpy and effective thermal conductivity separately for crumb and crust of partly baked bread (Hamdami, 2004). This study is an attempt to develop a mathematical two dimensional model which takes into account thermo-physical properties of fresh baked bread and heat and mass transfer by convection, radiation and evaporation in order to predict mass loss and time of cooling, and to show the influence of each kinds of heat transfer on the duration of cooling process.

Materials and Methods
Experiments were carried out with white pan bread which was baked from scratch in a laboratory and then cooled by natural and forced convections.
Dough was prepared based on the straight-dough bread making recipe (Finney, 1984) which is given in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Baker’s, %</th>
<th>Ingredients</th>
<th>Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Bread flour</td>
<td>1160</td>
</tr>
<tr>
<td>62</td>
<td>Water</td>
<td>742</td>
</tr>
<tr>
<td>2</td>
<td>Yeast (fresh)</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>NFDM</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>A.P. Shortening</td>
<td>35</td>
</tr>
<tr>
<td>6</td>
<td>Gran. sugar</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>Salt</td>
<td>23</td>
</tr>
<tr>
<td><strong>Total Weight</strong></td>
<td></td>
<td><strong>~2085</strong></td>
</tr>
</tbody>
</table>

Mixing was done in VMI spiral mixer (France). Dough was rounded and left for rest for 7 minutes at ambient temperature and final moulded using a Sorensen ’New Universal. Mark 2’ moulder (England) and then loaded into slightly greased unlidded rectangular tins (100x100x300 mm). Tins were placed in an Acrivan Proving Cabinets (UK) for final proofing (40 °C, 70% RH) for 1 hour. Baking was performed in a Frederick Bone gas-fired reel oven (UK) set at 244 °C for 30 min without steam injection with leads covered tins to achieve rectangular shape of final loaf.

Cooling of bread was considered as finished when the temperature at the bread geometrical centre reached 25 °C.

Temperature measurements in the middle of the crumb, at the crust surface and air near the surface were made by thermocouples connected to a Grant data-acquisition system (UK) each second during 12000 seconds.

Weight loss measurements were carried out using a Mettler PE 3600 precision scale; the volume of bread was measured by using of a laser volumeter. Such system allows to determinate the bread volume without any contact such as in the case of rapeseed displacement methods. The volume of the breads was measured just after baking, and in 3 hours after baking. Each bread sample was placed on a rotating plateau (60 rpm). A laser beam installed on a rotating arm was then used to measure the volume of the bread without contact. The accuracy of volume measurement using this equipment was estimated to ±0.4% according to 3 repetitions with the same loaf.

Bread moisture during chilling is determined gravimetrically by taking samples from the crumb and heating them for 1.5 hours at 130 °C.

Results and Discussion
Modelling is based on the Fourier’s second law for heat transfer and the Fick’s second law for the mass transfer.

Rectangular shaped bread loaf with dimensions 100 mm x 100 mm x 300 mm was chosen for model development. Two dimension geometry along x-y axis is shown on a Figure 1.
For modelling the grid containing 20 points in x and y coordinates, and the time step for 30 seconds are used.

The heat diffusion equation (1):

\[
\frac{\partial T}{\partial t} = \frac{\lambda(T)}{\rho_b c_p(T, W)} \left( \frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} \right),
\]

where:
- \( \lambda(T) \) – is the thermal conductivity, W (m K)\(^{-1} \);
- \( \rho_b \) – is the density of bread, kg m\(^{-3} \);
- \( c_p \) – is the specific heat of bread, J (kg K)\(^{-1} \).

As a boundary condition \( \lambda(T) \) is defined taking into account heat transfer by convection, radiation and evaporation.

\[
-\lambda(T) \left( \frac{\partial T}{\partial x} \right)_{x=L} = -\lambda(T) \left( \frac{\partial T}{\partial y} \right)_{y=L} = \alpha \left( T_s - T_{air} \right) + \varepsilon \sigma \left( T_s^4 - T_{air}^4 \right) + 
\]
\[
+ \beta H_{lg}(W) \left( p_s(T, W) - p_{ext}(T, W) \right),
\]

where:
- \( \alpha \) – is the heat transfer coefficient, W (m\(^2\) K)\(^{-1} \);
- \( \varepsilon \) – is the emissivity of bread surface;
- \( \sigma \) – is the Stefan–Boltzmann constant;
- \( T_{air} \) – is the air temperature near the bread surface, K;
- \( T_s \) – is the temperature of bread surface, K;
- \( \beta \) – is the mass transfer coefficient, m s\(^{-1} \);
- \( p_s, p_{ext} \) – are the partial vapour pressure on bread surface and near the bread surface, Pa.

\[
p_s = a_{ws} p_s^g,
\]

where:
- \( a_{ws} \) – is the water activity at the surface;
- \( p_s^g \) – is the saturating vapour pressure at the bread surface and can be found from
Antoine’s law as

\[ p_s = 133.3 e^{\left( \frac{18.3036 - 3816.44}{T_e - 46.13} \right)} \]  

(4)

Partial vapour pressure in the air near the bread surface \( p_{ext} \) depends on relative humidity of the ambient air \( H_{ext} \):

\[ p_{ext} = H_{ext} \ p_s \]  

(5)

where:

\( p_s \) is the saturating vapour pressure in the ambient air

\[ p_s = 133.3 e^{\left( \frac{18.3036 - 3816.44}{T_e - 46.13} \right)} \]  

(6)

The mass transfer equation:

\[ \frac{\partial W}{\partial t} = D(W) \left( \frac{\partial^2 W}{\partial x^2} + \frac{\partial^2 W}{\partial y^2} \right) \]  

(7)

where:

\( W \) is local moisture content, \( W_{surface} = 0.1 \);

\( D(W) \) is mass diffusion coefficient, m² s⁻¹.

\[ -D(W) \left( \frac{\partial W}{\partial x} \right)_{x=L} = -D(W) \left( \frac{\partial W}{\partial y} \right)_{y=L} = \beta(p_s(T,W) - p_{ext}(T,W)) \]  

(8)

The initial condition concerned mainly the temperature distribution in the crumb at the end of baking. The experimental temperature distribution was implemented in the code.

The initial temperature was constant and equal to \( T_0 \) in the sample, except close to surface where it was decreasing from \( T_{s0} \) to \( T_0 \) with a linear relationship through a 1 cm thickness layer.

The local water content was decreasing with a linear evolution from \( W_{max} \) in the center to \( W_0 \) at 1 cm under the surface, and then from \( W_0 \) to \( W_{min} \) until the surface.

The initial conditions parameters given in table 2.

### Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_0 )</td>
<td>372.00</td>
<td>K</td>
</tr>
<tr>
<td>( T_{s0} )</td>
<td>415.00</td>
<td>K</td>
</tr>
<tr>
<td>( W_0 )</td>
<td>0.70</td>
<td>g water / g dry matter</td>
</tr>
<tr>
<td>( W_{max} )</td>
<td>0.78</td>
<td>g water / g dry matter</td>
</tr>
<tr>
<td>( W_{min} )</td>
<td>0.10</td>
<td>g water / g dry matter</td>
</tr>
</tbody>
</table>

The solution of equations (1) and (2) based on the boundary and initial conditions derived from field experiments have allowed us to obtain a mathematical model of convective bread cooling.
Figure 2. Comparison between calculated and measured data of the temperature in the middle of the crumb

The two curves have some distinction; this indicates a lack of precision in the physical parameters of heat transfer. The quadratic average of the difference between the measured and calculated values was used to quantify the discrepancy between model and experiment (9).

\[
m = \sqrt{\frac{1}{N} \sum_{j=1}^{N} \left( T_{\text{exp}} - T_{\text{cal}} \right)^2} = 2.87,
\]

where:

\( N \) – is the number of measured points at the centre and on the surface.

Future work with the model will include improving of several physical parameters, such as thermal conductivity and mass diffusion coefficient of the bread, and also it is necessary to carried out modeling with a 3-D geometry. Received model was used for studying the effect of mass transfer coefficient on the time of cooling and mass changing. The influence of different heat transfer coefficient value on the calculated duration of cooling 1kg bread loaf with the two modes: with evaporation, convection and radiation, without evaporation (convection and radiation only), the mass of evaporated moisture, and duration of evaporation are given in Table.3.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat transfer coefficient ( a ), W (m(^2) K(^{-1}))</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>30.0</td>
</tr>
<tr>
<td>Time of cooling, min</td>
<td>139.0</td>
</tr>
<tr>
<td></td>
<td>106.0</td>
</tr>
<tr>
<td></td>
<td>89.0</td>
</tr>
<tr>
<td></td>
<td>80.0</td>
</tr>
<tr>
<td></td>
<td>73.0</td>
</tr>
<tr>
<td>Time of cooling (excluding evaporation), min</td>
<td>83.0</td>
</tr>
<tr>
<td></td>
<td>73.0</td>
</tr>
<tr>
<td></td>
<td>68.0</td>
</tr>
<tr>
<td></td>
<td>65.0</td>
</tr>
<tr>
<td></td>
<td>64.0</td>
</tr>
<tr>
<td>Mass loss, g</td>
<td>33.7</td>
</tr>
<tr>
<td></td>
<td>26.7</td>
</tr>
<tr>
<td></td>
<td>21.3</td>
</tr>
<tr>
<td></td>
<td>17.0</td>
</tr>
<tr>
<td></td>
<td>13.7</td>
</tr>
<tr>
<td>Time of mass changing, min</td>
<td>69.0</td>
</tr>
<tr>
<td></td>
<td>47.0</td>
</tr>
<tr>
<td></td>
<td>35.0</td>
</tr>
<tr>
<td></td>
<td>27.0</td>
</tr>
<tr>
<td></td>
<td>21.0</td>
</tr>
</tbody>
</table>

Conclusions
1. During the cooling process at low heat transfer coefficients the evaporation of moisture has a significant effect on the duration of cooling (40%). At high heat transfer coefficients the same effect on the duration of the process is small – only 12%. Number of evaporated moisture is low (1 to 3 percent of the mass loaf), and at high heat transfer
coefficients the moisture is evaporated during the first third of the process only, because of the rapid cooling of the surface. At low heat transfer coefficients the moisture is evaporated during almost the entire process.

2. The model showed good agreement with experimental data of the temperature changing during cooling process. For high cooling rates the heat evaporates the moisture only during the first third of the process, because of the rapid cooling of the surface. At low heat transfer coefficients the moisture is evaporated during almost the entire process.

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