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Dear participants,

The 9th Baltic Conference on Food Science and Technology „Food for consumer well-being" (FoodBalt 2014) will take place from 8th to 9th May, 2014 in the Latvia University of Agriculture (LLU). The conference is organized by the Faculty of Food Technology. The conference aims are to bring together master and doctoral students from Baltic countries, as well as students, established researchers from other countries, promoting the dissemination of new knowledge and allowing young scientists and students to meet professionals, contributing to the development of food science and technology area.

The conference plans to attract more than 120 delegates from 10 countries. The conference programme contains 4 Key lectures and 35 oral presentations over 7 sessions. Additionally, a total of 94 posters will be presented. The conference Organising Committee had received around 70 full paper submissions from 7 countries. A peer review process was enforced, with the help of experts who were members of the Conference Scientific Committee and researchers from Latvia University of Agriculture, all of them internationally recognized in one of the main conference topic areas. 69 papers were selected for publishing and published as reviews (4), original papers (57) and short communications (8).

On behalf of Organizing Committee I am honoured to welcome you at FoodBalt 2014 conference and to wish new experience, contacts and further cooperation activities in common projects and programmes. It would help you to achieve the excellence and recognition in food science area in Baltic region and in Europe, too.

Dr.sc.ing. **Inga Ciprovica**
The chair of the 9th Baltic conference on Food Science and Technology

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REVIEW

POTENTIAL OF IMAGE ANALYSIS BASED SYSTEMS IN FOOD QUALITY ASSESSMENTS AND CLASSIFICATIONS

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Abstract

Increasing life standards, developing technology, growing importance of food quality and safety lead food industry to search new analysis methods. In addition, high consumer expectation and increasing population forces manufacturers to find fast and accurate techniques. The present study was aimed to prepare a summary about computer aided image processing systems covering working principles and applications for different analysis of food products. Image processing systems have recently been considered in this extent and results have revealed that computer aided these techniques can provide all these needs in a non-destructive way for samples. These techniques can be adapted to a wide range of food and agriculture products like meat, bakery products, dairy products, vegetables but primarily fruits. Image processing can be utilized for different purposes related to these product groups. Size and shape based classification, defects detection, microbial safety, quality grading and variety determination are mainly investigated topics. Literature survey indicates favourable reported results. Generally small scale investigations have been presented, but some of them could find a place for industrial application with high success. Computer aided image processing has been taken increasing interests of researcher for its potential in the applications for food technologies.

In the present study it was aimed to prepare a summary about computer aided image processing systems covering working principles and applications for different analysis of food products. As a conclusion it could be said that computer aided image processing systems reveal high potential in food industry.

***Keywords*:** image processing, fast analysis method, food quality, classification.

Introduction

The developments in the technology, media and communication accompany the increasing awareness of consumers. Therefore, there are more expectations than ever. The situation forces the manufacturers to produce and present higher quality food and agricultural products to market. Moreover, these products have to satisfy sophisticated consumer desires. Computer imaging system is one of the methods serving the assurance for high quality food products.

Product quality is evaluated by a wide range of parameters including external, internal parameters. However, in some cases, sensory and safety scores gain higher importance than above ones. External quality parameters, such as surface colour, texture, presence of bruises and defects, are generally monitored and sorted manually by workers, whereas the internal quality parameters including firmness, pH value, soluble solid contents, titratable acidity are evaluated using common techniques. Sensory (e.g. sweetness, flavour) and food safety (e.g. pathogenic bacteria and faecal contamination, pesticide residues and other hazardous residues) characteristics influence general palatability of the products. However, the old fashion techniques are time consuming, destructive and unable to represent the whole batch (Zhang et al., 2012).

Unlike to traditional ones, computer imaging systems do not cause any damage on/in the product and they are rapid analysis techniques as well as being feasible for in-line process (Kim et al., 2007; Park et al., 2011). Being another advantage, these systems in this extent can be easily implemented for any analysis of an individual object and/or a batch of food and agriculture products, like intact fruits, even as they are on the yards (Stajnko et al., 2004). Against the traditional techniques, intensity of the analysed feature in a bulk is possible to figure out by imaging systems (ElMasry et al., 2012, Feng et al., 2013). Moreover, these systems also provide opportunity to perform rapid, hygienic, automated and objective inspections.

This paper represents an overview on camera imaging systems in the food industry. Basic knowledge is provided about steps and principles, some examples of applications are submitted as well.

Basic Steps of Image Processing and Analysis

Computer vision is a system designed with a few equipment and algorithm which are used to obtain information about objects of interest. The information could be utilized in order to shape classification, quality sorting, identification of internal and external features, etc.

Recognition processes of the specialities of the objects occur with a series of steps. Image acquisition, pre-processing, segmentation, representation and description, recognition and interpretation are basically the steps of the computer vision processes and every step must be implemented carefully, or else the results might be unsatisfactory (Gunasekaran, 1996).

Image acquisition is the stage for turning electronic signals from sensing device into numeric form. Ultrasound, X-ray and near infrared spectroscopy, displacement devices and document scanners, solid state charged coupled device (CCD) cameras are some of the sensors used to create images (Brosnan, Sun, 2004). Thermal imaging cameras (Gowen et al., 2010) and terahertz cameras are also used in image analyses systems (Lee, Lee, 2014). In order to get a high quality image which is a vital factor for sequent

steps, illumination and lighting arrangement, high quality optics and electronic circuitry must be prepared properly (Gunasekaran, 1996). Light sources vary according to purpose of using such as incandescent, fluorescent, lasers, X-ray tubes and infrared lamps (Brosnan, Sun, 2004).

Pre-processing stage includes one or more operations of noise reduction, geometrical correction, grey-level correction and correction of defocusing and aims to improve image quality (Shirai, 1987).

Image segmentation is one of the most important stages because accuracy of following step deeply related with this step. It is intended to separate the image into parts which have a strong relation with the object (Brosnan, Sun, 2004). After segmentation, the image generally represents a boundary or a region. First types of images are suitable for size and shape analyses while the latter one is used for determination texture and defects. The image representation should be chosen according to planned application (Gunasekaran, 1996).

Recognition and interpretation are generally performed using statistical classifiers or multilayer neural networks to provide information that is useful for process or machine control needed for quality sorting and grading (Brosnan, Sun, 2004). Fuzzy logic, decision tree and genetic algorithm are also used as learning techniques the same purposes (Du, Sun, 2006).

Applications in Food Quality Assessments and Classifications

Computer imaging systems can be used for sorting products, detecting defects, identifying internal and external characteristics of foods and inspecting food production equipment, etc. There are a wide range of applications for computer aided analysis systems and their popularities continuously increase. Because of increasing popularity and applicability, some food quality and food process applications were presented in this review.

Fruits and Vegetables

The external appearance of fresh fruits and vegetables is one of the factors affecting the consumer perception. Because the first examination of consumer is visual for quality features such as freshness, taste, decayed, maturity, it has vital importance how to present food and agriculture products to the market. Computer aided vision systems are considered as new tools which are implemented to meet the quality requirements depending on customers' demands. By this way it is possible to achieve shape classification, defect detection, quality grading and variety classification (Brosnan, Sun, 2004).

Yang et al. (2012) investigated the detection possibilities of faecal contaminations on the surface of Golden Delicious apples using hyperspectral line scan fluorescence imaging and developed a simple multispectral algorithm. A pair of violet red line lights equipped in order to excite the faecal contamination spots on the apples created in different dilutions. The algorithm utilized the four fluorescence densities at four wavebands (680, 684, 720 and 780). It was noted that more than 99% of the faecal spots could detected from the uncontaminated apple areas. It is feasible to use this system on high-speed apple processing line with the purpose of preventing food originated illness for food assurance and reduction of risks. Kim et al. (2007) built up a rapid online scanning system which works both with hyperspectral Vis/NIR reflectance and fluorescence in the Vis with UV-A excitation. The system was combined with a commercial apple sorting machine at a processing line speed over three apples per second. Great performance was acquired with a faecal detection rate of 100% (with no false positive) and 99.5% (with 2% false positive rate only) using fluorescence imaging and near-infrared reflectance, respectively. Another hyperspectral imaging system was developed by ElMasry et al. (2008) for detection of bruises on McIntosh apples in a recent study. The spectral region of the system was between 400 and 1000 nm however three wavelengths in the near infrared region (750, 820, 960 nm) gave promising results. It was noted that the bruised apples were successfully detected from the sound apples. Being different from previous studies, the system could recognize early bruises created just 1 hour before and could also recognize even the apples have different background colours (red, green or reddish).

Wang et al. (2011) examined the possibilities of detecting external insect infestation in jujube fruit. They utilized a hyperspectral reflectance imaging approach in the spectral region of 400–720 nm although three wavelengths (500, 650, 690 nm) gave the maximum discrimination. The results revealed that all of the intact cheeks and calyx-end regions classified correctly. More than 98.0% of the undamaged jujube fruits and 94.0% of the insect-infested jujube fruits were correctly identified and the overall classification success of the system was about 97.0%.

Taghizadeh et al. (2010) focused the evaluation of shelf-life of fresh white mushrooms (*Agaricus bisporus*) stored in different packaging materials. At the end of the study they managed to determine superiority of packaging materials compared to each other using hyperspectral imaging system. They were able to reduce the colour inspection time less than 1 minute without touching samples and also determined the distribution of quality in a batch which is not possible with classical colourimeters.

Due to hardness of selecting slight rottenness on the mandarins, workers carry out the issue manually under ultraviolet illumination. Often the rottenness is happening by infection of *Penicillium* sp. fungi. Unless the organisms are eliminated, they can spread to a large number of fruits and can cause great economical loss. Gómez-Sanchis et al. (2008) investigated the possibility of detecting fruits that were rotten due to *Penicillium digitatum.* The study aimed both preventing workers against the harmful effects of UV lights and detecting troubled mandarins in a faster and

more accurate way. Therefore they used a machine vision system, without any UV radiation illumination. The rate of success for sorting rotten mandarins from sounds was above 91% and this result indicated that the hyperspectral computer vision system was able to detecting damage caused by *Penicillium digitatum* in mandarins.

It is also possible to establish some internal quality attributes of fruits and vegetables without damaging via using computer vision system. A study conducted by ElMasry et al. (2007) indicated that moisture content, the total soluble solids and pH value of strawberry fruits could be determined by hyperspectral imaging in and near-infrared (400–1000 nm) regions with high correlation coefficients. Moreover, they conducted a texture analysis on the images based on grey-level co-occurrence matrix (GLCM) in order to determine the ripeness level of the strawberries. High classification accuracy of 89.61% was accomplished using the GLCM parameters. More recently, soluble solid content and titratable acidity, which are two of the internal quality properties indicating tomato flavour, determined by Flores et al. (2009) using near-infrared reflectance spectroscopy. The technique allows the examiner to analyse each tomato sample individually without destroying both in on-set and on-line systems and could accurately estimate tomato quality parameters. Fernandes et al. (2011) examined the determination of anthocyanin concentration of whole grape using hyperspectral imaging and neural network. Hyperspectral data was obtained from whole Cabernet Sauvignon grapes in the reflectance mode between the wavelengths from 400 to 1000 nm. At the end the study, they found a squared correlation coefficient of 0.65 between the estimated and traditionally analysed results.

One of the other techniques used for computer imaging is using thermal cameras. The thermal imaging method developed by Stajnko et al. (2004), in order to predict the number of apples in the orchard and measuring their diameter, brought out successful results. Coefficients of determination (R^2) were calculated from 0.83 to 0.88 for numbers of apples and were 0.68 to 0.70 for diameter of apples between manually measured and estimated numbers. It is noted that the rise in both of the correlation coefficient number were seen during the ripening period. Gan-Mor et al. (2011) utilized thermal imaging systems with the purpose of optimizing the high-temperature surface heat treatment which was employed against *Sclerotinia sclerotiorum* (Lib.) de Bary fungi in carrot packaging process. Excessive heating causes damages and discolouration whereas insufficient heating results in incomplete disinfection. The aim of the system was monitoring the process, regulating heat steam level, and gaining uniformity over carrots surface segments using a short, 3 s steam treatment. Reduction around 60–80% in post-storage phytotoxic colour change was achieved and sensitivity to post-storage soft rots by *Sclerotinia sclerotiorum* was lowered significantly.

Meat

Computer imaging systems are also applicable in the meat industry. These systems can be used for defining some quality features of fresh beef meats like surface colour, pH and tenderness, contaminants.

Park et al. (2011) successfully managed to detect small amount (about 10 mg) of faecal and ingesta contaminants on poultry carcasses by using real-time hyperspectral imaging system. This additional process was found not to affect real time process duration which was about 140 carcasses per min. ElMasry et al. (2012) determined some quality characteristics (pH, colour and tenderness) of fresh beef with traditional methods and modelled with their matching spectral values, gained by a hyperspectral imaging system in the near-infrared spectral region (900–1700 nm), using partial least square regression. However, the authors of this study stated that more samples were required for better examination and more robust modelling, and reported that the presented sampling was just enough to take satisfying results. Good performances were achieved and it was explained the system could be useful, reliable, non-destructive and rapid alternative to traditional analysis methods. Potential applicability under the visible light is one of the other advantages of the technique. Recently great accuracy was found in the study by Feng et al. (2013) where determination coefficients (R^2) of *Enterobacteriaceae* bacteria on chicken fillets were 0.89, 0.86 and 0.87 for the three wavelengths of 930, 1121 and 1345 nm, respectively, using near-infrared hyperspectral imaging analysis. The superiority of the imaging techniques compared to traditional analyses methods is that they allow displaying distribution of the analysed properties. On-line inspection of poultry carcasses is also feasible.

Other Foodstuff and Applications

Mokhtar et al. (2011) studied the in-line detection of crumbling, in-homogeneousness, unevenness in size, cracks and glutinous on the surface of pasta (stick together). Flat LED light source, a CCD camera were used and a production line monitoring system was developed accepting process analytical technology basis using a microcontroller of camera. The error in determination of stickiness, crumples and unevenness in size were 1%, 5% and 13%, respectively. It was also noted that being glutinous was easily detected.

Abdullah et al. (2000) investigated the possibilities of classification of muffins with respect to their colour. A classification algorithm used for separating dark from light samples using pre-graded and ungraded muffins. Classification correction rates were 96% of pre-graded and 79% of ungraded muffins when compared with visual inspection.

A computer vision system, developed by Kılıç et al. (2007) examined the classification of beans using artificial neural networks. Size and colour were the parameters for the application. Firstly, length and width were measured both by a calliper and the system. High correlation (r=0.984 and 0.971 for length and width,

respectively) was determined between the results. Secondly, the classification of beans into five classes was performed by human inspector and system. There were rate of correction of classify such that 99.3% of white beans, 93.3% of yellow–green damaged beans, 69.1% of black damaged beans, 74.5% of low damaged beans and 93.8% of highly damaged beans sorted correctly. The overall correct classification rate obtained was 90.6%.

Shafiee et al. (2014) developed a system for characterization of honey based on colour. Estimation of ash content, antioxidant activity and total phenolic content were intended using artificial neural networks. The correlation coefficients of predictions were 0.99, 0.98 and 0.87 for ash content, antioxidant activity and total phenolic content, respectively. They were found to be very promising. These results showed that such a system could effectively applicable for the industry.

The computer imaging systems can also be implemented in dairy industry. For instance a study was conducted by Fu et al. (2014) to determine melamine contamination in milk powder by a near-infrared hyperspectral imaging system. Three different spectral similarity analyses were performed to identify melamine particles in various concentrations. The study results revealed that system could detect melamine at very low concentrations (from 0.02% to 1%) in milk powder and authors also reported their providences that the method had potential to identify some kind of chemicals. Huang et al. (2014) aimed to obtain total chemical information of a milk powder mixed with three different components ($ZnSO_4$, lactose and melamine). In this study near-infrared micro-imaging system was employed to acquire information and distribution of three target components. The correlation coefficients for detection of melamine in various concentrations (30%, 10% and 5%, w/w) were all over 0.9. The success of determination was decreased and being less than 0.61 with reducing melamine concentration (1%, w/w), although this compound was still detectable. Encouraging results were also obtained for $ZnSO_4$ and lactose. In a previous study Eskelinen et al. (2007) investigated the possibility of quality and process control of Swiss cheese. 3D ultrasound imaging technique was conducted in order to acquire images. Cheese-eyes (gas holes), cracks and bulk cheese matrix could be distinguished with high success.

One of the newest technology was studied by Lee, Lee (2014) is based on using terahertz (THz) imaging. The inspection of foreign materials like metal objects in food products is commonly performed by X-ray radiation or ultrasonic detectors. If the food contains active compounds such as lactobacillus or fungus, there are concerns of radiation damage and residual radiation in specific materials. On the other hand, being free from radiation hazards, ultrasonic technology, especially low photon energy, is convenient for food applications. By improving resolution quality with a high-power gyrotron, a THz detector, and optical mirror system, a 0.8 mm resolution was achieved using a 0.4 THz transmission image. Consequently, it was demonstrated that it is possible to inspect foreign objects even in packaged food products using this technique in a non-destructive and radiation-free way unless the packaging material contains metal elements like aluminium (Lee, Lee, 2014).

Imaging systems are not used just for food and agriculture products and also used food processing surface. In their study Jun et al. (2009) tried to determine microbial biofilms on the surfaces in contact with food and agriculture products. They used a hyperspectral fluorescence imaging system. Maximum emission was identified at approximately 480 nm. The results indicated that it is possible to detect presence of microbial biofilm on the different surfaces such as stainless steel, polypropylene, formica and granite.

Conclusions

This paper represents basic knowledge about computer image analysis systems used in the food industry including general step of imaging processing and analyses with some examples on applications.

Computer imaging systems make possible to estimate some internal and external quality parameters and to enhance food and agriculture products safety and quality. Automated, rapid, hygienic, non-destructive and objective inspection can be accomplished with these systems. Having wide range of application field, being cheaper and usable in-line processes and their flexibilities make imaging systems more attractive.

It seems that further improvements in technology and continued development in computer imaging technology will bring out higher implementations understanding of these systems and allow providing growing needs of the food industry.

References

1. Abdullah M. Z., Aziz S. A., Dos Mohamed A. M. (2000) Quality inspection of bakery products using a color-based machine vision system. *Journal of Food Quality,* No. 23, p. 39–50.
2. Brosnan T., Sun D. W. (2004) Improving quality inspection of food products by computer vision - a review. *Journal of Food Engineering,* No. 61, p. 3–16.
3. Du C.-J., Sun D.-W. (2006) Learning techniques used in computer vision for food quality evaluation: a review. *Journal of Food Engineering,* No. 72, p. 39–55.
4. ElMasry G., Sun D.-W., Allen P. (2012) Near-infrared hyperspectral imaging for predicting colour, pH and tenderness of fresh beef. *Journal of Food Engineering,* No. 110, p. 127–140.
5. ElMasry G., Wang N., ElSayed A., Ngadi M. (2007) Hyperspectral imaging for nondestructive determination of some quality attributes for strawberry. *Journal of Food Engineering,* No. 81, p. 98–107.
6. ElMasry G., Wang N., Vigneault C., Qiao J., ElSayed A. (2008) Early detection of apple bruises on different background colors using hyperspectral imaging. *LWT-Food Science and Technology,* No. 41, p. 337–345.
7. Eskelinen J. J., Alavuotunki A. P., Hæggström E., Alatossava T. (2007) Preliminary Study of Ultrasonic

Structural Quality Control of Swiss-Type Cheese. *Journal of Dairy Science*, No. 90, p. 4071–4077.
8. Feng Y.-Z., ElMasry G., Sun D.-W., Scannell A. G. M., Walsh D., Morcy N. (2013) Near-infrared hyperspectral imaging and partial least squares regression for rapid and reagentless determination of Enterobacteriaceae on chicken fillets. *Food Chemistry*, No. 138, p. 1829–1836.
9. Fernandes A. M., Oliveira P., Moura J. P., Oliveira A. A., Falco V., Correia M. J., Melo-Pinto P. (2011) Determination of anthocyanin concentration in whole grape skins using hyperspectral imaging and adaptive boosting neural networks. *Journal of Food Engineering*, No. 105, p. 216–226.
10. Flores K., Sánchez M.-T., Pérez-Marín D., Guerrero J.-E., Garrido-Varo A. (2009) Feasibility in NIRS instruments for predicting internal quality in intact tomato. *Journal of Food Engineering*, No. 91, p. 311–318.
11. Fu X., Kim M. S., Chao K., Qin J., Lim J., Lee H., Garrido-Varo A., Pérez-Marín D., Ying Y. (2014) Detection of melamine in milk powders based on NIR hyperspectral imaging and spectral similarity analyses. *Journal of Food Engineering*, No. 124, p. 97–104.
12. Gan-Mor S., Regev R., Levi A., Eshel D. (2011) Adapted thermal imaging for the development of postharvest precision steam-disinfection technology for carrots. *Postharvest Biology and Technology*, No. 59, p. 265–271.
13. Gómez-Sanchis J., Gómez-Chova L., Aleixos N., Camps-Valls G., Montesinos-Herrero C., Moltó E., Blasco J. (2008) Hyperspectral system for early detection of rottenness caused by Penicillium digitatum in mandarins. *Journal of Food Engineering*, No. 89, p. 80–86.
14. Gowen A. A., Tiwari B. K., Cullen P. J., McDonnell K., O'Donnell C. P. (2010) Applications of thermal imaging in food quality and safety assessment. *Trends in Food Science & Technology*, No. 21, p. 190–200.
15. Gunasekaran S. (1996) Computer vision technology for food quality assurance. *Trends in Food Science and Technology*, No. 7, p. 245–256.
16. Huang Y., Min S., Duan J., Wu L., Li Q. (2014) Identification of additive components in powdered milk by NIR imaging methods. *Food Chemistry*, No. 145, p. 278–283.
17. Jun W., Kim M. S., Chao K., Lefcourt A. M., Roberts M. S., McNaughton J. L. (2009). Detection of microbial biofilms on food processing surfaces: hyperspectral fluorescence imaging study. **In:** *Sensing for Agriculture and Food Quality and Safety*, Proc. SPIE 7315.
18. Kılıç K., Boyacı İ. H., Köksel H., Küsmenoğlu İ. (2007) A classification system for beans using computer vision system and artificial neural networks. *Journal of Food Engineering*, No. 78, p. 897–904.
19. Kim M., Chen Y.-R., Cho B.-K., Chao K., Yang C.-C., Lefcourt A., Chan D. (2007) Hyperspectral reflectance and fluorescence line-scan imaging for online defect and fecal contamination inspection of apples. *Sensing and Instrumentation for Food Quality and Safety*, No. 1, p. 151–159.
20. Lee W.-H., Lee W. (2014) Food inspection system using terahertz imaging. *Microwave and Optical Technology Letters*, No. 56, p. 1211–1214.
21. Mokhtar A., Hussein M. A., Becker T. (2011) Monitoring pasta production line using automated imaging technique. *Procedia Food Science*, No. 1, p. 1173–1180.
22. Park B., Yoon S.-C., Windham W., Lawrence K., Kim M., Chao K. (2011) Line-scan hyperspectral imaging for real-time in-line poultry fecal detection. *Sensing and Instrumentation for Food Quality and Safety*, No. 5, p. 25–32.
23. Shafiee S., Minaei S., Moghaddam-Charkari N., Barzegar M. (2014) Honey characterization using computer vision system and artificial neural networks. *Food Chemistry*, No. 159, p. 143–150.
24. Shirai Y. (1987) *Three-Dimensional Computer Vision*. Berlin: Springer-Verlag.
25. Stajnko D., Lakota M., Hočevar M. (2004) Estimation of number and diameter of apple fruits in an orchard during the growing season by thermal imaging. *Computers and Electronics in Agriculture*, No. 42, p. 31–42.
26. Taghizadeh M., Gowen A., Ward P., O'Donnell C. P. (2010) Use of hyperspectral imaging for evaluation of the shelf-life of fresh white button mushrooms (Agaricus bisporus) stored in different packaging films. *Innovative Food Science & Emerging Technologies*, No. 11, p. 423–431.
27. Wang J., Nakano K., Ohashi S., Kubota Y., Takizawa K., Sasaki Y. (2011) Detection of external insect infestations in jujube fruit using hyperspectral reflectance imaging. *Biosystems Engineering*, No. 108, p. 345–351.
28. Yang C.-C., Kim M. S., Kang S., Cho B.-K., Chao K., Lefcourt A. M., Chan D. E. (2012) Red to far-red multispectral fluorescence image fusion for detection of fecal contamination on apples. *Journal of Food Engineering*, No. 108, p. 312–319.
29. Zhang R., Ying Y., Rao X., Li J. (2012) Quality and safety assessment of food and agricultural products by hyperspectral fluorescence imaging. *Journal of the Science of Food and Agriculture*, No. 92, p. 2397–240.

REVIEW

COMPOSITION OF CAROTENOIDS IN CALENDULA (*CALENDULA OFFICINALIS* L.) FLOWERS

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Abstract

Calendula (*Calendula officinalis* L.) belongs to the *Asteraceae* family. It is a medicinal plant and its flowers are used as important ingredient of pharmaceutical and food production. Most calendula research has been focused on extraction and pharmaceutical properties of bioactive compounds from flowers. Fresh calendula flowers are suggested to use as edible flowers but dried flowers – as an herbal tea and as condiment of food. Calendula accumulates large amounts of carotenoids in its flowers. The yellow and orange colour of petals is mostly due to the carotenoids and the shade depends on the quantity and composition of pigments. Carotenoids are known as biologically active compounds with multiple applications in therapy. It is important to humans as precursors of vitamin A and retinoids. Major factors that impact differences in the amount of total carotenoids in calendula flowers is reported to be the plant variety, colour of the ligulate and tubular florets, the place of cultivation and time of harvesting. Nowadays many important carotenoids are used as pigments – food colorants in the food industry. Carotenoids composition in calendula flowers has been recently begun to explore and in future the scientific research on calendula will be increased. In this review, existing information of carotenoids composition in calendula flowers depending of variety are explored, as well as carotenoids multi-activity and usage of calendula flowers are highlight.

***Keywords**:* pot marigold, edible flowers, calendula, variety, carotenoids.

Introduction

Calendula (*Calendula officinalis* L.) belongs to the *Asteraceae* family. Depending on variety and culture, the plants grow up to 60 cm. Flowers color varies from yellow to orange and they bloom from June until late autumn (Kemper, 1999; Mills, 1999). Calendula is a native of southern Europe but on commercial scale it is cultivated in all around the world. Calendula is a medicinal plant and its flowers are used as important ingredient of pharmaceutical, cosmetic and food production (Cromack, Smith, 1998; Rubine, Enina, 2004). Most calendula research has been focused on extraction and pharmaceutical properties of bioactive compounds from flowers. The name carotenoids, is derived from the fact that they constitute the major pigment in the carrot root, *Daucus carota*, are undoubtedly among the most widespread and important pigments in living organism. Carotenoids are the pigments responsible for the colors of many plants (Eldahshan, Singab, 2013). The yellow and orange color of flower petals is mostly due to the carotenoids and the shade depends on the quantity and composition of pigments (Faulks et al., 2005; Fernandez-Garcia et al., 2012; Tanaka et al., 2008; Zhu et al., 2010).

In this review, there are explored the composition of carotenoids in calendula flowers in order to compare existing information on this plant and its different varieties as well as highlight its multi-activity.

Characterization and health applications of carotenoids

Carotenoids are tetraterpenoids synthesized in plants and microorganisms. They can also be found in many animal species and are important colorants in birds, insects, fish and crustaceans. However, animals and humans cannot synthesize carotenoids and their level in body depends on dietary supply (Britton, 1995; Olson, Krinsky, 1995). Up to now, more than 750 different carotenoids have been isolated from natural sources and new carotenoids continue to be identified (Ramawat, Merillon, 2013). However, only about 40 carotenoids can be absorbed, metabolized, and used in human body. That number is reduced to 6 if the carotenoid profile that is usually detected in human blood plasma is considered. This group includes α- and β-carotene, lycopene, β-cryptoxanthin, zeaxantin and lutein, which are regularly presented in food list of human diet (Britton, Khachik, 2009).

From the chemical point of view, structurally carotenoids are divided into two major groups: carotenes and xanthophylls. Firstly, carotenes or exclusively hydrocarbon carotenoids only composed of carbon and hydrogen atoms, examples of these compounds are α-carotene, β-carotene, lycopene, etc. Secondly, xanthophylls are known as oxygenated hydrocarbon derivatives that contain at least one oxygen function such as hydroxyl, keto, epoxy, methoxy or carboxylic acid groups. Examples of these compounds are a zeaxanthin and lutein (hydroxy), spirilloxanthin (methoxy) and antheraxanthin (epoxy), etc. (Ramawat, Merillon, 2013).

Many research results shows, that the consumption of a diet rich in carotenoids has been epidemiologically correlated with a lower risk for several diseases (Faulks et al., 2005). Carotenoids are considered as important to humans as precursors of vitamin A and retinoids. The major provitamin A carotenoids in Western diet is β-carotene, but also α-carotene, and β-cryptoxanthin contribute to vitamin A supply and may prevent vitamin A deficiency (Olson, Krinsky, 1995; Het Hof et al., 2000). The importance of carotenoids in food goes beyond as natural pigments and biological functions and actions have increasingly been attributed

to these pigments (Faulks et al., 2005). Carotenoids in tissues reflect food choices too (Rock, 1997). Epidemiologic evidence links higher carotenoid intakes and tissue concentrations with reduced cancer and cardiovascular disease risk (Rock, 1997; Mayne, 1996). There is convincing evidence that carotenoids are important components of the antioxidant network (Rock, 1997). Photooxidative damage is suggested to be involved in the pathobiochemistry of several diseases affecting the skin and the eye, and carotenoids may protect light-exposed tissues.

Lutein and zeaxanthin are the predominant carotenoids of the retina and are considered to act as photoprotectants preventing retinal degeneration. The unique distribution, localization and high levels of both carotenoids within the macula lutea as well as their physicochemical properties make them suitable candidates for photoprotection (Laurence et al., 2000; Stahl, Sies, 2005; Richar, 2013). There are few reports on the toxicity studies of lutein and histopathological analysis of the organs supported the nontoxicity of lutein and its ester form (Harikumar et al., 2008).

β-carotene is one of the most abundant carotenoids in the human body. Comparing to carotenoids that can be metabolized into vitamin A, β-carotene has the highest provitamin A activity (Krinsky, Johnson, 2005). According to the researches, β-carotene is widely used as an oral sun protectant for the prevention of sunburn and has been shown to be effective either alone or in combination with other carotenoids or antioxidant vitamins (Sies, Stahl, 2004; Stahl, Sies, 2005; Berneburg, Krutmann, 2000; Stahl et al., 2000).

Protective effects are also achieved with a diet rich in lycopene. Many studies suggest that eating lycopene rich foods or having high lycopene levels in the body may be linked to reduced risk of cancer, heart disease, and age-related eye disorders. However, measures of lycopene intake have been based mainly on eating tomatoes and other products with high lycopene content, not on the use of lycopene supplements (Krinsky, Johnson, 2005; Rock, 1997; Sies, Stahl, 2004).

Carotenoids in calendula flowers

The several investigations of carotenoids compositions of calendula petals have been reported although the researches on it has been recently begun to investigate. Some report refers to qualitative aspects as separation and identification of carotenoids, and others to quantitative determination as total carotenoids. According to the researches, there are found out that calendula accumulates large amounts of different carotenoids in its flowers.

Bako et al. (2002) have studied the composition of carotenoids in calendula petals that were obtained from commercial garden in Pècs (southern Hungary) and found about 18 different types of carotenoids: neoxanthin, (9′*Z*)-neoxanthin, violaxanthin, luteoxanthin, auroxanthin, (9′*Z*)-violaxanthin, flavoxanthin, mutatoxanthin, (9′*Z*)-antheraxanthin, lutein, (9/9′*Z*)-lutein, (13/13′*Z*)-lutein, α-carotene, β-carotene. Kishimoto et al. (2005) were analyzed three orange flowered cultivars (‘Alice Orange’, ‘Orange Star’ and ‘Orange Zem’) and three yellow flowered cultivars (‘Alice Yellow’, Gold Star’ and ‘Golden Zem’) of calendula in their researches, grown in Tsukuba, Japan. They have identified 19 different carotenoids in their researches, such as, (8′*R*)-luteoxanthin, lutein-5,6-epoxide, flavoxanthin, (8R/8′*R*)-auroxanthin, (9′*Z*)-lutein-5,6-epoxide, lutein, antheraxanthin, (9*Z*)-lutein, (5′*Z*/9′*Z*)-rubixanthin, α-carotene, β-carotene, (5′*Z*)-rubixanthin, δ-carotene, (5*Z*/9*Z*/5′*Z*/9′*Z*)-lycopene, γ-carotene, (5′*Z*)-γ-carotene, (5*Z*/9*Z*/5*Z*)-lycopene, (5*Z*/9*Z*)-lycopene, all-E-lycopene. Two carotenoids of them ((5′*Z*)-γ-carotene and (5′*Z*/9′*Z*)-rubixanthin) have never been before identified. However, they have not identified numerous of carotenoids that have been found in by other researcher from other countries. From these results we can find out that the place of cultivation impact composition of different carotenoids in calendula flowers.

Carotenoids are generally responsible for petal colors in the yellow to red range and the wide range of petal colors in various varieties of calendula originates mainly from combinations of these pigments. Verghese (1998) has reported that marigold (*Tageteserecta*) flower petals are a significant source of the xanthophyll and have a much higher concentration of this pigment compared to other plant materials. Moehs et al. (2001) have reported that in marigold (*Tageteserecta*), the differences in petal color from pale-yellow to orange-red are caused by different levels of accumulation of yellow carotenoid lutein.

Kishimoto et al. (2005) have shown that distinctive differences in carotenoids content (characterized by HPLC chromatograms) were found between orange flowered varieties and yellow flowered varieties. In all, 19 carotenoids where observed in orange flowered varieties, and of these, 10 carotenoids were detected only in orange flowered varieties. They have found out that yellow colored petals don’t contain (5′*Z*/9′*Z*)-rubixanthin, α-carotene, (5′*Z*)-rubixanthin, δ-carotene, (5*Z*/9*Z*/5′*Z*/9′*Z*)-lycopene, γ-carotene, (5′*Z*)-γ-carotene, (5*Z*/9*Z*/5*Z*)-lycopene, (5*Z*/9*Z*)-lycopene, all-E-lycopene. Therefore we can consider that these carotenoids are responsible for the orange color formation of the petals. The similar results have shown Pintea et al. (2003) in their researches of four calendula varieties – two orange flowered (‘Double Esterel Orange’ and ‘Radio Extra Selected’) and two yellow flowered (‘Bonbon Abricot’ and ‘Double EsterelJaune’). They have found out that the composition of different carotenoids increases with color intensity, the dark orange variety being the richest one. The dark flowered variety (‘Double Esterel Orange’) is the richest in rubixanthin, lycopene and γ-carotene, but yellow flowered variety (‘Bonbon Abricot’) contains important amount of β-carotene, but very low amountof γ-carotene and it is especially low in lycopene. Kishimoto et al. (2007) have reported, that

orange and yellow flowered cultivars of *O. ecklonis* (*Compositae* family) showed the same composition, the proportions of three reddish carotenoids – 5′Z-γ-carotene, 5Z/9Z/5′Z-lycopene, and lycopene – were higher in the orange flowered cultivar. In general, there is found out that orange flowered cultivars of calendula accumulate more reddish carotenoids than yellow flowered varieties (Kishimoto, Ohmiya, 2009). The color intensity of orange flowered varieties is primarily due to lycopene, which is absent from yellow flowered varieties. Lutein and β-carotene were the most abundant carotenoids in the Italian type of calendula, grown in Ravenna, northern Italy (Piccaglia et al., 1999).

More reports refer only to quantitative determination as total carotenoids. The major factors that impact differences in the amount of total carotenoids in calendula flowers is reported to be the plant variety, color of the ligulate and tubular florets, the place of cultivation and time of harvesting (Bako et al. 2002; Kishimoto et al. 2005; Kishimoto et al. 2007; Kishimoto, Ohmiya, 2009; Pintea et al., 2003; Raal et al., 2009), as well as, environmental factors, such as soil mineral composition, temperature, oxygen and light (Piccaglia et al., 1999).

A wide range of calendula varieties were analyzed in Estonia. Raal et al. (2009) and Toom et al. (2007) have found out that the total carotenoids content varied in the investigated 42 samples from 200 to 3510 mg 100 g^{-1} dried flowers. The highest total carotenoids content (3510 mg 100 g^{-1}) was found in brownish-yellow flowered variety 'Golden Emperor' (Table 1.). The lowest sum of pigments was found in the variety 'Touch of Buff' (200 mg 100 g^{-1}), with a very unconventional combination of flowers color – its tubular florets were sorrel, but the ligulate ones were maroon in the top and cream colored in the bottom. In their studies, they have observed that usually canary yellow, carnation-yellow and yellow flowered cultivars contain total carotenoids of less than 1000 mg 100 g^{-1} while cultivars with orange flowers contain pigments between 1000–2000 mg 100 g^{-1} or even more. Pintea et al. (2003) have found the highest total carotenoids content in fresh flowers of orange flowered cultivar 'Double Esterel Orange' with a total content 276 mg 100 g^{-1}, but lowest in yellow-orange flowered cultivar 'Bonbon Abricot' – 48.2 mg 100 g^{-1}. Also Kishimoto et al. (2007) have found differences in total carotenoids content between orange and yellow flowered cultivars.

In general, that the richest in total carotenoids content are brownish-yellow and orange flowered calendula varieties. The total carotenoids content also depends on region of cultivation. The same variety grown in different regions has shown different content of total carotenoids. For example, variety 'Fiesta Gitana', grown in Central Estonia (Järvamaa) has shown 1060 mg 100 g^{-1},but towards to the south Estonia (Viljandimaa) total carotenoids content has increased to 1440.0 mg 100 g^{-1} (Raal et al., 2009). Pintea et al. (2003) have reported that the values of quantitative determinations are very different from one variety to another. Anyhow, the interconnection between calendula varieties and content of total carotenoids is not satisfactorily clear and therefore there more researches in future are needed.

Table 1

Amount of total carotenoids in flowers of calendula (*Calendula officinalis* L.) varieties

Variety (color of flowers)	Carotenoids, mg 100 g^{-1}	Place of cultivation (region, country)
Fresh flowers		
Alice Orange (orange)	170*	Tsukuba, Ibaraki, Japan
Alice Yellow (yellow)	125*	Tsukuba, Ibaraki, Japan
Bonbon Abricot (yellow-orange)	48**	Cluj-Napoca, Romania
Double EsterelJaune (lemon yellow)	97**	Cluj-Napoca, Romania
Double Esterel Orange (dark orange)	276**	Cluj-Napoca, Romania
Gold Star (yellow)	126*	Tsukuba, Ibaraki, Japan
Golden Gem (yellow)	118*	Tsukuba, Ibaraki, Japan
Orange Gem (orange)	107*	Tsukuba, Ibaraki, Japan
Orange Star (orange)	145*	Tsukuba, Ibaraki, Japan
Ponpon Orange (orange)	119*	Tsukuba, Ibaraki, Japan
Ponpon Yellow (yellow)	109*	Tsukuba, Ibaraki, Japan
Radio Extra Selected (orange)	112**	Cluj-Napoca, Romania
Dried flowers		
Apricot Beauty (dark orange-canary yellow)	530***	Järvamaa, Central Estonia
Apricot Beauty (yellow, top orange)	430***	Viljandimaa, Southern Estonia
Art Schades (canary yellow-light orange)	1780***	Järvamaa, Central Estonia
Art Schades (yellow-orange)	840***	Viljandimaa, Southern Estonia
Balls Orange (orange)	2000–2240***	Viljandimaa, Southern Estonia
Balls Orange (orange)	1150***	Järvamaa, Central Estonia
Balls Supreme (orange)	1750***	Viljandimaa, Southern Estonia
Cremeweiss (canary yellow)	370***	Viljandimaa, Southern Estonia
Cremeweiss (canary yellow)	270***	Pärnumaa, South-Western Estonia

Fiesta Gitana (orange- yellow-canary yellow)	1440***	Viljandimaa, Southern Estonia
Fiesta Gitana (orange- yellow-canary yellow)	1140***	Pärnumaa, South-Western Estonia
Fiesta Gitana (orange-yellow-canary yellow)	1060***	Järvamaa, Central Estonia
Golden Emperor (brownish-yellow)	3510***	Viljandimaa, Southern Estonia
Golden Emperor (brownish-yellow)	3420***	Järvamaa, Central Estonia
Kablouna (yellow-orange)	1460***	Viljandimaa, Southern Estonia
Kablouna (yellowish)	770***	Järvamaa, Central Estonia
KablunaOranz (orange)	1840***	Viljandimaa, Southern Estonia
Medetkos (orange)	890***	Pärnumaa, South-Western Estonia
NizkyPlnokvety (orange-canary yellow)	650***	Pärnumaa, South-Western Estonia
Orange King (orange)	1630–2750***	Viljandimaa, Southern Estonia
Pacific Beauty (goldenrod)	2100***	Järvamaa, Central Estonia
Pacific Beauty (orange-yellow)	760–1060***	Viljandimaa, Southern Estonia
Pacific Deep Orange (dark orange)	1190***	Pärnumaa, South-Western Estonia
Pacific Lemon Yellow (lemon yellow)	1480***	Viljandimaa, Southern Estonia
Pink Surprise (carnation-yellow)	960***	Viljandimaa, Southern Estonia
Pomyk (orange)	1680***	Pärnumaa, South-Western Estonia
PrinzesGenischt (yellow-orange)	1330***	Viljandimaa, Southern Estonia
RozovoiSjurpris (carnation-orange)	630***	Viljandimaa, Southern Estonia
RusskiiRazmer XXL (orange)	2970***	Viljandimaa, Southern Estonia
Tokaj (brownish-yellow)	1600***	Järvamaa, Central Estonia
Touch of Buff (top maroon, bottom cream-colored)	200***	Pärnumaa, South-Western Estonia
Touch of Red (orange from upper, brown from underside)	2050***	Viljandimaa, Southern Estonia
Touch of Red (orange from upper, brown from underside)	1610***	Järvamaa, Central Estonia
Unknown, Hungarian type of calendula (orange)	770****	Pècs, Southern Hungary
VysokyKablouna (canary yellow-yellow)	1560***	Pärnumaa, South-Western Estonia
ZeljonoeSertse (orange)	1490***	Viljandimaa, Southern Estonia
Zjelt'IGigant (goldenrod)	2510***	Järvamaa, Central Estonia

* Kishimoto et al., 2007; ** Pintea et al., 2003; *** Raal et al., (2009); **** Bako et al., 2002.

Usage of calendula flowers

Calendula is with huge potential in medicine, food and cosmetic production (Rubine, Enina, 2004; Sausserde, Kampuss, 2014). It is used as therapeutically plant from ancient time. In human medicine calendula flowers are used in tea, infusions, tinctures etc. (Loranty et al., 2010; Rubine, Enina, 2004). It was efficient for liver illness, cramps, ulcers, jaundice, hemorrhoids, internal healing, skin cancer treatment (Khalid, Jaime, 2012; Muley et al., 2009; Preethi et al., 2009) and also with wide spectra of action, such as, choleretic, antispasmodic, bactericidal, anticancer, wounds, frostbite, burns, acne. Calendula is applied in a form of lotion for skin illness, ulcerations, frostbites, burns, fungus (Jorge et al., 1996; Preethi et al., 2009). Extracts derived from dried flowers inhibit the replication of HIV-1 (Kalvatchev et al., 1997). In veterinary medicine the raw material of calendula flowers is used for the preparation of different tinctures (Grela, Sembratowicz, 1998).

Dried and fresh flowers of calendula are widely used as food. In many parts of the world people continues to prepare food with old traditions. More and more the assortments of foodstuffs produced begin to be markedly extended with edible flowers. And these flowers could be potential rich resources of natural antioxidants and carotenoids and could be developed into functional foods, which increases and improves the appearance, taste and aesthetic value of these foodstuffs (Tanji, Nassif, 1995; Mlcek, Rop, 2011; Li et al., 2014). According to the researches, calendula flowers accumulate high amount of different carotenoids. Since the content of these compounds are not very high in the majority of food stuffs (Mlcek et al., 2011), calendula flowers as edible flowers could be one of the sources of valuable substances. Fresh flowers are served as garnish and trimming of various meals and cold buffet food, and petals are used to decorate salads, sweet meals, fruit and ice-cream sundae, drinks, etc. In addition to the esthetic appearance they also correspond to specific taste and smell of served food (Rubine, Enina, 2004; Scherf, 2004). It has long been shown in human studies that fat enhances the absorption of carotenoids (Castenmiller, West, 1998). In recent studies, greater absorption of carotenoids was observed when salad was consumed

with full-fat than with reduced-fat salad dressing (Brown et al., 2004; Rodriguez-Amaya, 2010).
Calendula flowers can be also dried, soaked in alcohol or sugar, frozen either directly or in form of ice cubes, added in to cocktails, etc. The freeze drying is not cost effective but preserves the original appearance, color, shape, and gloss of flowers (Mlcek et al., 2011; Mohammad, Kashani, 2012; Rubine, Eniņa, 2004).
At the beginning of the food industry, pigments – natural of synthetic, were used to give an attractive appearance, perception of freshness, taste, and quality of food. Today, natural colorants are emerging globally due to the perception of its safer and eco-friendly nature (Pratheesh et al., 2009; Abbey et al., 2014). Carotenoids pigments are of interest to the food scientists, nutritionists and food industries due to their positive impact on human health and their economic benefits. Carotenoids are responsible for the attractive color of many plant foods, which is perhaps the first attribute that consumers assess when determining the quality and appearance of a product, and therefore conditions of its acceptability (Fernandez-Garcia et al., 2012).

Conclusions

Nowadays many important carotenoids are used as pigments – food colorants in the food industry Calendula accumulates large amount of different carotenoids in its flowers, therefore its promising plant for condiment of food production, as well as – food colorants in the food industry. Nowadays, a trend towards researches of calendula represents a challenge for food manufactures, because of its widely therapeutically applications, and diet rich in carotenoids represents lower risk for several diseases. The addition of many colors to healthy food and food products may become superfluous in the future. In collaboration of specialists of agriculture, bioengineering and food science it is possible to develop innovative techniques to produce foods that could be potential rich of natural carotenoids and to find out good colorants and to improve retention of total carotenoids pigments, as well as flavors and nutrients in cooked food.

References

1. Abbey J., Fields B., O'Mullane M. (2014) Food Additives: Colorants. *Encyclopedia of Food Safety*, Vol. 2, p. 459–465.
2. Bako E., Deli J., Toth G. (2002) HPLC study on the carotenoids composition of *Calendula* products. *Journal of Biochemical and Biophysical Methods*, Vol. 53, p. 241–250.
3. Berneburg M., Krutmann J. (2000) Photoimmunology, DNA repair and photocarcinogenesis. *Journal of Photochemistry and Pphotobiology, B: Biology*, Vol. 54, p. 87–93.
4. Britton G. (1995) Structure and properties of carotenoids in relation to functions, *The FASEB Journal*, Vol. 9, p. 1551–1558.
5. Britton G., Khachik F. (2009) Carotenoids in food. In: *Britton G., Liaaen-Jensen S., Pfander H.P. (Eds.) Carotenoids. Nutrition and Health*, Vol. 5, p. 45–66.
6. Brown M.J., Ferruzzi M.G., Nguyen M.L., Cooper D.A., Eldridge A.L., Schwartz S.J., White W.S. (2004) Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electro-chemical detection. *American Journal of Clinical Nutrition*, Vol. 80, p. 396–403.
7. Castenmiller J.J.M., West, C.E. (1998) Bioavailabilityandbioconversionof carotenoids. *Annual Review of Nutrition*, Vol. 18, p. 19–38.
8. Cromack H., Smith J. (1998) *Calendula officinalis* – production potential and crop agronomy in southern England. *Industrial Crops and Products*, Vol. 7, p. 223–229.
9. Eldahshan O.A., Singab A.N.B. (2013) Carotenoids. *Journal of Pharmacognesy and Phytochemistry*, Vol. 2, No. 1, p. 225–234.
10. Faulks R.M., Southon S. (2005) Chalanges to understanding and measuring carotenoids bioavailability. *Biochimica et Biophysica Acta*, Vol. 1740, p. 95–100.
11. Fernandez-Garcia E., Carvajal-Lerida I., Jaren-Galan M., Garrido-Fernanez J., Perez-Galvez A., Hornero-Mendez D. (2012). Carotenoids bioavailability from foods: From pigments to efficient biological activities. *Food Research International*, Vol. 46, p. 438–450.
12. Grela E.R., Sembratowicz I. (1998) Immunostimulatory activity of herbs. *Medycyna Weterynaryjna, Poland*, Vol. 54, No. 3, p. 152–158.
13. Harikumar K.B., Nimita C.V., Preethi K.C., Kuttan R., Shankaranarayana M.L., Deshpane J. (2008) Toxicity profile of lutein and lutein ester isolated from marigold flowers (*Tagetes erecta*). *International Journal of Toxicology*, Vol. 27, p. 1–9.
14. Het Hof K.H., West C.E., Weststrate J.A., Hautvast J.G. (2000) Dietary factors that affect the bioavailability of carotenoids. *The Journal of Nutrition*, Vol. 130, p. 503–506.
15. Jorge N.J., Fracasso J.F., Neves M.D.C.L.C., Santos L.E.D., Banuth V.L. (1996) Treatment of varicose ulcer and skin lesions with Calendula officinalis L. or Stryphnodendron barbadetiman Martius. *Revista De Ciencias Farmaceuticas*, Vol. 17, p. 181–186.
16. Kalvatchev Z., Walder R., Garzaro D. (1997) Anti-HIV activity of extracts from Calendula officinalis flowers. *Biomed Pharmacother*, Vol. 51, No. 4, p. 176–180.
17. Kemper K. (1999) Calendula (C*alendula officinalis*). The long wood Herbal Task Force and the Centre for Holistic *Pediatric Education and Research*. 767 p.
18. Khalid A.K., Jaime A.T.S. (2012) Biology of *Calendula officinalis* Linn.: Focus on pharmacology, biological activities and agronomic practices. *Medicinal and Aromatic Plant Science and Biotechnology*, Vol. 6, No. 1, p. 12–27.
19. Kishimoto S., Maoka T., Sumitomo K., Ohmiya A. (2005) Analysis of carotenoid composition in petals of calendula (*Calenula officinalis* L.). *Bioscience, Biotechnology, and Biochemistry*, Vol. 69, No. 11, p. 2122–2128.
20. Kishimoto S., Ohmiya A. (2009) Studies in carotenoids in the petals of composite plants. *Journal of the Japanese Society for Horticultural Science*, Vol. 78, No. 3, p. 263–272.
21. Kishimoto S., Sumitomo K., Yagi M., Nakayama M., Ohmiya A. (2007) Three routes to orange petal color via carotenoids components in 9 compositae species. *Journal*

of the Japanese Society for Horticultural Science, Vol. 76, No. 3, p. 250–257.
22. Krinsky N.I., Johnson E.J. (2005) Carotenoid actions and their relation to health and disease. *Molecular Aspects of Medicine*, Vol. 26, No. 6, p. 459–516.
23. Laurence M.R., Seema S.M., Jung H.C. (2000) Lutein and zeaxanthin concentrations in Rod Outer segment membranes from perifoveal and peripheral human retina. *Investigative Ophthalmology and Visual Science*, Vol. 41, No. 5, p. 1200–1209.
24. Li A.N., Li H.B., Xu D.P., Xu X.R. (2014) Total phenolic content and antioxidant capacities of 51 edible and wild flowers. *Journal of Functional Foods*, Vol. 6, p. 319–330.
25. Loranty A., Rembialkowska E., Rosa E.A.S., Bennett R.N. (2010) Identification, quantification and availability of carotenoids and chlorophylls in fruit, herb and medicinal teas. *Journal of Food Composition and Analysis*, Vol. 23, p. 432–441.
26. Mayne S.T. (1996) Beta-carotene, carotenoids, and disease prevention in humans, *The FASEB Journal*, Vol. 10, p. 690–701.
27. Mills S. (1999) *The essential Book of Herbal Medicine*. Penguin Books Ltd, Harmondsworth, Middlesex, 765 p.
28. Mlcek J., Rop O. (2011) Fresh edible flowers of ornamental plants – A new source of nutraceutical foods. *Trends in Food Science and Technology*, Vol. 22, p. 561–569.
29. Moehs C.P., Tian L., Osteryoung K.W., DellaPenna D. (2001) Analysis of carotenoids biosynthetic gene expression during marigold petal development. *Plant Molecular Biology*, Vol. 45, p. 281–293.
30. Mohammad S.M., Kashani H.H. (2012) Pot marigold (*Calendula officinalis*) medicinal usage and cultivation. *Scientific Research and essays*, Vol. 7, No. 14, p. 1468–1472.
31. Muley B.P., Khadabadi S.S., Banarase N.B. (2009) Phytochemical constituents and pharmacological activities of *Calendula officinalis* Linn (Asteraceae): A review. *Tropical Journal of Pharmaceutical Research*, Vol. 8, No. 5, p. 455–465.
32. Olson J.A., Krinsky N.I. (1995) Introduction: the colourful fascinating world of the carotenoids: important physiologic modulators, *The FASEB Journal*, Vol. 9, p. 1547–1550.
33. Piccaglia R., Marotti M., Chiavari G., Gandini N. (1999) Effects of harvesting date and climate on the flavonoid and carotenoid content of marigold (*Calendula officinalis* L.). *Flavour and Fragrance Journal*, Vol. 12, p. 85–90.
34. Pintea A., Bele C., Andrei S., Socaciu C. (2003) HPLC analysis of carotenoids in four varieties of *Calendula officinalis* L. flowers. *Acta Biologica Szegediensis*, Vol. 47, No. 1–4, p. 37–40.
35. Pratheesh V.B., Benny N., Sujatha C.H. (2009) Isolation, stabilization and characterization of xanthophyll from marigold flower – Tageteserecta-L. *Modern Applied Science*, Vol. 3, No. 2, p. 19–28.
36. Preethi K.C., Kuttan G., Kuttan R. (2009) Anti-inflammatory activity of flower extract of *Calendula officinalis* Linn. Andits possible mechanism of action. *Indian Journal of Experimental Biology*, Vol. 47, p. 113–120.
37. Raal A., Kirsipuu K., Must R., Tenno S. (2009) Content of total carotenoids in *Calendula officinalis* L. from different countries cultivated in Estonia. *Natural Products Communications*, Vol. 4, No. 1, p. 35–38.
38. Ramawat K.G., Merillon J.M. (2013) Tetraterpenes: Carotenoids. **In**: *Natural Products*. Vol. 1: Phytochemistry, Botany and Metabolism of alkaloids, phenolics and Terpenes. Ramawat K.G., Merillon J.M. (eds.). Germany: Springer Berlin Heidelberg, p. 3251–3283.
39. Richard L.R. (2013) Lutein, Zeaxanthin, and skin health. *American Journal of Lifestyle Medicine*, Vol. 7, No. 3, p. 182–185.
40. Rock C.L. (1997) Carotenoids: biology and treatment. *Pharmacology & Therapeutics*, Vol. 75, No. 3, p. 185–197.
41. Rodriguez-Amaya D.B. (2010) Quantitative analysis, *in vitro* assessment of bioavailability and antioxidant activity of food carotenoids – A review. *Journal of Food Composition and Analysis*, Vol. 23, p. 726–740.
42. Rubine H., Eniņa V. (2004) *Ārstniecības augi*.Rīga: Zvaigzne ABC, 344 lpp.
43. Sausserde R., Kampuss K. (2014) Calendula (*Calendula officinalis* L.) – promising medicinal plant. **In:** *Proceedings of the Scientific and Practical Conference "Harmonious Agriculture"*, Latvia, Jelgava, p. 161–165.
44. Scherf G. (2004) *Plane rostliny a jejichpouziti v kuchyni*. Praha: Beta-Dobrovsky, p. 8–15.
45. Sies H., Stahl W. (2004) Nutritional protection against skin damage from sunlight. *Annual Review of Nutrition*, Vol. 24, p. 173–200.
46. Stahl W., Heinrich U., Jungmann H., Sies H., Tronnier H. (2000) Carotenoids and carotenoids plus vitamin E protect against ultraviolet light-induced erythema in humans. *American Journal of Clinical Nutrition*, Vol. 71, p. 795–798.
47. Stahl W., Sies H., (2005) Bioactivity and protective effects of natural carotenoids. *Biochimica et BiophysicaActa (BBA) – Molecular Basis of Desease*, Vol. 1740, p. 101–107.
48. Tanaka Y., Sasaki N., Ohmiya A. (2008) Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. *The Plant Journal*, Vol. 54, 733–749.
49. Tanji A., Nassif F. (1995) Edible weeds in Marocco. *Weeds Technology*, Vol. 9, p. 617–620.
50. Toom M., Vares L., Kirsipuu K., Must R., Tenno S., Raal A. (2007) Content of the carotenoids in the pot marigold's sorts cultivated in Estonia. *European Journal of Pharmaceutical Sciences*, Vol. 32, No. 1, p. S. 25.
51. Verghese J. (1998) Focus on xanthophylls from TagetesErecta L. the giant natural complex-I. *Indian Spices*, Vol. 33, No. 4, p. 8–13.
52. Zhu C., Bai C., Sanahuja G., Yuan D., Farre G., Naqvi S., Shi L., Capell T., Christou P. (2010) The regulation of carotenoids pigmentation in flowers. *Archives of Biochemestry and Biophysics*, Vol. 504, p. 132–141.

REVIEW

HIGH-OXYGEN MODIFIED ATMOSPHERE PACKAGING OF ORGANIC MEATS?

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Abstract

In the last few years, it became widespread to package fresh meat for retail under modified atmosphere containing 70–80% oxygen ("high O_2-MAP"). Oxygen preserves the bright-red colour of oxymyoglobin while CO_2 (20–30%) inhibits obligatory aerobic bacteria such as psychrotrophic *Pseudomonas* spp., the common spoilage agents for unpackaged fresh meat. On the other hand, high oxygen partial pressure has been shown to cause some oxidative changes in the meat, resulting in sensory deviations.

Use of high O_2-MAP is also legally permitted for the packaging of organic meats. This may be regarded as being in conflict with the principle of "processing food with care" as stipulated by Article 6 of the Regulation (EU) 834/2007 on organic production and labelling of organic products. Therefore, the aim of our research was to provide information to decision-makers in the organic food chain on the necessity of high O_2-MAP for organic meats. Literature data from market research show that even consumers of organic food seek "convenience" in purchasing and preparing it. Hence, of the major associations of organic agriculture in Germany, only Demeter e. V. prohibits the use of high O_2-MAP. Literature data also indicate that undesirable changes of the meat could be delayed by reducing the oxygen content in the packages to about 40–50%, with only a slight reduction of shelf life. In our view, this would be a good compromise between "convenience" and eating quality.

Keywords: meat, packaging, modified atmosphere, organic.

Introduction

In various countries, there is a major trend that consumers tend to reduce the time spend for shopping and for preparing food in their households. The socio-economic reasons for it have been summarized by Schulze and Spiller (2008a) for Germany. As a consequence, an increasing percentage of food is sold in retail packages rather than "over the counter". This also applies for fresh meat. From 2000 to 2005, the market share of pre-packaged red meat increased from 23 to 42% (Schulze, Spiller, 2008b). In Europe, the percentage is similar (Belcher, 2006). The Fraunhofer-IVV Institute (Anonymous, 2013a) mentioned a market share for pre-packaged fresh meat (chilled or frozen) of 53% in 2010. Recent data are available for processed meats (hams, sausages) in Germany: From 2005 to 2012, the market share of pre-packaged products (excluding canned meats) increased from 62.3 to 65.3% (Anonymous, 2013b).

Appearance is a critical factor affecting buying decisions at the point of sale. The chemistry of meat colour has been reviewed by Manchini and Hunt (2005). A bright-red colour of oxymyoglobin is regarded as an indicator of freshness of meat, in particular, beef and pork (Carpenter et al., 2001; Becker et al., 2000; Robbins et al., 2003). At sufficient levels of oxygen, myoglobin is kept in this form whereas low oxygen partial pressure accelerates the formation of brownish metmyoglobin. Total exclusion of oxygen is difficult to achieve, and the resulting dark-red colour of myoglobin is less attractive, especially for pork. Hence, most pre-packaged fresh meat is displayed under oxygen-enriched atmospheres ("high O_2-MAP"). To avoid growth of aerobic spoilage bacteria such as *Pseudomonas spp*., carbon dioxide is included in the package at levels of 20–30%. As a "rule of thumb", high-O_2-MAP of fresh meat may double the time until spoilage by micro-organisms (see Sun, Holley, 2012).

It is common practice to package fresh meat under 70–80% oxygen ("high O_2-MAP") plus 20–30% CO_2. Such high oxygen levels, however, have been shown to cause some oxidative changes in the meat, resulting in sensory deviations.

Such changes occur in lipids and proteins. Thus, it may be argued that the consumer is misled because the meat looks fresher than it actually is. Accordingly, packaging meat in high O_2-MAP has been criticised by consumer organisations (Anonymous, 2013c).

According to Article 6 c and d of the Regulation (EC) 834/2007 on organic production and labelling of organic products (Anonymous, 2007), substances and processing methods should be excluded "that might be misleading regarding the true nature of the product", and the processing of food should be done "with care", preferably with the use of biological, mechanical and physical methods. Nevertheless, high O_2-MAP is also used for the packaging of organic meats.

Therefore, the aim of our research was to provide information to decision-makers in the organic food chain on the necessity of high O_2-MAP for organic meats, with focus on red meats (beef, pork).

Approach

We searched the literature on data about effects of high-O_2 MAP on meat quality, analysed the legal situation in the European Union, and interviewed representatives of the German "organic food sector" on the compatibility of high-O_2 MAP with the requirements of organic food processing.

Results and Discussion

1. Effect of oxygen levels in MAP on meat quality parameters

Colour

Table 1 summarizes data on atmospheres in MAP giving colour stability equal to high-O_2 MAP (70–80% O_2). It may be seen that the oxygen content in the package may be reduced to about 40–50% without major effects on colour and shelf life.

Table 1

Gas atmospheres giving a meat colour similar to high-O_2 MAP (70–80% O_2)

Product	Gas composition[1]		Storage		Ref.[3]
	O_2	CO_2	°C	days	
Beef steak	55	45	2	10	(1)
Beef steak	40	20	4	12	(2, 3)
Beef steak[2]	60	40	4	8	(4)
Ground beef	50	30	4	14	(5)
Pork chop	35	20	4	8	(6)
Pork retail cuts	20	20	2	10	(7)

[1] balance: nitrogen.
[2] aged 7 days, frozen and thawed before storage
[3] (1) Jakobsen, Bertelsen, 2000; (2) Zakrys et al., 2009; (3) Zakrys-Waliwander et al., 2011; (4) Resconi et al. 2012; (5) Esmer et al., 2011; (6) Zhang, Sundar, 2005; (7) Nitsch, 2012

Sensory deviations due to oxidative changes

Sensory deviations of meat packaged under high O_2 levels were frequently reported. Deficiencies include lack of meat aroma, rancidity, excess warmed-over flavour, as well as toughness and lack of juiciness (Lautenschläger, Müller, 2006; Dederer et al., 2014). In high-O_2 MAP, the rate of lipid oxidation is higher, as indicated by the concentrations of thiobarbituric acid-reactive substances (Jakobsen, Bertelsen 2000; John et al., 2004; Seyfert et al., 2004; Clausen et al., 2009; Kim et al., 2010). However, at least for beef steaks and ground beef, considerable less oxidative changes could be achieved by reducing the oxygen level in MAP to about 50% (Table 2).

A lack of tenderness of high-O_2 MAP meat is mainly due to cross-linking of myosin molecules (Lund et al., 2011; Kim et al., 2010). However, this effect could be minimized by reducing the oxygen level in MAP to 40% (Zakrys et al., 2009).

Table 2

Gas atmospheres in MAP minimizing oxidative changes in lipids

Product	Gas composition[1]		Storage		Ref.[3]
	O_2	CO_2	°C	days	
Beef steak	50	30	4	14	(1)
Ground beef	50	30	4	14	(2)
Pork chop	25	20	4	8	(3)

[1] balance: nitrogen.
[2] aged 7 days, frozen and thawed before storage
[3](1) Zakrys-Waliwander et al., 2011; (2) Esmer et al., 2011; (3) Zhang, Sundar, 2005.

Cholesterol oxidation

During storage of meat in high-O_2 MAP, cholesterol oxidation products (COP) are formed, in particular, 7β-hydroxy- 7α-hydroxy-, 7-keto- and β-epoxycholesterol (Ferioli et al., 2008; Boselli et al., 2009). In beef slices packaged in MAP containing 32% O_2 and exposed to light, levels were about twice as high as in meat packed in air (Boselli et al., 2009). There is some evidence from in-vitro experiments and from animal studies that uptake of these COP increases the risk of atherosclerosis (Anonymous, 2010a). On the other hand, the type of meat, the length of storage and the cooking process also affect the levels of COP, and it was concluded that meat stored in high-O_2 MAP contributes only little to the overall uptake of COP (Anonymous, 2010a). However, in view of the scarcity of data, it is advisable to minimize the formation of COP during meat storage and processing.

Premature browning

Consumers normally use greyish-brownish discoloration of meat as an indicator for doneness during cooking. Indeed, times and temperatures to achieve this are similar to those required for inactivation of pathogens such as salmonellae, shigatoxin-forming *Escherichia coli* (STEC) and *Campylobacter*. Various authors (Hunt et al., 1999; Seyfert et al., 2004; Clausen et al., 2009; Røsvoll et al., 2014) observed that meat packaged under high-O_2 atmospheres turn grey/brownish at lower cooking temperatures. This is due to the fact that oxymyoglobin is less stable to heat than myoglobin. "Premature browning" may result in a health hazard, especially to the consumer of hamburgers and similar cooked minced meats. For example, hamburgers looked "done" at internal temperature of about 60 °C. Stopping the cooking process at this stage will not inactivate STEC to a sufficient degree. In a survey, most consumers judge doneness by colour and preferred the batch with premature browning (Røsvoll et al., 2014). Reduction of oxygen levels in MAP to

50% did not completely eliminate this problem (Clausen et al., 2009).

2. Consumer attitudes and expectations

In response to open questions, German consumers (n=991) mention "freshness" as the main quality criterion for meat, followed by price and origin (Branscheid, 2008). "Colour" was mentioned by only 18% of all respondents, and by 23% of respondents stating that they eat meats daily. These results are in contrast to data clearly showing the major role of meat colour in buying decision (Becker et al., 2000; Robbins et al., 2003; Carpenter et al., 2001). Moreover, in open questions, only few consumers mentioned "best-before date" as a quality criterion. Therefore, it is plausible to assume that consumers trust their senses when buying meat and appearance (of the meat and the point of sale) is the main criterion.

In contrast, the best-before date was mentioned as an important criterion for their buying decisions by 87% of consumers participating in the German National Nutrition Survey II (Anonymous, 2008a). The results are based on questionnaires that eliminate most of the bias introduced by face-to-face or even telephone interviews and may reflect the reality more closely.

In May 2013, 1 002 German consumers were asked (by telephone interviews) about their attitude towards organic food (Anonymous, 2013d). Consumers regularly buying organic food mentioned regional origin (87%), animal welfare (85%), and absence of residues (83%) as the most important reasons. This confirms the importance of altruistic motivations, as also stressed by Padilla Bravo et al. (2013) on the basis of data from German National Nutrition Survey II. Of various parameters affecting buying decisions, "optimal freshness and quality" ranked first. 32% of the interviewees stated that they frequently (or even exclusively) buy organic meats. Consumers have more trust in the original producers of organic food, and in specialized shops but in contrast, they buy most organic food from supermarkets, and even from discounters, which are perceived as even less trustful. This indicates that, along with price, "convenience" in shopping is a major criterion even for the consumer of organic food (Padilla Bravo et al., 2013). However, many consumers may not always be ready to admit this.

3. Legal situation for conventional and organic meat

It is generally prohibited to mislead the consumer by insufficient or misleading information (see Article 7 of Regulation (EU) no. 1169/2011; Anonymous, 2011). Hence, the key question is whether or not the consumer mistakes "old" meat for fresh. To date, it is compulsory to label the use of "protective atmospheres" but not to indicate the composition of gas. In a document published by a working group of experts from the German official food control, it was stated that offering unpackaged meat treated with hyperbaric oxygen in order to restore its bright-red colour is only permitted if the consumer is appropriately informed about this treatment (Möllers et al., 2007).

Oxygen is a permitted food additive in the European Union. It is also listed in Annex VIII Section A of Regulation (EC) No. 889/2008 (Anonymous, 2008b) as a permitted additive for organic foods. German non-governmental associations for organic food generally prohibit the use of high-pressure oxygen treatment to restore the bright-red meat colour. Of the German associations, Bioland e.V. (Anonymous, 2010b) and Naturland e.V. (Anonymous, 2013e) permit high-O_2 MAP packaging of meat, arguing that O_2 only affects the colour of the surface. In contrast, Demeter e.V. approves only nitrogen and CO_2 as packaging gases (Anonymous, 2013f).

4. Alternatives to high-O_2 MAP

The available data indicate that reducing the oxygen level in MAP to about 40–50% still sufficiently stabilizes meat colour while reducing sensory deficiencies caused by oxidative processes. Other packaging methods for fresh meat have been reviewed by McMillin (2008) and O'Sullivan and Kerry (2010). Packaging in vacuum-skin packages may reduce oxygen levels so much that metmyoglobin formation is inhibited, and had a favourable effect on beef colour (Li et al., 2012). However, with many meat cuts (especially from pork), the dark-red colour of myoglobin is less attractive to the customer, and acid formation by lactic acid bacteria on the meat surface during storage may affect the globin moiety and lead to discolouration. The inclusion of carbon monoxide stabilizes the meat colour in the absence of oxygen but spoilage may be masked, and CO is unlikely to ever be approved for organic meat. Fat oxidation in meat packaged in high-O_2 MAP may be retarded in avoiding exposure to UV light (Anonymous, 2013a). Last but not least, it makes no sense to package "white meat" (e. g. many cuts from poultry) under oxygen. This also applies to poultry meat covered with skin.

Conclusions

In summary, using high-O_2 MAP with 70-80% oxygen appears in contradiction to the principle of "careful" processing of organic meat. On the other hand, there is clearly a market demand for fresh organic meats in convenient retail packages. A sound compromise could be to reduce oxygen levels in MAP to about 40-50% or even less.

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References

1. Anonymous (2007) Council Regulation (EC) No 834/2007 of 28 June 2007 on organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91. *Official Journal of the European Union*, Vol. L 207, p. 1–23.
2. Anonymous (2013c) *Fleisch unter Schutzatmosphäre verpackt - Oft rot statt frisch.* Vzbv Bundesverband, Berlin, Germany. Available: http://www.lebensmittelklarheit.de/cps/rde/xchg/lebensmittelklarheit/hs.xsl/1550.htm [last accessed on 14.01.2014].
3. Anonymous (2008a) *Die Nationale Verzehrsstudie II.* Max-Rubner-Institut, Karlsruhe, Germany. Available on http://www.mri.bund.de/NationaleVerzehrsstudie [last accessed 14.03.2014].
4. Anonymous (2008b) Commission Regulation (EC) No 889/2008 of 5 September 2008 laying down detailed rules for the implementation of Council Regulation (EC) No 834/2007 on organic production and labelling of organic products with regard to organic production, labelling and control. *Official Journal of the European Union*, Vol. L 250 , p. 1–8
5. Anonymous (2010a) *Keine gesundheitliche Gefährdung des Verbrauchers durch unter Sauerstoff-Schutzgas verpacktes Frischfleisch.* Stellungnahme 038/2010, Bundesinstitut für Risikobewertung, Berlin (Germany). Available on http://www.bfr.bund.de/cm/343/keine_gesundheitliche_gefaehrdung_des_verbrauchers_durch_unter_sauerstoff_schutzgas_verpacktes_frischfleisch.pdf.
6. Anonymous (2010b) *Bioland-Richtlinien für die Verarbeitung - Fleisch und Fleischerzeugnisse*. Bioland e.V., Mainz (Germany). Available: http://www.bioland.de/fileadmin/dateien/HP_Dokumente/Richtlinien/2010_11_23_RL_Fleisch_und_Fleischerzeugnisse.pdf [last accessed on 14.03.2014].
7. Anonymous (2011) Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers. *Official Journal of the European Union*, Vol. L 304, p. 18–63.
8. Anonymous (2013a) *Entwicklung einer MAP-Verpackung für Frischfleisch mit UV-Filterfolie.* Fraunhofer-Institut für Verfahrenstechnik und packung, Freising, Germany. Available: http://www.ivv.fraunhofer.de/de/geschaeftsfelder/lebensmittelqualitaet-sensorische_akzeptanz/map-verpackung.html [last accessed on 14.03.2014]
9. Anonymous (2013b) *Verzehr von Fleischerzeugnissen.* Deutscher Fleischerverband, Frankfurt, Germany. Available on http://www.fleischerhandwerk.de/cms/upload/pdf/GB2013_Fleischverzehr.pdf [last accessed on 14.03.2014].
10. Anonymous (2013d) *Ökobarometer*. Bundesministerium für Ernährung und Landwirtschaft, Germany. Available on http://www.bmel.de/oekobarometer2013.html [last accessed 14.03.2014].
11. Anonymous (2013e) *Naturland Richtlinien für Verarbeiter*. Naturland e.V., Gräfelfing, Germany. Available on http://www.naturland.de/fileadmin/MDB/documents/Richtlinien_deutsch/Naturland-Richtlinien_Verarbeitung_ gesamt.pdf [last accessed 14.03.2014].
12. Anonymous (2013f) *Richtlinie für die Anerkennung von Demeter-Fleisch- und Wurstwaren.* Demeter e.V., Darmstadt, Germany. Available on http://www.demeter.de/sites/default/files/richtlinien/VB_12-13_IV-4_FleischWurstwaren.pdf [last accessed 14.03.2014].
13. Becker T., Benner E., Glitsc K. (2000) Consumer perception of fresh meat quality in Germany. *British Food Journal*, Vol. 102, p. 246–266.
14. Belcher J. N. (2006) Industrial packaging developments for the global meat market. *Meat Science*, Vol. 74, p. 143–148.
15. Boselli E., Rodriguez-Estrada M. T., Fedrizzi G., Cabini M. F. (2009) Cholesterol photosensitised oxidation of beef meat under standard and modified atmosphere at retail conditions. *Meat Science*, Vol. 81, p. 224–229.
16. Branscheid W. (2008) Frische außerordentlich stark betont: Beweggründe deutscher Verbraucher beim Fleischeinkauf – Eine Untersuchung zur Bedeutung der regionalen Herkunft – Teil 1. *Fleischwirtschaft*, Vol. 88 (4), p. 94-97.
17. Carpenter C. E., Cornforth D. P., Whittier D. (2001) Consumer preferences for beef color and packaging did not affect eating satisfaction. *Meat Science*, Vol. 57, p. 359–363.
18. Clausen I., Jakobsen M., Ertbjerg P., Madsen N. T. (2009) Modified atmosphere packaging affects lipid oxidation, myofibrillar fragmentation index and eating quality of beef. *Packaging Technology and Science*, Vol. 22, p. 85–96.
19. Dederer, I., Troeger, K., Münch, S. (2014) Untersuchung der qualitativen Parameter von SB-Frischfleisch. *Fleischwirtschaft*, Vol. 94 (1), p. 88–92.
20. Esmer O. K., Irkin R., Degirmencioglu N., Degirmencioglu A. (2011) The effects of modified atmosphere gas composition on microbiological criteria, color and oxidation values of minced beef meat. *Meat Science*, Vol. 88, p. 221–226.
21. Ferioli F., Caboni M. F., Dutta P. C. (2008) Evaluation of cholesterol and lipid oxidation in raw and cooked minced beef stored under oxygen-enriched atmosphere. *Meat Science*, Vol. 80, p. 681–685.
22. Hunt M. C., Sørheim O., Slinde E. (1999) Color and heat denaturation of myoglobin forms in ground beef. *Journal of Food Science*, Vol. 64, p. 847–851.
23. Jakobsen M., Bertelsen G. (2000) Colour stability and lipid oxidation of fresh beef. Development of a response surface model for predicting the effects of temperature, storage time, and modified atmosphere composition. *Meat Science*, Vol. 54, p. 49–57.
24. John L., Cornforth D. P., Carpenter C. E., Sørheim O., Pettee B.C., Whittier D. (2004) Comparison of color and thiobarbituric acid values of cooked hamburger patties after storage of fresh beef chubs in modified atmospheres. *Journal of Food Science*, Vol. 69, 608–614.
25. Kim Y. H., Huff-Lonergan E., Sebranek J. G., Lonergan S. M. (2010) High-oxygen modified atmosphere packaging system induces lipid and myoglobin oxidation and protein polymerization. *Meat Science*, Vol. 85 (4), p. 759–767.
26. Lautenschläger R., Müller W.-D. (2006) Deutlicher Optimierungsbedarf bei MAP: Frischfleisch und Fleischerzeugnisse in Schutzatmosphärenpackungen – ein Statusbericht. *Fleischwirtschaft*, Vol. 96 (8), p. 41–45.
27. Li X., Lindahl G., Zamaratskaia G., Lundström K. (2012) Influence of vacuum skin packaging on color stability of beef longissimuslumborum compared with vacuum and high-oxygen modified atmosphere packaging. *Meat Science*, Vol. 92 (4), p. 604–609.

28. Lund M. N., Heinonen M., Baron C. P., Estévez M. (2011) Protein oxidation in muscle foods: a review. *Molecular Nutrition & Food Research*, Vol. 55, p. 83–95.
29. Mancini R. A., Hunt M. C. (2005) Current research in meat color. *Meat Science*, Vol. 71, p. 100–121.
30. McMillin K.W. (2008) Where is MAO going? A review and future potential of modified atmosphere packaging for meat. *Meat Science*, Vol. 80, p. 43–65.
31. Möllers M., Lohneis M., May M. (2007) Rechtliche Fragen bei der Behandlung von frischem Fleisch mit Sauerstoff. *Journal für Verbraucherschutz und Lebensmittelsicherheit*, Vol. 2, p. 516–517.
32. Nitsch P. (2012) Farbe von Schweinefleisch bei unterschiedlicher Sauerstoffkonzentration in der Schutzatmosphären-Packung. (Redness of meat colour in MAP depending on oxygen concentration) *Mitteilungsblatt Fleischforschung Kulmbach*, Vol. 51, no. 196, p. 97–103.
33. O'Sullivan M. G., Kerry J. P. (2010) Meat Packaging. **In**: *Handbook of Meat Processing*. F. Toldrá (ed.). Ames (Ia, USA): Wiley-Blackwell, p. 247–261
34. Padilla Bravo, C., Cordts A., Schulze, B., Spiller A. (2013) Assessing determinants of organic food consumption using data from de German National Nutrition Survey II. *Food Quality and Preference*, Vol. 28, p. 60–70.
35. Resconi V. C., Escudero A., Beltrán J. A., Olleta J. L., Sañudo C., del Mar Campo M. D. (2012) Color, lipid oxidation, sensory quality, and aroma compounds of beef steaks displayed under different levels of oxygen in a modified atmosphere package. *Journal of Food Science*, Vol. 77 (1), p. S10–S18.
36. Robbins K., Jensen J., Ryan K. J., Homco-Ryan C., McKeith F. K., Brewer M. S. (2003) Consumer attitudes towards beef and acceptability of enhanced beef. *Meat Science*, Vol. 65, p. 721–729.
37. Røssvoll E., Sørheim O., Heir E., Møretrø T., Olsen N. V., Langsrud S. (2014) Consumer preferences, internal color and reduction of shigatoxigenic Escherichia coli in cooked hamburgers. *Meat Science*, Vol. 96 (1), p. 695–703.
38. Schulze B., Spiller A. (2008b) Hat sich die Bedientheke überlebt? Verbrauchereinstellungen zu den Angebotsformen von Fleisch im LEH. In: *Zukunftsperspektiven der Fleischwirtschaft: Verbraucher, Märkte, Geschäftsbeziehungen*. A. Spiller, B. Schulze (eds). Göttingen: Universitätsverlag. p. 273–301. Available on http://www.uni-goettingen.de/de/document/download/3e790d965dbb4f5e1ae4031451945ba0.pdf/fleischwirtschaft.pdf [last accessed on 14.01.2014].
39. Schulze B., Spiller A. (2008a) Trends im Verbraucherverhalten: Ein Forschungsüberblick zum Fleischkonsum. In: *Zukunftsperspektiven der Fleischwirtschaft: Verbraucher, Märkte, Geschäftsbeziehungen.* A. Spiller, B. Schulze (eds). Göttingen: Universitätsverlag. p. 233–271. Available on http://www.uni-goettingen.de/de/document/download/3e790d965dbb4f5e1ae4031451945ba0.pdf/fleischwirtschaft.pdf [last accessed on 14.01.2014].
40. Seyfert M., Hunt M. C., Mancini R. A., Kropf D. H., Storda S. L. (2004) Internal premature browning in cooked steaks from enhanced beef round muscles packaged in high-oxygen and ultra-low oxygen modified atmospheres. *Journal of Food Science*, Vol. 69 (2), p. 721–725.
41. Sun X. D., Holley R. A. (2012) Antimicrobial and Antioxidative Strategies to Reduce Pathogens and Extend the Shelf Life of Fresh Red Meats. *Comprehensive Reviews in Food Science and Food Safety*, Vol. 11 (4), p. 340–354.
42. Zakrys P. I., O'Sullivan M. G., Allen P., Kerry J. P. (2009) Consumer acceptability and physiochemical characteristics of modified atmosphere packed beef steaks. *Meat Science*, Vol. 81, p. 720–725.
43. Zakrys-Waliwander P. I., O'Sullivan M. G., Walsh H., Allen P., Kerry J. P. (2011) Sensory comparison of commercial low and high oxygen modified atmosphere packed sirloin beef steaks. *Meat Science*, Vol. 88 (1), p. 198–202.
44. Zhang M., Sundar S. (2005) Effect of Oxygen Concentration on the Shelf-Life of Fresh Pork Packed in a Modified Atmosphere. *Packaging Technology and Science*, Vol. 18, p. 217–222.

REVIEW

PHYTOSTEROLS IN RICE BRAN AND USAGE OF RICE BRAN IN FOOD INDUSTRY

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Abstract

Phytosterols are very important for daily diet that can not be synthesized by human body. Main sources of phytosterols are vegetable oils, seeds, legumes and cereals. Phytosterols prevent cholesterol absorption therefore lower total and LDL cholesterol level in blood. Also plant sterols might protect against certain types of cancer such as colon, breast and prostate. Rice bran is the best source of total lipids and phytosterols based on raw material. Rice bran oil contains very high concentrations of cycloartenol and 2.4-methylenecycloartanol, which made up over 40% of the total phytosterols. Rice brain contains 12–22% oil, 11–17% protein, 6–14% fiber, 10–15% moisture, and 8–17% ash. Also, It is rich in vitamins, including vitamin E, thiamine, niacin, and minerals like aluminum, calcium, chlorine, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc. Furthermore, presence of antioxidants like tocopherols, tocotrienols, and γ-oryzanol also brighten prospects of rice bran utilization for humans as functional ingredient to reduce the life threatening disorders. To improve quality and nutrition of food product, rice bran can be evaluated as a potential food ingredient. It has been used in food as full-fat, defatted bran, bran oil, and protein concentrates. Rice bran is used by the food industry in the production of baked foods, snacks, crackers, breads, cereals, pastries, pancakes, noodles, muffins, biscuits. In this review, phytosterols found in rice bran which impacts on human health and the usage of rice bran in food industry are discussed in general terms.

Keywords: bioactive compound, cereals, phytosterol, rice bran.

Introduction

Phytosterols (including plant sterols and stanols) can not be synthesized by humans, and all plant sterols and stanols in the human body therefore originate from the diet. The class of sterols known as phytosterols is found mainly in plant cell walls and membranes. Sitosterol, campesterol, and stigmasterol were the major phytosterols in the lipid extracts. Phytosterols have hypocholesterolemic effects (Jong et al., 2003; Jiang and Wang, 2005). They are known to have several bio-active qualities with possible implications for human health (Normen et al., 2002). The difference of phytosterols which are a group of triterpens from cholesterols is the presence of a double bond within ethyl or methyl groups and within the side chains of them. Phytosterols can be found either in the free form and the esterified form. Phytosterols are classified either as -sterols or -stanols; according to the presence of the double bond within the position Δ5 (Bayrakçı, 2013).

Rice bran is the best source of phytosterols based on raw material. Rice bran, a part of the rice kernel that contains pericarp, aleurone, and subaleurone fractions, is a by-product of rice milling. It is estimated that the world annual production of rice bran amounts to 76 million tons. Rice brans, oils, and hulls contain a large number of bioactive compounds, with pigmented brans containing many more bioactive compounds than do white brans (Friedman, 2013).

Because of the increasing interest in relationship between health and food, results increasement of formulation studies about functional products. For this reason, stabilized rice bran or its components have been used as ingredients in various food matrices such as bread (Hu et al., 2009), cookies (Bhanger et al., 2008), pizza (de Delahaye et al., 2005), beverages (Faccin et al., 2009; Jeličić et al., 2008), tuna oil (Chotimarkorn et al., 2008). Rice bran is also used in meat emulsions and batter mixes industrially. In this study we will present brief overviews of phytosterols and selected recent studies on enrichment of some food products with rice bran.

Phytosterols and Chemical Structure

Phytostanols are a fully-saturated subgroup of phytosterols (contain no double bonds). Phytostanols occur in trace levels in many plant species and they occur in high levels in tissues of only in a few cereal species. Phytosterols can be converted to phytostanols by chemical hydrogenation. More than 200 different types of phytosterols have been reported in plant species. In addition to the free form, phytosterols occur as four types of "conjugates," in which the 3β-OH group is esterified to a fatty acid or a hydroxycinnamic acid, or glycosylated with a hexose (usually glucose) or a 6-fatty-acyl hexose (Moreau et al., 2002). The structures of sitosterol, sitostanol, campesterol and campestanol are shown in Fig. 1 (Gilbert et al., 2005).

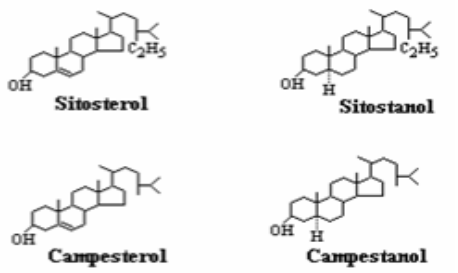

Figure 1. Chemical structures of some phytosterols (Gilbert et al., 2005)

Heath Effects and Resources of Phytosterols

Phytostanol; reducing blood cholesterol levels, as well as their other beneficial health effects, have been known for many years (Quilez et al., 2003). It was recognized in the 1950s that plant sterols lower serum concentrations of cholesterol (Pollak, 1953). Plant sterols might also protect against certain types of cancer such as colon, breast and prostate (Rao, Koratkar, 1997; Awad and Fink, 2000). Phytosterols can be found at widely varying concentrations in the fat-soluble fractions of seed, root stems, branches, leaves and blossoms. They are constituents of both edible and ornamental plants, including herbs, shrubs and trees. As natural constituents of the human diet, phytosterols are naturally found in all food items of plant origin, principally oils, but also pulses and dried fruits. Their content is highest in edible oils, seeds and nuts. The total contents are very variable and range from nearly 8 g kg^{-1} in corn oil to 0.5 g kg^{-1} in palm oil, with intermediate levels being found in commonly used oils. Tall oil contains a higher proportion of plant stanols than do vegetable oils. Phytosterols are of products based on vegetable oils. The refining process cited leads to a significant reduction in phytosterols in vegetable oils and it would therefore be very interesting to develop industrial methods which minimize these losses (Taşan et al., 2006). Main sources of phytosterols are wheat bran, wheat germ, durum wheat, rice bran, vegetables oils, seed and legumes (Clifton, 2002; Jiang, Wang, 2005). Germ and bran fraction of cereals, especially rice bran and wheat germ which have high level of oil, are known as best source of phytosterols. Rice bran contains %22.2 of phytosterol (Taşan et al., 2006). The dietary intake of phytosterols among and within different human population varies greatly, depending on the type and amount of plant foods eaten. Although cooking oils, margarine and peanut butter are the main sources of phytosterols in the diet, phytosterols are also consumed in seed, nuts, cereals and legumes (Clifton, 2002). The consumption of phytosterols can range between 170 mg per day in populations eating a Western diet and 360 mg per day in diets rich in vegetable products (Vries et al., 1997). The dietary intake of plant stanols is usually only about 50 mg/day unless the diet is supplement with tall oil, which is derived from conifers and is rich in sitostanol (Gilbert et al., 2005). The normal dietary intake of plant stanols is much less than that of plant sterols.

Rice Bran

Rice is one of the most important staple foods for a large part of human population. Global rice production is 645 million tons and this huge amount of production results large amount of rice by products (Kubglomsong, Threerakulkait, 2014; Al-Okbi et al., 2014). Rice bran is the major by-product generated during the milling processing (Kubglomsong, Threerakulkait, 2014). Brown rice is resulted from dehusking of raw rice and brown rice is covered by bran layers which namely, pericarp, testa and aleurone layers. Bran is obtained by de-branning or polishing process and constitutes 5–10% of brown rice. It's mainly composed of protein, fiber, oil, vitamins, minerals and starch which come from endosperm during polishing (Al-Okbi et al., 2014). It is also a good source of antioxidants such as polyphenols, tocopherols, tocotrienols and gamma-oryzanol which help in preventing the oxidative damage of body tissues and DNA. Many studies reported that rice bran has cholesterol lowering properties, cardiovascular health benefits and anti-tumor activity (Tuncel et al., 2014a). Table 1 shows the bioactive compounds of rice bran (Friedman, 2013). Jiang and Wang (2005) have studied to determine amount of phytosterols in some cereal by- product (rice bran, wheat bran, wheat germ, oat bran, oat germ, durum wheat, corn fine fiber).

Table 1

Bioactive compounds of rice bran (Friedman, 2013)

Phenolic and cinnamic acids	Anthocyanis, flavonoids	Steroial compounds	Polymeric carbohydrates
Caffeic acids	anthocyanin monomers, dimers, andpolymers	acylated steryl glucosides	arabinoxylan
Coumaric acid	apigenin	cycloartenol ferulate	glucans
Cathecins	cyanidin glucoside	campesterol ferulate	hemicellulose
Ferulic acid	cyanidin rutinoside	24-methylenecycloartenol ferulate	
Gallic acid	epicatechins		
Hyroxybenzoic acid			
Methoxycinnamic acid	eriodtyol	γ-oryzanol	
Sinopic acid	hermnetins	β-sitosterol ferulate	
Syringic acid	hesperetin	tocopherols	
Vanillic acid	isorhamnetins, luteolin, peanidin glucoside, tricin	tocotrienol	

For this purpose total lipid of this products are extracted and sterol profiles of these extracts were analyzed by gas chromatography. Rice bran is reported as the most lipid-rich material, with 22.2% total lipid extracted on a dry weight basis. In terms of raw material, the highest phytosterol content is also obtained from rice bran with 4.5 mg/g bran. Sitosterol, campesterol and stigmasterol are determined as major phytosterols in samples however different from these components, it is indicated that rice bran oil contains very high concentrations of cycloartenol and 24-methylenecycloartanol.

In a study that determined the antioxidant activity of different variety of two bran, three different extraction solvent (methanol, ethanol and ethyl acetate) were used shows that antioxidant pyhtocompounds from rice bran with methanol produces a significantly greater yield than ethanol and ethyl acetate. Presence of tocotrienols or synergistic effect of tocopherols and tocotrienols are reported as a reason of the strong antioxidant activity (Arab et al., 2011).

Rice bran contains 12–22% oil by weight (Sharif et al., 2014). Crude rice bran oil is rich in unsaturated linoleic and oleic fatty acids and bioactive compounds such as γ-oryzanol, phytosterols, tocopherols, and tocotrienols (Friedman, 2014). In addition to nutritious components and health benefits of rice bran oil, some properties such as good stability, appealing flavour and long fry life, provides the rice bran oil in shortening (Liang et al., 2014).

In spite of being an excellent nutrient source, raw rice bran is not suitable for human consumption because of rancidity problem. This problem mainly occurred by lipases and inactivation of the deteriorative enzymes is called stabilization. The employed stabilization approaches are extrusion, micro wave treatment, ohmic heating, dry heat treatment, gamma radiation, parboiling, toasting etc. (Tuncel et al., 2014b).

To evaluate rice bran as potential food ingredients and improve quality and nutrition, several studies were attempted. Especially most of the studies are available which are related to improve flour formulation of bread. Sekhan et al. (1997) prepared leavened pan bread with addition full fat and defatted rice bran flour at 5, 10 and 15%. Reduction in loaf volume and decreasing in overall acceptability of bread are recorded. In similar study, Lima et al. (2002) used full fat and defatted rice bran to evaluated functional behaviour of bread. Results showed that bread hardness, gumminess and chewiness increase depending on level of rice bran. Cohesiveness and springiness were not affected significantly. In another study, infrared stabilized rice bran is supplemented to white wheat, wheat bran and whole grain breads at levels of 2.5, 5.0 and 10.0 % by Tuncel et al. (2014a). For all types of breads, resilience and cohesiveness showed a significant decreasing trend with the inclusion of stabilized rice bran and a strong correlation was observed between these textural attributes. Crude fat and ash content of all bread types were increased with the inclusion of stabilized rice bran. It is observed that significant and gradual increase in insoluble, and thereby total dietary fiber content of pan breads. The same researchers also evaluated the effects of rice bran addition on the content of B vitamins, minerals and phytic acid content of breads. In all type breads, mineral, phytic acid and vitamin B content is significantly increased especially niacin. It is recorded that the antinutritive content of phytic acid should be taken into account (Tuncel et al., 2014b).

In production of corn flakes and tortilla chips, gelatinized corn flour was supplemented with rice bran from 10 to 30%. It was observed that the maximum breakdown viscosity and color quality was affected and sensory parameters decreased. Percentage of protein increased depending on the level of rice bran (Al-Okbi, 2014).

De delahaye et al. (2005) used stabilized rice bran flour in order to enrich wheat flour as a source of dietary fiber, in the preparation of frozen pizza and they also evaluated physical, chemical rheological and sensorial characteristics of the pizzas during the storage period of 2 months at -18 °C. It is observed that increasing the stabilized rice bran content results increase crude fat, ash and dietary fiber content of pizza dough. It is reported that the farinographic curves of pizza dough shows the development time, water absorption and stability decreased, while mixing tolerance index and departure time is not affected by enrichment level.

Conclusion

Phytosterols are defined as plant sterols which have important bioactive properties for human health. Because of lowering cholesterol level in blood, antibacterial, antifungal and antiulcer effects, phytosterols are being used in pharmaceutical and food industry. Rice bran as a by product of milling industry is a good source of phytosterols and also generally used as animal feed. Considering the amount of rice production in the world, the amount of by products will also be high level. In order to adding value to by product and producing healthy food, usage of rice bran is available. Due to nutritional profile, functional characteristics, apparent hipoallergenicity, it has many applications in a diet. In addition to them, using with different bioactive compounds might be able to provide multifunctional foods.

References

1. Al-Okbi S.Y., Husseın A.M.S., Hamed I.M., Mohamed D.A., Helal A.M. (2014) Chemical, rheological, sensorial and functional properties of gelatinized corn- rice bran flour composite corn flakes and Tortilla chips. *Journal of Food Processing and Preservation*, Vol. 38, p. 83–89.
2. Arab F., Alemzadeh I., Maghsoudi V. (2011) Determination of antioxidant component and activity of rice bran extract. *Scientia Iranica*, Vol. 18, No. 6, p. 1402–1406.

3. Awad A.B., Fink C.S. (2000) Phytosterols as anticancer
4. *The American Society for Nutritional Sciences*, Vol. 130, No. 9, p. 2127–2130.
5. Bayrakçı H. (2013) Tahıllarda bulunan fitosteroller ve fitostanoller. *Uluslararası 2. Helal ve sağlıklı Gıda kongresi, Gıda Bilimi ve Teknolojisi*, Kasım 7-10, Konya, Türkiye.
6. Bhanger M.L., Iqbal S., Anwar F., Imran M., Akhtar M., Zia-ul-Haq M. (2008) Antioxidant potential of rice bran extracts and its effects on stabilisation of cookies under ambient storage. *International Journal of Food Science and Technology*, Vol. 43, p. 779–786.
7. Chotimarkorna C., Benjakul S., Silalai N. (2008) Antioxidative effects of rice bran extracts on refined tuna oil during storage. *Food Research International*, Vol. 41, p. 616–622.
8. Clifton P. (2002) Plant sterol and stanols-comparison and contrasts. Sterol versus stanols in cholesterol-lowering: is there a different? *Atherosclerosis Supplements*, Vol. 3, p. 5–9.
9. De Delahaye E.P., Jimenez P., Perez E. (2004) Effect of enrichment with high content dietary fiber stabilized rice bran flour on chemical and fuctional properties of storage frozen pizzas. *Journal of Food Engineering*, Vol. 68, p. 1–7.
10. Faccin G.L., Vieira L.N., Miotto L.A., Barreto P.L.M., Amante E.R. (2009) Chemical, sensorial and rheological properties of a new organic rice bran beverage. *Rice Science*, Vol. 16, No. 3, p. 226–234.
11. Friedman M. (2013) Rice brans, rice bran oils, and rice hulls: Composition, food and ındustrial uses, and bioactivities in humans, animals, and cells. *Journal of Agricultural and Food Chemistry*, Vol. 61, p. 10626−10641.
12. Gilbert R., Thompson M.D., Grundy S.M. (2005) History and development of plant sterol and stanol esters for cholesterol-lowering purposes. *The American Journal of Cardiology*, Vol. 96, No. 1, p. 3–9.
13. Hua G., Huang S., Cao S., Maa Z. (2009) Effect of enrichment with hemicellulose from rice bran on chemical and functional properties of bread. *Food Chemistry*, Vol. 115, p. 839–842.
14. Jeličić I., Božanić R., Tratnik L. (2008) Whey -based beverages- a new generation of diary products. *Mljekarstro*, Vol. 58, No. 3, p. 257–274.
15. Jiang Y., Wang T. (2005) Phytosterols in cereal by-products. *Department of Food Science and Human Nutrition*, Vol. 82, No. 6, p. 439–444.
16. Jong A., Plat J., Mensink R.P. (2003) Metabolic effects of plant sterols and stanols. *The Journal of Nutritional Biochemistry*, Vol. 14, No. 7, p. 362–369.
17. Kubglomsong S., Theerakulkait C. (2014) Effect of rice bran protein extract on enzymatic browning inhibition in potato puree. *International Journal of Food Science and Technology*, Vol. 49, p. 551–557.

dietary components: evidence and mechanism of action.

18. Liang Y., Gao Y., Lin Q., Luo F., Wu W., Lu Q., Liu Y. (2014) A review of research progress on the bioactive ingredients and physiological activities of rice bran oil. *European Food Research and Technology*, Vol. 238, p. 169–176.
19. Lima I., Guaraya H., Champagne E. (2002) The functional effectiveness of reprocessed rice bran as an ingredient in bakery products. *Nahrung/Food*, Vol. 46, p. 112–117.
20. Moreau R.A., Whitaker B.D., Hicks K.B. (2002) Phytosterols, phytostanols, and their conjugates in food: structural diversity, quantitative analysis, and health-promoting uses, *Progress in Lipid Research*, Vol. 41, No. 6, p. 457–500.
21. Normen L., Bryngelsson S., Johnsson M., Evheden P., Ellegard L., Brants H., Andersson H., Dutts P. (2002) The phytosterol content of some cereal foods commonly consumed in Sweden and in the Netherlands. *Journal of Food Composition and Analysis*, Vol. 15, No. 6, p. 693–704.
22. Quilez J., Garcia-Lorda P., Salas-Salvado J. (2003) Potential uses and benefits of phytosterols in diet: present situation and future directions. *Clinical Nutrition*, Vol. 22, No. 4, p. 343–351.
23. Pollak, O.J. (1953) Reduction of blood cholesterol in man. *Circulation*, Vol. 7, p. 702-706.
24. Rao A.V., Koratkar R. (1997) Anticarcinogenic effects of saponins and phytosterols. *ACS Symposium Series*, Vol. 662, p. 313–324.
25. Sekhan K. S., Dhillan S. S., Singh N., Singh B. (1997) Functional suitability of commercially milled rice bran in India for use in different food products. *Plant Foods for Human Nutrition*, Vol. 50, p. 127–140.
26. Sharif M. K., Butt M. S., Anjum F.M., Khan S.H. (2014) Rice Bran: A novel functional ingredient. *Critical Reviews in Food Science and Nutrition*, Vol. 54, No. 6, p. 807–816.
27. Taşan M., Bilgin B., Geçgel Ü., Demirci A.Ş. (2006) Phytosterols as functional food ingredients. *Journal of Tekirdag Agricultural Faculty*, Vol. 3, No. 2, p. 154–159.
28. Tuncel N.B., Yılmaz N., Kocabıyık H., Uygur A. (2014a) The effect of infrared stabilized rice bran substitution on physicochemical and sensory properties of pan breads: Part I. *Journal of Cereal Science*, Vol. 59, No. 2, p. 155–161.
29. Tuncel N.B., Yılmaz N., Kocabıyık H., Uygur A. (2014b) The effect of infrared stabilized rice bran substitution on B vitamins, minerals and phytic acid content of pan breads: Part II. *Journal of Cereal Science*, Vol. 52, No. 2, p. 162–166.
30. Vries J.H.M., Jansen A., Kromhout D., Bovenkamp P., Staveren A., Mensink R.P., Katan M.P. (1997) The fatty acid and sterol content of food composites of middle-aged men in seven countries. *Journal of Food Composition and Analysis*, Vol. 10, p. 115–141.

EVALUATION OF NUTRITION VALUE OF ROMAN SNAIL'S (*HELIX POMATIA*) MEAT OBTAINED IN LATVIA

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Abstract

Roman snail (*Helix pomatia*) meat is a favoured product in many European countries as well as in other continents. Of late, its consumption is growing also in Latvia. Investigations about biochemical composition and nutritional value of snail meat are few. Therefore the following objective was set forth for our research: evaluate the nutrition value of the Roman Snail meat obtained in Latvia. Investigations were performed at the Research Institute of Biotechnology and Veterinary Medicine „Sigra", of Latvia University of Agriculture from 2011 to 2013. The chemical analyses of 35 samples were done. In the studied samples protein, amino acids, intramuscular fat, fatty acids and cholesterol content were by standard methods determined. The average protein content detected in snail meat samples were 12.86 mg 100 g^{-1} fat content 1.11 mg 100 g^{-1}. It was calculated the ratio of total saturated and polyunsaturated fatty acids, results are 20.39 and 44.06% of total fatty acids content respectively. Research shows that Snail meat is low in lipids. It could be recommended as meat with excellent nutritional qualities.

***Keywords*:** *Helix pomatia*, nutritional value, biochemical composition.

Introduction

Roman snail (*Helix pomatia*) is one of totally 91 terrestrial snail species found in Latvia. As part of the local fauna Roman Snail has been known since 16th century.

The snail meat is mainly consumed as delicacy characterized by a high dietetic value and excellent nutritious traits (Cîrlan, Sindilar, 2009). Research shows that it is rich in protein at the same time being low in lipids (Okonkwo, Anyaene, 2009; Ligaszewski et al., 2005; Miletic et al., 1991). Cultivation of Roman snails is rapidly growing in Latvia of late as one of alternative types of non-traditional agricultural production. The society of snail breeders already incorporates 200 snail farms. The breeders associate their market outlet with export of the snail meat. To ensure the competitiveness of locally produced snail meat on European markets, the research needs to be carried out on the quality of the obtained product and ways of improving it.

Objective of the research study: evaluation of the composition of fatty acids in pedal mass of Roman snails found in Latvia in the wild versus that of snails cultivated in a trial farm with an aim of use the results obtained for acquisition of high quality product with excellent organoleptic features.

Materials and Methods

The trial was performed in May-September of 2011 at the Roman Snail Research Facility of the Research Institute of Biotechnology and Veterinary Medicine „Sigra", of Latvia University of Agriculture LLU. The sampling was performed simultaneously from the wild and from snail farm - snails having received the special supplementary Roman snail feed and snails fed wheat meal and bran. The samples of the pedal mass of Roman snails were drawn three times per season: in spring (May), summer (July) and autumn (September). One aggregate sample consisted of 40–50 snails. After sampling, the snails were refrigerated for 24 hours (+4 °C). Post refrigeration the snails were slaughtered by mechanically breaking the shell and separating the pedal mass and visceral mass. The snail pedal fraction was analysed. The chemical analyses of 35 samples were done. Wild snail meat samples collected in the spring in May. In the studied samples protein, fat, cholesterol content, amino acids and fatty acids composition were determined.

Crude protein content was determined as total nitrogen content by Kieldahl method and using coefficient 6.25 for calculation (ISO 937:1974).

Intramuscular fat content was made by Sochlet method with hidrolysis procedure (boiling in the hidrocloric acid) using SoxCap 2047 and SOX TEH 2055 equipment (FOSS).

Amino acids. Dried, defatted meat samples were hydrolysed with 6N HCl in sealed glas tubes at10 °C for 23 h. Amino acids were detected using reversed-phase HPLC/MS (Waters Alliance 2695, Waters 3100, column XTerra MS C18 5 μm, 1×100 mm). Mobile phase (90% acetonitrile: 10% dejonized water) 0.5 mL min^{-1}, column temperature. 40 °C was used. The identity and quantitative analysis of the amino acids were assessed by comparison with the retention times and peak areas of the standard amino acid mixture.

Fatty acids. For the pedal fraction, the percentage of saturated (SFA), mono-unsaturated (MUFA) and poly-unsaturated (PUFA) fatty acids were determined. Previously homogenized meat samples were prepared for GLC (gas-liquid chromatography) analysis using direct saponification with KOH/methanol followed by a derivatization with (trimethylsilyl) diazomethane by the method of Aldai et al. (2006).

The statistical processing of data was performed with the data statistical processing software SPSS 17.0 (probability 95% or significance level – $p<0.05$). For

the evaluation of fatty acid level differences in different snail groups the two sampled populations T-test was used.

Results and Discussion

Study established in meat of wild Roman snails and breeding snails (*Helix* pomatia) amount of total protein and total fat (see Fig.1).

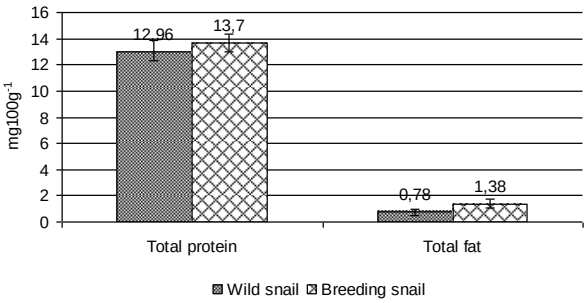

Figure 1. Total protein and total fat content (g 100 g^{-1}) in meat of wild snails and breeding snails

The content of crude protein for wild snails was 12.96±0.95 mg 100 $g^{-1}$and while the cultivated snails, wich are fed with concentrated feed protein was 13.70±0.66 mg 100 g^{-1}. In Roman snail meat total fat content is very low and can be considered as a dietetic product. The research of Fagbuaro et al. (2006) shows that in pedal mass of snails the crude protein content varies from 18.66±0.57% (*Limicolaria* spp.) to 20.56±0.05% (*Achachatina marginata*). The research results of Ligaszewski et al. (2005), on the other hand indicate that crude protein level in pedal mass of wild snail population was higher than that of snails cultivated in breeding facilities for all age groups, while protein level for wild snails at 2–3 years of age was essentially higher ($p<0.01$). Some authors (Okonkwo, Anyaene, 2009), examining *Helix pomatia* meat content in different regions of Lithuania have established the crude protein level from 11.51±0.03% to 16.60±0.03%. According to data of Zymantiene et al. (2006), the crude protein level in pedal mass of *Helix pomatia* is essentially ($p<0.001$) lower (14.15±0.76%) than that of pig meat (22.80±0.21). A relatively high protein content was established also in the pedal mass of the snail species *Archachatina*, *Archatina* and *Limiclaria* (Adeyeye, Afolabi, 1996).

At the same time in snails' meat it was also determined cholesterol level (Fig. 2).

Our sudy confirms that cholesterol content in meat of wild snail (150.93±11.56) and cultivated snail (174.69±0.21) was relatively high.

Differnces in cholesterol levels in wild snails meat and breeding snails meat is not essential ($p>0.05$). Different results were obtained in studies of Turkish scientists (Özogul, 2005). Studies with *Archachatina marginata* snail show relativly low cholesterol level.

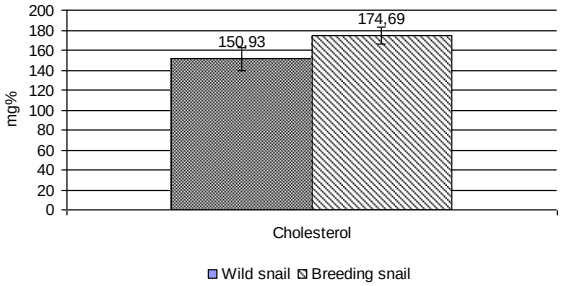

Figure 2. Cholesterol content (mg %) in meat of wild snails and breeding snails

The protein composition was determined and results assumed in Table 1.

Table 1

Amino acid content in snail pedal mass, mg 100 g^{-1}

Amino acid symbols	Wild snail (mean±SEM)	Breeding snail (mean±SEM)
*Val	0.441±0.08	0.378±0.02
*Leu	0.600±0.12	0.496±0.02
*Ile	0.408±0.08	0.308±0.02
*Phe	0.429±0.08	0.416±0.03
*Lys	0.567±0.13	0.409±0.02
*Thr	0.399±0,06	0.351±0.02
*Met	0.131±0.02	0.109±0.01
*Trp	0.417±0.01	0.849±0.01
Arg	0.465±0.06	1.146±0.63
Asp	0.748±0.11	0.651±0.03
Ser	0.365±0.05	0.319±0.02
Glu	1.110±0.16	1.002±0.07
Gly	0.541±0.08	0.502±0.04
Ala	0.559±0.08	0.550±0.03
Pro	0.426±0.07	0.394±0.02
Tyr	0.326±0.06	0.288±0.01
His	0.212±0.04	0.241±0.05
Hyp	0.451±0.01	0.845±0.01
ΣEAA	3.392±0.56	3.316±0.42

* – Essential amino acids (EAA)

The data summarized in Table 1 shows that wild snails meat has more aspartic acid (Asp), serine (Ser), glutamic acid (Glu), glycine (Gly), proline (Pro) and tyrosine (Tyr) than in meat of breeding snail, however the diferences between snails groups are not significant ($p>0.05$). Typically, the hydroxyproline (0.845 g 100 g^{-1}) has more in cultivated snails meat. The content of Leucine detected 0.600 g 100 g^{-1} in wild snail meat and 0.496 g 100 g^{-1} in cultivated snails meat. The content of Lysine detected 0.567 g 100 g^{-1} in wild snail meat and 0.409 g 100 g^{-1} in cultivated snails meat.

Helix pomatia meat is the greatest amount of non essential amino acid glutamic acid – 1.110 g 100 g^{-1} (wild snail) and 1.002 g 100 g^{-1} (cultivated snail).

Scientific literature references stating that it was snail meat contains essential amino acids leucine (Leu), izoleucine (Ile), fenilalanine (Phe) and tryptophane (Trp) (Imevbore, Ademosun, 1988; Imevbore, 1990; Stievenart, 1996; Ebenebe, 2000). Researchers believe that the snail meat contains very high quality protein (Ferhat et al., 2011).

The results of the fatty acid composition in pedal mass of the wild and breeding snails are summarised in Figure 3.

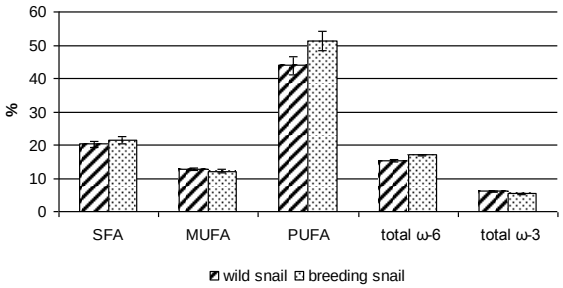

Figure 3. Comparison of fatty acid content (% of total fatty acids) in meat of wild snails and breeding snails

The research data revealed that there are significant differences in levels of the polyunsaturated fatty acids (PUFA) and long-chain poly-unsaturated ω-3 fatty acids (ω=3 PUFA) between wild and breeding snails meet samples ($p<0.05$).

The content of the poly-unsaturated ω-3 fatty acids (ω=3 PUFA) was slightly higher for the wild Roman snails – 6.03±0.29%, while the amount of ω-6 fatty acids (ω=6 PUFA) is higher for the cultivated snails - 16.36±0.16%. It was found that the meat of cultivated snails contained higher level of PUFA (51.17% on the average), than meat of the wild snails (44.06%). The content of MUFA (12.16±0.24%) and total ω-3 fatty acids (5.66±0.17%) in the meat of cultivated snails is lower, than in meat of the wild snails – MUFA 12.81±0.26% and total ω-3 fatty acids 6.03±0.29% ($p>0.05$). Content of SFA in the meat of the breeding snails detected 20.84±0.38% higher, than in meat of the wild snails – SFA 20.39±0.81 ($p>0.05$).

Research data of other scientists on the content of fatty acids both, in the pedal mass of wild *Helix pomatia* (Özogul et al., 2005), and *Helix aspersa maxima* (Çaġiltay et al., 2011; Milinsk at al. 2003) are available. In studies of Özogul et al. (2005), for wild *Helix pomatia* the saturated SFA are dominant (37.87%), while the poly-unsaturated PUFA are a minority (25.83%). In our case, the results are different: Roman snails (*Helix pomatia*) subjected to a feeding trial, showed higher levels of PUFA (44.06±2.59% and 51.17±1.65% accordingly) than SFA (20.39±0.80% and 20.84±0.38% accordingly). At the same time, the trials of Milinsk (2003) with feeding of plant oils, for snails *Helix aspersa maxima* have produced data which are very close to those obtained by us. The poly-unsaturated PUFAs are dominant (49.90–57.06%), while SFA content is relatively lower (22.20–26.26%). Different fatty acid contents have been obtained in trials of other authors (Çaġiltay et al., 2011) establishing PUFAs at the level of 34.38% and SFAs of 28.76% in pedal mass of *Helix aspersa*. However data on biochemical indicators of visceral mass of snails are noticeably less available.

Evaluating the levels of separate fatty acids, the highest content by percentage was established for SFAs – palmitic acid (C16:0) and stearic acid (C18:0), MUFA – oleic acid (C18:1 ω-9), and PUFAs – eicosadienoic acid (C20:2 ω-6) and eicosatrienoic acid (C20:3 ω-3) (see Table 2).

Table 2

Contents of separate fatty acids (%) in meat of Roman snails

Fatty acids	Wild snail (mean±SEM)	Breeding snail (mean±SEM)
C16:0	4.27±0.11	3.74±0.20
C18:0	11.62±0.50	12.92±0.85
C18:1 ω-9	11.47±0.26	10.88±0.47
C20:2 ω-6	11.92±0.32	13.43±0.35
C20:3 ω-3	14.24±0.21	14.38±0.33

SEM – Standard Error of Mean

The levels of fatty acids C20:2 ω-6 and C20:3 ω-3 were not significant higher in cultivated snail meat, than in wild snail meat.

Polyunsaturated fatty acids were found in the highest proportion by percentage, which determines also a higher PUFA amount. We found that the proportion of poly-unsaturated fatty acids for all snails is the highest.

Analysing separate fatty acids in pedal mass, we found that the content of α-linolenic acid (C18:3 ω-3) for wild snails was 1.78±0.29%, which is very close to indicators obtained in trials of Özogul (2005) – 1.87±0.01%. Different results however are obtained for snails *Helix aspersa*. According to Çaġiltay (2011) the proportion of α-linolenic acid for these snails is by 3.85% higher while Milinsk et al. (2006) has obtained indicators by 0.74% lower than for wild *Helix pomatia* in Latvian trial. Our initial research data certify that the fatty acid content found in pedal mass of Latvia's *Helix pomatia* is not always similar to fatty acid content established by other authors (Özogul et al., 2005, Çaġiltay et al., 2011): similar results have been found in pedal mass of *Helix aspersa* (Milinsk et al., 2003).

Conclusions

1. Roman snail meat protein content detected 12.96–13.70 g 100 g^{-1} including essential amino acids 3.1–3.4 mg 100 g^{-1} and it is evaluated as low in fat 0.78 g 100 g^{-1} (wild snails) and 1.38 g 100 g^{-1} (cultivated snails).
2. Snail meat in the greatest amounts of non essential amino acid glutamic acid (1.110 mg 100 g^{-1} found in wild snail meat and 1.002 mg 100 g^{-1} in cultivated snail) and least contain methionine (0.131 mg 100 g^{-1} found in wild snail meat and

0.109 mg 100 g^{-1} in meat obtained from cultivated snail).

3. The results on the whole, do not contradict to the data found in literature and they certify the richness of meat obtained from Roman snails of Latvia in polyunsaturated fatty acids (44.06–51.17% on the average) and its high biological value.
4. Cholesterol content in meat of wild snail (150.93±11.56 mg%) and cultivated snail (174.69±0.21 mg%) was relatively high.

References

1. Adeyeye E. I., Afolabi E. O. (2004) Amino acid composition of three different types of land snails consumed in Nigeria. *Food Chemistry*, Vol. 85, p. 535–539.
2. Aldai N, Osoro K, Barrón L. J. R, Nájera A. I. (2006) Gas–liquid chromatographic method for analysing complex mixtures of fatty acids including conjugated linoleic acids (cis9trans11 and trans10cis12 isomers) and long-chain (n-3 or n-6) polyunsaturated fatty acids:Application to the intramuscular fat of beef meat. *Journal of Chromatography*, A 1110, p. 133–139.
3. Akinnusi F. (2002) Comparative evaluation of fresh fruits leaves and concentrate feed on the growth and reproductive performance of the African land snail (*Arachachatina marginata*). *Proceedings of the 27th Annual Conference of the Nigerian Society for Animal production (NSAP)*, Federal University of Technology, Akure, (FUTA) p. 320–328.
4. Awesu M. O. (1980) The biology and management of the African gigant land snail (*Arachachatina marginata*). *M. Phil. Thesis, University of Ibadan*, 198 p.
5. Çağiltay F., Erkan N., Tosun D., Selçuk A. (2011) Amino acid, fatty acid, vitamin and mineral contents of the edible garden snain (*Helix aspersa*). *Journal of Fischeries Sciences*, Vol. 5, p. 354–363.
6. Cirlan A. F., Sindilar E. (2009) Observations regarding the physical and chemical composition of the meat from the *Helix pomatia* snail. *Journal Lucrări stiintifice – Medicină Veterinară*, Vol. 52, No. 11, Iss. 2, p. 860–862.
7. Ebenebe C. I. (2000) Mini-livestock production Nigeria. The present and future. In: *Proceedings 5th Annual conference ASAN*, Port Harcout, p. 19–22.
8. Fagbuaro O., Oso J. A., Edward J. B., Ogunleye R. F. (2006) Nutritional status of four species of giant land snails in Nigeria. *Journal of Zhejiang Uiversity Science*, Vol. 7(9), p. 686–689.
9. Ferhat C., Nuray E., deniz T., Arif S. (2011) Amino acid, fatty acid, vitamin and mineral contents of the edible garden snail (*Helix aspersa*). *Journal of Fisheries Sciences*, Vol. 5, p. 354–363.
10. Imevbore E. A., Ademosun A. A. (1988) The nutritive value the African giant land snails (*Archachatina marginata*). *Journal of Animal Production Research*, Vol. 8, p. 76–87.
11. Imevbore E. A. (1990) Management techniques in rearing African land snail (*Archachatina marginata*). *PhD thesis, University of Ibadan*, Nigeria.
12. Kalia G. A., Etela I. (2011) Nutritional and sensory profiling of the African giant land snail fed commercial-type and leaf-based diet in Rain Forest Ecology. African Journal of Food, Agriculture, *Nutrition and Development*, Vol. 23, p. 453–458.
13. Ligaszewski M., Łysak A., SurÓwka K. (2005) Chemical composition of the meat of *Helix pomatia* L. snails from the natural population and the derived breeding population. *Roczniki Naukowe Zootechniki*, Vol. 32, p. 33–45.
14. Milinsk M. C., Padre R. G., Hayashi C., Souza N. E., Matsushita M. (2003) Influence of diets enriched with different vegetable oils on the fatty acid profiles of snail. *Helix aspersa maxima*. *Food Chemistry*, Vol. 82, p. 553–558.
15. Miletic I., Miric M., Lalic Z., Sobajic S. (1991) Composition of Lipids and Proteins of Species of Molluscs, Marine and Terrestrial, from the Adriatic Sea and Serbia. *Food Chemistry*, Vol. 41, p. 303–308.
16. Okonkwo T. M., Anyaene L. U. (2009) Meat yield and the effects of curing on the characteristics of snail meat. *Journal of Tropical Agriculture, Food, Environment and Extension*, Vol.8, p. 66–73.
17. Stievenart C. (1996) Shell morphology, growth, reproduction and aestivation by African snail. Laborarory observation on *Archachatina marginata saturalis*, *Achatina achatina* and *Achatina fulica*. PhD Thesis, No 5, p. 206.

NUTRITIONAL CHARACTERISTICS OF WILD BOAR MEAT HUNTED IN LATVIA

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Abstract

Wild game meat is considered as significant source of healthy food, and its share in consumption in recent years, increasing in size. Investigations about biochemical composition of game meat, including wild boar (*Sus scrofa scrofa*) meat are not very much. Aim of our investigation was evaluate nutrition value of wild boar meet after hunting in Latvia. Nutritional characteristic of wild boar meat was based on the investigations carried out in different regions of Latvia. In the studied samples protein, amino acids, intramuscular fat, fatty acids, cholesterol and microelement content were determined. The average protein content detected in wild boar meat samples were 20.88 mg 100 g^{-1} fat content 3.45 mg 100 g^{-1}. It was calculated the ratio of total saturated fatty acids, ω-6 and ω-3, results are 42.98; 13.63 and 3.05% of total fatty acids content respectively. The content of microelements Fe and Zn in samples were 8.25 and 8.52 mg kg^{-1} it was higher than provides with meat of domestic animals. The results of investigation confirmed preference of wild boar meat in human health in comparison with beef or pork.

Keywords: game meat, nutritional value, dietetic product.

Introduction

Wild boar is a species that is utilised for food and sport hunting throughout the world and the potential of farming wild boars have stimulated interest in this species as a meat producer. In the last years consumption and assortiment of game meat products significantly increased. The public attention is especially paid to inhabitants health and value, through the consumption of wholesome food. Wild game meat characterized by high nutritional value and special sensory properties, desired by consumers is (Soriano et al., 2006; Rywotycki 2003) considered as significant source of healthy food. Since the amino acid composition of proteins from food animals is similar to human muscle and muscle makes up almost 50% of our body's weight, meat is an excellent source of the amino acids needed for growth, repair, and maintenance.

Similarly to other monogastric animals, meat fatty acid composition of wild boars depends on the diet provided (Di Matteo et al., 2003). This is also evident in depot fat from wild boars where, in contrast to ruminants, double bonds of fatty acids are not hydrogenated during digestion (Meyer et al., 1998). In nature, wild boars eat a great variety of indigenous plants, grains, seeds, roots, fruits, insects, earthworms, slugs and small mammals, with the bulk of food consumed consisting of plant material therefore their meat has balance in vitamins and microelements (Schley, Roper, 2003). The vitamin B_{12} is important for growth and development of human organism and can only be found in the food of animal origin. Investigations about biochemical composition of game meat, including wild boar (*Sus scrofa scrofa*) meat are not very much in Latvia.

Aim of our investigation was evaluate nutrition value of wild boar meet after hunting in Latvia.

Materials and Methods

Meat samples of wild boar (*Sus scrofa scrofa*) muscles (*m. logissimus lumborum*) were collected in the autumn-winter season. The investigations were conducted at the laboratory of Biochemistry and Microbiology of the Research institute of Biotechnology and Veterinary Medicine „Sigra". In the studied samples (n=12) protein, fat, ash and cholesterol content, amino acids and fatty acids composition and micronutrient amount were determined. Sample preparation was made in 48 hours after slaughtering or hunting. Meat samples of about 300 g were homogenized with BŰCHI B-400 (ISO 3100-1).

Protein content was determined as total nitrogen content by Kieldahl method and using coefficient 6.25 for calculation (ISO 937:1974).

Amino acids. Dried, defatted meat samples are treated with constant boiling 6N hydrochloric acid in an oven at around 110 °C for 23 h. Hydrolyzate diluted with 0.1% formic acid. Sample (2 mL) was filtered using siringe filter with 0.45 μm nylon membran. Amino acids were detected using reversed-phase HPLC/MS (Waters Alliance 2695, Waters 3100, column XTerra MS C18 5 μm, 1×100 mm). Mobile phase (90% acetonitrile: 10% deionized water) 0.5 mL min^{-1}, column temperature 40 °C. Data acquisition was done using programm Empower pro.

Intramuscular fat content was made by Sochlet method with hidrolysis procedure (boiling in the hydrochloric acid) using SoxCap 2047 and SOX TEH 2055 equipment (FOSS) (LVS ISO 1443:1973).

Cholesterol content was detected by Blur colorimetric method using spectrometer (Шманенков, 1973).

Fatty acids. Homogenized meat samples were prepared for GLC (gas-liquid chromatography) analysis using direct saponification with KOH/methanol followed by

a derivatization with (trimethylsilyl) diazomethane by the method of Aldai et al (2006) An ACME, model 6100, GLC (Young Lin Instrument Co.) equipped with a flame ionisation detector, and an Alltech AT-FAME analytical column (fused silica 30 m×0.25 mm i.d.) was used. The individual FAMEs (fatty acid methyl esters) were identified according to similar peak retention times using standard mixture Supelco 37 Component FAME Mix. The relative proportions of total saturated fatty acids and unsaturated and ω-6 fatty acids and ω-3 was calculated.

Micronutrient amount of meat are measured according to ISO 6869-2002. Methods are based on comparison of radiation absorption emmited by free metal atoms that are forming by spraying ashed sample and the concentrations of certain metal solutions in the flames. In the laboratory to determine the atomic absorption is used spectrometer Analyst 200.

The experimental design was randomised and data were evaluated by analysis was performed using SPSS 17. One-way ANOVA was used for comparison mean values. Statistical significance was declared at $p<0.05$. Research provides information on the biochemical composition of wild game meat: protein, fat, cholesterol, fatty acids, amino acids and trace amount that can eat any of us with 100 g of meat.

Results and Discussion

Obtained results of dry matter, protein, intramuscular fat, ash and cholesterol determined in wild boar meat samples assumed in Table 1.

Table 1

Biochemical composition of wild boar meat

Parameters	Wild boar meat	Standard deviation
Dry matter, %	25.38	0.62
Protein, %	20.88	2.99
Collagen content, %	30.24	1.42
Connective tissue protein, %	1.45	0.08
Intramuscular fat, %	3.45	1.67
Ash, %	1.14	0.13
Cholesterol, mg $100g^{-1}$	98.11	6.27

Content of protein in samples of wild boar meet was determined from 19.55% to 23.18%, average protein content 20.88±2.99%. The results of our investigation are similar with other research findings, where protein content in wild boar meat samples was reported 21.9% (Paleari et al., 2003) or 21.74–22.1% (Postolahe et al., 2011).

Results of investigation showed that the intramuscular fat content of wild boar meat determined from 1.63% to 4.27%, average content 3.45±1.67%. Scientific reports showed a wide range of results, depending of found feed intake the fat content determined: 1.23–4.27% (Quaresma et al., 2011), 3.5–5.2% (Zomborszky et al., 1996) or in the Poland 1.95% (Zmijewska, Korzeniowska, 2001).

The connective tissue protein, which in meat is also decisive for its tenderness, general contains 2.5–12.0% (Honikel, 2009). The connective tissue protein content determined in wild boar meet samples was 1.45%.

The meat samples of wild boar had higher cholesterol content – 98.11 mg 100 g^{-1} than determined in deer deer (74.23±2.49 mg 100 g^{-1}) or beef (67.92±7.99 mg 100 g^{-1}) samples (Strazdina, 2012). The cholesterol content in meat from wild boars hunted in Portugal was reported 55.6 mg 100 g^{-1} for female and 58.7 mg 100 g^{-1} for male (Quaresma et al., 2011)

The protein composition is more significant for human nutrition as protein content. Not all proteins have the same nutritional value; protein quality strongly depends on its amino acid composition and digestibility.

Table 2

Amino acid composition

Amino acid group	AA content, g 100 g^{-1}	Standard deviation
Σ Indispensible amino acids	7.30	1.12
Σ Partly indispensible amino acids	6.00	0.66
Σ Sulfur containing*	1.65	0.14

*(Phe + Tyr)

FAO/WHO recommended intake of total indispensable amino acids is 83.5 mg on kg of body weight per day (Essential amino acids) it is 5.8 g per human with body weight 70 kg

Since net protein utilization is affected by the limiting amino acid (the sum of phenylalanine and tyrosine or SCA), wild boar meat samples must be evaluated as source of protein with high biological value. The glutamic acid is the main one assigned meat taste. The content of glutamic acid determined 3.24 g 100 g^{-1} in wild boar meat samples.

The assumed essential amino acids in wild boar muscle protein – composition and scores are showed in Figure 1.

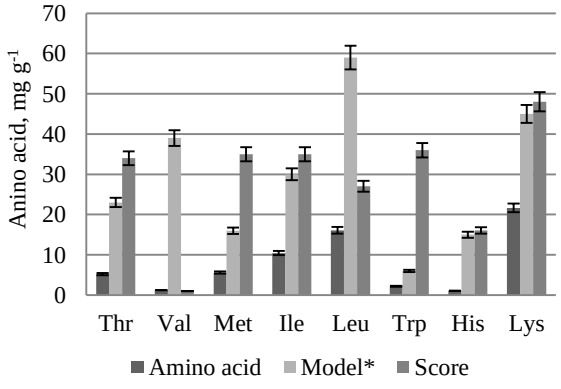

Figure 1. The sum of essential amino acids in wild boar muscle protein

*- model WHO/FAO/UNU (2007)

Amino acid score for SCA in the model protein defined 22, calculated score for wild boar meat is 75. The assumed amino acid score showed that biological value or the ability absorbed protein from wild boar meat to fulfil human amino acid requirements is high.
Evaluation of fatty acid composition of wild boar meat was made. Results of determined saturated, monounsaturated and polyunsaturated fatty acids demonstrated in Figure 2.

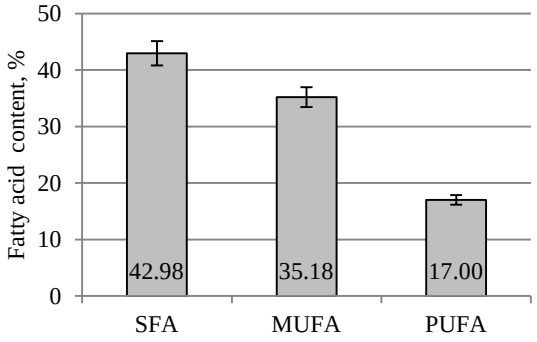

Figure 2. Fatty acid composition of wild boar meat samples, % of total fatty acids
SFA – saturated fatty acids, MUFA – monounaturated fatty acids, PUFA – polyunsaturated fatty acids

The results of investigation showed that average content of saturated fatty acids in wild boar meat assumed 42.98% of total fatty acids content. Saturated fatty acids content determined in pigs meat was higher – 45.97% (Jansons, Jemeljanovs, 2013), but not in wild deer meat – 33.34% (Strazdina et al., 2012). Scientific reports show lower saturated fatty acid proportion – 34.72% of total fatty acids in the *M. serratus anterior* from wild boars shot in the winter in a park in Hungary (Sales, Kotrba, 2013). Scientific reports show higher proportion of MUFA – 38–44% in wild boar meat (Quaresma et al., 2011, Razmaité et al., 2012) in comparison with our results. The proportion of polyunsaturated fatty acids determined in wild boar meat samples hunted in Latvia – 17% was in agreement with those obtained by Razmaité et al., – 17.9% PUFA determined in smples of male and 18.8% in samples of female (2012). Ratios ω-6 / ω-3 and PUFA / SFA showed in Table 3.

Table 3
Proportion of polyunsaturated fatty acids and ratios

Fatty acid	Wild boar meat	Standard deviation
ω-3, % of total fatty acid	3.05	0.21
ω-6, % of total fatty acid	13.63	0.20
ω-6/ω-3	6.13	0.31
PUFA/SFA	0.40	0.05

World Health Organization (2003) recommended ratio PUFA/SFA must be higher than 0.4 and scientific reports showed that domestic animals it has too low 0.1 (Wood et al., 2003). Quaresma reported higher ratio PUFA/SFA – 0.5 (males) to 0.6 (females) and higher ω-6 / ω-3 – 15.5 (females) to 17 (males) in wild boar meat hunted in Hungary because sum of ω-6 were detected 21–24% and sum of ω-3 just 1.4% (Quaresma et al., 2011).
Next to the favorable composition of fatty acids the game meat also contains B vitamins in large quantities. The following vitamins of the meat were examined: A, D, E, B_1, B_2, B_6, B_{12}, Niacin, Pantotenic, Folic acid, results are showen in Table 4.

Table 4
Vitamin content in wild boar meat, mg 100 g^{-1}

Parameters	Wild boar meat	Standard deviation
Fat-soluble vitamins		
Vitamin A	n.f.	0.00
Vitamin D	0.02	0.00
Vitamin E	0.38	0.03
Water-soluble vitamins		
Vitamin B_1	0.39	0.06
Vitamin B_2	0.26	0.02
Vitamin B_6	0.37	0.23
Vitamin B_{12}	0.01	0.02
Niacin	4.43	0.41
Pantotenic	0.68	0.04
Folic acid	0.01	0.00

n.f.-not found

In *M. psoas major* from wild boars, 71% of total vitamin E homologues were represented by α-tocopherol (Quaresma et al., 2011). Alpha-tocopherol was higher in adult males (19.2 μg g^{-1} meat) and females (18.1 μg g^{-1} meat), compared to youngsters (15.5 μg g^{-1} meat). Similarly, differences between maturity groups were detected in γ-tocopherol, with mean values of 1.75, 1.61 and 1.14 μg g^{-1} meat for adult males, adult females and youngsters, respectively. Alpha-tocopherol concentration in wild boar meat reported by Quaresma et al. (2011) was higher than concentrations of g 15.1–16.3 μg meat found by Jensen et al. (1997)
Red meat contains a number of B vitamins: thiamin (vitamin B_1), riboflavin (vitamin B_2), pantothenic acid, folate, niacin (vitamin B_3), vitamin B_6 and B_{12}. Meat, fish and animal-derived foods, such as milk, are the only foods that naturally provide vitamin B_{12}. For this reason, if you exclude such foods from your diet, you are at risk of having inadequate intakes. Red meat is a rich source of vitamin B_{12} (Mann, 2000) and about 35% of vitamin B_{12} intake comes from meat and meat products (Henderson, 2003). Dietary intakes of vitamin B_{12} are lower from vegetarian diets, and are particularly low in vegan diets (Phillips, 2005; Li et al., 2005) (which contain no animal foods), thus indicating the important contribution of meat and animal - derived products to B_{12} intake.
The following chemical parameters of the meat were examined: calcium, phosphorus, potassium, sodium, magnesium, iron, manganese, potassium, sodium,

magnesium showed in Table 5. The content of microelements Fe and Zn in samples were determined – 8.25 and 8.52 mg kg^{-1}. The amount of iron, phosphorus and potassium determined in the *m. longissimus dorsi* was 7.701 mg, 752 mg and 1114 mg per 100 grams of dry matter, respectively.

Table 5

The mineral content in wild boar meat

Parameters	Wild boar meat	Standard deviation
Macroelements, mg 100 g^{-1}		
Ca	3.22	0.21
P	91.15	0.31
K	930.71	3.17
Na	20.43	0.74
Mg	7.31	0.33
Fe	8.25	0.63
Trace elements, mg 100 g^{-1}		
Zn	8.52	0.45
Cu	0.04	0.00
Mn	0.03	0.00

According to Świergosz, Perzanowski, Makosz, and Biłek (1993), means for copper determine 6.4–7.4 mg kg^{-1} dry matter, iron 99.2–110.4 mg kg^{-1} dry matter and manganese 2.9–3.4 mg kg^{-1} dry matter did not vary to a large extent between two different regions in the south eastern part of Poland (Sales, Kotrba, 2013)

Conclusions

Result collected in the study revealed, that meat from wild boars, in many aspects, was characterised as higher quality in comparison to meat from domestic pigs. The percentage of proteins in meat is content 20.88±2.99%, and the proteins also have an above-average biological value- sum of Indispensible amino acids detected 7.30 g 100 g^{-1}. The most important vitamins are B_1 and B_2, as well as vitamin B_{12} that can be found only in food of animal origin. Nutritional value of game meat is completed with micro elements of vital essence: content of Fe, Zn was determined – 3.44 and 3.73 mg kg^{-1} respectively.

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References

1. Di Matteo S., Marsico G., Facciolongo A. M., Ragni M., Zezza F. (2003) Chemical and fatty acid composition of meat of wild boars fed on diets containing polyunsaturated fatty acids. *Italian Journal of Animal Science,* No. 2(Supplement 1), p. 418–420.
2. *Essential amino acids - HealthKnot health pages* [accessed on 2.09.2013.]. Available: http://www.healthknot.com/es sential_amino_acids.html
3. Henderson L., Irving K., Gregory J., Bates C. J., Prentice A., Perks J., Swan G., Farron M. (2003) *National Diet and Nutrition Survey. Adults aged 19 to 64 years.* London: The Stationery Office. Vol. 3. 160 p.
4. Honikel. K.O. (2009) Composition and Calories. **In**: Nollet, L.M., Toldra. (eds.): *Handbook of muscle foods analysis.* CRC Press-Book: eBook.ISBN 978-1-4200-4530-7 369-384 p. [accessed 04.02.2011].
5. Jansons I., Jemeljanovs A. (2013) Pork production. **In:** *Latvian population food producing meat characteristic.* Monograph. Research institute of Biotechnology and Veterinary Medicine "Sigra" Sigulda. 2013, p. 157–210. [in Latvian]
6. Li D., Siriamornpun S., Wahlqvist M. L., Mann N. J., and Sinclair A. J. (2005) Lean meat and heart health. *Asia Pacific Journal of Clinical Nutrition,* Vol. 14 No 2, p. 113–119.
7. Meyer H. H. D., Rowell A., Streich W. J., Stoffel B., Hofmann R. R. (1998) Accumulation of polyunsaturated fatty acids by concentrate selecting ruminants. *Comparative Biochemistry and Physiology, Vol.*°120 A,°p.°263–268.
8. Paleari M. A., Moretti V. M., Beretta G., Mentasti T., Bersanni C. (2003) Cured products from different animal species. *Meat sciences,* Vol. 63, No. 4, p. 485–489.
9. Phillips F (2005) Vegetarian nutrition. British Nutrition Foundation *Nutrition Bulletin* Vol. 30, p. 132–167.
10. Postolahe A. N., Ionescu O., Lazar R., Boisteanu P.C. (2011) Quality parameters of wild boar meat (sus scrofa ferus) harvested in northeastern Romania. *Lucrari Ştiintifice Medicina Veterinara,* Vol. XLIV(1), p. 213–222. [in Romanian]
11. Quaresma M. A. G., Alves S. P., Trigo-Rodrigues I., Pereira-Silva R., Santos N., Lemos J. P. C., Barreto A. S., Bessa R. J. B. (2011) Nutritional evaluation of the lipid fraction of feral wild boar (Sus scrofa scrofa) meat. *Meat Science,* Vol.89, p. 457–461.
12. Razmaitė V., Kerziene S., Jatkauskiene V., Nainiene R., Urbšiene D. (2009) Pork quality of male hybrids from Lithuanian Wattle pigs and wild boar intercross. *Agronomy Research,* Vol. 7. No. 1, p. 47–58.
13. Razmaitė V., Švirmickas G. J., Šiukščius A. (2012). Effect of weight, sex and hunting period on fatty acid composition of intramuscular and subcutaneous fat from wild boar. *Italian Journal of Animal Science,* Vol. 11, p. 174–179.
14. Rywotycki R. (2003) The influence of environment, mode of nutrition and animal species on level of nitrosamine contamination in venison. *Meat Science,* Vol. 65, p. 1045–1053.
15. Sales J., Kotrba R. (2013) Meat from wild boar (Sus scrofa L.): A review. *Meat science,* Vol. 94, p. 187–201.
16. Schley L., Roper T. J. (2003). Diet of wild boar Sus scrofa in Western Europe, with particular reference to consumption of agricultural crops. *Mammal Review,* Vol. 33, p. 43–56.
17. Soriano A., Cruz B., Gomez L., Mariscal C, Ruiz A. G. (2006) Proteolysis, physicochemical characteristics and free fatty acid composition of dry sausages made with deer (*Cervus elaphus*) or wild boar (*Sus scrofa*) meat: A preliminary study. *Food Chemistry,* Vol. 96, No.2, p. 173–184.
18. Strazdina V., Jemeljanovs A., Sterna V., Paeglitis D. (2012) Evaluation of nutrition value of deer meat

obtained in Latvian farms and wildlife. **In:** *Landbauforschung. vTI Agriculture and Forestry Research.* Special issue 362: 19.Tackling the Future Challenges of Organic Animal Husbandry. Proceedings of the 2nd Organic Animal Husbandry conference Hamburg/Trenthorst, Germany, p.°478–481.

19. Świergosz R., Perzanowski K., Makosz U., Bilek, I. (1993) The incidence of heavy metals and other toxic elements in big game tissue. *Science of the Total Environment,* Suppl., p. 225–231.
20. WHO/FAO/UNU (2007) *Protein and amino acid requirements in human nutrition*, Report of a Joint WHO/FAO/UNU Expert Consultation, World Health Organization Technical Report Series 935. WHO, Geneva.
21. World Health Organization (2003) *Diet, nutrition and the prevention of chonic diseases.* Report of a Joint WHO/FAO Expert Consultation, Geneva, 160.
22. Wood J. D., Enser M. (2003) Factors influencing fatty acids in meat and the role of antioxidants in improving meat quality. *British Journal of Nutrition*. Vol. 66. No. 1, p. 21–32.
23. Żmijewski T., Korzeniowski W. (2001). Technological properties of wild boars meat. *Electronic Journal of Polish Agricultural Universities* [accessed on 2.02.2014.]. Available online: *http://www.ejpau.media.pl/volume4/issue2/food/art-02.html*
24. Zomborsky Z., Sentmihályi G., Sarudi I., Horn P., Sabó C.S. (1996) Nutrient composition of muscle in deer and boar. *Journal of Food Science*, Vol. 61, p. 625–627.

DRIED VENISON PHYSICAL AND MICROBIOLOGICAL PARAMETERS CHANGES DURING STORAGE

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Abstract

Venison due to the lower fat content in muscles has gained increased popularity in recent years comparing to pork or beef. The aim of the current research was to determine changes in physical and microbiological parameters of dried venison during storage. For the experiments the meat (0.02×4.00×7.00 cm) pieces were marinated in "teriyaki sauce" marinade (composition: teriyaki sauce, sweet and sour sauce, taco sauce, soy sauce, American BBQ sauce hickory, sesame oil, garlic, garlic salt, tabasco red pepper sauce) at 4±2 °C temperature for 48±1h. To improve the meat textural properties sodium monophosphate (E339) was added in part of marinade too. After marinating meat samples were dried in microwave-vacuum drier MUSSON–1, packaged in vacuum pouches made from polymer film (PA/PE) with barrier properties and storage for 4 months at +18±1 °C temperature in dark place. The following physical and microbiological parameters of dried meat were evaluated after 35, 91 and 112 storage days using standard methods: pH (AACC 02-52), colour by Colour Tec PCM/PSM (CIE L*a*b* system), texture by TA. XT. plus Texture Analyser, the total count of mesophilic aerobic and facultative anaerobic microorganisms (Ref. Nr. 01–140), lactic acid bacteria (Ref. Nr. 01–135). Experimentally it was ascertained, that the shelf-life of dried venison with sodium monophosphate additive is 91 day and without sodium monophosphate additive – 112 days. Significant differences were detected in meat color and texture changes. However, dried meat color stability and texture is possible to provide during storage by adding of sodium monophosphate at marinade.

Keywords: venison, drying, microwave-vacuum drier.

Introduction

Venison due to the lower fat content in muscles has gained increased popularity in recent years. It is lower in calories, fat and cholesterol content comparing to lamb, pork or beef.

Colour, tenderness and juiciness are the most important parameters for consumers in meat (Vergara et al., 2003). Currently, marination is widely used by consumers and producers to improve meat tenderness and juiciness (Ergezer, Gocke, 2011).

Marination is the process of meat soaking or injecting a solution containing ingredients such as vinegar, lemon juice, wine, soy sauce, brine, essential oils, salts, tenderizers, herbs, spices and organic acids to flavour and tenderize meat products (Kargiotou et al., 2011; Pathania et al., 2010). The functionality of most marinades directly depends on their ingredients. The most common and important ingredients of acidic marinades are organic acid solutions (acetic acid, lactic acid, citric acid, etc.), vinegars, wine, or fruit juices (Burke, Monahan, 2003).

Drying is a method of food preservation that works by removing water from the food, which inhibits the growth of bacteria and has been practiced worldwide since ancient times to preserve food. A solar or electric food dehydrator can greatly speed the drying process and ensure more consistent results. Water is usually removed by evaporation (air drying, sun drying, smoking or wind drying). Bacteria, yeasts and moulds need the water in the food to grow, and drying effectively prevents them from surviving in the food (Bowser et al., 2009).

Hot-air drying has been to date the most common drying method employed for food materials. However, this method has many disadvantages, including poor quality of dried products, low energy efficiency and a long drying time. Microwave-vacuum drying is a novel alternative method of drying, allowing to obtain products of acceptable quality. It permits a shorter drying time and a substantial improvement in the quality of dried materials, in relation to those dried with hot air and microwaves drying method (Bondaruk et al., 2007).

Microwaves and radiofrequency waves are electromagnetic waves that are given in concrete frequency bands and both are enclosed into the dielectric heating (Knoerzer et al., 2006). Microwave energy is adequately combined with vacuum technology; it enables to keep temperatures within a low range (Yongsawatdigul, Gunasekaran, 1996). If a suitable control system is used, microwaves provide high quality products with less nutrient loss, more flavour retention, less colour change, a natural appearance (Drouzas, Schubert, 1996; Erle, Shubert, 2001).

The aim of the current research was to determine changes in physical and microbiological parameters of dried venison during storage.

Materials and Methods

The experiments were carried out at the Department of Food Technology, Latvia University of Agriculture, in Year 2013.

The object of the research

The meat of farmed red deer (*Cervus elaphus*) was obtained from a local farm Saulstari 1, located in Sigulda region, Latvia.

Components of marinade

Teriyaki sauce marinade (composition: teriyaki sauce, sweet and sour sauce, taco sauce, soy sauce, American BBQ sauce hickory, sesame oil, garlic, garlic salt, tabasco red pepper sauce) was used for venison marinating.

Meat marinating

Marinating process of the meat included the following steps:

- *Musculus longissimus lumborum* muscle from venison was manually divided into smaller pieces of the size 0.02×4.00×7.00 cm, and teriyaki sauce marinade was added. To improve the meat textural properties sodium monophosphate was added in 1.2% from total amount of marinade according to Ergezer and Gocke (2011).
- Prepared samples were marinated at 4±2 °C temperature for 48±1 h.

Meat drying and packaging

After marinating, the microwave-vacuum drier Musson-1 (OOO Ingriedient, Russia) (Vacuum microwave drier MUSSON-1, 2007) was used for venison drying.

Dry meat samples were packaged in the vacuum pouches made from polymer film (PA/PE) with barrier properties.

Dried meat storage conditions

Packaged dry meat samples were stored at dark place at 18±1 °C temperature for 112 days. Quality parameters of dry venison samples were analysed after 0, 35, 91 and 112 days of storage.

Standard methods for samples quality analysis

Microbiological parameters were analysed:

- the total plate count (TPC) of mesophilic aerobic and facultative anaerobic microorganisms according to the standard method LVS EN ISO 4833:2003
  (Ref. Nr. 01–140);
- lactic acid bacteria according to the standard method ISO 9332:2003 (Ref. Nr. 01–135).

The *pH* was measured using Jenway 3520 pH Meter (Jenway, AK). The pH electrode was dipped into a mixture of homogenized sample and distilled water (1:10), according to AACC02-52. For pH analyses, meats samples were homogenised using a household blender according to ISO 17604:2003 standard procedure.

Colour changes were determined with the *ColorTec PCM/PSM* – colorimeter (Accuracy Microsensors Inc., USA) – CIE L* a* b* system. Colour values were recorded as L* (brightness), a* (-a, greenness, +a, redness) and b* (-b, blueness, +b, yellowness).

Meat *tenderness* was evaluated by shear force using a TA. XT. plus Texture Analyser (Stabel Micro Systems, UK) equipped with a Warner-Bratzler blande.

Mathematical data processing

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

Results and Discussion

Technological parameters

During experiments optimal technological parameters for marinated meat (2.5±0.1 kg) drying in microwave-vacuum drier were developed: temperature – +36±2 °C and drying time – 80 min, one working cycle, pressure 7.5/9.3 kPa, container rotation speed – 6 rpm, the total energy input 2856 kJ kg^{-1} initial load.

Meat was dry until content of moisture in samples without and with sodium monophosphate accordingly achieved 25.08±0.66% and 34.78±0.60% corresponding to Yang et al. (2009).

Microbiological parameters

The total plate count (TPC) of mesophilic aerobic and facultative anaerobic microorganisms (CFU g^{-1}) in dry meat not regulates with European Commission regulation No. 2073/2005. Therefore for obtained data interpretation Russian sanitary and epidemiological regulations Enactment date of sanitary and epidemiological rules and regulations "Hygienic Requirements for Safety and Nutrition Value of Food Products / Sanitary Rules and Regulations (SanPin) 2.3.2.1078–01" with permissible limit as 1×10^4 CFU g^{-1} (Санитарно–эпидемиологические правила..., 2002) was used. Experimentally was detected that before drying TPC CFU g^{-1} of marinated meat without sodium monophosphate was 2.32 log CFU g^{-1} and with sodium monophosphate – 2.46 log CFU g^{-1}, obtained results was not exceed permissible level.

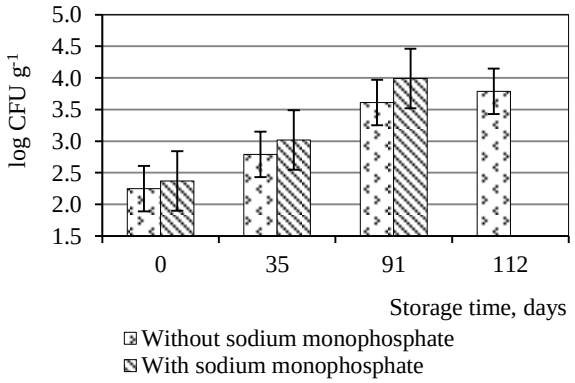

Figure 1. TPC dynamics of dried venison during storage

There are not found significant (p=0.117) changes of TPC of meat samples after drying.

Significant TPC changes of dry venison samples (Figure 1) with sodium monophosphate were indicated after 91 days storage, as a result the volume of TPC reach permissible level – 1×10^4 CFU g^{-1}, and it was 3.99 log CFU g^{-1}. Such changes could be explained by relatively higher moisture content in analysed meat samples. However in dry venison samples without sodium monophosphate TPC CFU g^{-1} reach permissible level after 112 storage days and it was – 3.79 log CFU g^{-1}. Therefore the recommendable dry venison with sodium monophosphate shelf-life

could be 91 days, but of venison without sodium monophosphate – 112 days.

The count of lactic bacteria (Fig. 2) in marinated venison without sodium monophosphate was 1.99 log CFU g^{-1}, however in samples with sodium monophosphate – 1.90 log CFU g^{-1}. After drying a count of lactic acid bacteria increases as follow in meat samples without sodium monophosphate until 2.00 log CFU g^{-1}, with sodium mono phosphate – 1.98 log CFU g^{-1} ($p>0.05$).

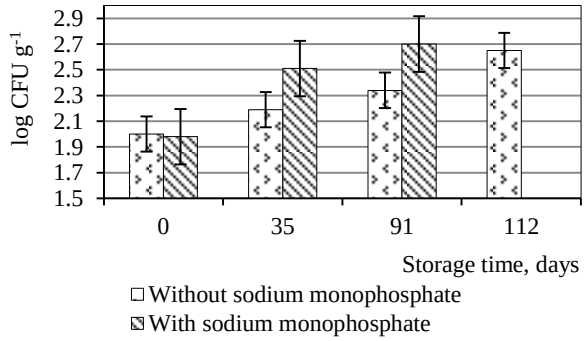

Figure 2. Lactic acid bacteria dynamics in dried venison during storage

There are not found significant differences in count of lactic acid bacteria between analysed dry venison samples during storage ($p=0.166$). From other side the higher count of lactic acid bacteria was established in dry meat with sodium monophosphate additive. Obtained results could be explained with higher moisture content in analysed dry meat samples with sodium monophosphate, that create favourable conditions for development of lactic acid bacteria. The changes of count of lactic acid bacteria very tightly connected with meat samples pH value as a result a pH value was less and the amount of lactic acid bacteria increases (Skandamis, Nychas, 2002).

Physical parameters

pH value of marinated venison without sodium monophosphate before drying was 5.14±0.06, but with sodium monophosphate – 5.32±0.04.

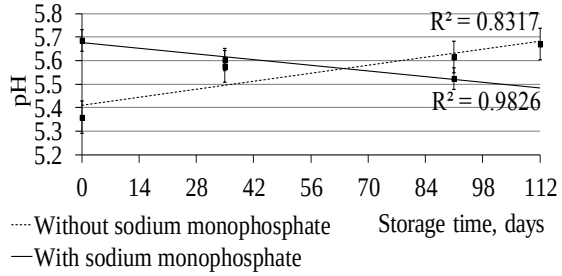

Figure. 3. Changes in pH values of dried venison during storage

In scientific literature is mentioned that in result of using of phosphate pH value of meat increase (Sheard, Tali, 2004; Murphy, Zerby, 2004).

pH changes of meat samples after drying was not significant ($p>0.05$). pH value of dry venison without sodium monophosphate was obtained as 5.36±0.01, with sodium monophosphate – 5.69±0.03. Significant differences between dry meat samples without/with sodium monophosphate in pH value was not established ($p>0.05$). An increase of count of lactic acid bacteria in meat during storage mainly depends on pH value decreasing in dry meat with sodium monophosphate.

Colour

The changes of colour parameters L*, a* and b* values of dry venison during storage are shown in Table 1.

Colour parameter L***** value of marinated meat with sodium monophosphate was higher comparing with marinated meat without sodium monophosphate; differences was not significant ($p>0.05$). L* value of marinated venison without sodium monophosphate was 30.66±1.60, but with sodium mono phosphate – 31.37±2.05. However, colour of meat stabilizes optimal pH value. Myoglobin is found in fresh meat, but after some hours of store at air ambiance or after of heat treatment process meat colour turn of brown or grey-brown, due to metmyoglobin formation. Meat colour stabilization is possible to prevent development of metmyoglobin.

Table 1

Effects of storage time on the colour values (L*, a*, b*) of dry venison

Parameter	Storage days	Dry venison meat	
		A	B
L*	0	31.05±2.71	30.42±5.17
	35	35.11±2.82	34.42±2.83
	91	36.89±2.81	34.85±3.84
	112	–	35.26±4.39
a*	0	8.45±9.68	7.99±1.51
	35	7.02±8.76	6.36±4.93
	91	6.16±7.65	5.78±8.46
	112	–	5.55±8.94
b*	0	5.03±4.64	6.19±7.30
	35	4.03±5.57	5.97±7.48
	91	3.98±1.19	5.26 ±4.40
	112	–	4.52±5.50

A – with sodium monophosphate; B – without sodium monophosphate.

After drying colour parameter L* value of meat decrease and meat samples turns darker, but obtained changes was not significant ($p>0.05$). The significant differences was established ($p<0.05$) in L* value between dry meat samples without/with sodium monophosphate in long period of storage time. Therefore, the experimental date indicates, that sodium monophosphate stabilizes colour of dry meat samples during storage.

Colour parameter a* value of marinated meat without sodium monophosphate (5.03±2.67) was lower comparing with marinated meat with sodium monophosphate (6.93±2.71), but detected differences was not significant ($p>0.05$).

Colour parameter a* values of meat samples without/with sodium monophosphate increased significantly (p=0.024; p=0.047) after drying, and it was 7.99±1.51 and 8.45±9.68 respectively. Such colour changes could be explained with moisture content decreases in meat samples after drying. As a result a colour of meat becomes more dark and red. Dry venison without sodium monophosphate color parameter a* value after 112 day of storage increase till 5.55±8.94 (significantly p=0.001), but of dry venison with sodium monophosphate a* colour parameter value after 91 day increase till 6.16±7.65, what is significant (p=0.0001). The O'Sullivan et al. (2002) indicated, that the colour parameter a* value usually was tightly connected with content of oxymyoglobin in meat. Therefore it is possible to conclude, that after heat treatment due to metmyoglobin formation colour parameter a* value decreases in meat. As a result meat colour turn of brown or grey-brown.

Colour parameter b* value of marinated venison without sodium monophosphate was obtained as 15.77±5.96, with sodium mono phosphate – 14.79±7.10 (p>0.05). After drying of marinated venison samples without/with sodium monophosphate colour parameter b* value decrease significantly (p=0.006; p=0.021) respectively, but differences between samples was not significant (p>0.05). b* colour parameter value of dry venison without sodium monophosphate was 6.19±7.30, with sodium monophosphate – 5.03±4.64. b* colour parameter value decrease significantly (p=0.0001) in dry venison samples without sodium monophosphate and in samples with sodium monophosphate (p=0.009) during storage (Table 1). After mathematical data processing significant differences was established between analysed samples without/with sodium monophosphate additive (p<0.05) during storage. It is necessary to indicate, according to the Franco et al., (2012) in scientific literature was not found explanation about b* colour parameter value changes in meat during storage.

Tenderness (shear force)

Experimentally it was detected that marinated meat with sodium monophosphate additive was softer then marinated meat without sodium monophosphate (p<0.05). Results indicated that marinated venison without sodium monophosphate tenderness was 23.42±6.72 N, but with sodium mono phosphate – 16.20±1.13 N or 1.45 times less.

Table 2

Effects of storage time on tenderness of dry venison

Parameter	Storage days	Dry venison meat	
		A	B
Shear force, N	0	95.33±9.46	120.77±7.71
	35	112.33±2.27	155.34±6.15
	91	151.61±4.54	292.63±9.65
	112	–	315.91±5.77

A – with sodium monophosphate; B – without sodium monophosphate.

After drying tenderness of meat samples increase significantly (p<0.05), because a content of moisture increase and finally structure mechanical properties of dried meat changed. Tenderness of dry venison without sodium monophosphate was 120.77±7.71 N, whereas with sodium monophosphate – 95.33±9.46 N (Table 2). During research it was proved that tenderness of meat samples with/without sodium monophosphate additive significantly increase (p<0.05) during storage. However dry meat samples with sodium monophosphate additive was softer (p>0.05), because it's higher pH value.

Conclusions

Experimentally it was ascertained, that the shelf-life of dried venison, packaged in vacuum pouches made from polymer film (PA/PE) with barrier properties and stored at a temperature +18±1 °C in the dark place, with sodium monophosphate additive could be 91 day and without sodium monophosphate additive – 112 days.

There are not found significant differences in pH values of analyses dried meat sample during storage comparing with the parameters of just dried meat.

Significant differences were detected in meat color and tenderness changes during meat storage. Dried meat color stability and structure mechanical properties possible provide by adding of sodium monophosphate at marinade.

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References

1. Bondaruk J., Markovski M., Błaszczak W. (2007) Effect of Drying Conditions on the Quality of Vacuum-Microwave Dried Potato Cubes. *Journal of Food Engineering*, Vol. 81, p. 306–312.
2. Bowser T.J., Frazier R.S., Weckler P.R., Kowalski S.J. (2009) Optimizing jerky drying time with minimal product impact. *The Open Food Science Journal*, Vol. 3, p. 79–83.
3. Burke R. M., Monahan F. J. (2003) The tenderisation of shin beef using a citrus juice marinade. *Meat Science*, No. 63, p. 161–168.
4. Drouzas A., Shubert H., (1996) Microwave application in vacuum drying of fruits. *Journal of Food Engineering*, Vol. 28, p. 203–209.
5. Ergezer H., Gocke R. (2011) Comparison of Marinating with Two Different Types of Marinade on Some Quality and Sensory Characteristics of Turkey Breast Meat. *Journal of Animal and Veterinary Advances*, Vol. 10, No. 1, p. 60–67.

6. Erle U., Shubert H. (2001) Combined osmotic and Microwave-Vacuum Dehydration of Apples and Strawberries. *Food Engineering*, Vol. 49, No.2, p. 193–199.
7. Franco D., Gonzalez L., Bispo E., Latorre A., Moreno T., Sineiro J., Sanchez M., Nunez M.J. (2012) Effects of calf diet, antioxidants, packaging type and storage time on beef steak storage. *Meat Science*, Vol. 90, p. 871–880.
8. Kargiotou C., Katsanidis E., Rhoades J., Kontominas M., Koutsoumanis K. (2011) Efficacies of soy sauce and wine base marinades for controlling spoilage of raw beef. *International Journal of Food Microbiology*, No. 28, p. 158–163.
9. Knoerzer K., Regier M., Schubert H. (2006) Microwave Heating: a New Approach of Simulation and Validation. *Chemical Engineering and Technology*, Vol. 9, No.7, p. 796–801.
10. Murphy M.A., Zerby H.N. (2004) Prerigor infusion of lamb with sodium chloride, phosphate, and dextrose solutions to improve tenderness. *Meat Science*, Vol. 66, p. 343–349.
11. O'Sullivan A., O'Sullivan K., Galvin K., Moloney A.P., Troy D.J., Kerry J.P. (2002) Grass silage versus maize silage effects on retail packaged beef quality. *Journal of Animal Science*, Vol. 80, p. 1556–1563.
12. Pathania A., McKee S. R., Bilgili S. F., Singh M. (2010) Antimicrobial activity of commercial marinades against multiple strains of *Salmonella* spp. *International Journal of Food Microbiology*, No. 139, p. 214–217.
13. Sheard P.R., Tali A. (2004) Injection of salt, tripolyphosphate and bicarbonate marinade solutions to improve the yield and tenderness of cooked pork loin. *Meat Science*, Vol. 68, p. 305–311.
14. Skandamis P.N., Nychas G.J.E. (2002) Preservation of fresh meat with active and modified atmosphere packaging conditions. *International Journal of Food Microbiology*, Vol. 79, p. 35−45.
15. Vacuum microwave drier MUSSON-1 (2007) Technical specification. Sankt-Petersburg, p.49 (in Russian).
16. Vergara H., Gallego L., García A., Landete-Casillejos T. (2003) Conservation of *Cervus elaphus* meat in modified atmospheres. *Meat Science*, No. 65, p. 779–783.
17. Yang H.S., Hwang Y.H., Joo S.T., Park G.B. (2009) The physicochemical and microbiological characteristics of pork jerky in comparison to beef jerky. *Meat Science*, Vol. 82, p. 289−294.
18. Yongsawatdigul J., Gunasekaran S., (1996) Microwave-vacuum drying of cranberries: Part 2. Quality evaluation. *Food Processing and Preservation*, Vol. 20, p. 145–156.
19. Санитарно–эпидемиологические правила и нормативы „Гигиенические требования к безопасности и пищевой ценности пищевых продуктов, СанПин 2.3.2.1078–01" (01.07.2002) / Enactment date of sanitary and epidemiological rules and regulations "Hygienic Requirements for Safety and Nutrition Value of Food Products. Sanitary Rules and Regulations (SanPin) 2.3.2.1078–01" (01.07.2002).

THE DIFFERENT PROTEIN SOURCES FEEDING IMPACT ON THE QUALITY OF PORK

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Abstract

The study was conducted to determine the quality of pork after feeding soybean meal and peas as protein sources to fattening pigs. The research was done in two pig farms in Latvia and four fattening pig groups were conducted (two in each holding). In holdings one pig group was soybean meal and the other peas group with 10 animals each. The pig groups received different amount of the protein feeds (soybean meal 15%, peas 15% and 28%) blended into a mixture of concentrated feed. The results showed that 15 and 28% inclusion of pea in finisher pig diets about 3–5% increase LWG. The higher pig ADG resulted on the increasing of fat in the body at the similar FC per 1 kg LWG. The IF about 0.68 and 0.48 kg were more in peas pig groups with significant differences ($p<0.001$) between soybean pig groups. For pigs, which fed peas, average backfat were of 14.3 mm, 2 and 1.3 mm more than the one of pigs in soybean groups. There were no differences of ash content and pH in *Longissimus dorsi* muscle, but moisture, fat, CP and cholesterol content were significantly different ($p<0.05$; $p<0.01$; $p<0.001$) between soybean and peas groups. Fat content in *Longissimus dorsi* muscle was 2.5 till 2.8% higher ($p<0.01$) for peas group pigs. The fatty acid content were not high, but were significantly ($p<0.001$, $p<0.01$, $p<0.05$) different with pigs of soybean groups. The highest values of nonessential amino acids were measured in glutamic (3.09–3.54 g). The lowest value were found out in tryptophane (0.245 g) and proline (0.37 g) in soybean group, but in peas groups the content of proline were (0.772–0.779 g) and was more twice times higher than tryptophan.

Keywords: pig, peas, nutrition, amino acid, pork.

Introduction

The main feed for pigs are cereal grains which commonly used as feed in industrial pig production. The cereal grains contain insufficient quantities of several of the indispensable amino acids such as lysine, threonine and sulfur containing amino acids (Sauer et al., 1977). Therefore, it is essential that the pigs' diet contain a supplementary source of these limiting amino acids. Soybean meal is often used as the main supplement of amino acids in pig feed (Jezierny et al., 2010). In temperate environments, soybeans are difficult to cultivate and the pig industry relies heavily on imported soybean meal. However, as the transportation cost for feed increases, pig producers will have to maximize the use of locally produced feedstuffs. Therefore, it is important that alternative sources of supplementary protein be developed. In temperate environments, the one crop which is potential for use in pig diets is the peas. Field peas (*Pisum sativum* L.) have been grown for centuries in many parts of the world. Historically, field peas have been produced mainly for human consumption, but during the last years, the industry has also found markets for field peas in pig feeding.

Because the goal of this study was to evaluate the effect of including different levels of pea in pig diets on performance of finisher pigs, carcass and meat quality.

Materials and Methods

Animals and housing. The research was done in two pig farms in Latvia. The four fattening pig groups were conducted (two in each holding), according of pig origin, age and liveweight. In holdings one pig group was (1 and 2) soybean meal and the other (1 and 2) peas group, with 10 animals each. The selected pigs were crossbreed – Large White×Landrace in both farms. Finisher pigs were housed on concrete floors with shavings and access to drinking water at all times. Each pig was allowed access to its individual feeder for 30 min twice daily. The trial was run for 50–54 days (FD) and concluded when the pigs reached an average weight of 94–113 kg.

Diets and Performance Measures. Commercial sources of pea (variety 'Respect') and soybean meal were obtained for the experiment. The soybean meal groups in both holdings received soybean meal 15% as alone protein feed in diet, but peas' groups pigs received 28% peas in first holding and 15% peas in second holding, for check out the peas amount influencing to pork quality. The protein feeds were blended into a mixture of concentrated feed. The other ingredients in diets were kept constant for all groups and included barley, wheat, triticale and trace element-vitamin premix. The pig feed mixture was prepared without crystalline amino acids. The diets were formulated to be isoenergetic for metabolize energy (ME), with the same crude protein (CP), Lysine (Lys) content (calculated for Feed Catalog), and to meet the minimum requirements of Ca and P by modifying the inclusion macroelements and vitamins (Table 1). Analysis of feed samples for dry matter, crude protein, crude fiber, fat, ash, Ca and P were determined in scientific laboratory of agronomik analysis of Latvia using such methods: for dry matter (DM) – forage analyses, USA, met. 2.2.1.1:1993, crude protein (CP) LVS EN ISO 5983-2:2009, crude fiber (CF) ISO5498:1981, fat ISO6492:1989, ash ISO 5984:2002/Cor1:2005, Ca LVS EN ISO 6869:2002, P ISO 6491:1998.

Individual body weight, feed consumption (FC) was recorded on a weekly basis.

Slaughter and carcass Quality Measurements. Pigs were slaugtered at a commercial slaughterhouse via

electrical stunning, followed by exsanguinations, and carcases were dehaired via scalding, eviscerated, and split vertically down the midline. Hot carcass weights were obtained and backfat depth was measured at a specific site [i.e., the head of the last rib, 6 cm from the mid back line, using a probe (Introscope Optimal Probe)]. The length of carcass (CL) was measured in a straight line from the forward edge of the first rib to the forward edge of the aitch bone and muscle-eye (LM) area with the planimeter. The internal fat (IF) was removed from carcass and weighed, the loin muscle (LMW) also was removed without fat from the left side of each group 3 pig carcases and weighed. Left side of carcass was devided into fractions for determination ham (HW), bone (BW) and meat weight (MW). The samples of meat were taken from the musculus *longissimus lumborum et thoracis* 24 hours post mortem and subsequently subjected to the chemical analysis. Chemical content of pork analyzed for such indices: fat content (LVS ISO 1443:1973), moisture (LVS ISO 1442:1997), crude protein content (CP) (LVS ISO937:1978), ash (ISO 936:1998), pH (LVS ISO 2917:2004), cholesterol (BIOR-T-012-132-2011), unsaturated fatty acids–alfa – linolenic (C18:3), arachidonic (C20:4), eicosapentaenoic (C20:5), linoleic (C18:2), oleic (C18:1) and palmitoleic (C16:1) (BIOR-T-012-131-2011). Amino acid composition of the muscle was analyzed with methods ISO13903:2005, but tryptophan content by method EN ISO 13904, HPLC. The content of each individual amino acid was calculated on g 100 g of wet matter basis.

Statistical Analysis. The data were processed with MS Excel mathematical program. The results were compared using t-test. t-test was carried out on the data for growth performance and meat quality.

Results and Discussion

Concentrated feed mixtures of chemical analyzes showed that the pigs received a full and balanced feed (Table 1). Based on fattening pig nutrition standards (NRC, 2012) the necessary crude protein is 14–16% with the lysine content 0.5–0.8% of the dry matter, depending on the liveweight (LW).

Table 1

The chemical content of feed in dry matter

Indices	First holding		Second holding	
	1 soybean group	1 peas group	2 soybean group	2 peas group
DM, %	88.89	88.00	88.02	87.47
ME MJ, kg	13.30	13.40	13.50	13.20
CP ,%	15.27	14.62	15.38	15.00
Lys, %	0.77	0.73	0.78	0.68
CF, %	4.36	4.67	3.46	3.71
Fat, %	1.74	1.67	2.43	1.95
Ash, %	5.57	4.89	4.32	2.76
Ca, %	0.90	0.79	0.63	0.62
P, %	0.59	0.56	0.49	0.46

Pig fattening results showed that pigs growth intensity was high in all study groups of pigs (Table 2).

The average daily liveweight gain (ADG) for the pigs were from 850±7.48 till 915±10.4 g, slightly higher in peas groups.

Although soybean meal protein digestibility is higher (87%) than pea protein (only 79%) (Jezierny et al., 2011), but about 5% higher and significantly different ($p<0.05$) live weight gain (LWG) showed the pig group in the second holding, which fed in the feed 15% peas, and in the first holding pig daily weight gain (28% peas in feed) was 3% higher compared to the soybean group (Table 2).

Table 2

Pig fattening results (n=40)

Results	First holding		Second holding	
	1 soybean group	1 peas group	2 second soybean group	2 peas group
Start LW, kg	46.6±0.43	46.3±0.42	68.0±0.76	68.4±0.45
End LW, kg	94.5±0.36	95.7±0.67	110.5±0.9[a]	113±0.74[a]
LWG, kg	47.9±0.77	49.4±0.95	42.5±0.64	44.6±0.71
% LWG	100	103	100	105
FD	54	54	50	50
ADG, g	887±21.8	915±10.4	850±7.48[b]	892±8.42[b]
FC per 1 kg LWG, kg	2.88	2.98	2.85	2.9

Values are presented as mean ±standard error. a,b $p<0.05$

For pigs the most important amino acid is lysine, which is necessary for building muscle tissue (Guoyao Wul et al., 2013). The digestibility of most amino acids in field peas is similar to that in soybean meal, but pea protein has relatively low concentration of methionine, cysteine and tryptophan. It is necessary to pay attention to the concentrations of these amino acids and often need to include crystalline sources. In our trial was not determined these amino acids in the pig diets because the higher pig daily gain may resulted on the increasing of fat in the body at the similar feed consumption per 1 kg liveweight gain. Results obtained for pigs confirm results from previous research demonstrating that there are no negative effects of including 36% field peas in diets fed to finishing pigs (Stein et al., 2004). Inclusion of 30% field peas is also was reported not to comprimise pig performance. The researchers results showed that even higher inclusion rates of peas may be used (66%) and that peas can substitute all the soybean meal in diets without negatively affecting performance and feed intake (Stein et al., 2006), in addition formulated of the digestible indispensable amino acids in pig diets is obligatory. The other research showed that including pea or faba bean in finisher pigs' diets

from 7.5–30% slightly reduced finisher daily gain, as tended to reduce over initial levels only (Smith et.al, 2013).

Carcass indicators show a tendency to accumulate more fat tissue in pigs, which fed pea in the feed. The internal fat about 0.68 and 0.48 kg were more in pea groups with significant differences ($p<0.001$) between soybean pig groups (Table 3). The significant differences was found also of meat weight in both holdings ($p<0.05$ and $p<0.001$), of muscle-eye area in first holding and of ham weight in second holding ($p<0.05$). The pigs which fed peas had a little smaller loin muscle areas (LM).

Table 3

Carcass traits (n=12)

Indices	First holding		Second holding	
	1 soybean group	1 peas group	2 soybean group	2 peas group
CW, kg	72.2±0.35	82.3±0.52	89.8±0.61	90.1±0.69
CL, cm	107±0.58	108.7±0.88	109±0.58	108.3±0.33
FT, mm	12.3±0.88	14.3±1.2	13±0.57	14.3±0.33
LM, cm^2	52.5±0.68[a]	50.5±0.21[a]	51.3±0.16	50.3±0.88
HW, kg	10.6±0.19	10.7±0.06	11.5±0.29[b]	12.5±0.19[b]
IF, kg	1.32±0.01[c]	2±0.05[c]	1.85±0.03[d]	2.33±0.04[d]
LMW, kg	2.88±0.04	2.89±0.09	3.22±0.02	3.23±0.04
BW, kg	9.9±0.06	13±0.05	13.4±0.12	10.8±0.15
MW, kg	62.3±0.23[e]	69.3±0.58[e]	76.4±1.21[f]	79.2±0.41[f]

Values are presented as mean ±standard error. a,b,f $p<0.05$ c,d,e $p<0.001$

For pigs, which fed peas, average backfat were of 14.3 mm, which is 2 and 1.3 mm more than the of soybean groups pigs. The results of the carcass traits of this experiment confirm previous studies. It has been reported from European studies that the lean meat is reduced and the backfat thickness is increased as the concentrations of dietary peas are increased (Stein et al., 2006). However, it was also demonstrated that this situation may be amelioreted by including crystalline Met and Trp in the diets. In other research showed that inclusion of 35% peas as a substitute for soybean meal had no detrimental effects on nutrient digestibility, pig performance and carcass traits (Castell et al., 1988), but in trial of researchers Gatel and Grosjan, (1990), the use of pea greater than 20% as alternative protein source in pig diets reduced performance.

There were no differences of ash content and pH in *Longissimus dorsi* muscle, but moisture, fat, crude protein and cholesterol content were significantly different between soybean and peas groups (Table 4). Fat content in *Longissimus dorsi* muscle was 2.5 till 2.8% higher ($p<0.01$) for peas group pigs than of soybean pig groups.

The similar results was found in research were peas included from 36–66% in pig groups diets, the pH and marbling of the *Longissimus dorsi* muscle and 10th rib bakcfat thickness were not influenced by treatment (Stein et al., 2006.

Interest in meat fatty acid composition stems mainly from the need to produce healthier meat, i.e., meat with higher ratio of polyunsatured (PUFA) to saturated fatty acids and a more favourable balance between n-6 and n-3 PUFA. In pigs, the drive has been to increase n-3 PUFA in meat and this can be achieved by feeding sources.

Table 4

Characteristics of *Longissimus dorsi* muscle (n=12)

Indices	Pig groups		
	1, 2 soubean groups	1 peas group	2 peas group
Moisture, %	72.3±0.47[a,e]	70.1±0.43[a]	69.2±0.55[e]
Fat, %	4.4±0.42[b,d]	6.9±0.21[b]	7.2±0.06[d]
CP, %	24.2±0.13[c,f]	22.9±0.35[c]	22.1±0.15[f]
Ash, %	1.27±0.09	1.2±0.06	1.13±0.03
pH	5.45±0.02	5.39±0.01	5.24±0.01
Cholesterol, mg 100 g	108.4±0.29[g,k]	105.0±0.54[g]	81.3±2.28[k]

Values are presented as mean ±standard error. a,e,c $p<0.05$; b,d,f,g $p<0.01$; k $p<0.001$

Only when concentration of alfa-linolenic (C18:3) approch 3% of neitral lipids or phospholipids are there any adverse effects on meat quality and flavour (Wood el al., 2003). Several papers have examined the effects of dietary oils containing a high propotion of 18:2 on the acid composition and quality of pigmeat, for example soya oil maize and sunflower, but not a peas. In present research the fatty acid content were not high, but were significantly ($p<0.001$, $p<0.01$, $p<0.05$) different with pigs of soybean groups (Table 5). High levels of PUFA are undesirable in pork, because they adversely affect consistency, storage stability and texture of the processed pork products.

The amino acids content was determined only in one sample of muscle from each pig group because the price of analyses was very high. The measured values of selected amino acids with different feeding in the groups are shown in Table 6.

Table 5

Unsaturated fatty acids content (% of total fatty acid methyl esters) in *Longissimus dorsi* muscle (n=12)

Fatty acids	Pig groups		
	1, 2 soybean group	1 peas group	2 peas group
C18:3	0.34±0.041	0.41±0.001	0.39±0.001
C20:4	0.3±0.001[a]	0.18±0.005	0.2±0.005[a]
C20:5	0.68±0.044	0.71±0.012	0.67±0.037
C18:2	4.3±0.058[c,b]	4.07±0.033[b]	3.93±0.033[c]
C18:1	46.8±0.31[d,e]	43.3±0.32[d]	40.2±0.38[e]
C16:1	3.16±0.09[f]	3.27±0.12	3.57±0.03[f]

Values are presented as mean ±standard error. b,f $p<0.05$; c,d $p<0.01$; a,e $p<0.001$

Table 6

Amino acid composition in *Longissimus dorsi* muscle (g 100 g^{-1})

Amino acids	Pig groups		
	1, 2 soybean groups	1 peas group	2 peas group
Tryptophan	0.274	0.245	0.268
Alanine	1.230	0.960	1.180
Arginine	1.320	0.870	1.270
Asparagine	1.990	1.310	1.950
Fenilalanine	0.921	0.670	0.833
Glicine	0.893	0.680	0.884
Glutamine	3.540	2.360	3.090
Histidine	0.898	0.850	0.879
Izoleicine	0.987	0.420	0.973
Leucine	1.700	1.190	1.650
Lysine	1.980	1.210	1.850
Proline	0.370	0.779	0.772
Serine	0.856	0.590	0.849
Tirozine	0.745	0.650	0.711
Treonine	0.997	0.620	0.983
Valine	1.040	0.450	1.010

The highest values in the group of nonessential amino acids were measured in glutamic (3.09–3.54 g), aspartic acid (1.31–1.99%) and leucine (1.19–1.70 g). The content of lysine was from 1.21 till 1.98 g. The lowest value were found out in the amino acids tryptophane (0.245–0.274 g) and proline (0.37 g) in soybean group, but in peas groups the content of proline were (0.772–0.779 g) and was more twice times higher than tryptophan. The biological value of meat is defined by fully valuable protein (Jukna et al., 2005). The full value of meat protein is calculating according to the relationship of irreplaceable amino acid tryptophan, which is found only in muscle tissue, and replaceable amino acid hydroxyproline, found only in connective tissue. Higher ratio shows more biologically valuable meat protein. The ratio tryptophan: hydroxyproline in pork was 7.9 (Ribikauskiene, 2003), but data in Table 6 evidently demonstrate that peas groups pork contained more proline than tryptophan.

Conclusions

The study indicates that 15 and 28% inclusion of pea in finisher pig diets about 3–5% increase liveweight gain. The higher pig daily gain resulted on the increasing of fat in the body at the similar feed consumption per 1 kg liveweight gain.

Carcass indicators show a tendency to accumulate more fat tissue in pigs, which fed pea in the feed. The internal fat about 0.68 and 0.48 kg were more with significant differences ($p<0.001$) between soybean pig groups. For pigs, which fed peas, average backfat were of 14.3 mm, 2 and 1.3 mm more than the pigs in soybean groups.

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The peas may replace soybean meal in diets fed to finishing pigs without negatively affecting pig performance that provided diets are balanced for concentrations of digestible indispensable amino acids.

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References

1. Castell A.G., Neden L. R. and Mount K. (1988) Potential of field pea (*Pisum sativum*) screenings as feed for market pigs. *Canadian Journal Animal Sciences*, Vol. 68, No. 3, p. 577–579.
2. Gatel F., Grosjean F. (1990). Composition and nutritive value of peas for pigs: a review of European results. *Livestock Production Science*, Vol. 26, Iss. 3, p. 155–175.
3. Guoyao Wu1, Fuller W. Bazer, Zhenlong Wu, Zhaolai Dai, Junjun Wang, Weiwei Wang, Bin Wang (2013). Dietary requirements for "nutritionally nonessential amino acids "by animals. **In***: Proceedings of the 11th World Conference on Animal Production*, China, Beijing, p. 71.

4. Jezierny D., Mosenthin R., Sauer N., Roth S., Piepho H.P., Rademacher M., Eklund M. (2011). Chemical composition and standardisal ileal digestibilities of crude protein and amino acids in grain legumes for growing pigs. *Livestock Science* Vol. 138, Issue 3, p. 229–243.
5. Jukna V., Maurucaite G., Kriksciunaite J., Rekstys V. (2005) Meat quality of Lithuanian white pigs in comparison to imported pig breeds. *Veterinarija ir Zootechnika*, Vol. 30, No. 52, p. 47–49.
6. NRC (2012). *Nutrient requirement of swine 11th edition*. Washington D.C.: National Academy Press, 400 p.
7. Ribikauskiene D. (2003) The influence of different breeds on the meat quality of crossbred pigs. **In**: *Procceedings of the 9th Baltic Animal Breeding Conference*, Sigulda, Latvia, p. 84–86.
8. Sauer W.C., Stothers S.C., Phillips G.D. (1977) Apparent availabilities of amino acids in corn, wheat and barley for growing pigs. *Canadian Journal Animal Sciences*, Vol. 57, No. 3, p. 585–597.
9. Smith L. A., Houdijk J.G.M., Homer D., Kyriazakis I. (2013) Effects of dietary inclusion of pea and faba bean as are placement for soybean meal on grower and finisher pig performance and carcass quality. *Journal of Animal Science*, Vol. 91, no 8, p. 3733–3741.
10. Stein H.H., Benzoni G., Bohlke R.A., Peters D.N. (2004) Assesmentof the feeding value of South Dakota grown peas (*Pisum sativum*) for growing pigs *Journal Animal Sciences*, Vol. 82, No. 9, p. 2568–2578.
11. Stein H.H., Everts A.K., Sweter K.K., Peters D.N.,Mardock R.I., Wulf D.M., Pederson C. (2006) The influence of dietary field peas (*Pisum sativum*) on pig performance carcass quality and the palability of pork. *Journal Animal Sciences, Vol.* 84, No. 11, p. 3110–3117.
12. Wood J.D., Richardson R.I., Nute G.R., Fisher A.V., Campo M.M., Kasapidou E., Sheard P.R., Enser M. (2003) Effects of fatty acids on meat quality: rewiew. *Meat Science*, Vol. 66, Iss. 1, p. 21–32.

ANTIMICROBIAL SUSCEPTIBILITY OF OXACILLIN-RESISTANT *STAPHYLOCOCCUS* SPP. ISOLATED FROM POULTRY PRODUCTS

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Abstract

The aim of this study was to investigate the prevalence of staphylococci in raw poultry products intended for human consumption and to determine antimicrobial susceptibility, particularly of oxacillin-resistant isolates. Poultry (chicken) liver as representative samples were randomly selected in different retail markets. Isolation of staphylococci was performed using general and selective nutrient media including Mannitol Salt Agar supplemented with cefoxitin as well as Brilliance MRSA2 Agar. Antimicrobial susceptibility testing was carried out using "Sensititre" plates for determination of minimal inhibitory concentrations. Interpretation of results was performed according to CLSI standard. Polymerase chain reaction was used for determination of *mec* genes.

Fifty samples from 70 tested were positive for *Staphylococcus* spp. (71%). In 30% of the isolated staphylococci, the growth was observed on selective media with cefoxitin as well as on MRSA2 Agar. The isolates from those samples were resistant to oxacillin (MIC≥0.5 mg mL^{-1}) however, the only one isolate harboured the *mec*A gene. All of the isolates with phenotypic resistance to oxacillin were susceptible to vancomycin, gentamicin, linezolid, daptomicin, rifampin and quinupristin/dalfopristin. The resistances of those isolates were observed to tetracycline (60%), erythromycin (40%), and fluoroquinolones (40%). The species of oxacillin-resistant staphylococci included *S. hyicus* (6), *S. hominis* (4), *S. haemolyticus* (1), *S. cohnii* (1), *S. lentus* (1), *S. warneri* (1) and *S. intermedius* (1). Oxacillin-resistant *Staphylococcus aureus* was not found. The obtained results demonstrated low correlation between phenotypic resistance to oxacillin and the presence of *mec* genes in staphylococcal isolates from the poultry products. Further studies need to be performed for investigation of this unusual finding.

Keywords: *Staphylococcus*, *mec*A, resistance, oxacillin, poultry.

Introduction

Staphylococcus spp. are significant bacteria in the etiology of avian diseases and may thus contaminate foods as a result of processed carcasses (Mead, Dodd, 1990; Pepe et al., 2006). Although enterotoxin-producing *S. aureus* is the most common cause of food-borne human illness throughout the world (Do Carmo et al., 2004; LeLoir et al., 2003) the other species such as *S. hyicus*, *S. sciuri*, *S. xylosus* or *S. cohnii* are also important, particularly because of carriage the genes encoding antimicrobial resistance (Aarestrup et al., 2000; Kawano et al., 1996). While staphylococci commonly occur on the skin and nasopharynx of healthy poultry (Mead, Dodd, 1990), they can survive, colonize, and persist at various processing stages in commercial poultry processing plants due to the expression of various key properties, including adhesion (Chaffey, Waites, 1987). Staphylococci are one of the most predominant groups during the slaughtering and processing of poultry, and they have been recovered from air samples (Ellerbroek, 1997), neck skin of chicken carcasses (Georgnaras et al., 1995; Olivier et al., 1996), and machinery surfaces (Mead et al., 1995; Huys et al., 2005). By this reason contaminated poultry products could be the source of possible transmission of different staphylococci species including resistant strains to humans, during food processing at home. The aim of this study was to investigate the prevalence of staphylococci in raw poultry products intended for human consumption, to determine species distribution and antimicrobial susceptibility, particularly of oxacillin-resistant isolates.

Materials and Methods

In 2013 seventy samples of raw poultry liver as representative samples intended for human consumption were randomly selected on different retail markets in Lithuania. The samples were delivered to the laboratory during 1-3 hours. Material was soaked into technically sterile plastic bags containing sterile Tryptone-Soya Broth (Oxoid, Thermo Fisher). Sterile cotton swabs were used for inoculation of outwash onto Sheep Blood Agar, Mannitol Salt Agar (Liofilchem, Italy), Mannitol Salt Agar supplemented with 4 mg L^{-1} cefoxitin (Sigma-Aldrich), Brilliance MRSA 2 Agar (Oxoid, Thermo Scientific) as well as on Contrast MRSA Broth (Oxoid, Thermo Scientific). Staphylococci up to the genus level were identified according to morphology characteristics, catalase production, gram-staining, susceptibility to furazolidone. PCR was used for identification of genus specific (16S) subunit. DNA material for molecular testing was obtained after bacterial lysis according to the extraction protocol prepared by the Community Reference Laboratory for Antimicrobial Resistance (Anonymous, 2009) with slight modifications. Briefly, bacterial colonies were taken with a bacteriological loop from the surface of Mueller Hinton Agar and transferred to phosphate buffered saline (pH 7.3). The content was centrifuged for 5 min at 13 000 rpm. Then the supernatant was discarded and the pellet was re-suspended in Tris-EDTA (TE) buffer. The suspension was heated using thermomixer (Biosan) in 100 °C degrees for 10 minutes. Boiled suspension was transferred directly on ice and diluted by 1:10 in TE.

Species identification was performed according to pigment and coagulase production, presence of protein

A and clumping factor as well as on biochemical properties using RapID Staph Plus (Thermo Scientific) identification system. In complicated cases species determination was performed by16S rRNA sequencing using ABI3730XL sequencer. The primers for obtaining of 16S rRNA product (1405 bp) as well as for sequencing were used as follows: forward - 5' GCTCAGGA(CT)GAACGCTGG 3' and the reverse - 5' AGACGATCCWTCAGTGAGC 3' (Couto, 2014). Sequences were analysed using Molecular Evolutionary Genetic Analysis software (MEGA, version 6). Basic local alignment search tool (BLAST) was used for comparison of obtained sequences with sequences presented in the database of National Center of Biotechnology Information.

Antimicrobial susceptibility testing was performed using the broth microdilution method. Sensititre® plates and ARIS 2X automated system (Thermo Scientific) were used with the following antimicrobials: daptomycin, ciprofloxacin, clindamycin, erythromycin, gentamicin, levofloxacin, linezolid, oxacillin, penicillin, co-trimoxazole, quinupristin/dalfopristin and rifampin. Interpretation of results was carried-out using manufacturers software (SWIN®) adapted to clinical breakpoints of Clinical and Laboratory Standards Institute (CLSI). The quality control strain *S. aureus* ATCC 29213 was included in each assay for validation purposes. Detection of *mec*A and *mec*A_{LGA251} (*mec*C) genes was performed by PCR described previously (Cuny et al., 2011; Anonymous, 2009).

Statistical analysis was performed using Microsoft Excel software. Comparison between categorical variables was calculated by chi-square and Fisher's exact test. Results were considered statistically significant if $p<0.05$.

Results and Discussion

Fifty samples from 70 tested were positive for *Staphylococcus* spp. (71%). In 30% of isolated staphylococci the growth was observed on Mannitol Salt Agar supplemented with cefoxitin as well as on MRSA2 Agar and/or Contrast MRSA Broth. The isolates from those samples were resistant to oxacillin (MIC≥0.5 mg L^{-1}) however the only one isolate harboured the *mec*A gene. All of the isolates phenotypically resistant to oxacillin were susceptible to vancomycin, gentamicin, rifampin, linezolid, daptomycin and quinupristin/dalfopristin. 40% of the isolates demonstrated resistance to at least one fluoroquinolone and erythromycin – the antimicrobials that are treated as critically important for humans. Attention should be paid to this unfavourable finding although it is in coincidence with our previous studies, where high frequency of resistance in other bacterial species (enterococci and *Escherichia coli*) isolated from poultry products has been detected toward fluoroquinolones and/or macrolides (Ružauskas et al., 2010a; Ružauskas et al., 2010b). Resistance to tetracycline was frequent and reached 60%. There are different data associated with *Staphylococcus* spp. isolated from poultry according to the resistance to tetracycline. For example, Shareef et al. (2009) found that 100% of the isolated *S. aureus* from layers were susceptible to tetracycline, while Jevinova et al., (2009) found that 22% of the *Staphylococcus* spp. from poultry meat demonstrated resistance to this antibiotic.

The distributions of minimal inhibitory concentration (MIC) of the oxacillin-resistant isolates are presented in Table 1.

Table 1

MIC distributions among the isolates (n=15)

	MIC distributions (%) (mg L^{-1})								
	0.12	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**
CIP			67	0	0	33			
CLI	47	20	7	13	7	7			
DAP	7	73	20	7	0				
ERY		0	60	0	0	0	40		
GAT				74	13	13	0	0	
GEN					100	0	0	0	0
LEV		47	20	0	7	13	13	0	
LZD				13	40	47	0	0	
OXA		0	53	33	7	0	7	0	
PEN	40	27	20	13	0	0	0	0	
SYN	0	13	47	20	20	0	0		
RIF			100	0	0	0	0		
TET					33	7	0	60	0
SXT			86	7	7	0	0		
VAN				93	7	0	0	0	0

Grey cells – susceptible; white cells – intermediate; dark cells – resistant

CIP – ciprofloxacin; CLI – clindamycin; DAP – daptomycin; ERY – erythromycin; GAT – gatifloxacin; GEN – gentamicin; LEV – levofloxacin; LZD – linezolid; OXA – oxacillin; PEN – penicillin; SYN – quinupristin/dalfopristin; RIF – rifampin; TET – tetracycline; SXT – co-trmoxazole; VAN – vancomycin.

The species of oxacillin-resistant staphylococci included *S. hyicus* (6), *S. hominis* (4), *S. haemolyticus* (1), *S. cohnii* (1), *S. lentus* (1), *S. warneri* (1) and *S. intermedius* (1). Oxacillin-resistant *Staphylococcus aureus* was not found. Identification of the aforementioned species using biochemical testing was reliable only for 60% of the isolates. For remaining cases sequencing of 16S rRNA was necessary for taxonomic verification.

The obtained results demonstrated low correlation ($p>0.05$) between phenotypic resistance to oxacillin and the presence of *mec*A gene in staphylococcal isolates from the poultry products. Recently Ba with co-authors (2014) found the similar findings in phenotypically oxacillin-resistant isolates of *Staphylococcus aureus*. Those strains didn't carried neither *mec*A nor *mec*C genes. The authors identified a number of amino acid substitutions present in the

endogenous penicillin binding proteins (PBP) in the resistant strains that were absent in closely related methicillin-susceptible strains. Possibly that substitutions of PBP could be the basis of resistance expression. There are no clear data about similar PBP in other *Staphylococcus* species up to date however, our findings suggest that similar penicillin binding proteins might be expressed in other *Staphylococcus* species as well. Interestingly, in 35 isolates of staphylococci (both coagulase-positive (CPS) and coagulase-negative (CNS) species) isolated from dogs recently (data not presented) we detected *mec*A gene in all of the isolates phenotypically resistant to oxacillin. However, the important aspect for the absence of *mec*A gene in some of the isolates in this study might be associated with different *Staphylococcus* species, where both CNS and CPS are presented. CLSI as well as European Committee on Antimicrobial Susceptibility Testing set oxacillin breakpoint as >0.25 mg L^{-1} only for CNS species, whereas some CPS species had no interpretation criteria. In any case, the results of this study are interesting, therefore further studies need to be performed for the investigation of this unusual finding associated with the absence of *mec* genes in oxacillin-resistant staphylococci prevalent in raw poultry products. Oxacillin-resistant species prevalence in poultry products is interesting as well as some of the species including *S. haemolyticus*, *S. hominis* and *S. cohnii* are prevalent in humans and are well-known because of the high antimicrobial resistance. This fact should be taken into account for safe food production.

Conclusions

Oxacillin-resistant staphylococci are prevalent in retail poultry products in Lithuanian markets, although oxacillin-resistant *S. aureus* has not yet been detected. The obtained results demonstrated low correlation between phenotypic resistance to oxacillin and the presence of *mec* genes in staphylococcal isolates from the poultry products. All oxacillin-resistant staphylococci were susceptible to vancomycin, gentamicin, linezolid, daptomycin, rifampin, co-trimoxazole and streptogramins. Resistance was observed toward fluoroquinolones (40%), macrolides (40%) and tetracycline (60%). Human-associated *Staphylococcus* species – *S. haemolyticus*, *S. hominis* and *S. cohnii* are prevalent in retail poultry products as well.

Acknowledgment

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References

1. Aarestrup F. M., Agers L. Y., Ahrens P., Jorgensen J. C., Madsen M., Jensen L. B. (2000) Antimicrobial susceptibility and presence of resistance genes in staphylococci from poultry. *Veterinary Microbiology*, No. 74, p. 353–364.
2. Anonymous. (2009) Protocol for PCR for *mec*A, *nuc* and 16S. *Community Reference Laboratory for Antimicrobial Resistance*. CRL course, Copenhagen, 20–22 April 2009.
3. Ba X., Harrison E. M., Edwards G. F., Holden M. T. G., Larsen A. R., Petersen A., Skov R L., Peacock S. J., Parkhill J., Holmes M. A. (2014) Novel mutations in penicillin-binding protein genes in clinical *Staphylococcus aureus* isolates that are methicillin resistant on susceptibility testing, but lack the *mec* gene. *Journal of Antimicrobial Chemotherapy*, No. 69, p. 594–597.
4. Chaffey B. J., Waites W. M. (1987) The adhesion of *Staphylococcus aureus* isolates from poultry-processing plants. *Journal of Applied Bacteriology*, No. 63, p. 17.
5. Couto N., Belas A., Couto V., Perreten V., Pomba C. (2014) Genetic relatedness, antimicrobial and biocide susceptibility comparative analysis of methicillin-resistant and–sesceptible *Staphylococcus pseudintermedius* from Portugal. *Microbial Drug Resistance* [Epub ahead of print].
6. Cuny C., Layer F., Strommenger B., Witte W. (2011) Rare occurrence of methicillin-resistant *Staphylococcus aureus* CC130 with a novel *mec*A homologue in humans in Germany. *PLoS ONE*, No. 6, e24360.
7. Do Carmo L. S., Cummings C., Linardi V. R., Dias R. S., De Suoza J. M., De Sena M. J., Dos Santos D. A., Shupp J. W., Pereira R. K., Jett M. (2004) A cause of massive staphylococcal food poisoning incident. *Foodborne Pathogens and Disease*, No. 1, p. 241–246.
8. Ellerbroek L. (1997) Airborne microflora in poultry slaughtering establishments. *Food Microbiology*, No. 14, p. 527–531.
9. Geornaras I., Dejesus A., Vanzyl E., von Holy A. (1995) Microbiological survey of a South-African poultry-processing plant. *Journal of Basic Microbiology*, No. 35, p. 73–82.
10. Huys G., D'Haene K, Eldere J. V., Holy A., Swings J. (2005) Molecular diversity and characterization of tetracycline-resistant *Staphylococcus aureus* isolates from a poultry processing plant. *Applied Environmental Microbiology*, No. 71, p. 574–579.
11. Jevinova P., Martonova M., Pipova M., Popelka P., Regecova I., nagy J. (2009) Tetracycline resistance of staphylococci isolated from game meat and mechanically deboned poultry meat. *Folia Veterinaria*, No. 53, p. 106–110.
12. Le Loir Y., Baron F., Gautier M. (2003) *Staphylococcus aureus* and food poisoning. *Genetics and Molecular Research*, No. 2, p. 63–76.
13. Kawamura Y., Hou X. G., Sultana F., Hirose K., Miyake M., Shu S. E., Ezaki T. (1998) Distribution of *Staphylococcus* species among human clinical specimens and emended description of *Staphylococcus caprae*. *Journal of Clinical Microbiology*, No 36, p. 2038–2042.
14. Kawano J., Shimizu A., Saitoh Y., Yagi M., Saito T., Okamoto R. (1996) Isolation of methicillin-resistant coagulase-negative staphylococci from chickens. *Journal of Clinical Microbiology*, No. 34, p. 2072–2077.
15. Mead G. C., Dodd C. E. R. (1990) Incidence, origin and significance of staphylococci on processed poultry. *Journal of Applied Bacteriology*. Symposium Supplement, No. 19, p. 81–91.
16. Mead G. C., Gibson C., Tinker D. B. (1995) A model system for the study of microbial colonization in poultry defeathering machines. *Letters of Applied Microbiology*, No. 20, p. 134–136.

17. Olivier M., Veary C. M., Cloete T. E., von Holy A. (1996) Microbiological status of selected chicken carcasses from a nonautomated poultry processing plant. *Journal of Basic Microbiology*, No. 36, p. 41–49.
18. Pepe O., Blaiotta G., Bucci F., Anastasio M., Aponte M., Villani F. (2006) *Staphylococcus aureus* and staphylococcal enterotoxin A in breaded chicken products: detection and behavior during the cooking process. *Applied Environmental Microbiology*, No. 72, p. 7057–706.
19. Ružauskas M., Šiugždinienė R., Krikštolaitis R., Virgailis M., Zienius D. (2010a) Prevalence and antimicrobial resistance of *E. coli* isolated from chicken liver sold in retail markets. *Veterinarija ir zootechnika*, No. 52, p. 67–72.
20. Ružauskas M., Šiugždinienė R., Sužiedėlienė E., Šeputienė V., Povilonis J. (2010b) Antimicrobial resistance of *Enterococcus* spp. spread in poultry products in Lithuania. *Journal of Food Safety*, No. 30, p. 902–915.
21. Shareef A. M., Mansour R. S., Ibrahim K. K. (2009) *Staphylococcus aureus* in commercial breeder layer flocks. *Iraqi Journal of Veterinary Sciences*, No. 23 (suppl.), p. 63–68.

DETERMINATION OF THE CONTENT OF COENZYMES Q_9 AND Q_{10} IN PORK MEAT FROM DIFFERENT BREEDS

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Abstract

Meat contains basic and essential nutrients to humans, and recently has been given a special attention to the presence of bioactive compounds.

The objective of this work was the simultaneous determination of coenzymes Q_9 and Q_{10} in pork meat, using HPLC. For this study were used 122 meat samples from different muscles and different breeds of pork. Besides, it was also evaluated the influence of animal breed and muscle type in the contents of CoQ_9 and CoQ_{10}.

The results showed that the breed influenced significantly the mean content of CoQ_9 (0.70 mg 100 g^{-1}) and CoQ_{10} (3.76 mg 100 g^{-1}) ($p<0.0001$), being this significantly higher in the meat from the breed 'Alentejano' when compared to the other breeds (Large White and Landrace). The type of muscle was also responsible for the variation in the levels of coenzymes in the samples analysed ($p<0.05$) and ($p <0.0001$) respectively.

From the results obtained it was concluded that the breed 'Alentejano' presented the highest contents in both coenzymes (CoQ_9 and CoQ_{10}) and that, among the muscles evaluated, the muscle *Semimembranousus (Sm)* showed higher concentrations of CoQ_{10}.

Keywords: chromatographic analysis, coenzyme Q_9, coenzyme Q_{10}, pork meat**.**

Introduction

The negative image often associated with meat and processed meat products is related to the presence of some components that can cause diseases to the health of the consumer, particularly high salt content, saturated fats, fatty acids and cholesterol responsible for the development of cardiovascular diseases, some cancers and obesity (Leunceford, 2008).

Meat also plays an important role in supplying our diet with minerals, such as iron, zinc, selenium, and vitamins of group B and E. Besides these basic nutritional components, studies have revealed that meat contains several bioactive compounds, such as conjugated linoleic acid (CLA), L-carnitine, carnosine, glutathione, taurine, creatine, coenzyme Q_9 and Q_{10}, among others (Toldrá, 2010).

The coenzymes Q contain a chromogenic nucleus (2,3-dimethoxy-5-methyl-1,4-benzoquinone) and a polyisoprenoid side chain in the 6-position which differs only in the number of isoprenoid units (Souchet, Laplante, 2007). The predominant form of ubiquinone in humans, animals, and fish is Co-enzyme Q_{10} (2,3-dimethoxy-5-methyl-6-decaprenylbenzoquino-ne), containing 10 isoprenoid units in the side chain (Linnane, 2007).

Coenzyme Q is an essential component of the electron transport chain electrons respiratory complexes I and II to respiratory complex III. Another important function of coenzyme Q is that of serving as a lipid soluble antioxidant protecting lipids, proteins and DNA from damage by reactive oxygen species (Santos, 2011).

In the respiratory chain in the mitochondria there are several coenzymes (coenzyme Q), as for example, CoQ_1 CoQ_2, CoQ_4, CoQ_6, CoQ_7, CoQ_8, CoQ_9 and CoQ_{10}. The Q_{10} is the most common form found in humans and most mammals, while coenzyme Q_9 is most often found in rats and guinea pig. On the other hand, the Q_6, Q_7 and Q_8 coenzymes are found in bacteria and yeasts (Sauer Shah, Laurindo, 2010).

The presence of coenzymes Q9 and Q10 in meat assumes a particular importance given their important roles on the human body as promoting health. However, the contents of these coenzymes may vary greatly according to breed, muscle type, sex, age, seasonal variations, and type of feed, among others.

Given the importance of natural antioxidants as preventing the damaging effect of free radicals formed during the cellular processes that are responsible for e.g. aging, cardiovascular disease and certain cancers, the objective of this work was to determinate the content of coenzymes Q_9 and Q_{10} in several samples of pork meat from different breeds by the method of HPL.

Materials and Methods

Sampling

In this study were used samples taken from meat originating from different animal breeds and different muscles. The pork samples corresponded to meat from 61 animals, from different breeds: Large White × Landrace (31 animals) and *'Alentejana'* (30 animals). From each animal, samples of muscles *Longissumus dorsi (Ld)* and *semimembranousus (Sm)* were removed, summing up a total of 122 samples analyzed.

The samples were homogenized and defatted, vacuum packed and kept frozen at -72 °C to perform the analysis.

Analytical Process

All the methodology inherent in the study and analysis of pork meat from different breeds was based on a technical protocol previously defined by Section of Meat and Meat Products from the National Institute for Agricultural and Veterinary Research (INIAV, 2013). These procedures were previously adapted to the study of meat and used for the analyses.

Extraction

For extraction were weighed 4 g of homogenized muscle sample, and cut into small pieces into glass petri dishes, in duplicate. Then, the Petri dishes were placed for about 24 hours in the lyophilizer (CHRIST BETA 1-8 K). The lyophilized samples were reduced to powder in a mortar placed in plastic centrifuge tube with cap and kept in a dry place until analysis. To the sample is added 10 mL of 0.15 M NaCl solution and then homogenized in a homogenization equipment (Polytron PT 3100) for 1 minute at 3500 rpm. After homogenization, was added 10 mL of absolute ethanol and stirred by vortex for 2 minutes (Vortex Heidolph REAX). Then, to the samples were added 15 mL of n-hexane and centrifuged again for 1 minute (Centrifuge Sorvall Instruments RC5C with SS-34 rotor). The extracts were then centrifuged at 3000 rpm at 4 °C for 5 minutes to separate the phases. The supernatant was removed to another tube, properly identified, and proceded to re-extraction of the pellet with another 15 mL of n-hexane, followed by further centrifugation. The supernatants from both centrifugations were combined. From this extract, 20 mL were used to evaporate on a rotary evaporator at 40 °C to dryness. The residue obtained was resuspended in 3 mL of 2-propanol and filtered through a syringe of 0.2 mm equipped with a PTFE filter (Acrodisc). Fifty microliters were injected into the HPLC system.

Analysis by high performance liquid chromatography (HPLC)

A Waters HPLC system consisting of a mark separation module Alliance 2487 and a detector Waters 2695 was used for the analyses. Empower Pro software was used to analyze the chromatograms obtained.

The separation of coenzymes Q_9 and Q_{10} was performed using a isocratic phase column Atlantis dC 18 4.6×150 mm, 3 μm, Waters mark at room temperature.

The mobile phase consisting of acetonitrile: tetrahydrofuran: water (55 : 40 : 5, v/v/v) was used at a flow rate of 1.5 mL min^{-1}. The coenzymes were detected in UV detector at 275 nm.

Analysis of Results

To evaluate the influence of the studied factors (breed and muscle) and their interactions on the variation of the levels of coenzyme Q_9 and Q_{10} there an Analysis of Variance was undertaken for 95% confidence level. When differences were significant for the race factor or interactions, the difference between means was assessed using the Tukey test.

The following factors were assessed:

- Influence of breed in the content of coenzymes Q_9 and Q_{10};
- Influence of muscle (*Ld* and *Sm)* in the content of coenzymes Q_9 and Q_{10};
- Influence of the interaction between muscle and breed.

Results and Discussion

Validation of analytical procedures

Determination of standard curves coenzymes Q_9 and Q_{10}

In order to proceed to quantification of the content of coenzymes in the samples, were prepared two solutions of coenzyme Q_9 and Q_{10}, which were injected into the HPLC in the form of various concentrations 0, 2, 5, 10, 20, 30, 50 (μg mL^{-1}), giving rise to the various "peaks" of different heights (AU), being the detection time 6.095 minutes to Q_9 and 8.065 minutes to Q_{10}.

With the integrations of "peaks" and the respective known concentrations of coenzymes, it was possible to obtain both standard curves, with their corresponding equations and correlation coefficients (r).

Determination of the Limits of Detection (LOD) and Quantification (LOQ)

The LOD and LOQ were determined by a sequence of analytical calculations to determine the standard deviation(s), using the specific formulas, from which were obtained the values in Table 1.

Table 1

Limits of detection and quantification of the process obtained

Type of coenzyme	LOD	LOQ
Coenzyme Q_9, mg 100 g^{-1}	0.343	1.040
Coenzyme Q_{10}, mg 100 g^{-1}	0.067	0.203

Determination of recovery rate

The calculus of the recovery rate is done using the peak obtained for the sample with the standard and the peak of the sample without the standard. This determination is made by means of the expression for the calculus of the recovery rate.

The results of the recovery rates made can be seen in Table 2.

Table 2

Recovery rates obtained in the process

Type of coenzyme	Recovery Rate
Coenzyme Q_9, mg 100 g^{-1}	50.00
Coenzyme Q_{10}, mg 100 g^{-1}	69.77

Simultaneous determination of coenzymes Q_9 and Q_{10} in pork meat

Coenzyme Q_9

Statistical analysis undertaken to the results of the determination of coenzyme Q_9 in pork meat, demonstrated significant differences between the breeds studied ($p<0.0001$) and between muscles *Ld* and *Sm* ($p <0.05$). With respect to the interaction between the two factors (Breed × Muscle) it was not found any significant influence.

Among the factors studied, the breed of the animal was that which exerted a significant influence over the content of coenzyme Q_9 in pork meat, with F value equal to 27.25.

According to Figure 1, it was possible to assess that there is a significant difference in the mean values ($p<0.001$). The samples obtained from animals of *'Alentejana'* breed showed an average content of coenzyme Q_9 higher (0.70±0.21 mg 100 g^{-1}) than samples of the animals *LW×LR* (0.52±0.19 mg 100 g^{-1}).

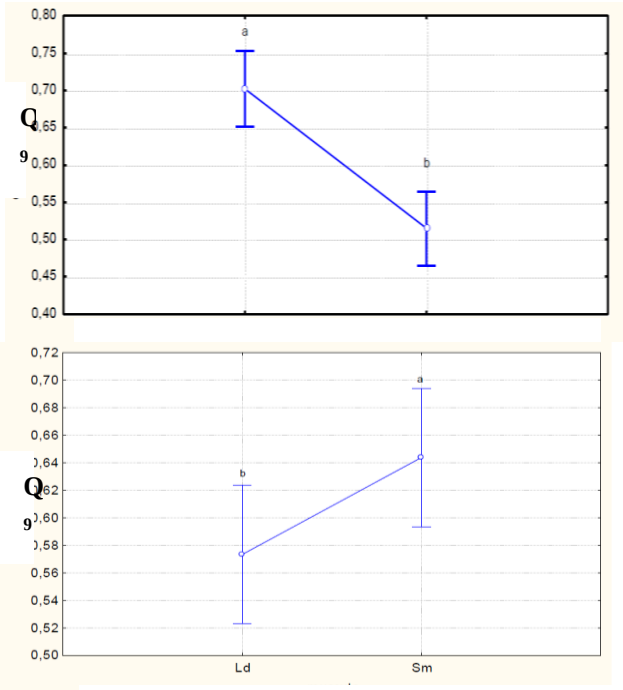

Figure 1. Influence of breed and muscle on the pork meat

With respect to the influence of the muscle type in the amount of Coenzyme Q_9, it was found a significant difference between the two muscles in the study (*Ld* and *Sm*). The *Sm* muscle showed a higher average (0.64±0.23 mg 100 g^{-1}), when compared with *Ld* muscle (0.57±0.21 mg 100 g^{-1}) (Figure 1).

Through the second graph of Figure 1, it was possible to ascertain that the average value for *Sm* CoQ_9 was significantly higher than *Ld*. A possible reason for this difference between the two types of muscle is due to the fact that muscle *Sm* belongs to the leg muscles, and the muscles in this location exert a greater physical effort for the animal's mobility.

The interaction between breed and muscle showed no significant influence on the results of coenzyme Q_9 in pork meat.

Coenzyme Q_{10}

In analyzing the results (ANOVA) of the content of CoQ_{10} in pork meat, it was found that all the factors studied: race, muscle, interaction of breed with the type of muscle, significantly influence the content of CoQ_{10} ($p<0.0001$).

Regarding the race factor, it was obtained a value of F equa to 226.78.

In the first graph of Figure 2 are presented the average values for coenzyme Q_{10} in meat from breeds *'Alentejana'* (3.75±1.44 mg 100 g^{-1}) and *LW×LR* (1.96±0.75 mg 100 g^{-1}). The results demonstrated that it is the meat of the *'Alentejana'* breed that has a higher content compared with that obtained in pork *LW×LR* (white pig) (Figure 2).

The muscle factor also exerted a high influence on the result, with a value of F equal to 213.46. It was also found that the *Sm* muscle (3.70±1.53 mg 100 g^{-1}) showed a higher mean value compared to *Ld* muscle (1.96±0.66 mg 100 g^{-1}). The difference between the two types of muscle examined can be observed in the second graph in Figure 2.

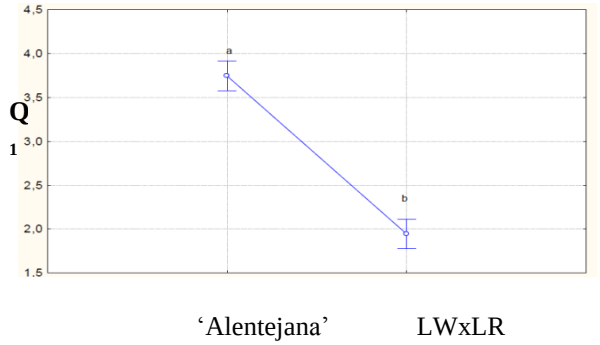

Figure 2. Influence of breed and muscle on pork meat (coenzyme Q_{10})-

This difference that occurred between muscles, as already mentioned in the analysis of CoQ_9, may be due to the type of muscle and breed. In fact, the animal exerts more physical effort with some of their muscles, which causes them to gain a greater oxidative character.

Doing the statistical analysis regarding the interaction of breed with muscle it was found to be a high-examined significant difference ($p<0.0001$), with value F =37.0.

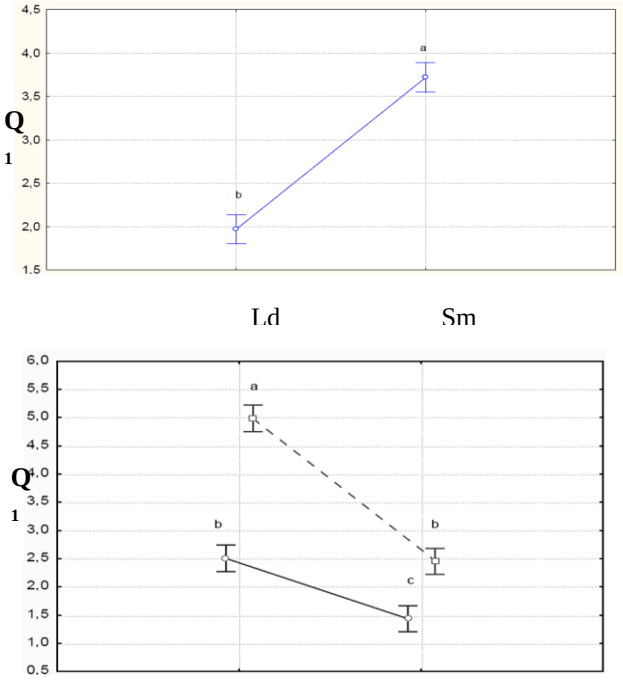

Figure 3. Influence of the interaction of breed with muscle in pork meat (coenzyme Q_{10})

Among all the conditions studied, the meat samples belonging to the *Sm* muscle of *'Alentejana'* pig breed (Figure 3), exhibited a higher average value. The mean value obtained for the samples of coenzyme Q_{10} in *Sm* muscle of the *'Alentejana'* breed was 4.99 mg 100 g^{-1}.
The values obtained for meat samples of *Sm* muscle from *LW×LR* breed and the values obtained for meat samples of *Ld* muscle from the *'Alentejana'* breed showed no significant difference (Figure 3). The *Ld* samples of animals from *LW×LR* breed showed the lowest mean level of CoQ_{10} (1.44 mg 100 g^{-1}), also revealing to be different from the medium content of all other conditions studied (Figure 3).
It was further observed that the highest values for CoQ_9 and CoQ_{10} occurred in the most oxidative muscle *(Sm*), which belongs to the leg muscle, where a greater physical effort is done having in mind the mobility of the animal. It is also known that the higher is the oxidative nature of the muscle, the largest will be the content of coenzymes Q_9 and Q_{10}, as found in this study.
Among the breeds analyzed that one which stood out for its high content of coenzyme Q_{10} was the sample of swine of the *'Alentejana'* breed.

Conclusion

This study aimed to determine the coenzymes Q_9 and Q_{10} in different meat samples. In view of the results obtained, it was evaluated the influence in the concentration of the coenzymes of the factors under study (breed, muscle and interaction between the two).
As regards pork meat, the results indicated that factors like breed and type of muscle exerted an influence on the content of coenzyme Q_9. However in the case of CoQ_{10} all the factors studied revealed to have a significant influence.

References

1. Araujo W. M. C., Montebello N. de P. (2006) *Carne & cia* (Vol 1). Senac.
2. Toldrá F. (2010) *Handbook of Meat Processing*. John Wiley & Sons.
3. Souchet N., Laplante S. (2007) Seasonal variation of Co-enzyme Q10 content in pelagic fish tissues from Eastern Quebec. *Journal of Food Composition and Analysis*, Vol. 20, No. 5, p. 403–410. doi:10.1016/j.jfca.2006.09.004
4. Sauer H., Shah A. M., Laurindo F. R. M. (2010). *Studies on Cardiovascular Disorders*. Springer.
5. Santos S. (2011). *Aspectos Bioquímicos e Moleculares da Coenzima Q10*. Universidade de Aveiro; Departamento de Biologia, Aveiro. Obtido de https://ria.ua.pt/bitstream/10773/7981/1/246586.pdf
6. Lunceford A. L. (2008). *The Effect of Coenzyme Q(10) Supplementation on Aging and Disease*. ProQuest.
7. Linnane A. W., Kios M., Vitetta L. (2007). Coenzyme Q10 – Its role as a prooxidant in the formation of superoxide anion/hydrogen peroxide and the regulation of the metabolome. *Mitochondrion*, *7*, *Supplement*, S51–S61. doi:10.1016/j.mito.2007.03.005.

THE EFFECTS OF pH, a_W, AND LACTIC ACID BACTERIA ON *LISTERIA MONOCYTOGENES* IN FERMENTED SAUSAGES

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Abstract

The survival of inoculated in fermented sausages *Listeria monocytogenes* strain was studied. The sausages were prepared with and without starter cultures. The survival limits of *L. monocytogenes* and lactic acid bacteria (LAB) were determined as colony forming units per gram (cfu g^{-1}) depending on water activity (a_w) and pH on 0, 1st, 3rd, 5th, 7th, 14th and 21st days of maturation. The decreasing water activity conditioned by moisture (weight) loss during ripening and pH decrease ensured negative polynomial growth rate of inoculated *L. monocytogenes* -0.27 lg (cfu g^{-1}) each day of ripening time, and -0.65 lg (cfu g^{-1}) on the first 7 days of maturation. A significant Pearson's correlation ($p<0.01$) was established between decreased values of *L. monocytogenes* count, a_w, salt concentration and LAB growth in sausages during the ripening period of 21 days. The main parameters, maintained negative exponential growth rate of *L. monocytogenes* in fermented sausages, are a_w value decrease and LAB (starter culture), which stopped *L. monocytogenes* growth at the beginning of fermented sausage maturation. If fermentation process went technically and hygienically correctly, the fermented (cold-smoked) sausages could be one of the safest meat products, because in real practice a low level contamination has been seen. The remaining count of *L. monocytogenes* in fermented sausage depends on the possible initial contamination level and could exceed the European Union regulation value 2.0 lg (CFU g^{-1}) for ready-to-eat products when contamination at first is more than lg 5.0.

Keywords: *Listeria monocytogenes*, lactic acid bacteria, fermented sausages, water activity, pH.

Introduction

Listeria monocytogenes is an ubiquitous bacterial pathogen that can be found in a large number of food products and can survive and multiply at refrigeration temperature (Lunden et al., 2003). Processed meat products such as fermented sausages are a part of major products associated with listeriosis (Thevenot et al., 2005). *L. monocytogenes* infection has a high mortality rate – 20–30% (Farber, Peterkin, 1991). In the United States, a zero tolerance of *L. monocytogenes* in ready-to-eat foods has been prescribed for several years (Shank et al., 1996), but the European Union regulation exceeded concentration of *L. monocytogenes* in ready-to-eat food to 100 colony forming units (CFU) per gram (Anonymous, 2005). Glass, Doyle (1989) found out that *L. monocytogenes* decreasing level in fermented sausages and ham would be 1–2 lg (sausages) in 14 days and 2–3 lg (ham) in 28 days. Because lactic acid bacteria (LAB) can grow under the same storage conditions as *Listeria* spp. (Bērziņš et al., 2007), many studies have been conducted to investigate if these gram-positive organisms can provide adequate competition against the pathogenic organisms that are also present.

The safety of fermented (cold smoked) sausages depends on the presence of factors such as concentration of sodium nitrite and salinity, relatively low water activity (a_w), low pH value, and application of probiotics (Lahti et al., 2001), like LAB used in fermented meat products (Bredholt et al., 2001). Liu et al. (2005) investigated that acid, alkali, and/or salt treatments, commonly used in food product processing, may not be sufficient to eliminate *L. monocytogenes*. Petran, Zottolla (1989) observed the growth of *L. monocytogenes* at the minimum a_w of 0.92. Below these minimum a_w levels, cell death is proportionate to water activity (Miller, 1992). According to literature sources available, some recent studies in food safety have investigated non-thermal processing of ready-to-eat food products, but there is little information about survival of *L. monocytogenes* found in different ripening stages of fermented sausages when main bacteria growth factors changed in time. Therefore, the aim of the study was to determine the survival limits of *L. monocytogenes* inoculated in manufactured fermented sausages depending on the LAB, water activity (a_w), and pH value changes in ripening time.

Materials and Methods

Individual pieces of raw sausages, before smoking at 28 °C, in initial weight mean value of 0.394 kg, were inoculated internally with a cocktail of local (wild) strains of *L. monocytogenes* (serotypes 1/2a and 4b) originally isolated from surfaces and meat products of the mother factory (Bērziņš et al., 2007). The inoculated samples were labelled and subjected to smoking and maturating processes. All manipulations with samples were done in laboratory conditions (20 °C, 75–80% RH). The measurements and tests were done on 0, 1st, 3rd, 5th, 7th, 14th and 21st day of maturation. Three batches of fermented (cold smoked) sausages were investigated (a total of 60 samples) and the mean values of lg (CFU g^{-1}) were estimated between each other, and in addiction of pH, moisture content, and water activity (a_w) changes at ripening time.

L. monocytogenes strains were incubated in half-Fraser base medium for 18 h at 37 °C. The fresh concentrated culture of the selected strains was measured by optical densities (densitometer DEN-1B, UK) and prepared with sterile half Fraser broth (CM0895, SR0166E, Oxoid) to obtain approximately 8.0 lg (CFU mL^{-1}), and then samples of dry sausages inoculated portionally

(1 mL of inoculate in 100 g of sample) randomly leading to beginning concentration of 6.0 lg (CFU g^{-1}).
Ingredients of a 100 kg fermented sausage raw material were: pork – 30 kg, beef – 10 kg, bacon – 35 kg, structural emulsion – 25 kg. Salt and species summary was 3.25 kg and starter culture – 0.02 kg ('Optistart Plus', prepared by Raps GmbH and Co.KG, Germany).
The smoking, fermentation, and ripening process were carried out in climatic chambers (models HR-6000 and HR-9000 'Sorgo' Austria) at 28 °C with a relative humidity of 95% on first 3 days, down to 75% RH and 14–15 °C on 4^{th} to 21^{st} day.
The determination of *L. monocytogenes* count, CFU g^{-1}, was done according to Standard ISO 11290-2:1198 A:2005 'Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of *L. monocytogenes*. Part 2: Enumeration method'. Each experimental batch was free of *L. monocytogenes* before culture inoculation, detected with standard method. The samples were analyzed by numbering *L. monocytogenes* on 0, 1^{st}, 3^{rd}, 5^{th}, 7^{th}, 14^{th} and 21^{st} days of maturation with the nine-tube most-probable-number (MPN) method. For analysis, 10 g of a carefully mixed fermented sausage sample were blended with 90 mL of sterile buffered peptone water in a laboratory blender (Stomacher 400, Interscience, France) for 1 min. Decimal dilutions were made to obtain samples of 1, 0.1, 0.01, 0.001, and 0.0001 g. To determine the MPN, three consecutive dilutions were used. Afterwards, 0.1 mL of each target dilution was spread on two LM-selective plates (PALCAM, Oxoid) and incubated for 48 h at 37 °C. For confirmation of *L. monocytogenes*, five typical colonies from two selective plates at each sampling time were streaked on sheep blood agar plates and incubated for 24 h at 37 °C. Catalase-positive, gram-positive rods, produced hemolysis on sheep blood agar (CAMP-test), were considered *L. monocytogenes* (McKellar, 1994). Total count (CFU g^{-1}) of *L. monocytogenes* in fermented sausage samples were calculated with classical formula given in Enumeration method standard.
The determination of LAB count, CFU g^{-1}, was done according to standard ISO 15214:1998 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of mesophilic lactic acid bacteria - Colony-count technique at 30 °C.
pH was measured at the same time as other measurements. 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. Meter calibration was done according to 2 point method with pH standard solutions 4.01 and 7.00.
Water activity was measured with PawKit (Decagon) water activity meter. Calibration of device was done with saturated NaCl (sodium chloride) 6.0 molal standard solution (0.760 a_w at 20 °C). Samples for water activity measurement were collected in original polyethylene vessels with caps and measured immediately after collecting.
The digital salt-meter "PAL-ES2" was used for direct measurement of NaCl cocentration in fermented sausages. The device have been calibrated with 2.50% NaCl solution AB250 from manufacturer (Atago, Inc. US) directly before measuring.
The determination of nitrite content in fermented sausages by ISO 2918:1975 standard was used for measuring nitrite on photometry method with 538 nm wavelength.
Statistical analysis. All experiments were reiterated three times, and tests were triplicated. The results represent the mean ± standard deviations (SD). Means were compared by Student's t-test. Differences were considered statistically significant when $p<0.05$. Statistical analysis was conducted with SPSS 17.0 (SPSS, Chicago, Ill., USA). Tables and chart figures were done by means of MS Excel 2007 appliances. To show the parameter changes in time, regression curves have been made for LAB, *L. monocytogenes*, a_w, and pH. The main factors, affecting the bacterial growth, have been calculated by correlation (SPSS, Factor analysis).

Results and Discussion

The results of the physicochemical parameters and bacterial analysis of the fermented sausages at the beginning and the end of the ripening time are reported in Table 1. The values of pH were about 4.6 in the final product - typical of medium acidity sausages, and this was the result of the classical trend of microbial growth in the fermented sausages, where LAB are increasing in numbers at the very beginning of the fermentations (Figure 1), producing acids and a decrease in the pH, followed in the phases of maturation by the activity of micrococci that are able to neutralize the acids produced (Comi et al., 2005). The value of water activity (a_w) showed a constant decrease during the maturation reaching final values of 0.80–0.82, and moisture of 251–258 g kg^{-1}. The final value of the salt content was around 40 g kg^{-1}, while the final nitrite about 9 mg kg^{-1} of NO_2^-. These changes of parameter were due to the effect of dehydration (Comi et al., 2005). Mean value of weight losses over 21 days of ripening, when relatively constant weight reached 75–76% of relative humidity of air (RH) in the climatic chamber, was approximately 110 and 117 g of samples with and without starter culture accordingly. Losses movement significantly ($p<0.001$) correlated with the mean value of moisture content.
Due to good adaptation of LAB to meat environment and their faster growth rates which were displayed during fermentation and ripening of sausages, they became the dominant microflora (Drosinos et al., 2005). The total count of LAB changes in fermented-cold-smoked sausages with and without starter culture is shown in Figure 1. Theoretically in sausage (A) by starter culture, calculated to 1 g sausage raw mass, lg 9.4 *Lactobacillus sakei* L110, lg 9.4 *Staphylococcus xylosus*, and lg 8.0 *Debaryomyces hansenii* were added.

Table 1

The measured parameters values on start 0th day (S) and finishing 21st day (F)

Parameters	Time	Samples with (A) starter culture	Samples without (B) starter culture
L.monocytogenes, lg (CFU g^{-1})	S	6.57±0.26	6.83 ± 0.12
	F	1.42±0.43	2.86±0.36
Total count of lactic bacteria, lg (CFU g^{-1})	S	5.72±0.18	3.35±0.15
	F	9.41±0.32	6.95±0.27
Mean sausage weight, kg	S	0.39±0.05	0.39±0.05
	F	0.28±0.05	0.28±0.05
Moisture content, %	S	34.78±0.40	39.43±0.38
	F	25.12±0.38	25.88±0.36
pH value	S	5.80±0.02	5.76±0.016
	F	4.67±0.016	4.59±0.015
a_w value	S	0.96±0.002	0.95±0.002
	F	0.82±0.003	0.80±0.003
Salt (NaCl) content, %	S	2.94±0.85	28.40±0.64
	F	3.98±0.75	40.20±0.82
Nitrite (NO_2^-) concentration, mg kg^{-1}	S	14.00±0.40	14.00±0.40
	F	9.00±0.30	9.00±0.30

The difference of detected LAB count between sausage A and B variants on 0th day was approximately lg 2.37, that evident of artificially increasing count of LAB in sausage A by approximately 100 times in comparison to sausage B.

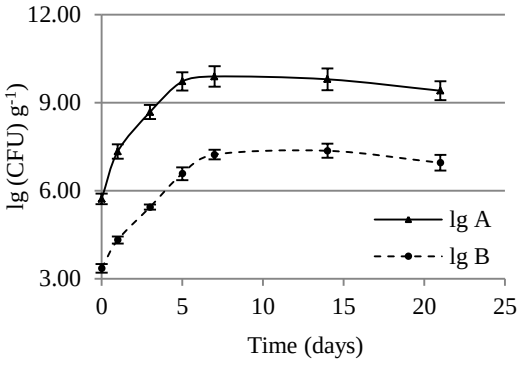

Figure 1. The polynomial changes of lactic acid bacteria count (lg values and SD values as±bars) in fermented sausages with (A) and without (B) starter culture during ripening (p<0.01)

The most significant regression of bacteria count during all time investigated has been shown by a polynomial graph curve (Figure 1). In the bacterial growth period on first 7 days the best conformity (R^2=0.98) to time, temperature, and interior factors are represented by linear regression in variant B (Figure 2).

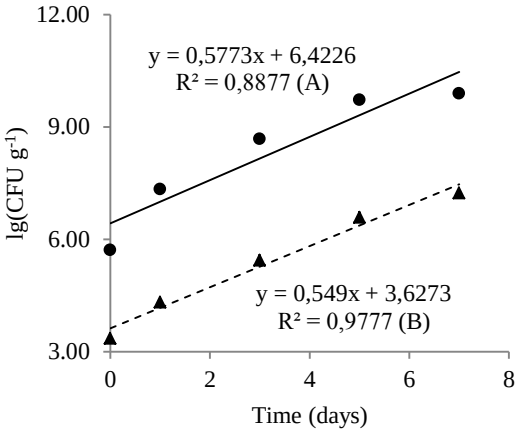

Figure 2. The linear regression trends of lactic acid bacteria count - lg (CFU g^{-1}) values in fermented sausages with ── (A) and without ····· (B) starter culture on first 7 days of ripening (p<0.05)

It can be seen in Figure 1 and Figure 2 that exponential phase of lactic bacteria growth stops on the 7th day of ripening, when a_w decreases below 0.92–0.90, and moves to stationary phase for next 7 days. The main species of LAB, detected before in the meat products prepared at the mother factory, and its minimal a_w value by Vermeiren, Debevere (2004), were 0.94 for *Lactobacillus brevis* and 0.92 for *Lactobacillus plantarum*, which have been detected as the main LAB species in experimental sausage samples too.

The main bacterial growth factor a_w minimal values for starter culture components are: 0.91 *L. sakei* (Leroy, de Vuyst, 1999), 0.86 *S. xylosus* (Terra et al., 2007), and 0.81 *D. hansenii* (Aggarwal, Mondal, 2009). These different requests of minimal a_w guaranty a constant level of pH during necessary ripening time.

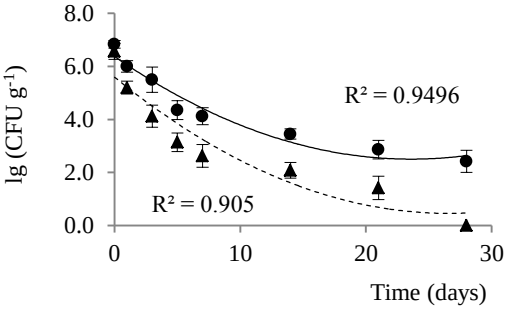

Figure 3. The polynomial regression trends of *L. monocytogenes* count - lg (CFU g^{-1})····· (A) with and ── (B) without starter culture in fermented sausages during ripening. ± SD values (p > 0.05)

Due to its water binding and ionic characteristics, salt affects the metabolism of a starter culture. The growth of lactic acid bacteria is sometimes enhanced in the presence of low content of sodium chloride (1 to 2%, 10–20 g kg^{-1}), but growth is clearly inhibited in the presence of NaCl content greater than 3% (30 g kg^{-1}) (Korkeala et al., 1992; Samapundo et al., 2010).

Homofermentative LAB is more resistant to sodium chloride than heterofermentative LAB are, and strains resembling *L. sakei* have been shown to be more resistant than other strains.

The initial *L. monocytogenes* inoculation concentration averaged 6.6–6.8 lg (CFU g^{-1}) was significantly ($p<0.01$) reduced at any ripening stage in both (A and B) sample variants (Figure 3). The sausage samples from this study had finally pH< 4.7 and a_w < 0.82. Such values guarantee no growth of *L. monocytogenes* (Vermeulen et al., 2007) and the rest count possibly depends on initial count.

In both batches (A and B) the decrease of detected *L. zmonocytogenes* count showed a negative linear regression curve during the first 7 days (Figure 4) with lg (CFU g^{-1}) decreasing rate lg -0.54 (A) and lg -0.39 (B).

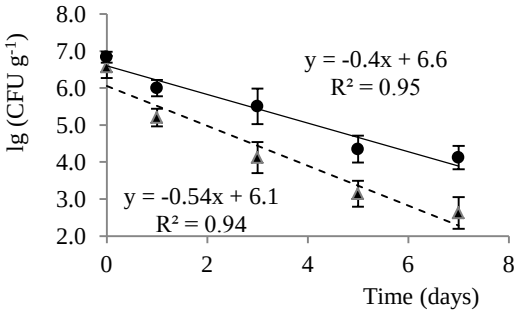

Figure 4. The linear regression trends of *L. monocytogenes* count - lg (CFU g^{-1})····· (A) with and —— (B) without starter culture in fermented sausages during the first 7 ripening days (p>0.05)

However, it can be said that *L. monocytogenes* were inhibited and did not exceed the growth in all observed ripening time (21 day). As it can be seen in Table 1, and Figure 4, the addition of starter culture hastened *L. monocytogenes* live cells, and detected count of *L. monocytogenes* decreased two times. All changes of the physic-chemical parameters, except salt content, were decreased, but all of them did not support *L. monocytogenes* growth. All parameter changes more or less correlated (Table 2) between each other, but water activity is the parameter which summarizes these changes, and that is why it can be conferred as the main factor which limited pathogen growth in food products.

The latest papers described that the growth of *L. monocytogenes* ceased at a cell concentration of about 10^2 CFU mL^{-1} when natural microflora of foods, such as lactic acid bacteria, entered stationary phase (Al-Zeyara et al., 2011).

pH, a_w, NaCl (g kg^{-1}), and NO_2^- (mg kg^{-1}) values changes are shown in double graph in Figures 5 and 6. The measurements of water activity show that *L. monocytogenes* growth would have been theoretically stopped on the 7th day of ripening when a_w decreased to 0.90 according to Vermeulen et al. (2007), but the observed results of bacterial count decrease made an idea of importance of a_w motion as the most significant factor against *L. monocytogenes* growing and survival in meat products.

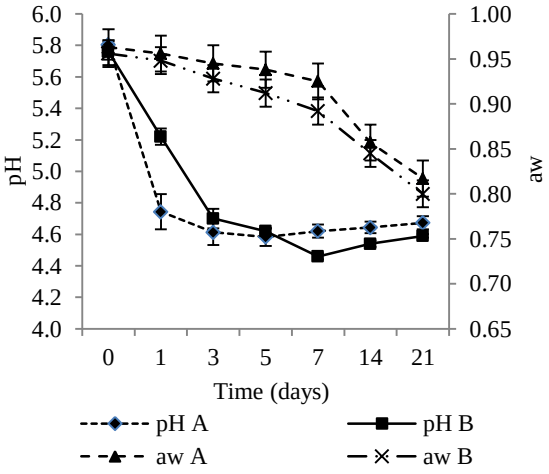

Figure 5. The changes of physico-chemical parameters: pH and a_w in fermented sausages with (A) and without (B) starter culture during 21 ripening days

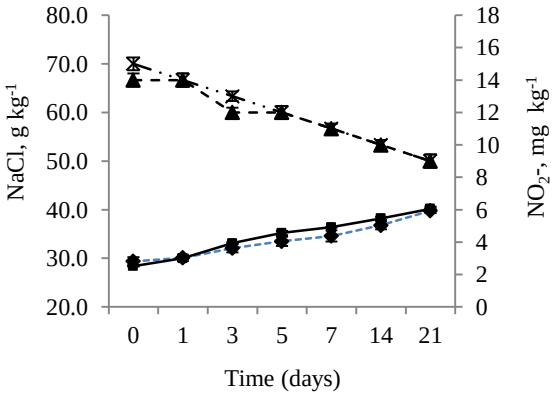

Figure 6. The changes of physic-chemical parameters: NaCl and NO_2^- in fermented sausages with (A) and without (B) starter culture during 21 ripening days

.....♦.... NaCl A, ----■--- NaCl B, - - ▲ - - NO_2^- A, -..-..x-..-.. NO_2^- B.

The samples of fermented sausages had a mean initial pH value of 5.80±0.02, which agrees with the results found by 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 4.67–4.56±0.02; this drop in pH was due to lactic acid production by the starter culture used for fermentation (Vermeiren, 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).

Table 2

The correlation (r) values and their significance (p) levels between measured physic-chemical parameters and changes of inoculated *L. monocytogenes* count

Parameters	A		B	
	r	p	r	p
Time (days)	-0.896	0.003	-0.963	0.000
lg LAB	-0.865	0.006	-0.873	0.005
pH	0.609	0.073*	0.765	0.023*
a_w	0.867	0.006	0.980	0.000
NaCl, %	-0.954	0.000	-0.981	0.000

*Correlation is not significant

Under the fermentation and maturation conditions in this work, the decrease of *L. monocytogenes* count in fermented sausage was less intense than reported by other studies (Työppönen et al., 2003; Tolvanen et al., 2008) where an expressive decrease was observed at the beginning of ripening process. This is probably due to a lower pH and water activity in the first days of maturation noted in other studies, and higher initial *L. monocytogenes* concentration in our experiments.

The mean values of the decrease rate in *L. monocytogenes* count in batch B are bigger than those Glass, Doyle (1989) found in sausages without added lactobacilli cultures.

No significant correlation was calculated in both experimental batches with and without starter culture between *L. monocytogenes* count and pH value. That could be explained by a relatively short time when pH value decreased to constant level and long stationary phase of pH value.

Conclusions

The main parameters, maintained negative exponential growth rate of *L. monocytogenes* in fermented sausages are a_w value decrease and lactic acid bacteria, which stopped *L. monocytogenes* growing at the beginning of fermented sausage maturation. If fermentation process goes technically and hygienically correctly, the fermented (cold-smoked) sausages could be one of the safest meat products, because in real practice we observed a low level of contamination.

The remaining count of *L. monocytogenes* in fermented sausage depends on the possible initial contamination level and could exceed the European Union regulation value 2.0 lg (CFU g^{-1}) for ready-to-eat products when contamination at first is more than lg 5.0.

References

1. Aggarwal M., Mondal A.K. (2008) *Debaryomyces hansenii*: An Osmotolerant and Halotolerant Yeast. **In**: *Yeast Biotechnology: Diversity and Applications*, T. Satyanarayana, G. Kunze (eds). Netherlands: Springer, p. 65–84.
2. Al-Zeyara S.A., Jarvis B., Mackey B.M. (2011) The inhibitory effect of natural microflora of food on growth of *Listeria monocytogenes* in enrichment broths. *International Journal of Food Microbiology*, Vol. 145, p. 98–105.
3. Anonymous (2005) Commission regulation (EC) No 2073/2005 of 15. November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union*, L338, p. 1–25.
4. Bērziņš A., Hörman A., Lundén J., Korkeala H. (2007) Factors associated with *Listeria monocytogenes* contamination of cold-smoked pork products produced in Latvia and Lithuania. *International Journal of Food Microbiology*, Vol. 115, p. 173–179.
5. Bredholt S., Nesbakken T., Holck A. (2001) Industrial application of an antilisterial strain of *Lactobacillus sakei* as a protective culture and its effect on the sensory acceptability of cooked, sliced, vacuum-packaged meats. *International Journal of Food Microbiology*, Vol. 66, p. 191–196.
6. Comi U., Iacumin K.R., Cattaneo C.C., Cocolin L. (2005) Characterisation of naturally fermented sausages produced in the North East of Italy. *Meat Science*, Vol. 69, Iss. 3, p. 381–392.
7. Drosinos M.M., Xiraphi G.M., Gaitis J.M. (2005) Characterization of the microbial flora from a traditional Greek fermented sausage. *Meat Science*, Vol. 69, Iss. 2, p. 307–317.
8. Farber J.M. Peterkin P.I. (1991) *Listeria monocytogenes*, a food-borne pathogen. *Microbiology and Molecular Biology Reviews*, Vol. 55, Iss. 4, p. 476-511.
9. Glass K.A. Doyle M.P. (1989) Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. *Applied and Environmental Microbiology*, Vol.55, Iss 6, p. 1565–1569.
10. Korkeala H., Alanko T., Tiusanen T. (1992) Effect of sodium nitrite and sodium chloride on growth of lactic acid bacteria. *Acta Veterinaria Scandinavica*, Vol. 33, Iss. 1, p.27–32.
11. Lahti E., Johansson T., Honkanen-Buzalski T., Hill P., Nurmi E. (2001) Survival and detection of *Escherichia coli* O157:H7 and *Listeria monocytogenes* during the manufacture of dry sausage using two different starter cultures. *Food Microbiology*, Vol.18, Iss. 1, p. 75–85.
12. Leroy F., de Vuyst L. (1999) The Presence of Salt and a Curing Agent Reduces Bacteriocin Production by *Lactobacillus sakei* CTC 494, a Potential Starter Culture for Sausage Fermentation. *Applied and Environmental Microbiology*, Vol. 65, Iss. 12, p. 5350–5356.
13. Liu D., Lawrence M.L., Ainsworth A.J., Austin F.W. (2005) Comparative assessment of acid, alkali and salt tolerance in *Listeria monocytogenes* virulent and avirulent strains. *FEMS Microbiology Letters*, Vol. 243, Iss. 2, p. 373–378.
14. Lunden J., Autio T., Korkeala H. (2003) Persistent and nonpersistent *Listeria monocytogenes* contamination in meat and poultry processing plants. *Journal of Food Protection*, Vol. 66, No. 11, p. 2062–2069.
15. McKellar R.C. (1994) Use of the CAMP Test for identification of Listeria monocytogenes. *Applied and Environmental Microbiology*, Vol. 60, No. 12, p. 4219–4225.
16. Miller A.J. (1992) Combined water activity and solute effects on growth and survival of *Listeria monocytogenes* Scott A. *Journal of Food Protection*, Vol. 55, Iss 3, p. 414–418.

17. Paleari M.A., Moretti V.M., Beretta G., Mentasti T., Bersani C. (2003) Cured products from different animal species. *Meat Science*, Vol. 63(4), p. 485-489.
18. Petran R.L., Zottola E.A. (1989) A study of factors affecting growth and recovery of *Listeria monocytogenes* Scott A. *Journal of Food Science*, Vol. 54(2), p. 458–460.
19. Samapundo S., Ampofo-Asiama J., Anthierens T., Xhaferi R., Van Bree I., Szczepaniak S., Goemaere O., Steen L., Dhooge M., Paelinck H., Dewettinck K., Devlieghere F. (2010) Influence of NaCl reduction and replacement on the growth of *Lactobacillus sakei* in broth, cooked ham and white sauce. *International Journal of Food Microbiology*, Vol. 143(1), p. 9–16.
20. Shank F.R., Eliot E.L., Wachsmuth I.K., Losikoff M.E. (1996) US position on *Listeria monocytogenes* in foods. *Food Control*, Vol. 7(4-5), p. 229–234.
21. Schillinger U., Kaya M., Lücke F.K. (1991) Behavior of *Listeria monocytogenes* in meat and its control by a bacteriocin-producing strain of *Lactobacillus sake*. *Journal of Applied Bacteriology*, Vol. 70(6), p. 473–478.
22. Terra N.N., Sossela de Freitas R.J., Cichoski A.J. (2007) Water activity, pH, moisture and growth of *Staphylococcus xylosus* during processing and storage of cured, matured and fermented pork shoulder. *Ciência e Tecnologia de Alimentos*, Vol. 27, Iss. 4, p. 2026–2031.
23. Thevenot D., Delignette-Muller M.L., Christieans S., Ver-nozy-Rozand C. (2005) Fate of *Listeria monocytogenes* in experimentally contaminated French sausages. *International Journal of Food Microbioogy*, Vol. 101. Iss. 2, p. 189–200.
24. Tolvanen R., Hellström S., Elsser D., Morgenstern H., Björkroth J., Korkeala H. (2008) Survival of *Listeria monocytogenes* strains in a dry sausage model. *Journal of Food Protection*, Vol. 71, Iss. 8, p. 1550–1555.
25. Työppönen S., Markkula A., Petaja E., Suihko M., Mattila-Sandholm T. (2003) Survival of *Listeria monocytogenes* in North European type dry sausages fermented by bioprotective meat starter cultures *Food Control*, Vol. 14, p. 181–185.
26. Vermeiren D., Debevere A. (2004) Evaluation of meat born lactic acid bacteria as protective cultures for the biopreservation of cooked meat products. *International Journal of Food Microbiology*, Vol. 96, Iss. 2, p. 149–164.
27. Vermeulen A., Gysemans K.P., Bernaerts K., Geeraerd A.H., Van Impe J.F., Debevere J., Devlieghere F. (2007) Influence of pH, water activity and acetic acid concentration on *Listeria monocytogenes* at 7 degrees C: data collection for the development of a growth ⁄ no growth model. *International Journal of Food Microbiology*, Vol. 114, Iss. 3, p. 332–341.
28. Visessanguan S.B., Panya C.K. Assavanig C. (2005) Influence of minced pork and rind ratios on physico-chemical and sensory quality of Nham-a Thai fermented pork sausage. *Meat Science*, Vol. 69, Iss. 2, p. 355–362.

MICROBIOLOGICAL QUALITY OF MEAT PREPARATIONS AND MEAT PRODUCTS

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Abstract

The aim of the research was to perform trend analysis to reveal probable gaps and shortcomings in monitoring of microbiological contamination of meat preparations and meat products produced in Latvia to highlight proposals for further improvements. The results on microbiological contamination of foodstuffs obtained in the frame of producers' self-control within HACCP procedures were used in the research. In total, 13 food types, including minced meat, breaded pork chop, different types of sausages, smoked meat products, aspic and liver pate were investigated. Using single factor analysis of variance (ANOVA) significant differences were revealed between food types, as well as large fluctuations in aerobic plate counts (APC) were demonstrated. According to the findings of the research, APC was significantly different (p=0.001) for sausages. The maximum mean APC (6.16±6.36 lg CFU g^{-1}), as well as maximum APC (6.68±6.36 lg CFU g^{-1}) was found for liver sausage. APC was largely variable for smoked meat products. Significant difference (p=0.01) was revealed with regard to APC for aspics tested one and six days after production. Hygiene indicatororganisms (*Escherichia coli*) and pathogens (e.g. *Salmonella spp*.) most often were detected in meat preparations. Presence of coliforms was detected in aspics and liver pate. The results of the research suggest that development and usage of guidelines of good manufacturing practice for purposeful monitoring of microbiological contamination risk of meat preparations and meat products is relevant to ensure high level of protection of consumers' health.

Keywords: microbiological contamination, meat preparations and products, good manufacturing practice.

Introduction

The safety of food must be assured by a preventative approach based on the application of a Hazard Analysis Critical Control Point (HACCP) at all stages of food chain. The HACCP system is a structured approach for identifying hazards and defining and implementing systems of adequate control. Risk-based programmes have been proved successful in achieving hazard control to the extent required for consumer protection. Microbiological examination of food and environmental samples is generally recommended to validate and verify the efficiency of foods safety and quality control (ICMSF, 2011; IFST, 1997).

Foodborne disease or microbiological spoilage of food can result from the failure or inability to control microorganisms at one or more stages of food production. Therefore, the microbiological testing at various stages of food production is relevant to reveal and understand the characteristic trends in distribution of microbiological contamination (ICMSF, 2011; Schaffner, 2007; Burlingame and Pineiro, 2007; IFST, 1997).

It should be mentioned that only one official regulation concerning the microbiological quality of meat preparations is stated in food safety legislation, namely, *Salmonella* spp. contamination must not be found in 10 g of minced meat and meat preparations intended for use after thermal processing. As regards ready-to-eat meat preparations, no legal requirement for microbiological safety is stated in the legislation (Commission Regulation 2073/2005).

To monitor microbiological quality of meat preparations and meat products guidelines and recommendations of international and national level have been developed in addition to legal requirements. According to the guidelines of good manufacturing practice the level of total microbiological contamination of raw meat and raw meat preparations should not exceed 10^5 (maximum 10^7) CFU g^{-1} and of thermally processed meat products – 10^4 CFU g^{-1}. *Escherichia coli* contamination in raw meat and raw meat preparations should not exceed 10^2 (maximum 10^4) CFU g^{-1} and *Enterobacteriaceae* and *Escherichia coli* contamination in meat products – 10^2 (maximum 10^4) and 10 (maximum 10^3) CFU g^{-1}, respectively (ICMSF, 2011; IFST, 1997).

It is commonly suggested that microorganisms can enter meat preparation like sausages from meat, spices, and other ingredients, as well as from processing environment, equipment, and handlers that can have a significant impact on the microbiological status of the end-products. In general, heating during technological processing of meat products is an effective tool to reduce microbial counts of end-products (Güngör, 2010).

Development of preventive food safety assurance systems comprises both the identification of important food safety hazards and the introduction of regular monitoring measures in critical control points of technological processes. It is widely recognised that management of technological processes should be based on detailed analysis of product characteristics and process conditions to assess the potential impact on quality and safety of the ready-to-eat foods (Schaffner, 2007; Burlingame, Pineiro, 2007).

The aim of the research was to perform trend analysis to reveal probable gaps and shortcomings in monitoring of microbiological contamination of meat preparations and meat products produced in Latvia to highlight proposals for further improvements.

Materials and Methods

The data on microbiological contamination of food products obtained in frame of producers' self-control within HACCP procedures (December 2012 – January 2014) were used to analyse microbiological quality of

meat preparations and meat products produced in Latvia. Food samples were taken at three meat processing companies and were stored at temperature +4 °C after sampling and during delivering to the laboratory. All samples of meat preparations were taken one day after production, but samples of meat products were taken on different days of shelf-life (namely, one to 11 days after production) to evaluate distribution of microbiological contamination.

During the research food products were divided into two compatibility groups: meat preparations (no thermal processing used during production) and meat products (thermally processed foodstuffs). In total 144 samples of meat preparations and 141 samples of meat products were analysed for the aerobic plate count (APC). 724 samples of meat preparations and products were analysed for presence of hygiene indicatororganisms (coliforms and *Escherichia coli*) and pathogens (*Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, and sulfite – reducing clostridia).

To perform the mathematical analysis of the analytical results, food products of the two compatibility groups were grouped into 13 compatibility types on the base of characteristic ingredients and/or production technology, namely: minced meat (110 samples, including pork, beef and mixed), hot-smoked meat products (33 samples), frankfurters and small sausages (33 samples), cooked sausages (29 samples), breaded pork chops (24 samples), aspics (19 samples), kebab or minced pork skewers with added spices (10 samples), semi-dried sausages (10 samples), hot-smoked sausages (six samples) liver sausages (four samples), semi-smoked sausages (four samples), liver pate (two samples), and cold-smoked sausages (one sample).

Following standards were used for testing of microbiological quality of meat preparations and products: standard LVS EN ISO 4833-1:2014 was used for detection of Aerobic plate counts (APC), standard LVS EN ISO 11290-2/A1:2007 amended by standard LVS EN ISO 11290-2:1998/A1:2005 – for testing of *Listeria monocytogenes*, standard GOST R 52815-2007 – for detection of *Staphylococcus aureus*, standard LVS EN ISO 6579:2003 amended by standard LVS EN ISO 6579:2003/AC:2006 – for testing of *Salmonella spp.*, standard GOST 29185-91 – for testing of presence of sulphite-reducing clostridia, standard LVS ISO 16649-2:2007 – for detection of presence of *Escherichia coli*, and standard GOST R 52816-2007 – for detection of coliforms in food samples.

The statistical analysis of the analytical data was performed using single factor analysis of variance (ANOVA). Results on microbiological testing of cold-smoked sausage were excluded from further statistical analysis because only one sample was tested. In cases when p-value was $p<0.05$, it was considered that features under research are mutually dependent with probability of 95%. The data of mathematical analysis were described with help of histograms.

Results and Discussion

The results of the mathematical analysis indicate that APC values for meat products and meat preparations are not significantly different (p=0.23) (Fig. 1 and Fig. 2).

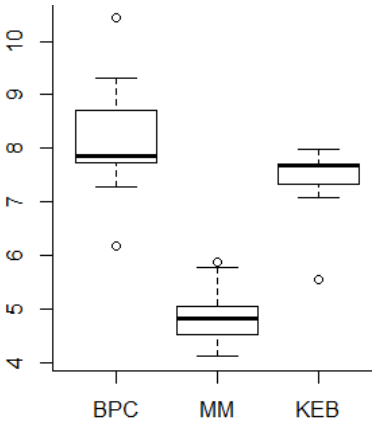

Figure 1. Minimum, maximum and median of APC levels in meat preparations (lg CFU g^{-1})
BPC – breaded pork chops, MM – minced meat, KEB – kebab

The findings of the research suggest that meat products, which are subjected to thermal processing during the production process, may still contain high numbers of microorganisms.

The results of the mathematical analysis indicate that the value of APC within the group of meat preparations (foodstuffs that have not undergone thermal processing) is substantially different (p=0.02) and dependent on the method of technological treatment. The highest maximum APC value (10.43±9.47 lg CFU g^{-1}) and the highest mean APC value (9.14±9.47 lg CFU g^{-1}) was found for samples of breaded pork chops (Fig. 1). Significant difference was not revealed for APC in kebab with added paprika and kebab with added herbs (p=0.15). Despite the fact that samples of meat preparations were taken and analysed only one day after production a huge variation between APC values was observed. Standard deviation of APC for breaded pork chops and minced meat was even higher than the mean value of APC (Table 1).

Standard deviation is usually used to describe the distribution in relation to the mean value. Basically, a large standard deviation means that the values in a statistical data set are farther away from the mean, on average. Thus, from mathematical point of view, a large standard deviation found in frame of the research reflects a large variation between APC values (or the existence of data with extreme values) for the types of meat preparations that were studied. From food safety viewpoint, the results that deviate significantly from the trend may indicate a tendency towards a situation which is out of control and may highlight the need for attention before control is lost. It is always very essential to understand the nature of potential hazards that may be presented by raw materials (ICMSF, 2011; Schaffner, 2007; IFST, 1997).

Table 1

Mean value, standard deviation and median of APC (lg CFU g^{-1}) for meat preparations and meat products

Type of meat preparation or product	Mean value	Standard deviation	Median
Minced meat	5.08	5.16	4.83
Kebab	7.64	7.48	7.67
Breaded pork chops	9.14	9.74	7.86
Cold-smoked sausages	1.00	-	1.00
Semi-dried sausages	3.01	2.85	3.10
Liver pate	3.51	1.85	3.51
Hot-smoked sausages	3.99	3.73	4.08
Cooked sausages	4.86	5.52	2.04
Frankfurters, small sausages	4.91	5.58	2.90
Smoked meat products	4.94	5.55	2.56
Semi-smoked sausages	5.12	5.41	3.51
Liver sausage	6.16	6.36	5.69
Aspic, day 1	4.56	4.73	4.19
Aspic, day 2	5.85	6.08	3.23
Aspic, day 6	8.33	8.40	8.24

The lowest maximum, minimum and mean APC values were found for minced meat samples. The mean value of APC for raw meat ($5.08x10^5 \pm 5.16x10^5$ CFU g^{-1}) is still in line with international guidelines of good manufacturing practice and Finnish recommendations to good quality of minced meat, however, the huge standard deviation suggests the large variability of microbiological quality of minced meat produced in Latvia. The maximum and mean APC values for breaded pork chops are well above the value recommended in the international guidelines (10^7 CFU g^{-1}) (ICMSF, 2011; IFST, 1997; Skrökki, 1997).

The results of the mathematical analysis indicate that the value of APC within the group of meat products (foodstuffs that have been thermally processed) is substantially different (p=0.0005) and dependent on the method of technological processing. As aspic samples were analysed on several days after production – one, two or six days after production – the APC values for aspics were excluded from the mathematical analysis.

The findings of the research suggest that microbiological quality of ready-to-eat meat products should be improved. The maximum and mean values of APC for liver sausages and semi-smoked sausages, as well as maximum APC values for frankfurters, small sausages and cooked sausages are not in line with international guidelines of good manufacturing practice (10^5 CFU g^{-1}) and are close to the maximum acceptable level (10^7 CFU g^{-1}) (ICMSF, 2011; IFST, 1997). The highest maximum APC (6.68±6.36 lg CFU g^{-1}) and the highest mean APC (6.16±6.36 lg CFU g^{-1}) was found for samples of liver sausage (Table 1, Fig. 2). High maximum and mean values of APC were also revealed for semi-smoked sausages and smoked meat products.

A huge variation between APC values was observed. Standard deviation of the mean values of APC for many meat products was much higher than the mean value of APC (Table 1). Like in case with meat preparations, the large standard deviations indicate that the APC values are largely dispersed and extreme values are farther away from the mean value. Thus, the large standard deviations found in frame of the research reflect a large variability of APC values within types of meat products that were studied. However, from food safety point of view the results that deviate significantly from the common trend may indicate a tendency towards a situation which is out of control and may highlight the need for attention before control is lost (ICMSF, 2011; Schaffner, 2007; IFST, 1997). Comparatively less variations in APC values were revealed for hot-smoked sausages and semi-dried sausages.

The results of the mathematical analysis indicate that the value of APC for aspics analysed one, two and six days after production is substantially different (p=0.01). In addition, the correlation coefficient r^2=0.75 indicates a strong positive correlation between level of APC and day of storage (Fig. 3). It is necessary to emphasize that the mean value of APC for aspics analysed six days after production (8.33±8.40 lg CFU g^{-1}) was about 300 times higher than for aspics analysed only one day after production (4.56±4.73 lg CFU g^{-1}). Maximum APC value that was observed for aspic tested six days after production was the highest one within the group of meat products (8.70±8.40 lg CFU g^{-1}).

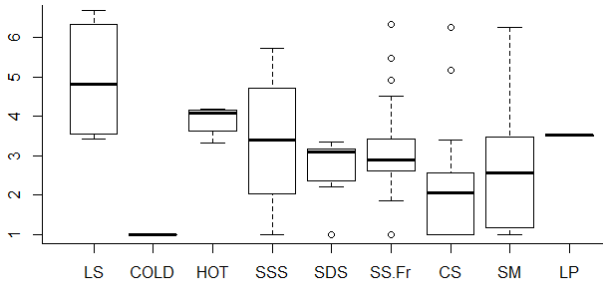

Figure 2. Minimum, maximum and median of APC levels in meat products (lg CFU g^{-1})

LS – liver sausages, COLD – cold-smoked sausages, HOT – hot-smoked sausages, SSS – semi-smoked sausages, SDS – semi-dried sausages, SS.Fr – small sausages and frankfurters, CS – cooked sausages, SM – smoked meat, LP – liver pate

The statistically significant difference (p=0.001) was found with regard to APC for different types of sausages. The minimum APC for the most types of sausages was <1 lg CFUg^{-1} with the exception of liver sausages and hot-smoked sausages, the minimum APC of which was 3.43±6.36 lg CFU g^{-1} and 3.32±3.73 lg CFU g^{-1}, respectively (Table 1, Fig. 2).

The maximum APC value (6.68±6.36 lg CFU g^{-1}) was found for liver sausage.

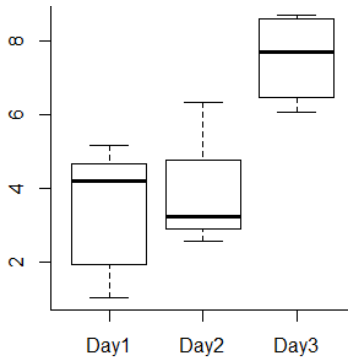

Figure 3. APC level in aspics lg CFU g^{-1}, referring to different days of storage

Day1 – one day after production, Day 2 – two days after production, Day 6 – six days after production

The research revealed a huge fluctuation between APC values for liver sausages produced in different factories, namely the APC values for liver sausage tested on the day of production (5.99 lg CFU g^{-1}) and for liver sausage tested two days after production (6.68 lg CFU g^{-1}) were much higher than for liver sausage the sample of which was analysed 10 days after production (3.43 lg CFU g^{-1}). Unlike for liver sausages, comparatively low APC was revealed for liver pate (3.52 lg CFU g^{-1}) even 11 days after production that may suggest more use of food preservatives.

Although in general it could be expected that APC for cooked meat products should be higher than for dried meat products, no significant difference was detected with regard to APC for smoked sausages and cooked sausages (p=0.55). The findings may suggest about inadequate parameters of technological processing or cross-contamination of smoked sausages after production.

Like for liver sausages a huge variation in APC values was found for smoked pork products produced in different factories; for example, APC varied greatly from <1 lg CFU g^{-1} to 6.26 lg CFU g^{-1} on the day of production of smoked pork products, as well as for sample tested on the day of production the APC value was much higher (6.26 lg CFU g^{-1}) than for several other samples tested 5 days after production (<1 lg CFU g^{-1}).

Hygiene indicatororganisms and pathogens were detected in 72 (10%) of 724 meat product samples tested.

In total, coliforms were tested in 77 samples of meat products, and only two samples – samples of aspic and liver pate – were positive. The presence of *Escherichia coli* (≥1 lg CFU g^{-1}) was detected in 28% of 188 samples tested. *Escherichia coli* were not found in thermally processed meat products. Most often *Escherichia coli* were detected in kebab samples, namely it was detected in 19 of 20 kebab samples tested. *Escherichia coli* were detected in 31 minced meat samples (in 23% samples of 135 samples tested). Statistically significant difference (p=0.008) was revealed in relation to *Escherichia coli* count for minced meat (mean value 2.04±1.94 lg CFU g^{-1}) and for kebab (mean value 3.11±3.40 lg CFU g^{-1}). The average *Escherichia coli* count in breaded pork chop was 1.70±1.45 lg CFU g^{-1}. The results indicate that *Escherichia coli* count in kebab exceed 10^2 CFU g^{-1} that is internationally recommended reference value of good hygiene practice (ICMSF, 2011; IFST, 1997).

Pathogenic microorganisms were mainly found in raw meat preparations. *Salmonella spp.* were detected in 8 minced meat samples of 282 samples of meat preparations and meat products tested. According to the results of *Salmonella* serotyping, *Salmonella typhimurium* were revealed in five tests.

Listeria monocytogenes were detected in 4 minced meat samples of 110 samples of meat preparations and meat products that were tested for the presence of *Listeria monocytogenes*.

Presence of *Staphylococcus aureus* was not detected in 29 samples of sausages and smoked meat. Sulphite – reducing clostridia were found in one sample of semi-dried sausage.

It should be taken into account that the potential for growth and/or toxin production of residual microbial population in the end-products depends on the types of organisms present in food and their ability to grow to a level of concern under the storage conditions applied during the shelf life (ICMSF, 2011; Schaffner, 2007; IFST, 1997). Therefore, the microbiological quality of raw meat and raw meat preparations, as well as methods of technological processing of meat and meat preparations is of great importance to control the microbiological contamination of ready-to-eat meat products.

The findings of the research demonstrate characteristic trends in prevalence of microbiological contamination in meat preparations and meat products produced in Latvia, including:

- high variability of microbiological quality of meat preparations and meat products was revealed during shelf-life of foodstuffs, suggesting that foods of significantly different microbiological quality are marketed;
- high numbers of microorganisms in thermally unprocessed meat preparations, especially in breaded pork chops were demonstrated, indicating that maximum and mean APC values can be well above the international guidelines of good manufacturing practice;
- also, high numbers of microorganisms in certain thermally processed meat products were detected, indicating that maximum and mean values of APC for liver sausages, semi-smoked sausages, as well as for frankfurters, small sausages and cooked sausages are not in line with international guidelines of good manufacturing practice;
- microbiological contamination of meat products was generally not dependent on the method of

technological processing, demonstrating that high risk products can be found within different meat products types – for example, statistically significant difference was not found between APC values of smoked and cooked sausages;

- the mean value of APC for aspics increased dramatically within six days after production, proving that setting of adequate "use by" is not carefully investigated;
- pathogenic and indicatororganisms were found in raw meat preparations, indicating that raw meat and raw meat preparations can be a source of pathogens and further thermal processing is therefore of great importance, including giving precise cooking instructions to final consumers on labels;
- the presence of coliforms was detected in aspics and liver pate, suggesting that occasionally presence of pathogens can not be excluded.

The most probable reasons of high microbial counts in meat preparations and meat products might be poor hygienic quality of raw meat and other ingredients, inadequate storage and thawing conditions, as well as contamination from technological equipment and via hands of personnel. In addition, the inadequate temperature-time parameters of thermal processing, cross-contamination of ready-to-eat products after production process or due to contaminated packaging material can enhance the microbial load of end-products.

A clear understanding of the effects of food handling practices and processing technologies on microorganisms and, in particular, on pathogens in foods, including their survival and growth characteristics is essential (ICMSF, 2011; Schaffner, 2007; IFST, 1997). The results of the research suggest that purposeful investigation of the microbiological quality of meat preparations and meat products with the aim to perform trend analysis of microbiological contamination should be carried out at the sites of production. In order to reduce presence, survival and multiplication of pathogens in end-products, verification of good manufacturing practice and monitoring procedures, as well as validation of overall HACCP procedure is of great importance.

Conclusions

A huge load of total microbiological contamination in meat preparations and meat products can lead to introduction of pathogenic microorganisms in foods therefore risk assessment on the base of trend analysis is relevant.

To conclude on bottle-necks during production of meat preparations and products, purposeful testing of indicatororganisms can be recommended. The analysis of trends in microbiological contamination of food and environmental samples should be used to improve theoretically developed HACCP plans at the level of individual food production companies. In addition, quality of marketing conditions should be studied by producers for better understanding of changes in microbiological quality of foods during shelf-life.

The results of the research suggest that development of guidelines on good manufacturing practice to explain common principles of trend analysis and purposeful monitoring of microbiological contamination of meat preparations and meat products is essential to ensure continuous improvement of microbiological quality of foods and thereby a high level of protection of consumers' health.

Acknowledgment

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References

1. Burlingame B., Pineiro M. (2007) The essential balance: risks and benefits in food safety and quality. *Journal of Food Composition and Analysis*, Vol. 20, Iss. 3–4, p. 139–146.
2. ICMSF (2011). Microorganisms in foods 8. Use of data for Assessing Process Control and Product Acceptance. New York: Springer. 400 S.
3. IFST (1997) Development and use of microbiological criteria for foods. *Food Science and Technology Today*, Vol. 11, Iss. 3, p. 137–176.
4. Güngör E., Gökoğlu N. (2010) Determination of microbial contamination sources at aFrankfurter sausage processing line. *Turkish Journal of Veterinary and Animal Sciences*, Vol. 34, Iss. 1, p. 53–59.
5. Schaffner D.W. (2007) *Microbial risk analysis of foods: Emerging Issues in Food Safety.*Washington, USA: American Society for Microbiology, 270 S.
6. Skrökki A. (1997) Hygienic quality of commercial minced meat as indicated by aerobic micro-organisms and Coliform bacteria. *Zeitschrift für Lebensmittel untersuchung und Forschung A*, Vol.204, Iss. 5, p. 391–394.
7. Commission Regulations (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. (2005). OJ L 338, 22.12.2005, p. 1–29.

THE INFLUENCE OF ENVIRONMENTAL CONDITIONS ON WINTER WHEAT WHOLEMEAL PROTEIN CONTENT AND RHEOLOGICAL PROPERTIES

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Abstract

The aim of this investigation was to clarify variation of protein content on winter wheat (*Triticum aestivum* L.) grain, water absorption and mixing properties of wholemeal dough, depending on harvest year weather conditions (2010–2012) and cultivar. Trial included winter wheat cultivars 'Bussard' and 'Zentos'. The farinograph water absorption (WA) and wholemeal dough mixing characteristics – dough development time (DDT), dough stability time (ST) and degree of softening (DS12) were tested by Brabender Farinograph (ICC 115/1). The data show that experimental year and cultivar significantly ($p<0.05$) affected protein content, farinograph water absorption and mixing properties of winter wheat wholemeal dough**.** The influence of year was confirmed on higher level for grain protein content and wholemeal dough stability time, compared with the cultivar effect. Cultivar had a much stronger effect on wholemeal dough water absorption, dough development time and degree of softening than year. Cultivar 'Bussard' wholemeal had higher protein content, water absorption, longer stability time and shorter degree of softening compared with 'Zentos'. The results demonstrate that the quality of the studied varieties meets the requirements for high-grade wheat for food consumption, and are suitable for wholegrain flour production and baking. The positive correlation (r=0.972) existed between protein content and dough stability. Protein content correlated negative (r=-0.893) with dough degree of softening. Dough stability time had negative correlation (r=-0.878) with degree of softening.

Keywords: winter wheat, protein, rheological properties, Farinograph.

Introduction

Wheat (*Triticum aestivum* L.) is a dominant cereal crop in the Latvia, and more and more is used in the preparation of bread made from whole wheat grain flour. Whole grain foods are an important part of the diet because they provide many nutrients (Kunkulberga et al., 2007b). The wheat kernels contain a germ, pericarp layers (outer and inner), seed coat, aleurone layer and starchy endosperm. The objective of milling is to separate the starchy endosperm from the kernel, and to ground it into flour. The aleurone layer, pericarp layer and seed coat form the bran. When white flour is produced, many important nutrients including dietary fibres are removed, because these components are mainly located in bran and germ (Dewettinck et al., 2008).

Quality parameters of winter wheat are not stable between production years because of the inconsistency of the variables, such as initiation of the growing season, distribution of rainfall and heat units available for crop growth during corresponding phases of plant growth and development (Linina, Ruza, 2004).

Protein content is commonly used as predictor of baking quality (Koppel, Ingver, 2010). The temperatures and water stress occurring during grain filling period affects changes in wheat protein aggregation (Daniel, Triboi, 2002). Grain protein content significantly varied depending on the differences among cultivars (Linina, Ruza, 2012). During ripening wheat needs sunny and warm weather and moderate moisture. These conditions secure biological maturity and acceptable technological and rheological properties of grain (Krejčirova et al., 2006).

Evaluation of rheological properties of flour by farinograph is popular among the millers, bakers, grain handlers and wheat breeders. The rheological characteristics reflect the dough properties during processing and the quality of the final product. Strong flours are characterized by a long dough development time, high stability with a small degree of softening, while poor flours weaken quickly, resulting in high degree of softening (Shahzadi et al., 2005).

Rheological properties of wheat mainly depend from cultivars diversity and the growth conditions. Panozzo (2000) reported that year meteorological conditions and cultivar interactions were significant for the dough rheological characteristics.

In Latvia there were no made investigations about winter wheat wholemeal rheological properties depending on weather conditions and cultivar till now.

Therefore, the aim of the present research was to investigate the influence of weather conditions in three harvesting years and two different cultivars on the content of wholemeal protein, and the farinograph water absorption, dough development time, stability time and degree of softening.

Materials and Methods

Study fields. Field experiments in years 2010, 2011 and 2012 were conducted at the Latvia University of Agriculture, Study and Research farm "Peterlauki" on silt loam brown lessive soil with close to neutral acidity (pH_{KCl} 6.9), medium high phosphorus and potassium, humus content 2.7 g kg^{-1}. Registered winter wheat (*Triticum aestivum* L.) bread cultivars from Germany 'Bussard' and 'Zentos' were sown after black fallow. Both cultivars are with high bread – making quality. Phosphorus and potassium fertilizers were applied in

autumn P_20_5 – 72 kg ha^{-1} and K_20 – 90 kg ha^{-1}. Nitrogen (N), was applied N60 – 150 kg ha^{-1} in spring after resumption of vegetative growth. Grain was harvested at full ripeness; sampling procedure for grain quality evaluation was performed according to the standard ICC 101/1 for obtaining average sample.

Weather data collection. The air temperature in investigation years (Table 1) in April was by 0.8–2.5 °C higher compared with long-term average observations; also May was by 0.3–1.3 °C warmer, which promoted plant growth and development. Average daily temperature in June 2010 and 2011 was warmer by 0.9–2.0 °C which contributed to the accumulation of protein. In 2012 air temperature was lower than 1.1 °C, compared to long-term average data. Temperature in the grain filling period (July), which is most decisive for grain quality formation, was in 2010 by 4.4 °C warmer and by 2.7 °C warmer in 2011, while in 2012 only by 1.2 °C higher than the long-term average mean data.

Table 1

Weather conditions during the field experiment

Month	Average temperature ºC			
	2010	**2011**	**2012**	**LTM***
April	6.2	7.9	6.4	5.4
May	12.6	11.6	12.2	11.3
June	16.1	17.2	14.1	15.2
July	21.2	19.5	18.0	16.8
Average	14.0	14.1	12.7	12.2
	Sum of precipitation, mm			
April	48	32	106	40
May	85	56	45	51
June	60	78	95	75
July	298	179	197	82
Average	122	86	111	62

*LTM–long-term mean

Water availability has effect on wheat grain quality. Precipitation in April 2010 and 2011 was close to long-term average, but in 2012 by 265% more than long-term means data.

May in 2010 was wet, when precipitation was 164% higher than the long-term average for this month, in 2011 and 2012 precipitation was close long-term mean data for this month.

Precipitation in June 2010 and 2011 was close to long-term mean; but in 2012 by 126% more than long-term means data. July in 2010, 2011 and 2012 was very rainy, respectively by 365%, 219% and 241% exceeded the long-term averages data.

Technological properties of wheat. The protein content and rheological properties of wholemeal wheat were determined at the Latvia University of Agriculture, in the Grain and Seed Research Laboratory and Laboratory of Food analysis. Grains were milled to wholemeal using Laboratory Mill 3100 (Perten Instruments, Sweden) at a particle size of 0.8 mm. Wheat technological properties were analyzed in duplicate. Grain protein content (PC) was calculated multiplying total nitrogen content by factor 5.7 determined by Kjeldahl method (ICC 105/2; Kjeltec system 1002, Foss Tecator AB, Sweden). The farinograph water absorption (WA, 14%) and dough mixing characteristics–dough development time (DDT), dough stability time (ST) and degree of softening (DS12) were tested by Brabender Farinograph with a mixer using 300 g of flour, with slow blade rotation speed 63 rpm and measurement control system software 2.5.17 (Brabender, Germany; ICC 115/1).

Statistical analysis. Experimental data evaluation was done using two – factor analysis of variance by Fisher's criteria and least significant difference ($LSD_{0.05}$) were applied to estimate the effects of year (weather conditions) and cultivars. Component of variance ANOVA for each quality characteristic were expressed as percentage to illustrate the relative impact of each source to the total variance. Correlation analysis between protein content and wholemeal rheological properties was also carried out.

Results and Discussion

Protein content (PC) is one of more important indicators of grain quality for the bread making industry (Ruzgas, Liatukas, 2008). Grains with protein content 12–13% are suitable for bread making; however, grains with higher protein content traditionally are used for improving the properties of lower quality grains. Grain processing companies in Latvia are limited requirements for A quality class wheat is protein contents >145 g kg^{-1}, first class > 140 g kg^{-1}, second class >130 g kg^{-1} and third class – > 120 g kg^{-1}.

Average data in our experiment (3 years) suggest (Table 2) that protein content in cultivar 'Bussard' grains was 143.4 g kg^{-1}, it was statistically significantly higher compared to 'Zentos' (122.6 g kg^{-1}). Grain protein content significantly varied depending on the cultivars and meteorological conditions (Ruza et al., 2002; Kunkulberga et al., 2007a; Skudra, Lininа, 2011).

Table 2

Winter wheat wholemeal protein content, g kg^{-1}

Cultivar,	Year, factor B			Average
factor A	**2010**	**2011**	**2012**	$LSD_{0.05\ A}$=1.1
Bussard	150.1	155.3	124.9	143.4
Zentos	131.5	133.8	102.5	122.6
Average B	140.8	144.5	113.7	×
$LSD_{0.05\ B}$ = 1.4				
$LSD_{0.05\ AB}$ = 2.0				

The protein content of both wheat cultivars in years 2010 and 2011 was consistent with the requirements of food wheat, while protein content in variety 'Bussard' grains was significantly higher than mentioned in A class requirements. The average temperature from

April to July during the years 2010 and 2011 exceeded 14 °C and this was favourable for protein synthesis, therefore, in cultivar 'Bussard' grain average protein content was 153.6 g kg^{-1}, while in 'Zentos'– 132.7 g kg^{-1}. Similar scientific results were obtained in the trial Dotnuva (Lithuania) Cesevičiene and co-authors (2009), they conclude, that warmer weather with more sunny days is more favourable for the accumulation of protein content in wheat grains.

The lowest protein content was identified in both analysed cultivars in year 2012. Grain quality mainly was affected by the weather conditions during the ripening period (Cesevičiene, 2012). Cool (average air temperature in growing season 12.7 °C) and rainy weather in 2012 during grain filling – ripening stage was adverse for protein accumulation. In cultivar 'Bussard' grain average protein content was 124.9 g kg^{-1} and corresponded to third class, especially low protein content accumulated in the cultivar 'Zentos' grain – 102.5 g kg^{-1}.

In our experiment, according to Fisher's criteria, the weather conditions and cultivar had a significant ($p<0.05$) impact on the grain protein content. Grain protein content did not significantly depend either on the year–cultivar interaction.

Similarly, the analysis of variance for two cultivars and 3 experiment years suggest that winter wheat grain protein content by 63.3% depended on weather conditions (year), but the influence cultivar was also remarkable – 36.4% (Table 3). Influence of the year was most remarkable also in the investigation with 15 winter and 14 spring wheat cultivars in the years 2004–2007 in Estonia (Koppel, Ingver, 2008).

Table 3

Impact factors of winter wheat wholemeal quality indices, %

Source of variation	PC	WA	DDT	ST	DS12
Year	63.3	28.1	37.4	59.1	28.1
Cultivar	36.4	66.4	51.8	37.8	68.9
Year/cultivar interraction	0.2 ns.	3.1 ns.	10.6	3.0	2.1

PC–protein content, WA–water absorption; DDT–dough development time; ST–dough stability; DS12–degree of softening, ns–not significant.

Dough quality is one of the most important features enabling one to predict the final bread making value of the winter wheat cultivar (Liatukas et al., 2012). The elasticity of the dough was measures by Brabender's farinograph, whose operations are based on physical methods. The diagram (farinogram) shoved direct indexes: water absorption, dough development time, stability and degree of softening.

Water absorption (WA) is the most important parameter measured by farinograph, it indicated as the amount of water need to develop the standard dough of 500 farinograph unit (FU) at the peak of the curve. Stronger wheat flours have the ability to absorb and retain more water as compared to weak flours (Mis, 2005).

Average data suggest (Table 4), that wholemeal made from 'Bussard' absorbed water (721 g kg^{-1}) on average by 34 g kg^{-1} more than 'Zentos' (687 g kg^{-1}) and this is statistically significantly higher. In reports of Mašauskiene and Cesevičiene (2006) with winter wheat a white bread flour Type-550 water absorption for cultivar 'Zentos' was 591–631 g kg^{-1}, however according to Koppel and Ingver (2010) 550–650 g kg^{-1} WA is appropriate for yeast bread. Wholemeal flour need larger water content as compared to white flours, in experiment of Haridas Rao and other (1989) it was 704–825 g kg^{-1}, similar results we obtained in our experiment too.

It has been shown that water absorption increased with increasing of PC content (Zaidul et al., 2002; Shahzadi et al., 2005, Constantinescu et al., 2011). In our experiment the average PC in wheat grain cultivars 'Bussard' and 'Zentos' was higher in year 2011, (respectively 155.3 and 133.8 g kg^{-1}) as a result WA was higher too, respectively 704–734 g kg^{-1}, similar results also found by Varga with colleges (2003).

Table 4

Winter wheat wholemeal water absorption, g kg^{-1}

Cultivar, factor A	Year, factor B			Average $LSD_{0.05\ A}$=6.4
	2010	2011	2012	
Bussard	715	734	712	721
Zentos	672	704	686	687
Average B	694	719	699	×
$LSD_{0.05\ B}$ = 7.9				
$LSD_{0.05\ AB}$ = 11.2				

The cultivar and weather conditions (harvesting year) had a significant ($p<0.05$) impact on the wholemeal WA, while cultivar–year interaction does not affect WA significantly (Table 3). WA was mostly affected by the cultivar (66.4%) however; the year factor was also remarkable (28.1%). In Koppel and Ingver (2010) experiments with 11 winter wheat cultivars in the years 2005-2009, WA dependency on year complete to 63.6% but the influence of cultivar – 24.3%.

Dough development time (DDT) indicates the relative strength of the wheat flour and can also reflect the level of water absorption in the test. Therefore, if the dough development time is shorter, the less time is needed to mix the dough (Sabovics, Straumite, 2012). In the present experiments, the dough development time of both analyzed cultivars was high (Table 5).

A dough development time of wholemeal from 'Bussard' was 4.75 min in average, and this is by 1.11 min lower compared with 'Zentos' (5.86 min). Sabovics and Straumite (2012) found similar results in Latvia: they tested wholemeal triticale properties by farinograph and determined dough development time as 5.95 min, while Mašauskiene and Cesevičiene (2006) found that wheat dough development time for cultivar 'Zentos' white flour was 2.25–3.43 min.

Table 5

Winter wheat wholemeal dough development time, min

Cultivar, factor A	Year, factor B			Average $_{LSD\ 0.05\ A}$=0.05
	2010	2011	2012	
Bussard	5.55	4.67	4.05	4.75
Zentos	5.99	6.33	5.27	5.86
Average B	5.77	5.50	4.66	×
$LSD_{0.05\ B}$ = 0.06				
$LSD_{0.05\ AB}$ = 0.09				

Prabhasankar et al., (2002) and Shahzadi et al., (2005) found that wholemeal dough require a longer development time compared to white flour because it contains bran.

The weather conditions in the investigations years, cultivars and year–cultivar interaction had a significant ($p<0.05$) impact on the wholemeal DDT. DDT was more affected by the cultivar (51.8%) than by the year (37.4%) and lowest impact belonged to interaction cultivar-year (10.6%).

Dough Stability (ST) indicates the time when the dough maintains maximum consistency and a good indication of dough strength (Karaoglu, 2011). ST is measure that is expected by baking industry for producing yeast bread. Good quality dough could be stable for 4–12 min. Satisfactory ST is about 6 min.

Average dough stability (Table 6) of cultivar 'Bussard' wholemeal dough was 9.01 min, while it for 'Zentos' was 6.77. The dough stability should relate with the cultivars peculiarities mainly (Cesevičiene et. al., 2012).

Table 6

Winter wheat wholemeal dough stability time, min

Cultivar, factor A	Year, factor B			Average $_{LSD\ 0.05\ A}$=0.07
	2010	2011	2012	
Bussard	9.95	9.99	7.11	9.01
Zentos	6.94	8.54	4.82	6.77
Average B	8.45	9.26	5.96	×
$LSD_{0.05\ B}$ = 0.08				
$LSD_{0.05\ AB}$ = 0.12				

Dough stability is an important indicator for flour strength which is based on the quantity and quality of dough protein (Kučerova, 2005). In our experiment it was determined that if the protein content in grain was higher (year 2011), the dough stability made from both cultivars 'Bussard' and 'Zentos' wholemeal was longer – 9.99 and 8.54 min respectively, similar results Varga et. al., (2003) was found.

The weather conditions in the investigations years, cultivars and year–cultivar interaction had a significant ($p<0.05$) impact on the wholemeal ST (Table 3). ST was most markedly affected by the year (59.1%) but influence of a cultivar was also reliable (37.8%), while the influence year–cultivar interaction was small 3.0%, this is agreement with earlier reports of Kopel and Ingver (2010).

Degree of softening (DS12) is the difference between the centre of the curve at the end of the dough development time and the centre of the curve 12 minutes after this point. Dough mixing qualities are considered satisfactory when the degree of softening is below 70 FU. When this value exceeds 110 FU, the dough is considered to be weak (Mašauskiene, Cesevičiene, 2006). Average degree of softening of 'Bussard' wholemeal was 23.8 FU where the degree of softening 'Zentos' was 43.0 FU (Table 7). Therefore the dough softening depends on the cultivar genetic characteristics (Mašauskiene, Cesevičiene, 2006) which were confirmed in the present experiment.

Table 7

Winter wheat wholemeal dough degree of softening, FU*

Cultivar, factor A	Year, factor B			Average $_{LSD\ 0.05\ A}$=2.34
	2010	2011	2012	
Bussard	17.4	24.0	30.1	23.8
Zentos	36.8	39.0	53.3	43.0
Average B	27.1	31.5	41.7	×
$LSD_{0.05\ B}$ = 2.87				
$LSD_{0.05\ AB}$ = 4.05				

***FU–farinograph units

In 2012, when the protein content in grain was lower (compared with 2010 and 2011), the dough softening of analyzed wholemeal made from both cultivars 'Bussard' and 'Zentos' was higher–30.1 and 53.3 FU respectively, acquired results agree with report of Varga et al., (2003). The year, cultivar and year – cultivar interaction had a significant ($p<0.05$) impact on the wholemeal DS12 (Table 3). DS12 mostly depending on cultivars (68.9%), influence of a year was 28.1% while both factors influence was only 2.1%. Protein content is often used as indirect indicator of baking quality–higher protein content means that the dough stability is higher, compare with lower protein content (Kopel, Ingver, 2010).

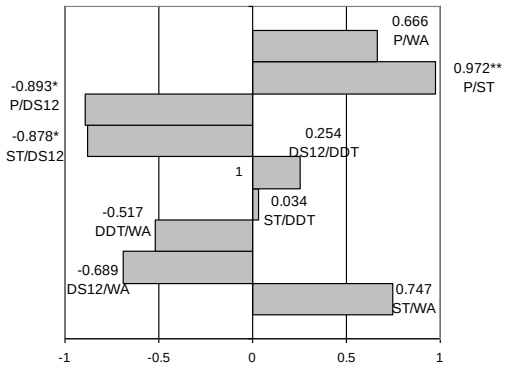

Figure 1. Correlation coefficient (r) **between quality indices**

r**–is significant at 99%; r*–at 95% level of probability. PC–protein content, WA–water absorption; DDT–dough development time; ST–dough stability; DS12–degree of softening.

In our investigation a tendency was found, namely, if the grain protein content is higher the dough formation time is longer, the dough stability is higher, and the dough softening is lower.

The wheat wholemeal quality parameters were assed by correlation analysis too (Figure 1).

A statistically significant positive correlation was found between PC and ST r=0.972**, R^2=0.945, a regression equation y=9.192x+60.48, these results are in accordance with studies made by Kopel and Ingver (2010), and Ceseviciene et al., (2012).

Preston (2001) found that the correlation between PC and WA was statistically significant (r=0.51), however in our investigation relationship between PC and WA was higher (r=0.66), but not significant.

PC showed high negative relationship between DS12 described by the regression equation y=–1.341x+177.83, and correlation coefficient r=0.893*, R^2=0.798.

Mikos and Podolska (2012) found that higher degree of softening indicated lower dough stability. Our results are in agreement with this as a strong negative relationship was evident between DS12 and ST r=–0.878*, R^2=0.771, y=–0.139x+12.55.

Conclusions

In the present research it was found that wheat cultivars with differences in their genetic as well as weather conditions significantly influence protein formation in grains. Higher protein content was found in the wheat samples in harvest years with warmer growing conditions (2010 and 2011).

Winter wheat rheological properties demonstrate that the quality of the studied varieties correspond to the requirements for high-grade wheat for food consumption, and are suitable for wholegrain flour production and bread baking.

Winter wheat cultivars ‘Bussard’ and ‘Zentos’ are different in their farinograph curve shapes. ‘Bussard’ wholemeal had higher water absorption, longer stability time and shorter degree of softening, compared with ‘Zentos’.

Cultivar had a much stronger effect on wholemeal dough water absorption, dough development time and degree of softening than weather conditions in investigations years. The influence of environmental conditions on higher content of protein in winter wheat grain was confirmed as well as wholemeal dough stability time, compared with the cultivar effect.

The strong correlation was found between protein content and dough stability time. Degree of softening had negative correlation with protein content. Dough stability correlated negatively with degree of softening.

Acknowledgement

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References

1. Ceseviciene J., Leistrumaite A., Paplauskiene V. (2009) Grain yield and quality of winter wheat varieties in organic agriculture. *Agronomy Research,* Vol. **7** (I), p. 217–223
2. Ceseviciene J., Slepetiene A., Leistrumaite A., Ruzgas V. (2012) Effects of organic and conventional production systems and cultivars on winter wheat technological properties. *Journal of the Science of Food and Agriculture*, Vol. 92 (14), p. 2811–2818.
3. Constantinescu G., Dabija A., Buculei A., Rebenciuc I. (2011) Evaluation of cereal cultivar impact on bread quality. *Journal of Agroalimentary Processes and Technologies, Vol.* 17 (4), p. 473–476.
4. Daniel C., Triboi E. (2002) Changes in wheat protein aggregation during grain development: effects of temperatures and water stress. *European Journal of Agronomy*, Vol. 16, p. 1–12.
5. Dewettinck K., Van Bockstaele, F., Kuhne, B., Van de Walle, D., Courtens, T.M., Gellynck, X. (2008) Nutritional value of bread: influence of processing, Food interaction and consumer perception. *Journal of Cereal Science*, No. 48, p. 243–257.
6. Haridas Rao P., Leelavathi K., Shurpalekar S.R. (1989). Effect of damaged starch on the chapatti–making quality of whole wheat flour. *Cereal Chemistry*, Vol. 66 (4), p. 329–333.
7. Karaoglu M.M. (2011) Dough characteristics of wheat flour milled from wheat grains stored in spike form. *International Journal of Food Science and Technology*, Vol. 46 (9), p. 1905–1911
8. Koppel R., Ingver A. (2008) A comparison of the yield and quality traits of winter and spring wheat. *Latvian Journal of Agronomy*, Vol.11, p. 83–89.
9. Koppel R., Ingver A. (2010) Stability and predictability of baking quality of winter wheat. *Agronomy Research*, Vol. 8 (III), p. 637–644.
10. Krejčirova L., Capouchova I., Petr J., Bicanova E., Kvapil R. (2006) Protein composition and quality of winter wheat from organic and conventional farming. *Zemdirbyste-Agriculture*, Vol. 93 (4), p. 285–296.
11. Kučerova J. (2005) The effect of sites and years on the technological quality of winter wheat grain. *Plant, Soil and Environment*, Vol. 51 (3), p. 101–109.
12. Kunkulberga D., Ruza A., Linina A., Galoburda R. (2007a) Evalution of wholegrain flour baking properties depending on variety. *Food Chemistry and Tehnology*, 2007, Vol. 41, Iss. 2, p. 24–29.
13. Kunkulberga D., Klava D., Ruza A., Zirvite I., Riekstina A. (2007b) Development of wholegrain products. **In:** *Proceedings of 4 International congresses "Flour - Bread'07*". 6th Croatian congress of cereal technologists, Croatia, Opatija, p. 300–305.
14. Linina A., Ruza A. (2004) Variety and environment effects on quality traits in Latvian-grown winter wheat. **In***: 4 International Crop Science Congress*, Brisbane, Australia, [accessed on 10.09.2012.]. Available at: www.cropscience.org.au/icsc2004/poster/5/1/2/1051_anda.htm
15. Liniņa A., Ruža A. (2012) Cultivar and nitrogen fertilizer effects on fresh and stored winter wheat grain quality indices. *Proceedings of the Latvian Academy of Sciences*, Vol. 66 (4/5), p. 177–184.

16. Mašauskiene A., Cesevičiene J. (2006) Variation in Winter wheat grain qualities as affected by NK fertilisation and grain storage period. II. Dough mixing properties by Brabender Farinograph. *Proceedings of the Latvia University of Agriculture*, Vol. 16 (311), p. 59–67.
17. Mis A. (2005) Influence of chosen factors on water absorption and rheological properties of gluten of bread wheat (*Triticum aestivum* L), *Acta Agrophysica*, (In Polish), Vol. 128 (8), p. 1–120.
18. Mikos M., Podolska G. (2012) Bread–making quality of old common bread (Triticum aestivum ssp vulgare L) and spelt (Triticum aestivum ssp. spelta L.) wheat cultivars. *Journal of Food, Agriculture and Environment,* Vol.10, Iss. (3 and 4, p. 221–224.
19. Panozzo J.F. (2000) Cultivar and environmental effects on quality characters in wheat. II Protein. *Australian Journal of Agricultural Research*, Vol. 51, p. 629–636.
20. Prabhasankar P., Manohar R.S., Gowda L.R. (2002) Physicochemical and biochemical characterization of selected wheat cultivars and their correlation to chapatti making quality. *European Food Research and Tehnology*, Vol. 214, p. 131–137.
21. Preston K.R., Hucl P., Townley-Smith T.F., Dexter J.E, Williams P.C., Stevenson S.G. (2001) Effects of cultivar and environment on farinograph and Canadian short process mixing properties of Canada western red spring wheat. *Canadian Journal of Plant Science*, Vol. 81 (3), p. 391–398.
22. Ruza A., Linina A., Kreita Dz. (2002) Sustainable production and plant growth: Interaction between crop yield and quality of winter wheat. **In:** *Environment and Crop Production.* Enfield: Science Publishers Inc. p. 31–39.
23. Ruzgas V., Liatukas Ž. (2008) Breeding of winter wheat for end-use quality. *Latvian Journal of Agronomy*, Vol. 11, p. 135–140.
24. Liatukas Ž., Ruzgas V., Razbadauskiene K., Brazauskas G., Koppel R. (2012) Winter wheat cultivars 'Kovas DS', 'Zunda DS', 'Vikaras DS', 'Kaskada DS' for high input farming; development and characterization. *Zemdirbiste-Agriculture,* Vol. 99, Iss. 3, p. 225–264.
25. Shahzadi N., Butt M.S., Rehman S.U., Sharif K. (2005) Rheological and Baking Performance of Composite Flours. *International Journal of Agriculture and Biology*, Vol. **7** (1). p. 100–104.
26. Skudra I., Linina A. (2011) The influence of meteorological conditions and nitrogen fertilizer on wheat grain yield and quality. **In:** *The 6 Baltic Conference on Food Science and Technology "Innovations for food science and production" Foodbalt–2011;* Conference Proceedings, Latvia, Jelgava, p. 23–26.
27. Sabovics M., Straumite E. (2012) Rheological properties of triticale (Triticosecale Wittmack) flour blends dough. **In:** *Annual 18 International conference*: *Research for Rural Development,* Conference Proceedings, Vol. 1, p. 143–148.
28. Varga B., Svecnjak Z., Jurkovic Z., Kovacevic J., Jukic Z. (2003) Wheat grain and flour quality as affected by cropping intensity. *Food Technology and Biotechnology*, Vol. 41 (4), p. 321–329.
29. Zaidul I.S.M., Karim A.A., Manan D.M.A., Ariffin A., Norulaini N.A.N., Omar A.K.M. (2002) Study of rheological profile analysis related to texture for mixtures of sago-wheat gel. *Internation Journal of Food Properties*, Vol. 5, Iss. 3, p. 585–598.

SOME CHEMICAL, YIELD AND QUALITY PROPERTIES OF DOMESTIC OAT CULTIVARS

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Abstract

Oat *(Avena sativa* L.) is one of the cereal crops cultivated in climate temperate zones. It is well known as a healthy food in the world, because of its unique biochemical structure. Nowadays the quality of grain for consumers has become important especially in terms of lipids and β-glucan content. The aim of this study was to characterize the yield, volume weight, 1000 kernel weight, husk content and kernel size distribution for two naked and three husked oat cultivars. Some quality analyses were determined, such as protein, starch, lipid and β-glucan contents. Investigations were carried out at the State Stende Cereal Breeding Institute in 2012 and 2013. The obtained results showed significant differences among naked and husked oat cultivars in all tested parameters for example lipid content for husked oat cultivars varied from 58.1–66.8 g kg^{-1}, but for naked oat 92.3–108.8 g kg^{-1}. β-glucan content among husked oat cultivars varied from 3.81–3.85 g 100 g^{-1}, but naked oat breeding line '33793' reached 5.07 g 100 g^{-1}. Little variation between years was detected as well. Research showed that naked oat characterized with better quality and insignificant husk content, what is preferable for consumers', but kernel size uniformity and yielding abilities of husked oat cultivars would be preferable reasons for farmers and food producers to choose them as a raw material. Overall naked oat could be better for food production from consumers' side, but food producers are made to choose husked oat cultivars because of the requirements of production techniques.

Keywords: naked oat, husked oat, quality.

Introduction

Oat *(Avena sativa* L.) is an important crop produced in climate temperate zone and distinct among the cereals due to its multifunctional characteristics and nutritional profile. It is used both for human and animal nutrition. Before using in human nutrition, oat was used for medicine purposes. Oat is a nutritious source of protein, carbohydrate, fiber, vitamins and minerals. Currently, discussion on oat grain dietetic value and suitability for the production of functional foods is more frequently mentioned in scientific literature and scientific projects (Biel et al., 2009).

Grain yield, volume weight and 1000 kernel weight and husk content are the most important economic traits mentioned by the oat consumers, because the end-product outcome is due to these traits when oat grain is processed (Sadiq Butt et al., 2008).

Oat kernel size uniformity is an important parameter for the oat milling industry because the processing of oats for human food generally involves size separation of kernels into different streams before dehulling. Oat spikelets may contain one, two, tree, or more kernels, and the main kernel is always larger than others. Larger oat grains can be dehulled at slower rotor speeds than smaller oat grains, it is because an oat kernel with a larger mass will possess more energy of inertia when impacting the walls of the impact dehuller than smaller oat grains at the same rotor speed. So it is better if oat cultivar is characterized with larger kernel fraction or more of the same size grains (Doehlert et al., 2006b, Doehlert et al., 2004).

Oat breeders through hybridization and selection have improved yielding ability potential of oat varieties; they have developed oat varieties dwarfed in length and more resistant to lodging. On consumers'side lower standards are set forward regarding biochemical composition of grain: protein, lipids, β-glucan, starch contents in grain, though dietetic value of oats is just due to these traits (Wood, 2007). Protein is considered as the most important nutrient for humans and animals as well. The average protein content of cereal grains covers a relatively narrow range (8–11%) variations, however, are quite noticeable. The main part (approximately 40%) of kernel structural component is starch. Because of its unique properties, starch is important for the textural properties of many foods, in particular bread and other baked goods. It is located only in endosperm and is present in granular form. Oat contains relatively high amounts of lipids (approximately from 7%) compared to other cereal grains (Sadiq Butt et al., 2008).

Among the main compounds associated with health-promoting effects in cereals is dietary fiber which is found only in plant origin foods. It consists of both – soluble and insoluble fiber. Both types are important for human health (Manthey et al., 1999). Water-soluble fiber in cereals is composed of non-starchy polysaccharides such as β-glucan also called lichenin. It is presented particularly in barley (3–7%) and oat (3.5–5%), whereas less than 2% β-glucan is found in other cereals. Some of the oat constituents are valuable ingredients or starting materials for several types of products (Brindzova et al., 2008). Oat β-glucan has received the most attention and has a number of potential uses (Wood, 2007).

The aim of this study was to characterize the yield, test weight, 1000 kernel weight, husk content and kernel size distribution for two naked and three husked oat cultivars.

Materials and Methods

Field trials and investigation were carried out at the State Stende Cereals Breeding Institute (SSCBI) in 2012 and 2013. Five local oat cultivars (int. al. two

naked oat and three husked oat cultivars) were used for this research. The soil of the site was sod-podzolic in both, the humus content – 18 g kg^{-1} (2012), 20 g kg^{-1} (2013), the soil pH KCl – 6.2 (2012), 6.6 (2013) , the available for plants content of phosphorus P – 42 mg kg^{-1} (2012), 39 mg kg^{-1} (2013), and that of potassium K – 59 mg kg^{-1} (2012), 53 mg kg^{-1} (2013). The pre-crop was barley for both years. All agro-technical operations were carried out at optimal terms according to the weather conditions during the vegetation period and depending on the plant development phases. Seed rate was 500 seeds per 1 m^2. Before cultivation of the soil, a complex mineral fertilizer was applied: N-51, P–30, and K – 42 kg ha^{-1}. Variants were arranged in four replications with plot size 10 m^2 in a randomized block design.

The temperature and moisture conditions provided good oat field germination in 2012 and 2013 and were shown in Figure 1 and Figure 2, respectively.

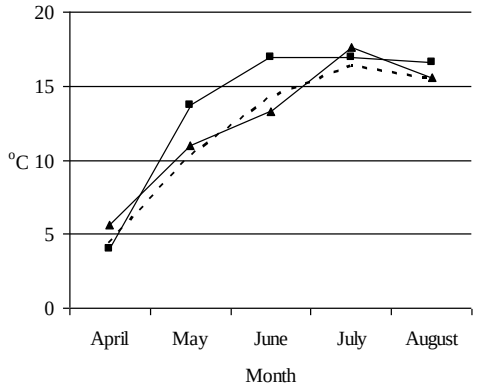

Figure 1. The mean daily temperature at SSCBI
▲ – year 2012, ■ – year 2013, - - - - – long term average

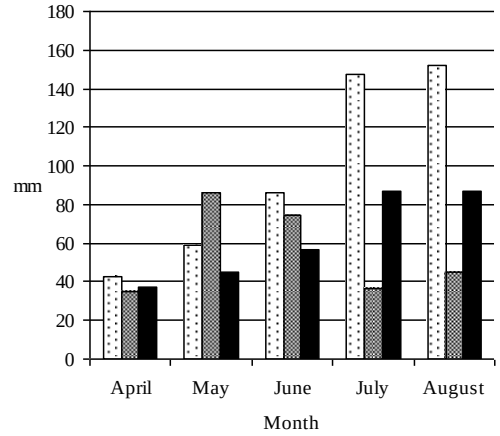

Figure 2. Sum of precipitation at SSCBI
▒ – year 2012, ▒ – year 2013, ■ – long term average

The mean daily temperature in 2012 was close to long term average, but weather observations in 2013 were high above it. Vegetation period in 2012 characterized by abundant rainfall and mean values of all months exceeded the long-term observed monthly norm. Harvesting was delayed approximately by ten days because of heavy rainfalls at first decade of August. The opposite situation was in 2013. Precipitation in May was excellent for germination, and lack of moisture in end of vegetation period ripened grains in panicles.

Mean samples from all replications (0.5 kg) were taken for testing with Infratec Analyser 1241 (test weight, protein, starch, β-glucan and lipid content) performed at the State Stende Cereals Breeding Institute. 1000 kernel weight was detected by standard method LVS EN ISO 520:2011. Kernel size classifications were carried out with SORTIMAT separator machine. Cleaned sample of 100 g to be weighed on a balance accurate to 0.01 g and then placed onto the top sieve. The sieving period was set for 3 minutes, recommended by producers. The sieves were used with diameters of 2.5 and 2.2 mm. With a weighed batch of 100 g, the percentage proportion was then obtained by weighing the individual fractions. Husk content was determined by four samples of 5 g of each cultivar, separating husk from kernel manually and weighed, percentage proportion was calculated.

The obtained results were statistically processed by MS Excel program package using the methods of descriptive statistics. ANOVA procedures were used for data analysis.

Results and Discussion

Quality

Nowadays, oat in Latvia has been widely studied as a raw material for human diet. For using oat meals in human nutrition, it is necessary to investigate its quality and thereby suitability. In this study, protein, starch, lipid and β-glucan contents were determined as quality parameters for naked and husked oat cultivars, the results were shown in Table 1.

Table 1

Quality of naked and husked oat cultivars (means calculated according to two years results, g kg^{-1}±sd on dry matter basis)

Cultivar	Protein*	Starch*	Lipids*
Naked oat cultivars			
S-156	168.8±0.13^{ab}	299.8±1.26^{b}	108.8±0.21^{ab}
33793	163.9±0.15^{ab}	349.8±0.87^{b}	92.3±0.16^{ab}
Husked oat cultivars			
'Arta'	114.8±0.10^{b}	462.8±0.17^{ab}	58.1±0.09^{b}
'Laima'	100.8±0.06^{b}	458.5±0.20^{ab}	65.6±0.23^{b}
'Stendes Dārta'	100.9±0.07^{b}	456.5±0.23^{ab}	66.8±0.16^{b}

*difference are significant between naked and husked oat cultivars with the level of $p<0.05$, ^{a} trait mean values significantly higher comparing husked and naked oat cultivars, ^{b} differences significant between years.

Starch is the major storage carbohydrate of cereals and an important compound for human nutrition. Starch contents of naked oats were significantly ($p<0.05$)

lower than that of husked oat cultivars. Mean starch content for naked oats was 324.8 g kg^{-1}, but for husked oats – 459.3 g kg^{-1}. Opposite results were reported in the study of Givens et al. (2003), who were found that husked oat cultivars had significantly lower starch contents than that of naked ones (400 g kg^{-1} and 580 g kg^{-1}, respectively). Starch content in oat grain is subjected to variety, nitrogen treatment and weather conditions. In this study, nitrogen treatment was the same in both years, but chosen varieties and meteorological conditions might affect starch contents according to our results.

Protein and lipid contents of naked oat cultivars were significantly high ($p<0.05$). Lipids in foods are an important nutritional factor and their profile may play a crucial role as concerns the stability of cereal products (Brindzova et al., 2008). Oat grain has soft kernel and lipid distributed throughout the seed, which makes the milling process more difficult than wheat and corn. To prevent from atmospheric oxidation, the oat is hydrothermally treated before processing (Sadiq Butt et al., 2008). The high lipid content is not desirable for food producers, but crucial component for human diet, because of consistence of vitamin E and fatty acids, which is located in lipids. The highest lipid content (108.8 g kg^{-1}) was observed with naked oat breeding line of S-156. Conciatori et al. (2000) determined mean lipid content for naked oat as 116.4 g kg^{-1} which was close to our results. The lowest lipid content (66.8 g kg^{-1}) was observed with husked oat variety of Stendes Dārta, similar result obtained with common oat as 72 g kg^{-1} (Koehler and Wieser, 2013). Lipid content in oat cultivars is strongly dependent on meteorological conditions of sowing year which was indicated by Givens et al. (2003). It was also seen in our research, where lipid content for naked oat cultivars was significantly ($p<0.05$) higher in 2013. Significant difference ($p<0.05$) was observed only for naked oat cultivars.

The highest protein content (168.8 g kg^{-1}) among naked oat cultivars was detected with the breeding line of S-156. Among the husked oat cultivars, the highest protein content (114.8 g kg^{-1}) was obtained with the sample of Arta. In the literature, for husked oat, average protein content is reported as 115.0 g kg^{-1}, for naked oat, it was reported as 143.4 g kg^{-1} (Biel et al., 2009). Protein content in oat grain is dependent on mostly agro-meteorological conditions: variety, nitrogen treatment, sowing date and weather conditions (Givens et al., 2003). That is the reason why significant differences were found between husked and naked oat cultivars, also between varieties and years.

β-glucan, which is a soluble dietary fiber, is an important component of oat grain with health promoting effect. It could have beneficial role in gastrointestinal diseases, lowering of cholesterol level, promoting heart health, preventing diabetes and even cancer (Daou, Zhang, 2012). When naked and husked oat cultivars were compared, husked oat had significantly lower β-glucan content, as shown in Figure 3. The cultivar of Stendes Dārta had the highest β-glucan content among the husked oat cultivars, like 3.85 g 100 g^{-1}. The naked oat breeding line of 33793 had the highest β-glucan content (5.07 g 100 g^{-1}) among all cultivars studied. In the literature, β-glucan contents for oats were reported between 2.3–8.5 g 100 g^{-1}. Cultivars studied in this research demonstrated average results compared to data reported previously.

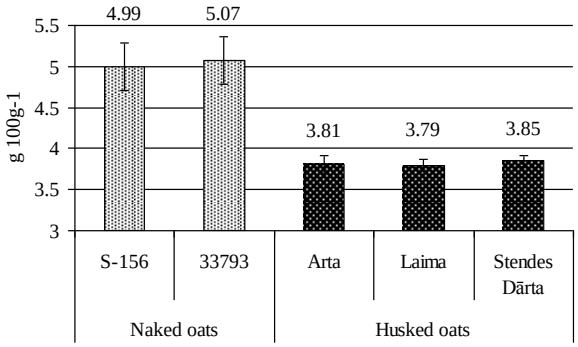

Figure 3. β-glucan (g 100 g^{-1}) contents of oat cultivars

– naked oat cultivars, – husked oat cultivars

Productivity

Productivity parameters used in this study were yield, 1000 kernel size, test weight and husk content. Yield results shown in Table 2 varied from 4.31 t ha^{-1} to 5.30 t ha^{-1} for husked oat genotypes, but for naked ones the highest yield was obtained with the sample of S-156 as 3.69 t ha^{-1}. Zute et al. (2010) reported husked oat yield about 5.02 t ha^{-1} between 1993 and 2009 years. Same as quality parameters, yield is also dependent on meteorological conditions.

Table 2

Yield and husk contents of naked and husked oat cultivars

Cultivar	Yield*, t ha^{-1}	Husk content*, %
Naked oat cultivars		
S-156	3.69±0.33	0.06±0.02 [b]
33793	3.20±0.06	0.10±0.02 [b]
Husked oat cultivars		
Arta	4.31±0.31 [a]	23.23±0.65 [ab]
Laima	5.30±0.02 [a]	26.44±0.18 [ab]
Stendes Dārta	5.25±0.04 [a]	26.14±0.75 [ab]

*difference are significant between naked and husked oat cultivars with the level of $p<0.05$, [a] trait mean values significantly higher comparing husked and naked oat cultivars, [b] differences significant between years

Husk content of oat grain is about 25–30 % of the seed. The grain is dehulled before use, whereas husk after processing may be used in food industry. Unprocessed husk contains silicate particles, which are harmful to nature and can irritate the mouth, esophagus and gastrointestinal tract (Sadiq Butt et al., 2008). The highest husk content was detected with the cultivar of Laima, as 26.4% (Table 2). Husk contents of naked oat

cultivars were not significant. Yield consists of husk and groat weight. Although naked oat cultivars had low amount of husk, their groat yields were close to the yield of husked oat cultivars.

1000 kernel weight is a parameter which characterizes kernel weight. The results showed that husked oat cultivars have significantly higher ($p<0.05$) 1000 kernel weight comparing with naked oat cultivars and variation between years was significant as well. Volume weight is most commonly used to evaluate grain quality. Volume weight was significantly ($p<0.05$) higher for naked oat cultivars also observed by Doehlert et al. (2001), and also its value was influenced by sowing year.

Kernel size distribution

Kernel size uniformity test showed that the distribution of kernel size was dependent mostly on cultivar and two distribution classes (>2.5 and <2.2) on sowing year (Table 3).

Table 3

Kernel size distribution (%) of husked and naked oat cultivars (sieve hole diameters of 2.5 and 2.2 mm)

Cultivar	>2.5	2.5–2.2	<2.2
		2012*	
Naked oat cultivars			
33793	17.6[b]	42.0	40.4[ab]
S-156	18.5[b]	44.7	36.8[ab]
Husked oat cultivars			
Stendes Dārta	47.7[ab]	40.5	11.8[b]
Arta	63.0[ab]	27.6	9.4[b]
Laima	36.3[ab]	51.9	11.8[b]
Cultivar	**>2.5**	**2.5–2.2**	**<2.2**
		2013*	
Naked oat cultivars			
33793	14.1[b]	39.8	46.0[ab]
S-156	12.1[b]	42.8	45.1[ab]
Husked oat cultivars			
Stendes Dārta	55.7[ab]	40.3	4.0[b]
Arta	67.4[ab]	27.1	5.4[b]
Laima	44.5[ab]	49.8	5.7[b]

*difference are significant between husked and naked oat cultivars with the level of $p<0.05$, [a] trait mean values significantly higher comparing husked and naked oat cultivars, [b] differences significant between years.

With larger kernels (>2.5 mm) characterized as husked oat cultivars. For example 63.0% of kernels in 2012 and 67.4% of kernels in 2013 were observed in class of >2.5 mm for the cultivar of Arta. For food production, such cultivar with larger grains is more suitable than others, because it requires lower rotor speed in dehulling process (Doehlert et al., 2004). As the husked oat spikelets may contain 1 to mostly 3 florets, naked oat spiklets consists of up to 12 florets (Doehlert et al. 2006a). It means that the kernels of naked oat cultivars are smaller comparing with husked oat. As we mentioned that kernel size distribution is quite dependent on cultivar, the results of other scientists were much different from ours. Doehlert et al. (2004) worked with four sieve classes, like 3.18, 2.58, 2.38, 1.98 mm slot sieves. The most of husked cultivars were characterized as small grains (2.38–1.98 mm slot) in that research. Kernel size uniformity is an important character for food producers, but it is hard to find cultivars with required quality parameters, such as large grains.

Conclusions

For all tested parameters, there were significant ($p<0.05$) differences between naked and husked oat cultivars. The naked oat cultivars exhibited better quality parameters, for example they had the highest protein and the lowest starch contents. The lipid contents of naked oat cultivars was higher than the reported literature findings, however β-glucan level failed to meet expectations and average value was obtained in studied years. Productivity of tested cultivars showed that yield, husk content and 1000 kernel weight results of husked oat cultivars were higher than naked oat cultivars. Besides, volume weight results of naked oat cultivars were higher than that of husked oat cultivars. Such parameters, like larger grain size, yield and lower lipid content could be main reasons for food producers to choose husked oat cultivars. When the low processing requirements is considered, the naked oat cultivars can be more valuable for food industry.

Acknowledgment

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References

1. Biel W., Bobko K., Maciorowski R. (2009) Chemical composition and nutritive value of husked and naked oats grain. *Journal of Cereal Science*, Vol. 49, p. 413–418.
2. Brindzova L., Čertik M., Rapta P., Zalibera M., Mikulajova A., Takācsovā M. (2008) Antioxidant activity, β-glucan and lipid contents of oat varieties. *Czech Journal of Food Science*, Vol. 26(3), p. 163–173.
3. Conciatori A., De Stefanis E., Redaelli R., Sgrulletta D. (2000) Chemical characterization of some kernel trait in naked oat genotypes. *Journal of Genetics and Breeding*, Vol. 54, p. 299–302.
4. Daou C., Zhang H. (2012) Oat beta-glucan: its role in health promotion and prevention of diseases. *Comprehensive Reviews in Food Science and Food Safety*, Vol. 11, p. 355–365.
5. Doehlert C.D., Jannink J.L., McMullen M.S. (2006b) Kernel size variation in naked oat. *Crop Science*, Vol. 46, p. 1117–1123.
6. Doehlert C.D., McMullen M.S., Hammond J.J. (2001) Genotypic and environmental effects on grain yield and quality of oat grown in North Dacota. *Crop Science*, Vol. 41, p. 1066–1072.
7. Doehlert C. D., McMullen M. S., Jannink J .L., Panigrahi S., Gu H., Riveland N.R. (2004) Evaluation of

oat kernel size uniformity. *Crop Science*, Vol. 44, p. 1178–1186.
8. Doehlert C.D., McMullen M.S., Jannink J.L. (2006a) Oat grain/groat size rations: A physical basis for test weight. *Cereal Chemistry*, Vol. 83(1), p. 114–118.
9. Givens D.I., Davies T.W., Laverick R.M. (2004) Effect of variety, nitrogen fertiliser and various agronomic factors on the nutritive value of husked and naked oats grain. *Animal Feed Science and Technology*, Vol. 113, p. 169–181.
10. Koehler P., Wieser H. (2013) Chemistry of cereal grains. **In:** *Handbook on Sourdough Biotechnology*. M. Gobbetti, M. Gänzle (eds.). US: Springer, p. 11–45.
11. Manthey F.A., Hareland G.A., Huseby D.J. (1999) Soluble and insoluble dietary fiber content and composition in oat. *Cereal Chemistry*, Vol. 76 (3), p. 417–420.
12. Sadiq Butt M., Tahir-Nadeem M., Kashif Iqbal Khan M., Shabir R. (2008) Oat: unique among the cereals. *European Journal of Nutrition*, Vol. 47, p. 68–79.
13. Wood P.J. (2007) Cereal β-glucans in diet and health. *Journal of Cereal Science*, Vol. 46, p. 230–238.
14. Zute S., Vīcupe Z., Gruntiņa M. (2010) Factors influencing oat grain yield and quality under growing conditions of West Latvia. *Agronomy Research*, Vol. 8 (Speciel Issue III), p. 749–754.

LIPID COMPOSITION OF OAT GRAIN GROWN IN LATVIA

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Abstract

Oat (*Avena sativa* L.) is a well known annual crop in temperate climates. It is recognised as a healthy food containing significant amounts of fat-soluble vitamin E and polyunsaturated fatty acids in the world. There are few investigations of lipid composition in connection with human health in Latvia. Therefore the aim of this study was to characterize the composition of lipids of same oat varieties to grown in Latvian condition. Investigations were performed at the State Stende Cereal Breeding Institute. In the studied samples content of fat, composition of fatty acids and vitamin E (α-tocopherol) were determined, same as ratio most significant for human health like PUFA/SFA and ω-3/ ω-6 were calculate. The total fat was made by Soxlet method, fatty acids by LVS CEN ISO/TS 17764-1:2007 and content α-tocopherol determination was made using high-performance liquid-chromatography. The high concentration was determined as mg kg dry matter. The obtained results showed a wide range of fat content among varieties, it varied from 4.9 to 10.5 g 100 g^{-1}. The content of α-tocopherol in oat grain was determined 8.5–12.3 mg kg^{-1}, and the sum of unsaturated fatty acids accounted 78–81.5% of total fatty acids content. The ratio P/S varied from 2.2 to 2.4. Result of evaluation leads to conclusion that lipid of oat grain are rich with biologicaly significant substances.

***Keywords*:** oat for human health, fat content, fatty acids, vitamin E.

Introduction

Oat is recognised as a healthy food containing significant amounts of fat-soluble vitamin E and polyunsaturated fatty acids in the world.

The fat content of oat grain is the highest among grains and varied from 4.2 to 11.8 g 100 g^{-1}, in comparison with wheat (2.1–3.8 g 100 g^{-1}), rice (2.0–3.1 g 100 g^{-1}), millet (4.0–5.5 g 100 g^{-1}), barley (3.3–4.6 g 100 g^{-1}), rye (2.0–3.5 g 100 g^{-1}) (Zhou, 1999). Total lipid in oat grains averages 4–6 % when based on relatively few varieties. However, larger ranges are apparent when more genotypes are considered, the range of 3.1 to 11.6% was found among more than 4 000 US entries in the world collection (Zhou, 1999). A high lipid content of 15.5% in a US experimental line of groats was reported by Hartunian – Sowa and White, 1992). Morphologically, oat can be classified as husked and naked; naked oat is nutritionally richer than the common husked oat.

Vitamin E activity is provided by the tocopherols and tocotrienols, which together create tocols. Tocols are fat soluble and form part of the total lipid. The vitamin activity of tocols is associated with their antioxidant function *in vivo*. The highest level of vitamin E activity is produced by α-tocopherol, following by β-tocopherol and α-tocotrienol, which have 40% and 30% of the activity of α-tocopherol, respectively on an equal weight basis (Welch, 1995; Zielinski et al., 2001; Jackson et al., 2008). Alpha-tocopherol is a major antioxidant component in crude oat unaltered when the lipid is refined (Webster, 1986). Among the Latvian genotypes, the variety 'Arta' had the highest α-tocopherol concentration – 7.5 mg kg^{-1}, and there was no significant difference between years (Berga et al., 2012).

The fatty acid composition of oat lipids is bound as to oat breeders so to nutritionists because of the nutritional significance of fatty acids. Separate fatty acids (FA) also have different impact on human health. Many studies estimate the beneficial effects of polyunsaturated (PUFA) and monounsaturated fatty acids (MUFA) (Chillard et al., 2000). Linoleic, oleic and palmitic acids are major oat lipid constituents (Brindzova et al., 2008). The proportion of unsaturated fatty acids is about 75% of total and content of palmitic acid as main of saturated fatty acids determined 14–17% (Saastamoinen, 1989).

To compare the potential impact of fat on human health often used fatty acids ratio in the scientific literature. The most commonly used is ratio – sum of polyunsaturated fatty acids to sum of saturated fatty acids – PUFA / SFA. World Health Organization (2003) recommended ratio PUFA/SFA must be higher than 0.4. The decrease in ratio between n-6 and n-3 FA, as well as increase in ratio between stearic / palmitic acids (C18:0 / C16:0) and oleic / stearic acids (C18:1c / C18:0) are desirable for the prevention of number of diseases (Gebauera et al., 2005).

The lipid content of oat grain depends on genetic and environmental factors as well as the method of determination (Zhou, 1999). The limiting heritabilities for fat content is high and polygenic (Karow, Rosberg, 1984)

The aim of this study was to characterize the composition of lipids of same oat varieties to grown in Latvian condition**.**

Materials and Methods

Investigations were performed at the State Stende Cereal Breeding Institute. The material consisted of 7 oat genotypes (int. al. 4 husked and 3 naked). In the studied samples fat, α-tocopherol content and fatty acids composition were determined.

Fat content was made by Sochlet method.

Vitamin E. Mean samples from all replications (0.5 kg) were taken and milled for α-tocopherol determination in the Laboratory of Food and Environmental Investigations of the Institute of Food Safety, Animal

Health and Environment BIOR, using high-performance liquid-chromatography. The method is often used for detection of α-tocopherol in diet samples. Absorption was measured at 292 nm. Chromatography was carried out in a C18 column using methanol / water liquid (98 / 2 v/v). The concentration was determined as mg kg^{-1} in dry matter.

Fatty acids. Previously homogenized samples were prepared for GLC (gas-liquid chromatography) analysis using direct saponification with KOH/methanol followed by a derivatization with (trimethylsilyl) diazomethane. Alltech AT-FAME analytical column was used. The carrier gas (He) flow rate was 2 mL min^{-1}. The injector and detector temperatures were 225 °C and 250 °C, respectively. Peaks were identified using standard mixture Supelco FAME Mix C4-C24, Sigma Aldrich. The concentration was determined as % of total fatty acids.

The content of different fatty acids (FA) was calculated according to formulas 1–3:

$$SFA = \sum C14:0;\ C16:0;\ C18:0;\ C20:0; C22:0 \quad (1)$$

$$MUFA = \sum C16:1, C18:1, C20:1 \quad (2)$$

$$PUFA = \sum C14:0; C16:0; C18:0; C20:0; C22:0 \quad (3)$$

The obtained results were statistically processed using descriptive statistics in the MS Excel software package. Correlation defined as medium close if 0.5<r>0.79. Differences between the groups were tested for significance ($p<0.05$) by ANOVA.

Results and Discussion

Obtained results of fat and α-tocopherol contents in analyzed oat grain samples assumed in the Table 1.

Table 1

Average fat and α-tocopherol contents of oat grain

Genotipes /Lines	fat, g 100 g^{-1}	α-tocopherol	
		mg kg^{-1}	% of fat
Naked oat cultivars			
S-156	8.9	10.3	0.012
33793	7.9	10.2	0.013
33805	10.7	9.0	0.009
Husked oat cultivars			
'St. Līva'	4.0	9.0	0.019
'Laima'	5.65	4.2	0.007
'Arta'	5.32	5.8	0.011
'St. Dārta'	5.67	3.3	0.012

As showed in the table 1, fat content same as content of α-tocopherol in grain samples differed significantly among oat varieties ($p<0.05$). Varieties of husked oat 'Stendes Līva' and 'Arta' had smallest fat content – 4.0 and 5.32 g 100 g^{-1} respectively. The richest in fats was grains of naked oat breeding line '33805'. Data of other investigations show that fat content of oat varied from 5–9% (Morrison, 1979) and 4.2–11.8 g 100 g^{-1} (Zhou, 1999).

Content of α-tocopherol in oats determined from 3 to 12.3 mg kg^{-1}, the richest was grains of breeding lines 'S-156' and '33793' with average α-tocopherol contents 10.3 mg kg^{-1} and 10.2 mg kg^{-1} respectively. Relatively richest with vitamin E was fat of variety 'St.Līva', with α-tocopherol concentration 0.019% of fat content. Previous investigations among the Latvian genotypes show that the variety 'Arta' had the highest α-tocopherol concentration – 7.5 mg kg^{-1} (Berga, 2012). Oat genotypes bred in Latvia did not show a marked difference when compared with material of foreign origin. The difference between husked and naked oat genotypes can be explained by the differences in sample structure.

Correlation between fat and α-tocopherol content reflected in Figure 1.

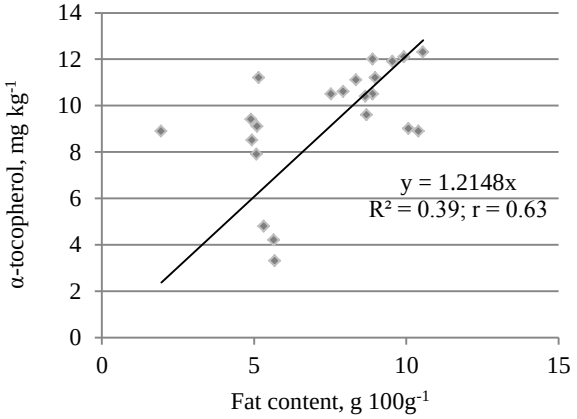

Figure 1. Corelation between fat and α-tocopherol content in oat grain samples

Correlation between fat and α-tocopherol content is not strong r=0.63, in husked oats correlation was calculate r=0.83, but in naked oats r=0.57. Our study confirms that just 39% of α-tocopherol changes could explain with fat content in oat grains, more than 60% explain with other factors such as oats variety impact.

The lipid fraction of the oat grain determines in large measure nutritional quality via the fatty acid composition. The content and degree of unsaturation of the oat total fatty acid was higher in winter sown than in spring sown crops, indicating that low temperatures cause a higher synthesis of unsaturated fatty acids in oats (107).

Fatty acid composition of husked oat grain same as ratio PUFA/SFA, C18/C16; C18:1/C18 assumed in Table 2 and fatty acid composition of naked oat grain with ratios assumed in Table 3.

Obtained results showed that content of oleic acid was determined 36.2–40.4% and content of linoleic acid 38.4–41.6%. Data of other investigations show that content of oleic acid in oat grain varieties determined from 37.2 to 42.1% and content of linoleic acid 38.6–42.5% (Saastamoinen, 1989).

Table 2

Fatty acid composition of husked oat grain, % of total

Fatty acids	Laima	Arta	St.Līva	St.Dārta
C14:0	0.2	–	–	–
C16:0	16.1	16.8	16.4	17.1
C18:0	1.7	2.5	2.3	2.1
C18:1	36.2	37.5	37.6	37.8
C18:2(n-6)	41.6	40.1	39.8	39.4
C18:2	1.2	1.0	1.0	1.1
C18:3(n-3)	1.2	0.9	1.3	0.9
C20:0	0.2	0.2	0.2	0.2
C20:1	0.8	0.7	0.8	0.6
C20:5	0.4	0.2	0.4	0.3
C22:0	0.1	0.1	0.1	0.1
PUFA/SFA	2.4	2.2	2.3	2.2
C18/C16	0.11	0.15	0.14	0.12
C18:1/C18	21.3	15.0	16.4	19.2

Table 3

Fatty acid composition of naked oat grain, % of total

Fatty acids	S-156	33793
C14:0	–	–
C16:0	17.4	15.5
C18:0	1.7	2.4
C18:1	37.9	40.4
C18:2(n-6)	39.1	38.4
C18:2	1.2	0.9
C18:3(n-3)	1.1	0.9
C20:1	0.7	0.7
C20:0	0.2	0.2
C20:5	0.3	0.2
C22:0	0.1	0.1
PUFA/SFA	2.2	2.3
C18/C16	0.10	0.15
C18:1/C18	22.3	16.8

The main saturated fatty acids increased cholesterol level in human blood are myristic acid and palmitic acid. Results of investigation showed that myristic acid determined 0.2% in oats of 'Laima' and content of palmitic acid in oat varieties determined 15.5–17.4%. The reviewed data showed higher content of myristic acid (0.2–4.9%) and palmitic acid (13.2–28.0%) in oat grains.

The evaluation of ratios significant for human health showed that ratio PUFA/SFA of analysed oat samples varied from 2.2 to 2.4, it was 5–6 times higher as WHO (2003) suggested and did not differed among oat varieties ($p>0.05$).

As mentioned before, the increase in ratio between stearic / palmitic acids (C18:0 / C16:0) and oleic / stearc acids (C18:1c / C18:0) are desirable for the prevention of number of diseases. The calculated ratio C18:0 / C16:0 varied from 0.10 to 0.15 and higher was calculated in husked oat variety 'Arta' same as naked oat breeding line '33793'. Evaluation of ratio C18:1 / C18:0 shoved that more healthy are husked oat samples 'Laima' same as naked oat samples 'S-156'.

Assumed saturated, mono unsaturated and polyunsaturated fatty acids in oat grains are showed in the Figure 2.

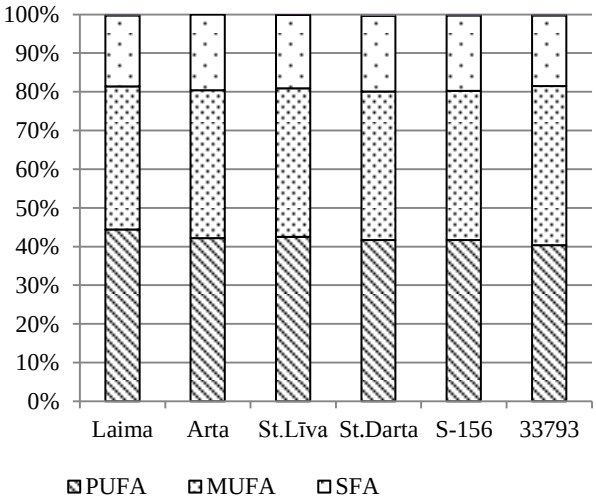

Figure 2. Proportions of fatty acids groups in oat grains

The sum of saturated fatty acids varied from 18.2% to 19.6% of total fatty acids and it was not differed among varieties ($p>0.05$). As showed in Figure 2, the sum of monounsaturated fatty acids accounted 40.4–44.4% and the sum of polyunsaturated fatty acid 37–41.1% of total fatty acids.

The sum of unsaturated fatty acids accounted 78–81.5% of total fatty acids. Saastamoinen et al. (1989) had determined mean content of unsaturated fatty acids about 75%, content of palmitic acid 14–17% for oat varieties which was close to ours.

Conclusions

The fat content was determined from 4 g 100 g^{-1} – in husked oat grains to 10.7 g 100 g^{-1}in naked oat grain samples. There were observed significant ($p<0.05$) difference in fat content among oat varieties.

Content of α-tocopherol in oats determined from 3 to 12.3 mg kg^{-1}, the richest was grains of naked oats breeding lines 'S-156' and '33793' with average α-tocopherol contents 10.3 mg kg^{-1} and 10.2 mg kg^{-1} respectively.

The sum of unsaturated fatty acids accounted 78.0–81.5% of total fatty acids. Result of evaluation leads to conclusion that lipid of oat grain are rich with biologicaly significant substances.

Acknowledgment

This work was supported by the Latvian State Research Program in Agro – biotechnology, and the European

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References

1. Berga L., Zute S. (2012) Variability in α-tocopherol concentration of husked and naked oat genotypes. **In**: *Proceedings of the Latvian academy of Sciences*. Section B, Vol. 66, No 1/2, p. 26–29.
2. Brindzova L., Čertik M., Rapta P., Zalibera M., Mikulajova A., and Takacsova M. (2008) Antioxidant activity, beta-glucan and lipid contents of oat varieties. *Czech Journal of Food Sciences*, Vol. 28, p.163–173.
3. Chilliard Y., Ferlay A., Nansbridge R. M., Doreau M. (2000) Ruminant milk fat plasticity:nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. *Annales de Zootechnie*, Vol. 49. p.181–205.
4. Gebauera S., Harris W.S., Kris-Ethertona P.M., Ethertonc T.D., (2005) Dietary n-6:n-3 Fatty Acid ratio and Health. *Healtful lipids*. AOCS Press, CRC.
5. Jackson E.W., Wise M., Bonman J.M., Obert D.E., Hu G., Peterson D.M. (2008) QTLs Affecting α-tocotrienol, α-tocopherol and total tocopherol concentrations detected in the Ogle/TAM O-301 Oat Mapping Population. *Crop Science*, Vol. 48, p. 2141–2152.
6. Karow R.S., Rosberg R.A. (1984) Oil composition in parental, F1 and F2 populations of two oat crosses. *Crop science*, Vol. 24, p. 629–632.
7. Morrison W. R. (1978) Cereal lipids. **In**: Advances in Cereal Science and Technology, Vol. 2. Y. Pomeranz, ed. *American. Assoiation of. Cereal Chemists*. St. Paul, MN p. 228–348.
8. Saastamoinen M., Kumpulainen J., Nummela S. (1989) Genetic and environmental in oil content and fatty acid composition of oats. *Cereal Chemistry*, Vol. 66, p. 296–300.
9. Thro A. M., Frey K. J., Hammond E. G.,(1985) Inheritance of Palmitic, Oleic, Linoleic, and Linolenic Fatty Acids in Groat Oil of Oats. *Crop Science*, Vol. 25, p. 40–44.
10. Webster, F.H. (1986). Oats. **In:** *Chemistry and Technology*. USA, American Association of Cereal Chemists. 220 p.
11. Welch, R.W. (1995) *The Oat Crop: Production and Utilization.* London, Chapman & Hall. 516 p.
12. World Health Organization (2003). Diet, nutrition and the prevention of chonic diseases. **In:** Report of a Joint WHO/FAO Expert Consultation, Geneva,160 p.
13. Zielinski H., Ciska E., Kozlowska H. (2001) The cereal grains: Focus on vitamin E. *Czech. Journal of Food Science*, Vol. 19, p. 182–188.
15. Zhou M X, Robards K, Glennie-Holmes M, Helliwell S. (1999) Oat Lipids. *Journal of American Oil Chemistry Science*, Vol. 79, p. 585–592.

OAT HULLS AND SEA BUCKTHORN POMACE – A POTENTIAL SOURCE OF ANTIOXIDANTS FOR HEMPSEED OIL

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Abstract

Hempseed oil, as well as other oils containing polyunsaturated fatty acids, is subjected to oxidation processes caused by air, heat or light. Usually these processes are suppressed by addition of various synthetic phenol type antioxidants. The aim of current research was to increase oxidative stability of vegetable oils with natural antioxidants. Herein, we demonstrate that the oxidative stability can be increased with natural antioxidants present in oat hulls and sea buckthorn pomace – by-products of food processing. Extracts from pomace and hulls were prepared by maceration of ground plant material in the hempseed oil. The extraction was accelerated by ultrasound. It was established that the highest amount of polyphenols in extracts of both plant materials can be achieved within 30 min; further increase of extraction time sometimes even reduced the total amount of polyphenols. The highest amount of polyphenols in sea buckthorn extracts was 7.73±0.29 mg GAE 100 g^{-1}, but in oat hull extracts – 4.63±0.21 mg GAE 100 g^{-1}, when extracts were prepared by ultrasonification of hempseed oil containing 5% wt of plant material. Various amounts of plant material additives were used for extraction under optimized conditions. The highest antioxidant activity (expressed as ratio of time when peroxide value of sample and blank reaches 48 meq O_2 kg^{-1}) – 1.51±0.05 and 1.40±0.06 to 1.44±0.02, respectively – had extracts obtained from 1% wt additive of sea buckthorn pomace or 2.5–5.0% wt additive of oat hulls. The prepared hempseed oil extracts demonstrated higher oxidative stability than hempseed oil containing 0.02% additive of synthetic antioxidant BHT.

Keywords: hempseed oil, oat hull, sea buckthorn pomace, oxidative stability.

Introduction

Oats (*Avena sativa* L.) demonstrate antiatherogenic properties in *in vitro* assays, animal experiments and human studies, exhibit anti-inflammatory and antioxidative action (Andersson, Hellstrand, 2012; Pazyar et al., 2012). Oats reduce coronary heart disease (Harris, Kris-Etherthon, 2010; Truswell, 2002) *via* different mechanisms – decreasing serum low-density lipoprotein cholesterol, blood pressure and improving glucose and insulin response (Harris, Kris-Etherthon, 2010). Due to the skin protecting effect against ultraviolet rays, oats are used in cosmetic formulations (Pazyar et al., 2012; Kurtz, Wallo, 2007). Unique oat polyphenols – avenanthramides – have strong antioxidant activity *in vivo* and *in vitro*, exhibit anti-inflammatory, antiproliferative and anti-itching activity, which may provide additional protection against coronary heart disease, colon cancer and skin irritation (Meydani, 2009; Guo et al., 2009).

Sea buckthorn (*Hippophae rhamnoides* L.) is widely used due to its biological activity: oil (Kumar et al., 2011), fruits and leaves (Suryakumar, Gupta, 2011; Xu et al., 2011) are used to treat cancer and disorders of skin, as well as cerebral-cardiovascular and immune systems. Sea buckthorn is applied to lower cholesterol, platelet aggregation, blood pressure and blood sugar (Xu et al., 2011). The oil absorbs ultraviolet light and promotes healthy skin (Stobdan et al., 2013; Vernet, 2006).

Due to the great biological properties of oats we studied oat grains as a source of natural antioxidants to enhance oxidative stability of various vegetable oils. Previously we have clarified, that additives of oat grains improve the oxidative stability of vegetable oils more than 3.5 times (Jure et al., 2010); other authors (Xing, White, 1997) have established that methanolic extracts of oat hulls dramatically reduced the formation of peroxides during storage of the soybean oil. Our current studies are devoted to the examination of oat hulls as an alternative source of antioxidants for stabilization of hempseed oil; the impact of natural antioxidants extracted from oat hulls and pomace of sea buckthorn is compared. The usage of these sources of natural antioxidants is sustainable and economically beneficial, as both of them are by-products of food industry.

Materials and Methods

Materials

Hempseed oil (HO) was obtained by cold-pressing method at *DUO AG* Ltd. Sea buckthorn pomace (SB) was supplied by farm *Puntiņi* as the by-product of juice manufacturing. Oat hulls (OH) were purchased from Rigas Dzirnavnieks JSC.

Preparation of hempseed oil extracts

Ground sea buckthorn pomace or oat hulls were mixed with hempseed oil [ratio of plant material and hempseed oil was 1:99, 1:39 or 1:19 (wt:wt)]. The mixture was treated with ultrasound (for the time of ultrasonification see Table 1). The temperature was 16 to 29 °C in the sonification bath. The plant material after the extraction was separated by centrifugation at 3000 rpm. Each extract was prepared triplicate.

Preparation of the extracts of polyphenols

The hempseed oil extract (4 g) was shaken with 80% ethanol (10 mL) at room temperature in dark for 24 h by orbital shaker *Biosan OS-10*. The ethanol extract was used for further analysis of polyphenols according to the Folin-Ciocalteu method (Mierina et al., 2011). The total amount of phenolic compounds (TAP) was calculated from calibration curve (R^2=0.9936) and was expressed as mg of gallic acid equivalents per 100 g of oil (mg GAE 100 g^{-1} oil); calibration curve consisted

from 5 points and was linear in the range of obtained results. The absorption of all solutions was measured with single beam scanning UV/Visible spectrometer *Camspec M501*.

Antioxidant activity

The hempseed oil extracts of oat hulls and sea buckthorn pomace were kept under accelerated oxidation conditions in thermostat at 40 °C in dark. The samples (30 g) were filled in Petri dishes with diameter 10 cm. The oxidation process was characterized with peroxide value. Antioxidant activity (AA) was expressed according to the equation:

$$AA = \frac{t_{extract}}{t_{blank}} \quad (1)$$

where

$t_{extract}$ and t_{blank} is the time when peroxide value reaches 48 meq O_2 kg^{-1} for hempseed oil extract of plant material and hempseed oil without additive, respectively.

Analogously the impact of 0.02% butylated hydroxytoluene (BHT) additive on oxidative stability of hempseed oil was detected.

Antiradical activity

2,2-Diphenyl-1-picrylhydrazyl DPPH (2 mL, 200 μM solution in isooctane) and hempseed oil extract of sea buckthorn or oat hulls (2 mL, 40, 30, 20 or 10 mg mL^{-1} solution in isooctane) were mixed; after 30 min absorption ($Abs_{extract}$) of solution was measured at 515 nm. The blank (Abs_{blank}) was prepared as above mentioned, pure isooctane was used instead of the solution of hempseed oil extract. The inhibition of DPPH (INH, %) was expressed according to the equation:

$$INH = \frac{Abs_{blank} - Abs_{extract}}{Abs_{blank}} \times 100\% \quad (2)$$

In order to find out the concentration that inhibits 50% of DPPH, the curves were constructed between the concentration of hempseed oil extract in isooctane and inhibition of DPPH (%).

Peroxide and acid values

Peroxide and acid values were determined according to standard LVS EN ISO 3960 and LVS EN ISO 660, respectively.

Statistical analysis

Statistical analysis was carried out using Microsoft Excel software package. Standard deviation was calculated by linear least squares regression. All measurements were carried out at least triplicate.

Results and Discussion

Hempseed oil is characterized with high amount of polyunsaturated (especially, linoleic) fatty acids. The ratio of *n-6* and *n-3* fatty acids is 3:1; therefore implementation of hempseed oil into the diet would shift the ratio to the recommendations of various nutrition societies (Matthäus, Brühl, 2008). The oil is widely used in cosmetic formulations due to the presence of *γ*-linolenic acid (Da Porto et al., 2012). Besides that, the oil contains high amount of tocopherol – even higher than in flax and canola seed oil (Teh, Birch, 2013). Unfortunately it has to be taken into consideration that the linoleic and linolenic acids are highly susceptible to oxidation. Also the presence of high amounts of chlorophyll promotes the oxidation (Matthäus, Brühl, 2008).

Both sea buckthorn and oat are rich sources of antioxidants. Sea buckthorn oil (Kumar et al., 2011), fruits and leaves (Suryakumar, Gupta, 2011) are well known sources of tocopherols, tocotrienols, carotenoids, phytosterols, vitamin C, phenolic compounds and trace elements. Main oat antioxidants are vitamins, carotenoids, phenolic compounds including phenolic acids and flavonoids, phytoestrogens, phytosterols, inositol phosphates, glutathione and melatonin (Zieliński et al., 2012; Peterson, 2001). Oat is almost exclusive source of *N*-cinnamoylanthranilates called avenanthramides (Peterson, 2001). Oat hulls and sea buckthorn pomace can increase oxidative stability of polyunsaturated hempseed oil, as well as make it even more advantageous for cosmetic purposes.

Usually extracts of oat and sea buckthorn antioxidants are prepared using various organic solvents. Such extraction procedure has several disadvantages – flammability of organic solvents and necessity to evaporate solvents require special equipment, but solubility of such extracts in vegetable oils may be problematic. Besides that toxicity and harmfulness of organic solvents must be kept in mind when the extract is envisaged for food or cosmetic industry. We already described preparation of "ready to use" extracts having increased oxidative stability obtained from sea buckthorn seeds and pomace (Seržane et al., 2012) and grains of oats (Jure et al., 2010) by extraction with vegetable oils. Usually the extraction was carried out at room temperature for 24 h or the extract was prepared using various cold-pressing techniques.

It is well known that antiradical activity of hempseed oil can be increased if the seeds before the oil extraction are treated with ultrasound for 20 to 40 min (Da Porto et al., 2013). In order to enhance the extraction as well as to use conditions that can increase the oxidative stability of hempseed oil we examined ultrasound accelerated process. Ground plant material and hempseed oil were mixed in ratio 1:19 (in the previous studies we clarified that the optimal ratio for various plant materials, including oat grains and sea buckthorn pomace was 5% additive to the vegetable oil) and treated with ultrasound for various time from 10 min to 1 h (see Table 1). Ultrasonification had small impact on the quality of hempseed oil – immediately after the extraction peroxide and acid values of extracts were similar in most of the cases; ultrasonification slightly increased peroxide value of extracts in comparison with samples prepared by cold-pressing (HO-2, SB-11). Both the amount of plant material

additive and the duration of ultrasonification had negligible effect on the peroxide value. The effectiveness of extraction was characterized by the total amount of polyphenols (see Table 2) – ultrasonification raised TAP value from 4.8 to 7.7 mg GAE 100 g^{-1} oil in case of sea buckthorn extracts (within 30 min) and to 5.5 mg GAE 100 g^{-1} oil for extracts of oat hulls (within 20 min).

Table 1

Hempseed oil extracts of sea buckthorn pomace and oat hulls

Abbr.	AmA	TU	PV	AV
OH-1	5.0	10	5.24±0.36	5.05±0.32
OH-2	5.0	20	6.06±0.20	4.51±0.09
OH-3	5.0	30	5.59±0.59	4.44±0.05
OH-4	5.0	40	6.47±0.61	4.25±0.04
OH-5	5.0	50	3.88±1.31	4.48±0.14
OH-6	5.0	60	4.37±0.96	4.35±0.07
OH-7	1.0	30	5.20±0.63	4.26±0.01
OH-8	2.5	30	3.07±0.13	4.48±0.12
SB-1	5.0	10	4.59±0.42	4.64±0.07
SB-2	5.0	20	4.05±0.27	4.43±0.17
SB-3	5.0	30	4.21±0.41	4.62±0.41
SB-4	5.0	40	4.48±0.88	4.46±0.01
SB-5	5.0	50	4.42±0.62	4.28±0.04
SB-6	5.0	60	4.09±0.27	4.43±0.05
SB-7	1.0	30	5.82±0.73	4.39±0.16
SB-8	2.5	30	4.52±0.32	4.45±0.04
HO-1	0	30	5.74±0.94	4.40±0.40
HO-2	0	0	4.32±1.54	4.79±0.88
SB-11	5.0	0*	2.38±0.53	3.45±0.85

AmA – Amount of additive in the mixture of plant material and hempseed oil, %. TU – Time of the ultrasonification, min. * – The extract was prepared according to the known cold-pressing method (Poiss, 2004).

When irradiation was carried out just for 10 min (OH-1, SB-1), TAP did not change in comparison with sonicated hempseed oil without any additive (HO-1), most probably due to the partial degradation of antioxidants both in the plant material and hempseed oil. The continued (more than 30 min) ultrasonification of the samples generally slightly decreased the total amount of polyphenols: e. g., the difference between cold-pressed hempseed oil and sonicated (for 30 min) oil was 0.6 mg GAE 100 g^{-1} of oil. The increase of TAP during the short ultrasonification can be explained both with more complete extraction process and formation of endogenous antioxidants, the nature and amount of which may depend on sonication time (Da Porto et al., 2013). TAP increased with the amount of plant material used for the extraction; the difference of the TAP was about 2.4 mg GAE 100 g^{-1} of oil for the extracts obtained from 1 and 5% additive of sea buckthorn (SB-7 and SB-3). The amount of oat hulls additive did not show strong regularity and had less impact on TAP – the maximal value was reached with 5% additive by 60 min ultrasonification, but similar TAP had extract obtained only within 20 min.

Table 2

TAP and antioxidant activity of extracts of sea buckthorn pomace and oat hulls

Abbreviation	TAP	AA
OH-1	4.85±0.37	1.50±0.03
OH-2	5.47±0.10	1.30±0.02
OH-3	4.63±0.21	1.44±0.02
OH-4	4.65±0.04	1.42±0.17
OH-5	4.74±0.09	1.37±0.04
OH-6	5.58±0.28	1.36±0.04
OH-7	5.32±0.32	1.32±0.02
OH-8	4.60±0.24	1.40±0.06
SB-1	4.86±0.17	1.26±0.03
SB-2	7.07±0.19	1.27±0.06
SB-3	7.73±0.29	1.32±0.02
SB-4	6.68±0.20	1.31±0.04
SB-5	6.63±0.26	1.37±0.04
SB-6	6.73±0.15	1.35±0.04
SB-7	5.33±0.24	1.51±0.05
SB-8	6.08±0.88	1.42±0.02
HO-1	4.86±0.15	–
HO-2	5.51±0.05	1.00
SB-11	4.78±0.44	0.75±0.02

In order to evaluate the impact of extraction conditions and the amount of oat hulls and sea buckthorn pomace on the oxidative stability of hempseed oil, all samples were kept under accelerated oxidation conditions in Petri dishes at 40 °C. The accelerated oxidation was continued until the peroxide value of the sample reached at least 48 meq O_2 kg^{-1}. The curves of peroxide values were used to estimate antioxidant activity of the prepared extracts – in most of the cases antioxidant activity varied from 1.3 to 1.5 (see Table 2); such activity is considered as low or very low (Ramamoorthy, Bono, 2007). Nevertheless the effect of sea buckthorn and oat hulls is comparable to the activity of synthetic antioxidant BHT – 0.02% additive of BHT increases oxidative stability of hempseed oil 1.5 times. The oxidative stability of hempseed oil extracts did not increase proportionally to TAP value – extracts of sea buckthorn pomace demonstrated strong antioxidant-prooxidant effect; any correlation between TAP and AA was not observed for the extracts of oat hulls. The acid values of all samples at the end of experiments did not exceed 5 mg KOH g^{-1}. The extracts that were prepared under ultrasound accelerated conditions demonstrated better AA and higher TAP than the extract SB-11 obtained by cold-pressing (method described by Poiss (2004)) of mixture of hempseeds and sea buckthorn pomace.

Antiradical activity against DPPH was measured for the extracts obtained by 30 min ultrasonification (Table 3). The most common solvent for DPPH analysis is alcohol (mainly ethanol or methanol). Nevertheless extensive studies of the mechanism of antioxidant interaction with free radicals have revealed that this mechanism strongly depends on the properties of the solvent. It is established that the reaction of phenols with DPPH in alcoholic media goes as sequential proton loss and electron transfer (SPLET) (Litwinienko, Ingold, 2003; Foti et al., 2004). It is well known that oxidation of vegetable oils proceeds *via* homolytic cleavage of C-H bond mainly at *bis*-allylic and allylic positions of fatty acid moiety (Belitz et al., 2004). In order to avoid SPLET process during DPPH analysis we carried out our experiments in isooctane – a solvent that supports hydrogen atom transfer (HAT) mechanism (Litwinienko, Ingold, 2007). Hempseed oil extracts of oat hulls demonstrated better antiradical activity against DPPH in comparison to extracts of sea buckthorn pomace (Table 3).

Moderate correlation existed between inhibition of DPPH and total amount of polyphenols in extracts of sea buckthorn pomace – the antiradical properties of these extracts generally rose with the increase of TAP. Such correlation was not observed in case of extracts of oat hulls. Medium linear correlation was found both for hempseed oil extracts of sea buckthorn pomace and oat hulls between inhibition of isolectronic peroxyl and DPPH radicals; the first one was expressed as antioxidant activity and the second – as concentration (mg mL^{-1}) of oil extract in isooctane that inhibits 50% of DPPH with initial concentration 100 μM (abbreviated as IC_{50}).

Table 3

Antiradical activity of hempseed oil extracts

Hempseed oil extracts of oat hulls		Hempseed oil extracts of sea buckthorn pomace	
Extract	**IC_{50}, mg mL^{-1}**	**Extract**	**IC_{50}, mg mL^{-1}**
OH-3	8.4±0.2	SB-3	7.8±0.4
OH-7	8.0±0.1	SB-7	9.0±0.0
OH-8	7.7±0.0	SB-8	9.1±0.1

Conclusions

Our studies demonstrate that ultrasound is an effective mean to accelerate extraction of sea buckthorn pomace and oat hulls with hempseed oil. Even within 30 min the maximal total amount of polyphenols can be reached and such extracts demonstrate acceptable oxidative stability. Luck of direct correlation between total amount of polyphenols and antioxidant activity, as well as between TAP and antiradical activity of extracts let us suggest that beside polyphenols other compounds present in extracts have a strong influence on oxidative stability of hempseed oil.

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References

1. Andersson K. E., Hellstrand, P. (2012) Dietary oats and modulation of atherogenic pathways. *Molecular Nutrition and Food Research*, Vol. 56, p. 1003–1007.
2. Belitz H.-D., Grosch W., Schieberle P. (2004) Lipids. **In:** *Food Chemistry*. 3rd ed. Berlin, Heidelberg, New York: Springer-Verlag, p. 157–244.
3. Da Porto C., Decorti D., Tubaro F. (2012) Fatty acid composition and oxidation stability of hemp (*Cannabis sativa* L.) seed oil extracted by supercritical carbon dioxide. *Industrial Crops and Products*, Vol. 36, p. 401–404.
4. Da Porto C., Natolino A., Decorti D. (2013) Effect of ultrasound pre-treatment of hemp (*Cannabis sativa* L.) seed on supercritical CO_2 extraction of oil. *Journal of Food Science and Technology*, doi: 10.1007/s13197-013-1143-3.
5. Foti M. C., Daquino C., Geraci C. (2004) Electron-transfer reaction of cinnamic acids and their methyl esters with the DPPH radical in alcoholic solutions. *Journal of Organic Chemistry*, Vol. 69, p. 2309–2314.
6. Guo W., Kong E., Meydani M. (2009) Dietary polyphenols, inflammation, and cancer. *Nutrition and Cancer*, Vol. 61, p. 807–810.
7. Harris K. A., Kris-Etherton P. M. (2010) Effects of whole grains on coronary heart disease risk. *Current Atherosclerosis Reports*, Vol. 12, p. 368–376.
8. Jure M., Seržane R., Strēle M., Mieriņa I. (2010) Improvement of the oxidative stability of vegetable oils with lipophilic oat extracts. **In:** *8th Euro Fed Lipid Congress "Oils, Fats and Lipids. Health & Nutrition, Chemistry & Energy"*; Conference Materials, Germany, Munich, p. LAMI014.
9. Kumar R., Phani Kumar G., Chaurasia O. P., Singh S. B. (2011) Phytochemical and pharmacological profile of seabuckthorn oil: a review. *Research Journal of Medicinal Plants*, Vol. 5, p. 491–499.
10. Kurtz E. S., Wallo W. (2007) Colloidal oatmeal: history, chemistry and clinical properties. *Journal of Drugs in Dermatology*, Vol. 6, p. 167–170.
11. Litwinienko I., Ingold K. U. (2003) Abnormal solvent effects on hydrogen atom abstractions. 1. The reaction of phenols with 2,2-diphenyl-1-picrylhydrazyl (dpph) in alcohols. *Journal of Organic Chemistry*, Vol. 68, p. 3433–3438.
12. Litwinienko G., Ingold K. U. (2007) Solvent effects on the rates and mechanisms of reaction of phenols with free radicals. *Accounts of Chemical Research*, Vol. 40, p. 222–230.
13. Matthäus B., Brühl L. (2008) Virgin hemp seed oil: an interesting niche product. *European Journal of Lipid Science and Technology*, Vol 110, p. 655–661.
14. Meydani M. (2009) Potential health benefits of avenanthramides of oats. *Nutrition Reviews*, Vol. 67, p. 731–735.

15. Mierina I., Serzane R., Strele M., Moskaluka J., Seglina D., Jure M. (2011) Extracts of Japanese quince seeds - potential source of antioxidants. **In:** *6th Baltic Conference on Food Science and Technology "Innovations for Food Science and Production"*; Conference Proceedings, Latvia, Jelgava, p. 98–103.
16. Pazyar N., Yaghoobi R., Kazerouni A., Feily A. (2012) Oatmeal in dermatology: a brief review. *Indian Journal of Dermatology, Venereology and Leprology*, Vol. 78, p. 142–145.
17. Peterson D. M. (2001) Oat antioxidants. *Journal of Cereal Science*, Vol. 33, p. 115–129.
18. Poiss G. (2004) *Production of multi-component vegetable oils using cold pressing method*, LV Patent 13200.
19. Ramamoorthy P. K., Bono A. (2007) Antioxidant activity, total phenolic and flavonoid content of *Morinda citrifolia* fruit extracts from various extraction processes. *Journal of Engineering Science and Technology*, Vol. 2, p. 70–80.
20. Seržane R., Strēle M., Mieriņa I., Jure M. (2012) Stabilization of hempseed oil with natural antioxidants. **In:** *Abstracts of the Riga Technical University 53rd International Scientific Conference: Dedicated to the 150th Anniversary and the 1st Congress of World Engineers and Riga Polytechnical Institute / RTU Alumni: Section: Material Science and Applied Chemistry*, Latvia, Riga, p.76.
21. Stobdan T., Korekar G., Srivastava R. B. (2013) Nutritional attributes and health application of sea buckthorn (*Hippophae rhamnoides* L.) – a review. *Current Nutrition and Food Science*, Vol. 9, p. 151–165.
22. Suryakumar G., Gupta, A. (2011) Medicinal and therapeutic potential of sea buckthorn (*Hippophae rhamnoides* L.) *Journal of Ethnopharmacology*, Vol. 138, p. 268–278.
23. Teh S.-S., Birch J. (2013) Physicochemical and quality characterizations of cold-pressed hemp, flax and canola seed oils. *Journal of Food Composition and Analysis*, Vol. 30, p. 26–31.
24. Truswell A. S. (2002). Cereal grains and coronary heart disease. *European Journal of Clinical Nutrition*, Vol. 56, p. 1–14.
25. Vernet A. (2006) Sea buckthorn (*Hippophae rhamnoides* L.). *Phytotherapie*, Vol. 4, p. 125–129.
26. Xing Y., White P. J. (1997) Identification and function of antioxidants from oat groats and hulls. *Journal of the American Oil Chemists` Society*, Vol. 74, p. 303–307.
27. Xu Y.-J., Kaur M., Dhillon R. S., Tappia P. S., Dhalla N. S. (2011) Health benefits of sea buckthorn for the prevention of cardiovascular diseases. *Journal of Fuctional Foods*, Vol. 3, p. 2–12.
28. Zieliński H., Archemowicz B., Przygodzka M. (2012) Antioxidants in cereal grains. *Food. Science Technology. Quality*, Vol. 19, p. 5–26.

INFLUENCE OF DIFFERENT SELENIUM CONCENTRATIONS ON THE PROTEIN AND STARCH CONTENT IN RYE MALT

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Abstract

The research object was rye malt. Experiments were carried out at the Faculty of Food Technology of the Latvia University of Agriculture. The main aim of this study was to investigate the influence of different selenium concentrations on the content of protein and starch in rye malt. The protein, starch, also hectolitre mass were tested by Grains Analyzer Infratec 1241 (Sweden), the Falling number was analysed using Hagberg-Perten method, according the ISO 3093:2009. Rye grain of 95% viability were soaked and germinated at temperature +6±2 °C for 3 days, using sodium selenate Na_2SeO_4 solutions (Se concentration in solution 3 mg L^{-1}, 5 mg L^{-1}, 10 mg L^{-1}), dried in the drier for 24 hours at temperature from 70 to 112 °C. The germination of grain with deionised water served as a control. The obtained results showed that the increase of selenium concentration in solution significantly decreases hectolitre mass (from 60.6 to 59.3 kg hL^{-1}), the protein (from 10.28 to 10.02 mg 100 g^{-1}dm), starch (from 68.7 to 66.9 mg 100 g^{-1}dm) content and falling number (from 90.5 to 81.0 s) in rye malt comparing with control sample.

Keywords: rye malt, selenium, protein, starch.

Introduction

During recent years significant progress has been made in the understanding of the processes of uptake and pathways of various chemical elements in plants (Shtangeeva et al., 2011; Kranner, Colville 2011).

Selenium (Se) is not an essential element for plants; it is an essential micronutrient for both humans and animals. More then 20 different selenoproteins have been characterized, including glutathione peroxidases and thioredoxinreductase, which are involved in controlling tissue concentrations of highly reactive oxygen-containing metabolites. The consumption of food provides the principal route of Se intake for the general population. The Food and Agriculture Organization of the United Nations (FAO, 2001) recommends a daily Se-allowance for humans of between 6 and 42 μg day^{-1} depending on age and gender (Bitterli at al., 2010). Average Se concentration in cereals amounts 0.024 mg kg^{-1} of grain dry matter, ranging from 0.006 to 0.122 mg kg^{-1}, for barley 0.06 mg kg^{-1}, for wheat 0.011 mg kg^{-1} on the average (Dūma, 2006). Plant Se accumulation can vary more than two orders of magnitudes at a given soil Se concentration for a specific form of soil Se (i.e. "native" soil Se, Se added as selenate, or as selenite) among different plant taxa. Differences observed in the transfer of Se from soil into plants appear to result primarily from differences in the solubility of Se species in soil and only to a minor degree from differences in plant uptake efficiency among these species (Shtangeeva et al., 2011; Bitterli at al., 2010).

Plant roots can take up Se in form of selenate, selenite and also organo-Se compounds, e.g., selenocysteine and selenomethionine. Selenium is not available for plant uptake in the form of colloidal elemental Se or metal selenides (Kranner, Colville 2011; Bitterli et al., 2010).

It is important to remember that under ordinary conditions, each plant part may have its own characteristic concentrations of elements; therefore, comparisons of element concentrations in plants may not be referred to the plant as a whole but should refer to the same plant parts (e.g. roots, or leaves, or seeds). Lastly, may assume that not only concentration of one or another element in a particular plant part of any two plant species growing in the same environment may differ significantly but relations between elements in the plants may also be different (Shtangeevaa et al., 2011).

Rye (*Secale cereale* L.) is an important source of whole grain foods in Eastern and Northern European diets (Katina et al., 2007). Rye malt is a natural food product produced by germinating rye grains (Luoto et al., 2012; Siwela et al., 2010).

Germinated cereals / pseudocereal or sprouts are believed to have a greater nutritive and physiological value than cereal and pseudocereal grains and their products (Donkor et al., 2012). During germination (i.e. hydrothermal treatment in ambient conditions) the biosynthetic potential of grains is exploited and a number of hydrolytic enzymes are synthesised. The reactions in germinating grain lead to structural modification and the synthesis of new compounds, some of which have high bioactivity and can increase the nutritional value and stability of the grains (Kaukovirta-Norja at al., 2004; Gomand at al., 2011).

Plant derived foods provide an important source of proteins and dietary minerals. This is especially true in developing countries where plant foods are a predominant portion of the diet. The concentrations of some minerals, especially iron, zinc, iodine, and selenium, are inherently low in plants as opposed to animal derived foods. As a result, more than 3 billion people worldwide suffer from micronutrient malnutrition (Waters, Sankaran, 2011).

Protein content shows grain suitability for processing. The temperatures and water stress occurring during grain filling period affects changes in rye protein aggregation. Usually in whole grain rye flour contains 8–13% proteins. The starch composition of cereals grains plays a major part in the digestibility and bread-making quality of flour. Generally in whole grain rye flour contains 56–70% starch.

Hectolitre mass (HM) is often used as an index of milling potential. The HM can be used as a silo management tool to optimize the storage space in the silo. The moisture content, weathering, kernel size, density, and packing factors affect hectolitre mass. (Zarina, 2012). The minimum hectolitre mass requirement for intervention of rye in the European Union (EU) is 68.0 kg hL^{-1}.

Hagberg falling number (hereinafter falling number) is one of the most important grain quality indices of rye. The Hagberg falling number is an indicator of α–amylase activity and a measurement of how far the break-down of starch has progressed in the kernel through enzymatic activities. It is expressed in seconds (s) and generally provides a measure of α-amylase enzyme activity in the grain. A high falling number indicates minimal activity, whereas a low falling number indicates more substantial enzyme activity (Buchanon, Nicholas 1980). Alpha–amylase activity depends on weather conditions, especially precipitation. Under rainy conditions, the grains of wheat germinate in the ear either before or at harvest-ripeness, known as sprouting in the ear (Kunkulberga et al., 2007).

There is match investigation about selenium uptake, accumulation, biofortification, translocation, enrichment, toxicity, and tolerance in higher-plants (Shtangeeva et al., 2011; Kranner, Colville 2011; Bitterli, 2010;Waters, Sankaran, 2011), but information about selenium influence on the content of starch, protein, hectolitre mass and falling number in rye malt is scarce. The aim of this research was to investigate the influence of different selenium concentrations on the protein and starch content, grains hectolitre mass and falling number in rye malt.

Materials and Methods

Plant material

The object of the research was rye grain (variety ´Kaupo´) from Ltd. Naukšēni (Latvia) harvested in 2011. Rye grain were soaked and germinated at temperature +6±2 °C for 3 days, using sodium selenate Na_2SeO_4 solutions (Se concentration in solution was 3 mg L^{-1}, 5 mg $L^{-1}$and 10 mg L^{-1}). The germination of grain with deionised water served as a control. After germination, all sprouts were dried for 24 h at a temperature from 70 to 112 °C; then they were grounded.

Determination of protein content, starch and hectolitre mass

The content of protein, starch and hectolitre mass of rye malt grains were determined by a near-infrared spectroscopy Grain Analyzer Infratec 1241. The contents of protein and starch where calculated based on grain dry matter (dm).

Determination of Falling number

The Hagberg falling number – α-amylase activity – was measured by the Hagberg-Perten method using a Perten Instruments (Sweden) „Falling number 1500" assessed to ISO 3093:2009. The Falling Number laboratory measurements with some modifications are conducted according to a standardized method aimed at measuring the viscosity of a mixture of 6g milled wheat with 1 g miled rye malt and 25 mL water placed in a bath at 100 °C (Gaaloul et al., 2011).

Mathematical data processing

The results were processed by mathematical and statistical methods. The statistical analyses of data were carried out using Microsoft Excel for Windows 7.0 (Microsoft Corporation, Redmond, WA). Mean value, standard deviations and significant value were calculated. p-values<0.05 were regarded as significant.

Results and Discussion

Hectolitre mass

The influences of different selenium concentrations on the hectolitre mass of rye malt are present in Figure 1.

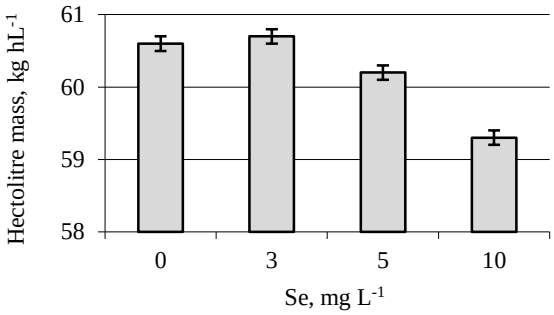

Figure1. The influence of different selenium concentrations on hectolitre mass of rye malt

Analyzing obtained results we can see that significantly changes of rye malt hectolitre mass depends on selenium concentration in solution ($p<0.05$). The relative decreases for 2.2% of hectolitre mass in rye can be observed at selenium concentration in solution 10 mg L^{-1} ($p=0.00$).

Protein content

The obtained results have shown that content of protein in rye malt changes significantly ($p<0.05$) using for germination solutions with different selenium concentrations. These results are shown in Figure 2.

The content of protein in rye malt significantly decreases for 2.5% comparing with control sample using solution where selenium concentration was 5 and 10 mg L^{-1} ($p=0.00$). Accordingly, if the concentration of selenium in solution was 3 mg L^{-1} the decrease was 2% ($p=0.001$). The decrease of protein and starch content could be reason for the changes of hectolitre

mass and it shows the increase of proteolytic enzymes activity in rye malt.

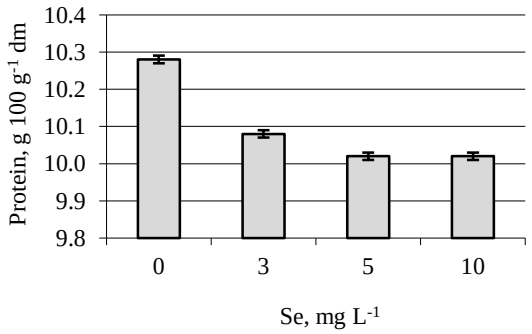

Figure 2. The influence of different selenium concentrations on the content of protein in rye malt

The results of research relate to Rakcejeva (2007) statement that the proteins were split by proteolytic enzymes, as a result of which the total protein content decreased.

Starch content

Starch is the major carbohydrate of ryes. The starch content is limited mainly to the endosperm, and contents between 57.1 and 65.6 g 100 g^{-1} of dry matter (dm) are reported in rye grains (Hansen et al., 2004). Starch consists of a mixture of amylose (AM) and amylopectin (AP). Typical levels of AM and AP in cereal starches are 22–28% and 72–78% (Gomand et al., 2011). The changes of starch content in rye malt using solutions with different selenium concentrations are shown in Figure 3.

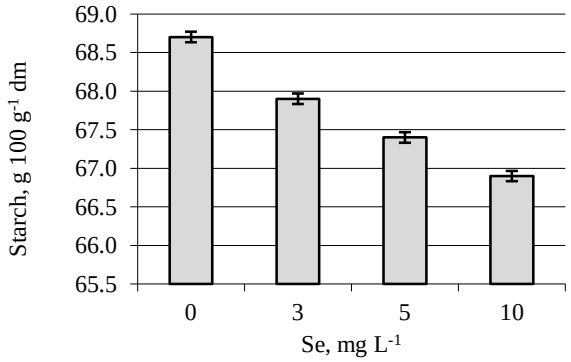

Figure 3. The influence of different selenium concentrations on the content of starch in rye malt

Significant differences in starch content were found in two analyzed samples ($p<0.05$) (Fig. 3). The obtained results showed that content of starch in rye malt depend on the selenium concentration. It decreases with increasing selenium concentration in solution. At selenium concentration 5 and 10 mg L^{-1} the content of starch in rye malt relative decreases for 1.9% and 2.6% compared with control sample, but at selenium concentration in solution 3 mg L^{-1} these changes were not so significant ($p=0.240$). These changes can be explained that the different selenium concentration affect the activity of amylolytic enzymes as a result of which the total starch content decreased in analyzed samples.

Hagberg Falling number

The potential activity of α-amylase in rye flour characterized Hagberg Falling Number (FN). The Hagberg Falling Number is an internationally recognized measure that allows the indirect determination of α-amylase activity. FN is the major quality attribute of rye grain. FN low values reflect high α-amylase levels causing the loaves of bread to be discoloured, sticky and of poor resilience and texture (Gaaloul et al., 2011). If the rye grain is harvested during the optimum time and not sprout-damaged normally has a FN in the range of 150 s, when weather conditions was dry and sunny the FN are 300 s and higher. The minimum FN requirement for intervention of rye in the EU was 100 s (Hansen et al., 2004).

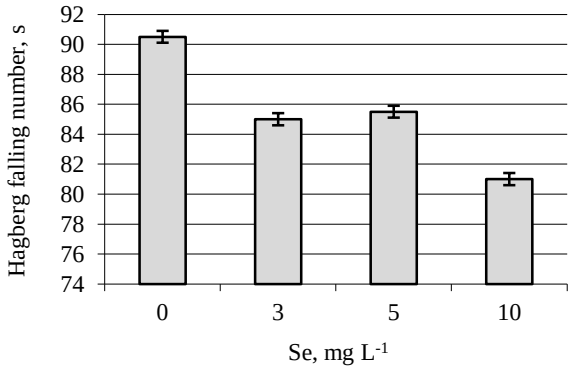

Figure 4. The influence of different selenium concentrations on the falling number of rye malt

The influence of different selenium concentrations on the falling number of rye malt is shown in Figure 4. The obtained results have shown that all analyzed Se concentrations significantly promoted the decrease of rye malt falling number and the highest decreases was observed when selenium concentration was 10 mg L^{-1} ($p<0.05$). In this case the falling number of rye malt decreases for 10.5% comparing with control sample. Results of present research show the different selenium concentration positive affects the activity of amylolytic enzymes and relative decreases the falling number.

Conclusions

The different selenium concentration in solutions affects the composition and of rye malt. The most significant change in rye malt was observed at selenium concentration in solution 10 mg L^{-1}. The relative content of protein and starch decreases for 2.5% and 2.6% compared with control sample it gives evidence about that degradation. The degradation of protein and starch affects the hectolitre mass, the relative decreases of hectolitre mass was 2.2% at selenium concentration in solution 10 mg L^{-1}. The Hagberg Falling Number decreases for 10.5% (selenium contration in solution 10 mg L^{-1}) that characterized the increase of amylase activity.

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References

1. Bitterli C., Bañuelos G.S., Schulin R. (2010) Use of transfer factors to characterize uptake of selenium by plants. *Journal of Geochemical Exploration*, Vol. 107, p. 206–216.
2. Buchanon A.M., Nicholas E.M. (1980) Sprouting, alpha amylase and bread making quality. *Cereal Research Communication*, Vol. 8, p. 23–28.
3. Donkor O.N., Stojanovska L., Ginn P., Ashton J., Vasiljevic T. (2012) Germinated grains – Sources of bioactive compounds. *Food Chemistry*, Vol. 135, p. 950–959.
4. Dūma, M., Kārkliņa, D. (2006) Fortified wheat grains with microelement selenium. ***In***: International Scientific Conference Proceedings „Research for Rural Development 2006", 17–20 May, Jelgava, Latvia, p. 210–213
5. FAO, WHO (2001) Human vitamin and mineral requirements. *Report of a Joint FAO/WHO Expert Consultation*, Bangkok, Thailand, Food and Nutrition Division, FAO, Rome.
6. Gaaloul I., Riabi S., Ghorbel R. E. (2011) Implementation of ISO 22000 in cereal food industry "SMID" in Tunisia. *Food Control*, Vol. 22, p. 59-66.
7. Gomand S.V., Verwimp T., Goesaert H., Delcour J.A. (2011) Structural and physicochemical characterisation of rye starch. *Carbohydrate Research*, Vol. 346, p. 2727–2735.
8. Hansen H. B., Møller B., Andersen S. B., Jørgensen J. R., Hansen A. (2004) Grain characteristics, chemical composition, and functional properties of rye (*Secale cereale* L.) as influenced by genotype and harvest year. *Agricultural and Food Chemistry*, Vol. 52, p. 2282–2291.
9. Katina K., Liukkonen K. H., Kaukovirta-Norja A., Adlercreutz H., Heinonen S.M., Lampi A. M., Pihlava J. M., Poutanen K. (2007) Fermentation-induced changes in the nutritional value of native or germinated rye. *Journal of Cereal Science*, Vol. 46, p. 348–355.
10. Kaukovirta-Norja A., Wihelmson A., Poutanen K. (2004) Germination: a means to improve the functionality of oat. *Journal of Agricultural and Food Science*, Vol. 13, p. 100–112.
11. Kranner I., Colville L. (2011) Metals and seeds: Biochemical and molecular implicationsand their significance for seed germination. *Environmental and Experimental Botany*, Vol. 72, p. 93–105.
12. Kunkulberga D., Ruza A., Linina A., Galoburda R. (2007) Evalution of wholegrain flour baking properties depending on variety. *Food Chemistry and Tehnology*, Vol. 41 (2), p. 24–29.
13. Luoto S., Jiang Z., Brinck O., Sontag-Strohma T., Kanerva P., Bruins M., Edens L., Salovaara H., Loponen J. (2012) Malt hydrolysates for gluten-free applications: Autolytic and praline endopeptidase assisted removal of prolamins from wheat, barley and rye. *Journal of Cereal Science*, *V*ol. 56, p. 504–509.
14. Rakcejeva T, Skudra L (2007) Biological value changes in grain during biological activation time. *LLU raksti, nr. 18 (313),* p. 25–33.
15. Shtangeeva I., Steinnes E., Lierhagen S. (2011) Macronutrients and trace elements in rye and wheat: Similarities and differences in uptake and relationships between element. *Environmental and Experimental Botany*, Vol. 70, p. 259–265.
16. Siwela M., Taylor R.N., J. de Milliano A.W., Duodu K. G. (2010) Influence of phenolics in finger millet on grain and malt fungal load, and malt quality. *Food Chemistry*, Vol. 121, p. 443–449.
17. Waters B.M., Sankaran R.P. (2011) Moving micronutrients from the soil to the seeds: Genes and physiological processes from a biofortification perspective. *Plant Science*, Vol. 180, p. 562–574.
18. Zariņa L. (2012) Test weight of rye depending of crop rotation. [accesed on 01.02.2014.]. Available at : http://stendeselekcija.lv/konference/jaunumi/data/augsupl ades/files/2.%20Zarina_Test%20weight pdf

COMPARISON OF DIETARY FIBRE CONTENT IN DIFFERENT FIBRE SOURCES

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Abstract

Dietary fibre is an important component of human's nutrition. It is the common name for all carbohydrate components occurring in foods that are non-digestible in the human small intestine. It is known that deficiency of dietary fibre in food, provoke disturbance of intestinal tract, for example, constipation and that different fibre sources have different composition and quantities of dietary fibre. Therefore the task of research was to investigate the content of total, soluble and insoluble dietary fibre in different fibre sources such as wheat and rye bran, defatted flaxseeds, chicory and Jerusalem artichoke powder.

The results of research showed that total dietary fibre content in different fibre sources ranged between 41.76% and 59.42%, where the lowest TDF content was determined for wheat bran and the highest for Jerusalem artichoke powder. The content of TDF in defatted flaxseeds and Jerusalem artichoke powder was significantly higher comparing with other samples ($p<0.05$). Whereas dried chicory and Jerusalem artichoke powder were important sources of soluble dietary fibre, which significantly differed from other analysed samples ($p<0.05$). The significant part of SDF content made inulin, which contain in dried chicory was 94.3% of total SDF and in Jerusalem artichoke powder – 97.7%. The significantly higher amount of IDF was determined in wheat and rye bran, defatted flaxseeds ($p<0.05$) comparing with dried chicory and Jerusalem artichoke powder.

Keywords: bran, flaxseeds, chicory, Jerusalem artichoke, soluble and insoluble fibre.

Introduction

The food industry is facing the challenge of developing new food products with special health – enhancing characteristics, since the beneficial effects of healthy diet on the quality of life are wildly recognized. These functional materials come from a wide variety of plant sources which provide important nutraceutical components that may be used in food systems (Lee et al., 2004). Dietary fibre is an important component of human's nutrition. These health benefits include reduction in bowel transit time (Gear et al., 1981; Brennan, Cleary, 2005), prevention of constipation (Odes et al., 1993), reduction in risk of colorectal cancer in vitro (Rowland, 1995; Verghese et al., 2002), production of short chain fatty acids (Wasan, Goodland, 1996; Scharlau et al., 2009) and promotion of the growth of beneficial gut micro flora (Brennan, Cleary, 2005; Bosaeus, 2004). Fibre is naturally present in cereals, fruits, pulses, vegetables; it can be produced from sources that might otherwise be considered waste products. For example, soy hulls, defatted flaxseeds, oat hulls, spent brewer's grain and waste portions of fruits and vegetables (Katz, 1997). Dietary fibre is the common name for all carbohydrate components occurring in foods that are non-digestible in the human small intestine. These components include non-starch polysaccharides, resistant starch, and resistant oligosaccharides with three or more monomeric units, and other non-digestible, but quantitatively minor, components when naturally associated with dietary fibre polysaccharides, especially lignin. Dietary fibre components consist of two major classes: (1) water soluble polymers (SDF), such as pectins and gums, and (2) water insoluble materials (IDF), in which cellulose, hemicelluloses and lignin are included (Lebesi, Tzia, 2011). The Institute of Medicine (USA) recommends that children and adults consume 14 g of fibre for every 1 000 calories of food they eat each day. That means a person who eats 2 500 calories each day should get at least 35 g of fibre daily, while a person who eats 1 700 calories each day needs somewhat less fibre – about 24 g. Here are general fibre intake recommendations for different age groups and genders. These recommendations are based on the average daily calorie intake for people in these age and gender groups. Individuals who consume more or fewer calories than this average should adjust their fibre intake accordingly (Institute of Medicine, Food and Nutrition Board, 2005). In 2010 EFSA set European dietary reference values for nutrient intakes, where is written that a daily intake of 25 g of dietary fibre is adequate for normal bowel function in adults. In Latvia man consumes 20.2 g per day, women 15.8 g per day (Joffe et al., 2009). It is less than recommended daily intake of fibre. The part of nutritionists and diet experts suggest that 20–30% of daily fibre intake should come from soluble fibre (Institute of Medicine, Food and Nutrition Board, 2005).

Bran represents the outer parts of grains including the pericarp and surrounding cuticle, the testa and the aleurone layer. Depending on the milling process, commercial bran preparations also contain variable amounts of starchy endosperm and germ (Kamal-Eldin et al., 2009). Wheat bran are widely commercially available, are well characterised with respect to their composition and properties, especially with regard to their fibre components (Luhaloo et al., 1998). Wheat bran is the best known source of insoluble dietary fibre which helps to prevent and control bowel problems and to lower cancer risk (Verma, Banerjee, 2010). Whereas rye consumption inhibits breast and colon tumour growth in animal models, lower glucose responses in diabetics, and lowers the risks of death from coronary heart diseases (Verma, Banerjee, 2010).

Defatted flaxseeds contain circa 30% dietary fibres of which of one third is viscous and the majority of the flaxseed water-extractable dietary fibre (the mucilage) belongs to heterogenic polysaccharides. Viscous dietary fibre has been shown to induce an increased sensation and lowered energy intake at the following meal (Archer et al., 2004). Defatted flaxseeds are commercially available as the product rich in fibre, proteins, and polyunsaturated fatty acids. This dietary source is from oil pressing, using cold pressing method. Defatted flaxseeds are dietary fibre source, which are primarily recognised as a rich source of alfa linolenic acid and plant lignin and have as such been proposed to play a role in cardiovascular disease prevention (Bloedon, Szapary, 2004).

Chicory (*Chicorium intybus*) is one of the earliest known and most widely used raw materials for the manufacture of coffee substitutes (Pazola, 1987). The major component of chicory root is inulin which is a polymer of fructose with (2-1) glycoside linkages. Inulin is soluble in water and not hydrolyzed by human digestive enzymes, it is expected to behave like a soluble (Gibson et al., 1995). Inulin is used in the food industry not only as a source of dietary fibre (Flamm et al., 2001), but also as a functional food ingredient since it affects biochemical and physiological processes resulting in better health and reduction in the risk of many diseases in humans beings (Kaur, Gupta, 2002).

Jerusalem artichoke (*Helianthus tuberosus* L.) is cultivated mainly for use as green or ensiled forage, as a cover crop in marginal areas and to produce sugars (especially fructose) and fructans (inulin), which are used as food or for various chemical, electronicand pharmaceutical applications (Bosticco et al., 1989; Maijer, Mathijssen, 1991). Jerusalem artichoke is one of the most important candidates for use as a raw material for the industrial production of biological fructose and inulin. It is a particularly interesting and suitable crop, for southern European countries and especially in low-requirement environments (Paolini et al., 1998; D'Egidio et al., 1998).

It is known that deficiency of dietary fibre in food, provoke disturbance of intestinal tract, for example, constipation and that different fibre sources have different composition and quantities of dietary fibre. Therefore the task of research was to investigate the content of total, soluble and insoluble dietary fibre in different fibre sources such as wheat and rye bran, defatted flaxseeds, chicory and Jerusalem artichoke powder.

Materials and Methods

The research was performed at the Food technology laboratory of the Department of Food Technology of Latvia University of Agriculture and at the laboratory of Microbiology and Biotechnology Department of the Faculty of Biology of Latvia University.

Materials used in the research were wheat bran (Farm "Paukulnieki", Latvia), rye bran (Farm "Paukulnieki", Latvia), defatted flaxseeds with fat content less than 10% ("Iecavnieks and Co", Latvia), dried chicory ("RA5", Latvia) and Jerusalem artichoke ("Herbe", Latvia). Wheat and rye bran as well dried chicory were milled for analysis.

The content of total, soluble and insoluble dietary fibre was determined by AOAC Official Method: 991.43; Total, Soluble and Insoluble Dietary Fiber in Foods Enzymatic-Gravimetric Method (Phospate buffer) and using the FOSS Analytical Fibertec E 1023 System.

The content of inulin was determined by AOAC Official Method 999.03 and by AACC Official Method 32.32.

The analyses were performed in triplicate. The differences in the content of total, soluble and insoluble dietary fibre were analyzed using the analysis of variance (ANOVA). t-test was applied to compare the mean values, and p-value at 0.05 was used to determine the significant differences.

Results and Discussion

The total dietary fibre (TDF) content in different fibre sources such as wheat and rye bran, defatted flaxseeds, dried chicory and Jerusalem artichoke powder is given in Table 1. The obtained results were compared with literature data for analysis.

Table 1

Comparison of total dietary fibre content in analyzed samples with literature data, %

Samples	Total dietary fibre (TDF)		Authors
	Research data	Literature data	
Wheat bran	41.76±1.54	39.9–53.1	Kamal-Eldin et al., 2009
Rye bran	42.28±0.98	41.1–47.5	Kamal-Eldin et al., 2009
Defatted flaxseeds	57.41±1.03	22.33[b]	Schakel et al., 2001
Dried chicory	46.10±1.09[a]	80.00[c]	Schittenhelm, 1996
Jerusalem artichoke powder	59.42±1.21[a]	62.88[a]	Gedrovica, 2012

[a] Total dietary fibre content with inulin
[b] Total dietary fibre content in raw flaxseeds
[c] There is indicated only inulin

Total dietary fibre (TDF) content in different fibre sources ranged between 41.76% and 59.42%, where the lowest TDF content was determined for wheat bran and the highest for Jerusalem artichoke powder. Comparing the research data with literature it was established that obtained results were close to data given in literature except defatted flaxseeds and dried chicory. The differences of TDF content in flaxseeds could be explained with various used materials: raw flaxseeds (data of literature) and defatted flaxseeds (data of research). The literature data of TDF content in defatted flaxseeds was not available. The differences of

TDF in dried chicory could be chained with various factors such as chicory sort, growing conditions, season, and region as well treatment technology.

The results of dispersion analysis established that there were no significant differences of TDF content among wheat and rye bran as well dried chicory ($p>0.05$). Whereas the content of TDF in defatted flaxseeds and Jerusalem artichoke powder was significantly higher comparing with other samples ($p<0.05$). Evaluating the dietary fibre content there is important the amount of soluble and insoluble dietary fibre in product. Therefore the content of soluble and insoluble dietary fibre in analysed fibre sources is shown in Figure 1.

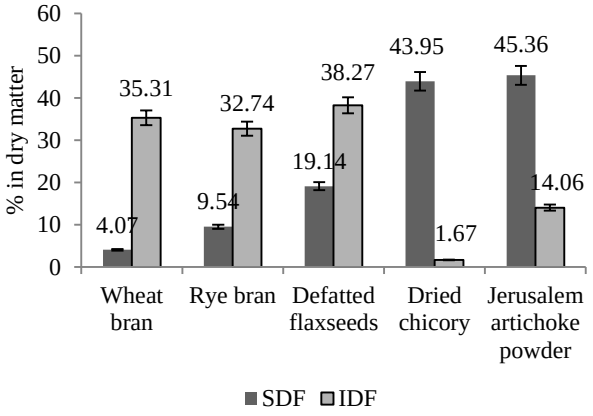

Figure 1. Content of soluble and insoluble dietary fibre in different fibre sources

SDF – soluble dietary fibre, IDF – insoluble dietary fibre

The obtained results showed that dried chicory and Jerusalem artichoke powder were important sources of soluble dietary fibre (SDF), which significantly differed from other analysed samples ($p<0.05$). The significant part of SDF content made inulin, which contain in dried chicory was 94.3% of total SDF and in Jerusalem artichoke powder – 97.7%. Due inulin contain in dried chicory and Jerusalem artichoke powder the both fibre sources have been attempted in different products attributed to their health benefits. Inulin possesses the typical properties of fibre – reduction of transit time in bowel, decreasing of pH in colon, beneficial effect on blood indices (Roberfroid, 1993). The SDF contain in rye bran confirm the conclusions of Åman et al. (1997) research that rye fibre contains soluble fibre 11–12% , i.e., in the form of arabinoxylan (9%) and β-glucan (2–3%).

Evaluating the content of insoluble dietary fibre (IDF) in analysed samples there was determined significantly higher amount of IDF in wheat and rye bran, defatted flaxseeds ($p<0.05$) comparing with dried chicory and Jerusalem artichoke powder. The obtained results correspond to Verma and Banerjee (2010) investigations that wheat bran is the best known source of insoluble dietary fibre.

The present study showed that different fibre sources had various amount of soluble and insoluble dietary fibre. Therefore it is important to mix different fibre sources for producing new functional products with advisable soluble and insoluble dietary fibre correlation.

Conclusions

Total dietary fibre (TDF) content in different fibre sources ranged between 41.76% and 59.42%, where the lowest TDF content was determined for wheat bran and the highest for Jerusalem artichoke powder. The content of TDF in defatted flaxseeds and Jerusalem artichoke powder was significantly higher comparing with other samples ($p<0.05$). Whereas dried chicory and Jerusalem artichoke powder were important sources of soluble dietary fibre, which significantly differed from other analysed samples ($p<0.05$). The significant part of SDF content made inulin, which contain in dried chicory was 94.3% of total SDF and in Jerusalem artichoke powder – 97.7%. The significantly higher amount of IDF was determined in wheat and rye bran, defatted flaxseeds ($p<0.05$) comparing with dried chicory and Jerusalem artichoke powder.

References

1. Åman P., Nilsson M., Andersson R. (1997) Positive health effects of rye. *Cereal Foods World,* Vol. 42, p. 684–688.
2. Archer B.J., Johnson S.K., Devereux H.M., Baxter A.L. (2004) Effect of fat replacement by inulin or lupin-kernel fibre on sausage patty acceptability, postmeal perceptions of satiety and food intake in men. *British Journal of Nutrition,* Vol. 91, p. 591–599.
3. Bloedon L.T., Szapary P.O. (2004) Flaxseed and cardiovascular risk. *Nutrition Reviews,* Vol. 62, p. 18–27.
4. Bosaeus I. (2004) Fibre effects on intestinal functions (diarrhoea, constipation and irritable bowel syndrome). *Clinical Nutrition Supplements,* Vol. 1(2), p. 33–38.
5. Bosticco A., Tartari E., Benati G. (1989) The Jerusalem artichoke (*Helianthus tuberosus* L.) as animal feeding and its importance for depressed areas. *Journal of Agricultural Medicine and Community Health,* Vol. 119, p. 98–103.
6. Brennan C.S., Cleary L.J. (2005) The potential use of cereal (1-3, 1-4)-beta glucans as functional food ingredients. *Journal of Cereal Science,* Vol. 42, p. 1–13.
7. D'Egidio M.G., Cecchini C., Cervinig T., Donini B., Pignatelli V. (1998) Production of fructose from cereal stems and polyannual cultures of Jerusalem artichoke. *Industrial Crops and Production*, Vol. 7, p. 113–119.
8. Dietary Reference Intakes: energy, carbohydrates, fiber, fat, fatty acids, cholesterol, protein and amino acids. *Institute of Medicine; Food and Nutrition Board,* 2005. Washington (DC): National Academies Press. 1319 p.
9. Flamm G., Glinsman W., Kritchevsky D., Prosky L., Roberfroid M. (2001) Inulin and oligofructose as dietary fiber: a review of the evidence. *Critical Reviews in Food Science and Nutrition,* Vol. 41, p. 353–362.
10. Gear J.S., Brodribb A.J., Ware A., Mann J.I. (1981) Fibre and bowel transit times. *British Journal of Nutrition,* Vol. 45, p. 77–82.
11. Gedrovica I. (2012) Pastry products enriched with dried Jerusalem artichoke (*Helianthus tuberosus* L.) powder. *Promotion work for acquiring the Doctor's degree of Engineering Sciences in sector of Food Science*, 143 p.

12. Gibson G.R., Beatty E.B., Wang X., Cummings J.H. (1995) Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology*, Vol. 108, p. 975–982.
13. Joffe R., Ozoliņš G., Šantare D., Bartekvics V., Mike L., Briška I. (2009) *Latvijas iedzīvotāju visaptverošais pārtikas patēriņa pētījums, 2007-2009*. [*The National Food Consumption Survey of Latvia, 2007–2009*] Rīga: PVD Nacionālais diagnostikas centrs, PVD Pārtikas Centrs. 115 p. (in Latvian).
14. Kamal-Eldin A., Lærke H.N., KnudsenK.E.B., Lampi A.M., Piironen V., Adlercreutz H., Katina K., Poutanen K., Åman P. (2009) Physical, microscopic and chemical characterisation of industrial rye and wheat brans from the Nordic countries. *Food and Nutrition Research,* Vol. 53, doi:10.3402/fnr.v53i0.1912
15. Katz F. (1997) Putting the function in functional food. *Food Process*, Vol. 57(2), p. 56–58.
16. Kaur N., Gupta A.K. (2002) Applications of inulin and oligofructose in health and nutrition. *Journal of Biosciences*, Vol. 27, p. 703–714.
17. Lebesi D.M., Tzia C. (2011) Effect of the addition of different dietary fiber and edible cereal bran sources on the baking and sensory characteristics of cupcakes. *Food and Bioprocess Technology,* Vol. 4(5), p. 710–722.
18. Lee S., Inglet G.E., Carriere C.J. (2004) Effect of Nutrim oat bran and flaxseed on rheological properties of cakes. *Cereal Chemistry*, Vol.81, p. 637–642.
19. Luhaloo M., Mårtensson A-C., Anderson R., Åman P. (1998) Compositional analysis and viscosity measurements of commercial oat brans. *Journal of the Science of Food and Agriculture,* Vol. 76, p. 142–148.
20. Maijer W.J.M., Mathijssen E. (1991) The relation between flower initiation and sink strength of stems and tubers of Jerusalem artichoke. *Netherlands Journal of Agriculture Science*, Vol. 39(2), p. 123–135.
21. Odes H.S., Lazovski H., Stern I., Madar Z. (1993) Double-blind trial of a high dietary fiber, mixed grain cereal in patients with chronic constipation and hyperlipidemia. *Nutrition Research,* Vol. 13(9), p. 979–985.
22. Paolini R., Prncipi M., Del Puglia S., Rocchi C. (1998) The effect of harvest time and mechanical weed control on the yield of Jerusalem artichoke (Helianthus tuberosus L.). *Italian Journal Agronomica*, Vol. 2, Iss. 2, p. 91–99.
23. Pazola Z. (1987) The chemistry of chicory and chicory-product beverages. **In:** *Coffee. Related beverages*. Vol. 5. R.J. Clarke, R. Macrae (eds.). New York, NY: Elsevier Applied Science Publishers Ltd., p. 19–57.
24. Roberfroid M. (1993) Dietary fiber, inulin and oligofructose: a review comparing their psysiological effects. *Critical Reviews in Food Science and Nutrition*, Vol. 33, Iss. 2, p. 103–148.
25. Rowland I.R. (1995) Toxicology of the colon: role of intestinal microflora. **In:** *Human colon bacteria: Role in nutrition, physiology and pathology*. G.R. Gibson, G.T. Macfarlane (eds). Boca Raton: CRC Press, p. 155–174.
26. Schakel F., Pettit J., Himes J.H. (2001) Dietary fiber values for common foods. **In:** *The CRC handbook of dietary fiber in human nutrition.* 3rd ed. G.A. Spiller (eds). London: CRC, 711 p.
27. Scharlau D., Borowicki A., Habermann N. (2009) Mechanisms of primary cancer prevention by butyrate and other products formed during gut flora-mediated fermentation of dietary fibre. *Mutation Research*, Vol. 682(1), p. 39–53.
28. Schittenhelm S. (1996) Productivity of root chicory, Jerusalem artichoke and sugar beet. **In**: *Proceedings of the Sixth Seminar on Inulin*. A. Fuchs, A. Van Laere (eds). Belgium, Leuven, p. 100–103.
29. Verghese M., Chawman C.B., Williams, D.R. Rao. (2002) Dietary inulin suppresses azoxymethane-induced preneoplastic aberrant crypt foci in mature Fisher 344 rats. *The Journal of Nutrition*, Vol. 132(9), p. 2804–2808.
30. Verma A.K., Banerjee R. (2010) Dietary fibre as functional ingredient in meat products: a novel approach for healthy living – a review. *Journal of Food Science and Technology*, Vol. 47(3), p. 247–257.
31. Wasan H.S., Goodland R.A. (1996) Fiber-supplemented foods may damage your health. *Lancet,* Vol. 348, p. 319–320.

DETERMINATION OF THE OPTIMAL RATIO OF RECIPE INGREDIENTS IN THE PROCESS OF DESIGNING CONFECTIONERY PRODUCTS

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Abstract

The aim of this study was to develop new recipes of confectionery products for functional purposes in accordance with predetermined criteria of optimization.

The paper formulates the objective of recipe optimization relating to the class of multicriteria nonlinear programming problems, and shows the algorithm developed for its solution. As an example, the article gives the formulation and solution of the problem that involves designing candy recipes with mixed jelly-whipped body. The choice of ratio between layers of combined body enriched with functional ingredients was carried out according to four criteria (energy value, carotenoids, dietary fiber, and the cost price of the finished product) under the given constraints on technological and consumer product characteristics.

The solution of the multicriteria problem identified a number of effective points belonging to the area of Pareto. The final choice of possible options was made on the basis of the ratio "price–quality" tailored to meet the daily requirements of micronutrients and organoleptic evaluation of the product.

Adjustment of the layers provides a wide range of jelly-whipped candies with original organoleptic characteristics and a given set of micronutrients that can be recommended for different categories of the population. Varying the composition of recipe mixtures from possible set of raw ingredients, enriching them with micronutrients, can influence on the physiological effect of finished products.

The proposed approach can be recommended for recipe designs of complex multicomponent food systems with specified set of characteristics in terms of multiple alternatives of ingredient composition and interchangeability of raw materials.

Keywords: recipe calculations, multicriteria optimization.

Introduction

Increasing the competition in raw materials and food markets, the growing need for products of functional purposes, a variety of raw ingredients in the market foreground issues on the design of new recipes and their corrections depending on fluctuations in raw material parameters.

According to modern ideas the concept of „food design" includes the development of models describing the steps for creating products of specified quality and representing mathematical relationships which reflect different functional connections between technological, economic and other parameters of raw ingredients, desired characteristics of finished products (objective function) and a number of constraints arising from the requirements of normative documents (Киселев, Першина, 2009; Муратова, Толстых et al., 2010) .

One of the important objectives in the design of new types of functional foods for various categories of the population is to provide the optimal set of recipe ingredients and ratios in multicomponent food systems. The difficulty of solving this problem is related to the fact that:

- raw material base of the modern food industry employs thousands of ingredients that differ in functional, technological, physical, chemical and organoleptic characteristics;
- semi-finished products should have a given complex of technological properties;
- finished products must comply with the balanced nutrition formula, respond to medical and biological requirements, have high consumer properties and be affordable to the public.

The need to consider a variety of recipes, to take into account properties of semi-finished and finished products according to the criteria and limits for each stage of designing a new product requires the involvement of mathematical support, software algorithms and the use of automated systems as essential tools for solving the problem of determining the optimal ratio of recipe ingredients (Muratova et al., 2010; Дворецкий et al., 2012).

Materials and Methods

To maximize the use of micronutrient complex of different chemical nature that is a part of functional additives of plant origin, and to create a broad product line of candies a combination of candy mass that have different composition and colloid-chemical properties can be used. As functional additives we used powdered semi-finished products prepared from local plant raw materials with different dispersion from 0.14 to 0.5 mm. Pumpkin and carrot powders containing vitamins of groups A, B, C, E, carotenoids and other vitamin-like substances, dietary fibre, macro- and micronutrients (potassium, calcium, iron, magnesium) have been received through combined convective-vacuum impulse drying that allows to keep all biologically valuable substances included in raw.

For enriching products with functional additives we used whipped cream and jelly candy mass with gelatinous consistency and low, compared to other confectionery, energy value. The composition of ingredients for preparing the whipped mass included sugar, agar, syrup, egg white, carrot powder, and citric acid. The composition of jelly mass included sugar, syrup, pectin, pumpkin powder, citric acid, sodium citrate.

When creating recipes we considered the possibility of chemical interaction between ingredients and chose combinations and methods of application ensuring their maximum safety during production and storage as well their high bioavailability.

During the research we applied commonly used and special research methods for studying the properties of raw materials, semi-finished and finished products. The experimental data were processed by methods of mathematical statistics using software packages MS Office Exel, MathLab 7.1. When designing formulations used the method of multi-objective optimization using LPτ sequences (Соболь, Статников, 2006).

It is necessary to consider the problem of designing confectionary recipes based on combining jelly and whipped candy mass with gelatinous consistency and the same moisture content. As the functional ingredient we used pumpkin powder (3 wt.%) in the jelly mass and carrot powder (10 wt.%) in the whipped mass (Muratowa, Smolikhina, 2013).

The set of alternative raw ingredients and main production stages of candies with combined whipped-jelly bodies for functional purposes are well known. By varying the composition of recipe mixtures and enriching them with micronutrients we can achieve certain physiological effects. For designing multicomponent food products object-oriented approach is offered to use. A distinctive feature of the object-oriented approach to recipe design of multicomponent food systems is presenting the recipe in a hierarchical structure.

Results and Discussion

It is important to determine the recipe $x=(x_1, x_2, \ldots . x_n)$ of the finished product in which the basic consumer characteristics (food, biological and energy value, etc.) and cost price of the finished product $F(x)=(f_1(x), f_2(x), \ldots, f_m(x))$ reach the optimal values, and other consumer characteristics (microbiological indicators, shelf life, safety performance) $g_j(x), j=\overline{1,\gamma}$ satisfy the requirements of the technical specification for developing new food recipes, i.e. $g_j(x) \le 0, j=\overline{1,\gamma}$.

The problem of mathematical formulation of optimal recipe design is as follows:

$$x^* = \arg\min_{x \in X} \{F(x)\} \qquad (1)$$

Restrictions on other consumer characteristics of the finished product:

$$g_j(x) \le 0, j=\overline{1,\gamma} \qquad (2)$$

and the ratio of raw ingredients and semi-finished products:

$$\underline{x_i} \le x_i \le \overline{x_i}, i=\overline{1,n} \qquad (3)$$

The formulated problem belongs to the class of multiobjective problems in nonlinear programming. The problem was solved on the basis of the most-informative presentation of the searched area for compromise solutions by points filling the area X according to the method of I.M. Sobol. Algorithm for solving this problem is presented in the paper (Дворецкий et al., 2012).

Solving the problem of optimal recipe design of jelly-whipped candies enriched with functional ingredients (1)-(3) supposes using the applied criteria: carotenoids, $f_1(\gamma, x)$; dietary fibre content, $f_2(\gamma, x)$; energy value $f_3(\gamma, x)$, cost price of the finished product $f_4(\gamma, x)$.

Characteristics of the whipped and jelly layers are shown in Table 1.

Objective functions are calculated by the formula:

$$f_i(x) \equiv \sum_{j=1}^{2} c_{ij} x_j, i=\overline{1,4} \qquad (4)$$

where $x_j, j=\overline{1,2}$ – percentage of the whipped and jelly layer, respectively; $c_{1,j}, j=\overline{1,2}$ – energy value of constituent layers, kcal; $c_{2,j}, j=\overline{1,2}$ – carotenoids, mg; $c_{3,j}, j=\overline{1,2}$ – dietary fiber, g; $c_{4,j}, j=\overline{1,2}$ – cost price, rubles.

Table 1

Characteristics of jelly and whipped layers

Range of admissible values, wt.%	Energy, kcal	BAS content		Cost, rubles
		Carotenoids, mg	Dietary Fiber, g	
Jelly layer, 10.0–90.00	20.1–321.0	0.01–0.56	0.40–6.20	6.60–58.90
Whipped layer, 10.0–90.00	28.4–463.0	0.50–7.50	0.02–0.38	11.60–103.50

As a result of solving the multicriteria problem (1)–(3) many effective points (γ, x) of Pareto area (F) were built, which may be recommended for different recipes of jelly-whipped candies with high nutritional value taking into account consumer preferences:

1) γ= 2,3, F = (2,6; 11,76; 363,0; 80,29) – low energy value and high content of dietary fibre – can be recommended for the diet;
2) γ= 1, F = (4,09; 8,22; 380,0; 91,1) – balanced composition and average cost – for mass consumption;
3) γ= 0,4, F = (5,38; 4,6; 386,0; 100,35) – high energy value and high antioxidant content – can be recommended for sports nutrition.

The final choice of the above mentioned options was made on the basis of the ratio "price–quality" tailored to meet the daily requirement of micronutrients and organoleptic evaluation of the product. Adjustment of the layers provides a wide range of jelly-whipped candies with original organoleptic characteristics and a given set of micronutrients (Figs. 1 and 2).

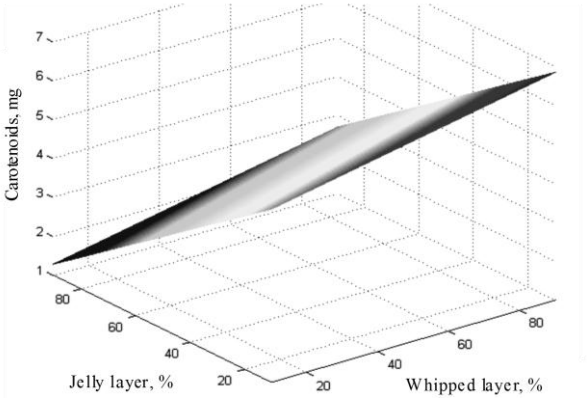

Figure 1. Dependence of the carotenoid content on the ratio of body layers

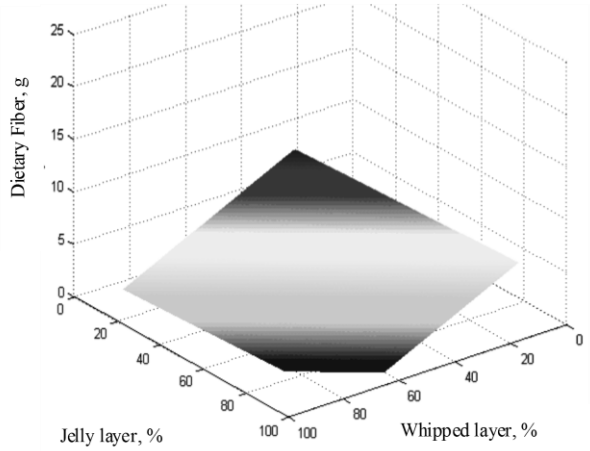

Figure 2. Dependence of the pectin content on the ratio of body layers

Table 2

Food and energy value of jelly-whipped candies

Indicator	Food value			
	Control	With the addition of vegetable powders and the ratio of the body layers		
		30:70	50:50	70:30
Energy value per 100g of candies, kcal (kJ)	400.0 (1676.0)	386.0 (1617.3)	380.0 (1592.2)	363.0 (1520.9)
Proteins, g	1.80	2.08	1.85	1.61
Fat, g	15.18	17.71	15.17	12.64
Carbohydrates, g	62.99	54.29	58.28	62.27
BAS	Per 100g (satisfying the norm of physiological needs, %)			
Dietary fibre, g	0.4 (7.7)	4.6 (21.3)	8.2 (41.1)	11.8 (58.8)
Vitamin C, mg	0.4 (0.54)	12.8 (18.2)	10.0 (14.2)	6.7 (9.6)
Carotenoids, mg	0.02 (0.4)	5.3 (89.7)	4.1 (68.2)	2.6 (43.3)

Taking into account the established and well-known rules of biologically active substances norm losses during technological processing and storage we calculated food and energy value of jelly-whipped candies with the addition of vegetable powders and different ratios of the body layers (Table 2).

For the calculation of multi component food recipes we used an automated system „Multifaz" based on the object-oriented approach (registration number 2011616793) and developed by the staff of Tambov State Technical University.

A distinctive feature of the object-oriented approach to the recipe design of multicomponent food systems is presenting the recipe as a hierarchical structure (Fig. 3).

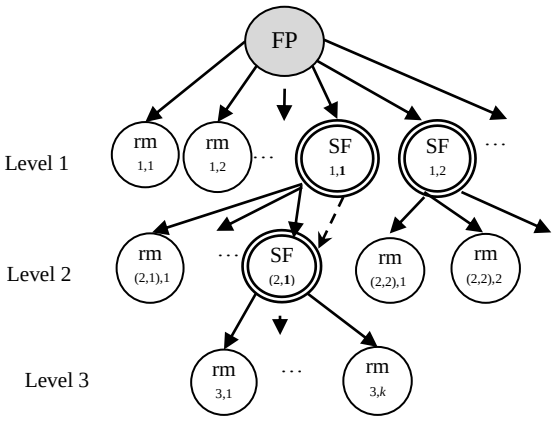

Figure 3. Diagram of program modes for calculating the single-phase recipe

Each of the top in the hierarchical structure is an object (the finished product, semi-finished product, raw materials). Every hierarchy level corresponds to a specific stage of manufacturing the food product and may have its own individual number of tops positioned lower in the hierarchy. Figure 3 shows a three-level hierarchy of calculating recipes, where the first index – the level number, the second index – the number of recipe component mixture. If on one or another recipe level several semi-finished products are used, their first index becomes composite and is denoted as a list (*i*, *j*), where *i* – the level number; *j* – the serial number of the semi finished product on the *i* level. This composite index is used below in the hierarchy (shown by the dashed arrow).

The algorithm for calculating multiphase food product recipe begins with the calculation of the final level in the longest branch of the hierarchical structure of the calculation. The initial data for calculation of the last level are: the consumption on downloading all kinds of raw materials and semi-finished products in kind; the loss of dry substances; the predetermined amount of finished products equal to 1 t.

Functional diagram of the automated information system is presented in Figure 4 (Муратова, Толстых, 2010). The system consists of information database

including recipe components and semi-finished products, calculation and optimization modules. Information module is a collection of data for recipe components and semi-finished products.

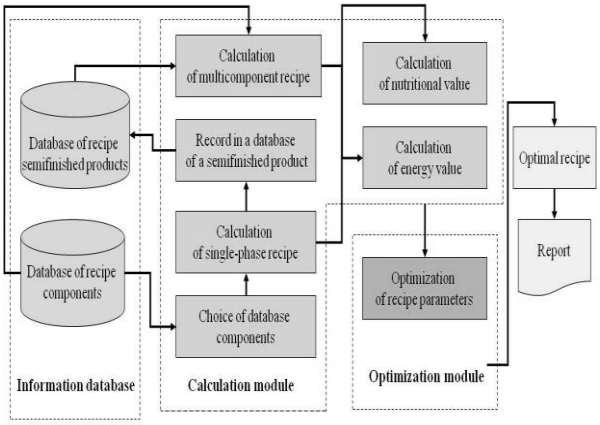

Figure 4. Functional diagram of the system „Multifaz"

The calculation module is used for selecting and recording components into the database information module, making calculations of single-phase and multiphase recipes, as well as food and energy value. The optimization module performs the selection of recipe components according to food and energy value in terms of restrictions on the cost price (Figure 5)

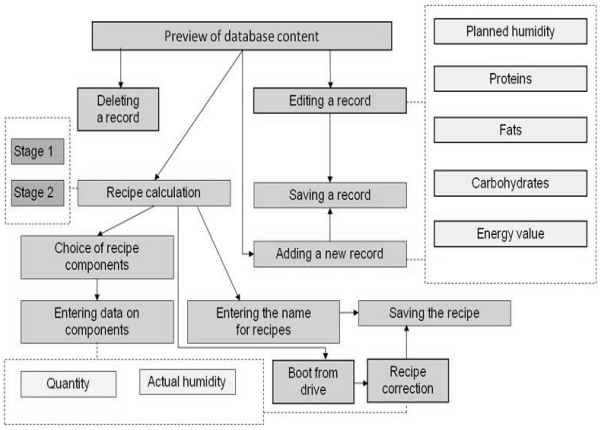

Figure 5. Diagram of program modes for the calculation of single-phase recipe

To construct the recipe hierarchy from the list of raw semi-finished components it is necessary to select semi-finished products from which the product is made and set the amount for the load. Then each recipe of semi-finished products is prepared with indicating solids content and the possibility of adding new recipe components to the list. Next the loading characteristic is set for each component of the raw material. The general hierarchy view of the wafers recipe for functional purposes is shown in Figure 6.
Pressing the button „Calculation" we get raw material consumption for cooking semi-finished products in accordance with this recipe, as well as a summary recipe of the designed product.

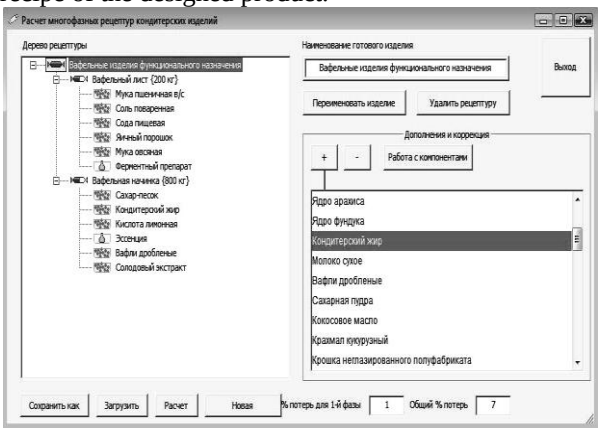

Figure 6. General view of the recipe tree of products for functional purposes

Calculation module of the automated system "Multifaz" has been tested and used in the educational process during the study of the discipline „Design of combined food" and during performing the final qualification work of bachelors, specialists and masters. The development of software module for optimizing recipes on food and energy value is now in progress.

Conclusions

Computer-aided recipe design noticeably allows to accelerate the calculation and optimization of complex multi component food products, to create products with predetermined chemical composition, nutritional value and functional orientation, to respond quickly to changes in the properties and types of raw ingredients.

Acknowledgment

The authors thank Doctor of Engineering, Professor S.I. Dvoretskiy for advice on the use of mathematical modeling for recipe design and optimization of complex multicomponent food products, Ph. D. Associate Professor S.S. Tolstykh for the development of algorithms and software for the calculation of recipes and Ph. D. E. Yu. Voyakina for the translation of the article.

References

1. Muratowa E.I., Smolikhina P.M. (2013) The use of regional raw materials for creating confectionery products for functional purposes **In:** *Proceeding of international scientific and technical Conference after Leonardo da Vinci. Vol. 1*. – Berlin: Wissenschaftliche Welte. V., p. 177-185.
2. Дворецкий С.И., Муратова Е.И., Толстых С.Г., Толстых С.С. (2012) Разработка модуля оптимизации рецептур продовольственных продуктов в составе автоматизированной информационной системы. *Вестник Воронежского государственного университета инженерных технологий*, Vol. 3. p. 59–64.

3. Киселев В.М. Эволюционная методология проектирования функциональных продуктов питания / В.М. Киселев, Е.Г. Першина // *Пищевая промышленность*, 2009. Vol. 11, p. 57–59.
4. Муратова Е.И., Толстых С.Г. (2010) Проектирование рецептур кондитерских изделий. *Тамбов: Изд-во ГОУ ВПО ТГТУ*. 32 с.
5. Муратова Е.И., Толстых С.Г., Леонов Д.В. (2010) Разработка автоматизированной информационной системы расчета рецептур многокомпонентных продуктов питания **In:** *Международная научно-практическая ежегодная заочная конференция «Аспекты ноосферной безопасности в приоритетных направлениях деятельности человека»*, ФГБОУ ВПО Тамбовский государственный технический университет, Тамбов, с. 113-117.
6. Соболь И.М., Статников Р.Б. (2006) Выбор оптимальных параметров в задачах со многими критериями. *М.: Дрофа.* 175 с.
7. Толстых С.Г., Смолихина П.М., Муратова Е.И., Дворецкий С.И. (2013) Решение задачи оптимизации рецептур конфет с комбинированными корпусами **In:** *XXVI Международная научная конференция Математические методы в технике и технологиях – ММТТ-26*, Ангрaрск: Ангарск. гос. технол. акад.; Иркутск: Иркут. гос. ун-т, с.168–170.

INTERACTION OF SUGAR CONFECTIONERY SHERBET QUALITY PARAMETERS DURING STORAGE TIME IN DIFFERENT PACKAGING

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Abstract

Essential importance for milk pomade confectionery quality assurance during storage time is alternate design of appropriate packaging technologies and materials, wherewith in this research in order to substantiate scientifically the optional shelf life of sherbet, different packaging materials in air ambiance, modified atmosphere (MAP), and active packaging with oxygen absorbers were used to approve their conformity for providing the main physical parameters and microbiological security during long term storage of investigated sugar confectionery. Shelf life can be extended significantly in conventional polymer packaging with high barrier properties: to 10–12 weeks in air ambience OPP and Multibarrier 60 HFP material; to 16 weeks in MAP 100% CO_2 and Multibarrier 60 HFP, metallised BOPET/PE and Aluthen material, exceeding neither experts' accepted hardness of 300 N nor permissible total plate count of microorganisms' level (TPC $g^{-1} \leq 10^4$).The sherbet hardness in active packaging after 16 weeks of storage in Multibarrier 60 HFP is 261.18±8.32 N, in comparison with MAP 100% CO_2 environment, it is by 11.5% lower, which is valued as positive.

Keywords: confectionery, sherbet, quality, packaging.

Introduction

Nowadays confectionery products are especially popular among children and elderly people, thus the popularity and consumption of these products are increasing (Duran et al., 2009). The European candy's market is fragmented. Leading market partners are *Mars* (14.3%), *Nestle S.A* (8.9%), and *Cadbury plc* (8.6%), the remaining part of market fill small producers – 68.3%. Leading partners in confectionery market of Latvia are Join-Stock Company *Laima*, Ltd *Skrīveru saldumi*, Ltd Pure *Chocolate*, and SIA Bona *Dea*. Join-Stock Company *Laima* is the largest producer of confectionery in Latvia, and at present *Laima* is the major sweet manufacturer in the Baltic (Laima, 2013).

With development of the choice of sugar confectionery products, most actual becomes the question about the preservation of a shelf-stable quality of the above mentioned products for a longer time, convenient use for consumers as well as opportunity to export the product more successfully (Brown, 2011).

At present in Latvia, confectionery products are sold in two ways – as bulk products which are weighed at the trading place, and as products packed at the manufacturing enterprises in a certain size of commercial packaging. Consistency of the product quality is affected significantly during shelf life by environment that surrounds the product. In oxygen environment, irreversible changes take place in foodstuffs, for instance fats and oils oxidize and become bitter, vitamin content reduces, colour changes, product loses its aromatic substances, and aerobic microorganism growth takes place. A decrease of foodstuff quality in presence of oxygen is also facilitated by increased temperature, moisture, light, especially ultraviolet light. During the course of time, more and more new opportunities are created how to preserve food quality, and scientists suggest new technologies in the packaging industry (Labuza et al., 2004; Subramaniam, 2000; Sucharzewska et al., 2003).

Important factor influencing the confectioneries shelf life is moisture migration from product and its permeation through packaging accordingly affecting the physical-chemical indices of product (Ergun et al., 2010; Willis, 1998). Ergun, Lietha and Hartel indicated that moisture content losses of candies produced on sugar bases have fundamental importance for quality maintenance. Producers should improve packaging technologies to eliminate oxygen and moisture destructive effect.

Nowadays, one of the most perspective methods how to extend the product shelf life is packaging in modified environment – vacuum or modified atmosphere packaging (MAP), where oxygen content is decreased to minimum but the carbon dioxide content is increased allowing to extend the storage time of the product by some days or even several months (Ahvenainen, 2010; Devlieghere, Debevere, 2003; Lagaron, López-Rubio, 2010). Active packaging is an innovative packaging technology, reducing the oxygen content below <0.1%, thus increasing the storage time of foodstuffs (Gibis, Rieblinger, 2011).

In order to provide the product quality constancy and extend its shelf life, it is recommended to use packaging materials with high barrier properties as well as to seek for new developments, the latest generation of biomaterials and active packaging technologies.

Scientists Londhe, Pal un Raju have investigated the shelf life of Asian sweet stuff – brown *peda* by packaging interventions, in conventional cardboard boxes, modified atmosphere and vacuum packaging techniques during storage for 40 days at 30±1 °C and concluded that brown *peda* could be best preserved in vacuum packaging without appreciable quality loss (Londhe et al., 2012). Brown *peda* is candy with low moisture and high sugar content, highly of same kind like sugar confectionery sherbet.

In this research in order to substantiate scientifically the optional shelf life of sherbet, different packaging materials in air ambiance, modified atmosphere

(MAP), and active packaging with oxygen absorbers were used to approve their conformity for providing the main physical parameters and microbiological security during long term storage of investigated sugar confectionery.

Materials and Methods

The object of the research was milk pomade sweet – sherbet with crunchy peanut chips, produced by stockholder Laima, Latvia. Dimensions of one piece of sherbet in average was 40×40×8 mm, mass 30±1 g.

Sherbet was packed by two pieces in a package, the total weight per package – 60±2 g. The size of each bag was 80×120 mm. Samples were packed in air ambience, vacuum packaging, MAP, and in active packaging with oxygen absorbers, stored at room temperature 18.0±3.0 °C, relative air moisture (RH) 40%. Samples were analyzed before packaging and after 2, 4, 6, 8, 10, 12, 14, and 16 weeks of storage.

At present research the most widely used traditional packaging technologies (in air ambience, vacuum and MAP) in conventional packaging materials (Table 1) and innovative (environmentally friendly) materials (Table 2) were studied; also oxygen absorbers in active packaging were applied, and its effect on the quality of sugar confectionery products during the storage time was estimated.

Table 1

Characteristics of conventional packaging materials used in experiments

Packaging material	Composition	Thickness, μm
OPP	Single layer, transparent OPP	40±2
Multibarrier 60 HFP	Transparent laminate, APA/TIE/PA/EVOH/PA/TIE/PE/PE	60±2
BIALON 50 HFP	Frosty white, laminate, BOPA/PE	50±3
BIALON 65 HFP	Transparent laminate, BOPA/PE	65±3
PP	Single layer, transparent PP	40±2
met.BOPET/PE	Metallised laminate BOPET/ALU/PE	65±2
Aluthen	Metallised laminate PET/ALU/PE	80±2

*Packaging in air ambience***.** At present, the most often commercially applied packaging of sugar confectionery products is air ambiance in cardboard boxes, transparent PP bags and metallised PP pouches.

Vacuum packaging. Products were put into initially from polymer film thermally sealed bag; after that, the air was removed or vacuum was created, and then the bag was sealed hermetically (Robertson, 2011).

Modified Atmosphere Packaging. For food packaging, MAP of carbon dioxide CO_2 (E 290) and nitrogen N_2 (E 941) were used, supplied by AGA, Ltd. In experiments, the following gas mixtures were used: 30% CO_2 and 70% N_2; 70% CO_2 and 30% N_2; 100% CO_2. The product was put into initially of different materials made polymer film bags; then, the air was removed from the bags and replaced by gas mixture prepared in gas mixer KM100-2MEM, and the package was hermetically sealed.

Table 2

Characteristics of biodegradable packaging materials used in experiments

Packaging material	Composition	Thickness, μm
BIO NVS	Single layer, transparent, cellophane based biodegradable NVS film	25±1
Ceramis®-PLA	PLA coated with SiOx Highbarrier properties	60±2
met. NatureFlex 23NM	Metallised / coating / cellulose film / coating	23±2
Nativia™ NTSS-30	Transparent BOPLA film	30±2
Nativia™ NZSS-20	Metallised BOPLA pouch	20±2
ECOLEAN film	Single layer, white, 40% Ca / 60% PE	78±4
BIO NVS film	Single layer, transparent, cellophane based NVS	25±1

For reduced oxygen packaging (ROP) creation (O_2 – 0%) in pouches an iron based oxygen scavenger sachets of 100 cc obtained from Mitsubishi Gas Chemical Europe Ageless® were used (Ageless, 2011). The samples were hermetically sealed by MULTIVAC C300 vacuum chamber machine and stored at the room temperature of +21.0±1 °C, (controlled by MINILog Gresinger electronic) and about 40% RH for 12 weeks under day and night conditions. The materials for experiments were selected with different water vapour transmission rate and various thicknesses. To achieve a more active oxygen removal, the active absorber was combined with MAP (100% CO_2)

The following mechanical and physical characteristics were analyzed:

1. The dynamics of gas composition in a hermetically sealed package headspace during the storage time was measured as a percentage of oxygen and carbon dioxide by a gas analyser OXYBABY® V O_2/CO_2.
2. Moisture content accordant at the storage time was determined by using verified balance KERN (Germany) with precision ±0.001g; mass loss calculation (%) – were determined by weighing on the electronic scales.
3. Hardness for freshly manufactured sherbet samples was determined as cutting force (in N) by using

TA-XTplus Texture Analyser. Cutting force was determined for six small sherbet samples from each it piece. A special probe with knife edge for a cut test HDP/BSK blade set with knife was applied. The maximum cutting force (in N) was detected at the deformation rate 10 mm s^{-1} and distance 10 mm. The samples were cut right through, in order to check whether any different structural characteristics (peanut pieces) were present under the knife inside the product or on the surface. Plotting force (in N) versus storage time (in weeks), the hardness change of sherbet stored in each gas composition in the package as well as for each packaging material was calculated. The maximum cutting force (N) was used as an index for the cut test.

4. For determination of mesophyll aerobic and facultative anaerobic microorganism colony forming unit count (TPC) a standard method was used LVS EN ISO 4833:2003. For determination of yeasts and moulds colony forming units count LVS ISO 21527-2:2008 was used.
5. Statistical analysis. Figures and Tables were developed and calculations were carried out with *MS Excel* program and *SPSS 16* statistics program. The hypotheses were checked with a *p* value method. Factors were estimated as significant when the *p* value was $<\alpha_{0.05}$. For interpretation of results it was accepted that α=0.05 with 95% of confidence if not indicated otherwise (Arhipova, Bāliņa, 2006; Bower, 2009).

Results and Discussion

Following the assessment of analysed quality parameters of sugar confectionery product sherbet samples (in 30% CO_2 + 70% N_2, 70% CO_2 + 30% N_2, 100% CO_2), as optimal packaging environment there was accepted 100% CO_2. In order to find out the effect of the packaging materials on the sherbet, the dynamics of the product hardness during the storage time in air ambience (Fig. 1) and MAP 100% CO_2 (Fig. 2) in different packaging materials are compared. Using a five-point hedonic scale, the hardness level of samples was previously determined when still the product is good for consumption. The initial sherbet hardness have been determined 55.80±2.96 N. Sensory evaluation recognized that the product could be considered usable for consumption up its hardness 300 N. The effect of the packaging material on the hardness during the storage is significantly different ($p<0.05$). The sample kept in a cardboard box, during two weeks of storage had already reached 300 N. Sherbet packaged in biodegradable packaging materials could be stored 2 to 6 weeks; while in other packaging materials, sherbet could be stored from 6 to 16 weeks. Comparing the effect of packaging technologies on the dynamics of sherbet hardness during the storage, it has been approved that the sherbet storage time in one and the same packaging material is different (Fig. 3). If the recommended shelf life in a cardboard box was 2 weeks, then in *Multibarrier 60 HFP* it was ranging from 8 to 16 weeks. The shelf life in *Multibarrier 60 HFP* packaging and 100% CO_2 ambience could be provided to 16 weeks, in addition its hardness was below 300 N. However in the active packaging using oxygen absorbers during the same time its hardness was by 11.5% less; that could be assessed as positive result.

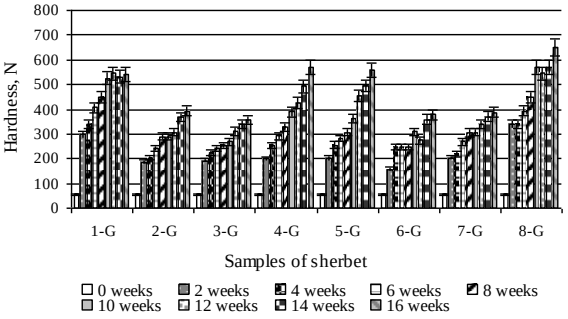

Figure 1. The dynamics of sherbet hardness during the storage time in air ambience

1-G – cardboard box; 2-G – OPP; 3-G – *Multibarrier 60 HFP*; 4-G – *BIALON 50 HFP*; 5-G – *BIALON 65 HFP*; 6-G – *ECOLEAN*; 7-G – PP; 8-G – *BIO NVS*

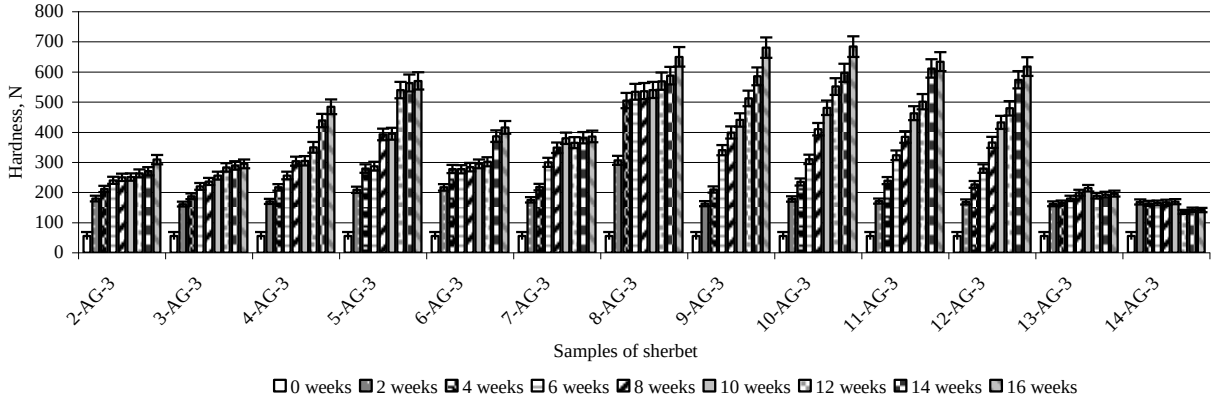

Figure 2. The dynamics of sherbet hardness in MAP (100% CO_2) during the storage time

2-AG-3 – OPP; 3-AG-3 – *Multibarrier 60 HFP*; 4-AG-3 – *BIALON 50 HFP*; 5-AG-3 –*BIALON 65 HFP*; 6-AG-3 – *ECOLEAN*; 7-AG-3 – PP; 8-AG-3 – *BIO NVS*; 9-AG-3 – *Ceramis®-PLA*; 10-AG-3 – met. *NatureFlex 23NM*; 11-AG-3 – *Nativia™ NTSS-30*; 12-AG-3 – *Nativia™ NZSS-20*; 13-AG-3 – met. BOPET/PE; 14-AG-3 – *Aluthen*

Table 3

The dynamics of sherbet sample moisture content during the storage time in air ambience, %

Samaple	Storagetime, weeks						
	0	2	6	8	12	14	16
1-G	3.40±0.12	2.27±0.10	1.67±0.09	1.63±0.09	1.50±0.09	1.53±0.09	1.31±0.08
2-G	3.40±0.12	3.05±0.12	2.74±0.11	2.56±0.11	2.55±0.11	2.29±0.10	2.26±0.10
3-G	3.40±0.12	3.17±0.12	2.95±0.11	2.81±0.11	2.55±0.11	2.40±0.10	2.20±0.10
4-G	3.40±0.12	2.61±0.11	2.46±0.10	2.31±0.10	2.14±0.10	2.14±0.10	2.03±0.10
5-G	3.40±0.12	2.82±0.11	2.56±0.11	2.36±0.10	2.19±0.10	2.00±0.10	1.80±0.09
6-G	3.40±0.12	3.14±0.12	2.95±0.11	2.92±0.11	2.50±0.10	2.28±0.11	2.20±0.10
7-G	3.40±0.12	3.27±0.12	2.68±0.11	2.53±0.11	2.30±0.10	2.19±0.10	2.08±0.10
8-G	3.40±0.12	2.51±0.11	1.99±0.09	1.66±0.09	1.48±0.08	1.18±0.08	1.10±0.08

1-G – cardboard box; 2-G – OPP; 3-G – *Multibarrier 60 HFP*; 4-G – *BIALON 50 HFP*; 5-G – *BIALON 65 HFP*; 6-G – *ECOLEAN*; 7-G – PP; 8-G – *BIO NVS*

Table 4

The dynamics of sherbet sample moisture content in MAP (100% CO_2) during the storage time, %

Samaple	Storagetime, weeks						
	0	2	6	8	12	14	16
2-AG	3.40±0.12	2.82±0.11	2.46±0.10	2.43±0.10	2.48±0.10	2.41±0.10	2.35±0.10
3-AG	3.40±0.12	2.98±0.11	3.25±0.12	2.98±0.11	2.63±0.11	2.62±0.11	2.59±0.11
4-AG	3.40±0.12	2.93±0.11	2.66±0.11	2.56±0.11	2.39±0.10	2.12±0.10	2.03±0.10
5-AG	3.40±0.12	2.82±0.11	2.44±0.10	2.35±0.10	2.02±0.10	1.77±0.09	1.71±0.09
6-AG	3.40±0.12	3.14±0.12	2.78±0.11	2.63±0.11	2.71±0.11	2.45±0.10	2.19±0.10
7-AG	3.40±0.12	3.62±0.12	2.66±0.11	2.70±0.11	2.58±0.11	2.53±0.11	2.49±0.10
8-AG	3.40±0.12	2.81±0.11	2.10±0.10	1.93±0.09	1.41±0.08	1.31±0.08	1.28±0.08
9-AG	3.40±0.12	2.87±0.11	2.55±0.11	2.19±0.10	2.03±0.10	1.59±0.09	1.19±0.08
10-AG	3.40±0.12	3.26±0.12	2.55±0.11	2.21±0.10	1.68±0.09	1.26±0.08	1.23±0.08
11-AG	3.40±0.12	2.92±0.11	2.52±0.11	2.21±0.10	1.88±0.09	1.47±0.08	1.20±0.08
12-AG	3.40±0.12	3.04±0.12	2.65±0.11	2.27±0.10	1.95±0.09	1.66±0.09	1.21±0.08
13-AG	3.40±0.12	3.15±0.12	3.22±0.12	3.16±0.12	3.29±0.12	3.22±0.12	3.21±0.12
14-AG	3.40±0.12	3.16±0.12	3.20±0.12	3.26±0.12	3.28±0.12	3.27±0.12	3.27±0.12

2-AG – OPP; 3-AG – *Multibarrier 60 HFP*; 4-AG – *BIALON 50 HFP*; 5-AG-3 –*BIALON 65 HFP*; 6-AG – *ECOLEAN*; 7-AG – PP; 8-AG – *BIO NVS*; 9-AG – *Ceramis®-PLA*; 10-AG – met. *NatureFlex 23NM*;11-AG – *Nativia™ NTSS-30*; 12-AG – *Nativia™ NZSS-20*; 13-AG – met. BOPET/PE; 14-AG – *Aluthen*

Table 5

The dynamics of sherbet sample moisture content in *Mutibarrier 60* film during the storage, %

Samaple	Storagetime, weeks						
	0	2	6	8	12	14	16
G	3.40±0.12	3.17±0.12	2.95±0.11	2.81±0.11	2.55±0.11	2.40±0.10	2.20±0.10
V	3.40±0.12	3.07±0.12	2.66±0.11	2.59±0.11	2.58±0.11	2.43±0.10	2.29±0.10
AG-1	3.40±0.12	2.83±0.11	2.62±0.11	2.54±0.11	2.58±0.11	2.51±0.11	2.43±0.10
AG-2	3.40±0.12	2.83±0.11	2.62±0.11	2.54±0.11	2.58±0.11	2.51±0.11	2.43±0.10
AG-3	3.40±0.12	2.98±0.11	3.25±0.12	2.98±0.11	2.63±0.11	2.62±0.11	2.59±0.11
AG-3-A	3.40±0.12	3.30±0.12	3.05±0.12	2.84±0.11	2.78±0.11	2.78±0.11	2.76±0.11

K – cardboard box; G – air ambience; V – vacuum packaging; AG-1 – 30% CO_2+70% N_2; AG-2 – 70% CO_2+30% N_2; AG-3 – 100% CO_2; AG-3-A – 100% CO_2 + oxygen absorber

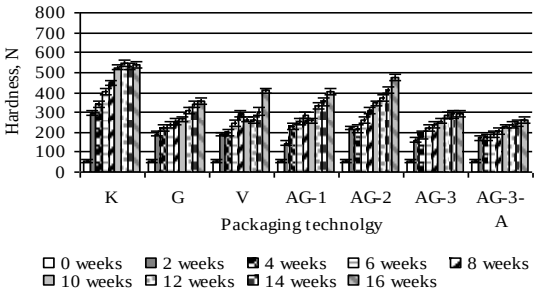

Figure 3. The dynamics of sherbet hardness in Multibarrier 60 HFP material during the storage

K – cardboard box; G – air ambience; V – vacuum packaging; AG-1 – 30% CO_2+70% N_2; AG-2 – 70% CO_2+30% N_2; AG-3 – 100% CO_2; AG-3-A – 100% CO_2 + oxygen absorber

The dynamics of sherbet moisture content during storage in air ambiance and different packaging materials is represented in Table 3. The initial moisture content of sherbet is 3.40±0.12%, which during storage step by step reduces generates hardening of milk pomade confectionary. The highest moisture content decrease in sherbet samples has been observed in air ambiance and cardboard box (1-G) bulk packaging, as well as in cellulose based biodegradable film *BIO NVS* (8-G) packaging. The moisture content of samples in *BIALON* (4-G; 5-G) and *BIO NVS* (8-G) packaging already after 2 weeks decrease up to 2.51–2.82%, and following up the storage till 16 weeks the moisture decreases below 2.03%. Moisture content of samples packed in PP film pouches during 16 weeks decreased up to 2.08±0.10%. The least decrease of moisture content at the same time is observed in OPP (2-G), *Multibarrier 60 HFP*; (3-G) and *ECOLEAN* (6-G) film packed samples, where it reduced till 2.26±0.10%. The dynamics of sherbet moisture content during storage in MAP (100% CO_2) and different packaging materials is represented in Table 4. The influence of biodegradable films is tremendous. Respectively in *BIO NVS* (8-AG-3), *Ceramis®-PLA* (9-AG-3), *NatureFlex 23NM* (10-AG-3); *Nativia™ NTSS-30* (11-AG-3) un *Nativia™ NZSS-20* (12-AG-3) film packaged samples during storage time 16 weeks the moisture content reduced till 1.19±0.10 to 1.28±0.14%. Accordingly better the moisture of samples remained in conventional *BIALON 65 HFP* (5-AG-3) packaging – till 1.71±0.11%, *BIALON 50 HFP* (4-AG-3) –l till 2.03±0.05%, OPP (2-AG-3), *Multibarrier 60 HFP* (3-AG-3), *ECOLEAN* (6-AG-3) and PP (7-AG-3) respectively till 2.35±0.11%, 2.59±0.16%, 2.19±0.08% and 2.49±0.11%. The samples in met. BOPET/PE (13-AG-3) and *Aluthen* (14-AG-3) packaging disparate ($p<0.05$) from all other investigated samples, and the changes in their moisture content during storage are minimal – from initial moisture content 3.40±0.12% till 3.21±0.12% during 16 weeks of storage.

The moisture dynamics of sherbet samples in *Mutibarrier 60 HFP* packaging material with high barrier properties is influenced by various packaging technologies (Table 5). The moisture content of sherbet in cardboard boxes and air ambiance during 16 storage weeks decreased till 1.31±0.08%, while in *Multibarrier60 HFP* packaging and MAP it ranged within 2.20 to 2.76%. Application of packaging materials with high barrier properties could cat down the moisture migration and increase the shelf life of sherbet (Romeo et al., 2010). The verity alike this is expressed in studies of Londhe, Pal and Raju (2012) concerning Asian candies *Peda*, which were packed applying different packaging technologies. As a main problem of *Peda* hardening they consider packaging in air ambiance and cardboard boxes without barriers.

Linear regression analysis affirms that close correlation exists between the sherbet hardness and moisture content apart from the packaging technology and material (Fig. 4). The dynamics of moisture content, in its turn, was affected by the packaging material barrier properties, environment composition within the packaging, and presence of oxygen which could be regulated by the oxygen absorbers (active packaging).

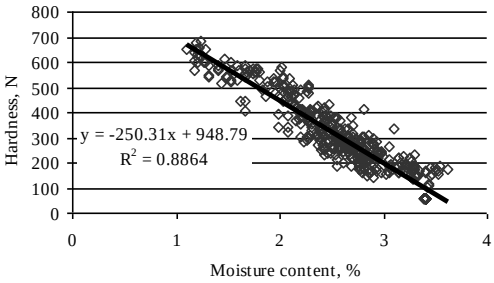

Fig.4. Correlation of the hardness with moisture content of sherbet

Research group under the guidance of scientist Romeo (2010) quoted his authorities and justified linkage among moisture content and hardening of product (Romeo et al., 2010). Regression equation obtained by computerized data processing prove on the fact if product moisture content will be reduced by 1%, the hardness will grow by 250 N. The variance analyses performed of scientists Londhe, Pal and Raju (2012) showed on the moisture content disparity among packaging technologies and storage time. Migration of moisture from the product permeating through packaging to environment could be reduced by selecting appropriate packaging material and conditions (Subramaniam, 2000).

The shelf life of sherbet could be extended significantly using packaging materials with high barrier properties: to 10–12 weeks in OPP and *Multibarrier60 HFP* materials in air ambiance; to 16 weeks in *Multibarrier 60 HFP*, metallised BOPET/PE and *Aluthen* materials in MAP (100% CO_2) ambience; as well as to 16 weeks using oxygen absorbers (active packaging) in metallised BOPET/PE and *Aluthen* materials not exceeding the estimated hardness 300 N. The sherbet hardness in active packaging after 16 weeks in *Multibarrier 60 HFP* was determined 261.18±8.32 N, in metallised BOPET/PE – 146.01±7.54 N and in *Aluthen* – 117.61±8.64 N. By the side of packaging in MAP (100% CO_2), in active

packaging it is for 11.5%, 20% and 35.7% less that can be assessed as positive. Biodegradable polymers with improved barrier properties provide a short-time shelf life of sherbet in air ambience maximum to 4 weeks, in MAP (100% CO_2) – to 6 weeks.

The maximum permissible TPC in sugar confectionery sherbet could be ≤4 log CFU g^{-1} (SanPin, 2002). After 16 weeks of storage this level has been exceeded only in samples packed in cardboard boxes and OPP film in air ambiance. Close to this level, aerobic and facultative anaerobic microorganisms grow in biodegradable *Ceramis®-PLA* and *NatureFlex 23NM* packaged sherbet samples. In *Multibarrier 60 HFP*, met. BOPET/PE and *Aluthen* packaging, the growth of microorganisms in sherbet samples occurs more slowly. In experimentally analyzed sherbet samples during the storage time, the number of yeast and mould colony forming units has been estimated. The admissible level of both yeasts and moulds is 50 CFU g^{-1}. In any of the tasted samples throughout the storage time, the number of TPC, yeast and mould colony forming units does not exceed the admissible level.

Conclusions

Linear regression analysis prove a close correlation between the sherbet hardness and moisture content in all the studied packaging types, while the dynamics and intensity of moisture content in the product during the storage time are affected by the packaging material barrier properties.

The desideratum hardness of sherbet is 300 N, which in cardboard box packaging establishes already after two weeks of storage. Shelf life can be extended significantly in conventional polymer packaging with high barrier properties: to 10–12 weeks in air ambience OPP and *Multibarrier 60 HFP* packaging; to 16 weeks in MAP 100% CO_2 and *Multibarrier 60 HFP*, met. BOPET/PE and *Aluthen* material, exceeding neither experts' accepted hardness nor permissible total plate count of microorganisms' level (TPC $g^{-1} \leq 10^4$).

The sherbet hardness in active packaging after 16 weeks of storage in *Multibarrier 60 HFP* is by 11.5% lower than in MAP 100% CO_2, which is valued as positive. Biodegradable polymers with improved barrier properties provide the shelf life of sherbet in air ambience maximum to 4 weeks, in MAP 100% CO_2 maximum to 6 weeks.

References

1. *Ageless Oxygen Absorbing Sachet.* [Accessed on 2.03.2014.]. Available: http://www.conservation-by-design.co.uk/productdetails.aspx?id=404&itemno=SUAGEL1000.
2. Ahvenainen R. (2010) Active and intelligent packaging: An introduction. **In:** *Novel Food Packaging Technologies*. R. Ahvenainen (eds.). Cambridge, U.K: Woodhad Publishing, p. 5–21.
3. Arhipova I., Bāliņa S. (2006) *Statistika ekonomikā un biznesā.* Risinājumi ar SPSS un Microsoft Excel. 2. izdevums. Rīga: Datorzinību Centrs, 364 lpp.
4. Bower J.A. (2009) *Statistica Methods for Food Science: Introductory Procedures for the Food Practitioner*. J.A. Bower (eds.) Oxford, UK; Ames, Iowa: Wiley-Blackwell, 307 p.
5. Brown A.C. (2011) Candy **In:** *Understanding Food: Principles and Preparation*. A.C. Brown (eds). Hardcover: Wadsworth Publishing Company, p. 518–534.
6. Devlieghere F., Debevere J. (2003) MAP, product safetyand nutritional quality. **In:** *Novel Food Packaging Techniques*. R. Ahvenainen (eds). Cambridge, UK: Woodhead Publishing Limited, p. 208–230.
7. Duran A., Tuzen M., Soylak M. (2009) Tracemetalcontents in chewing gums and candies marketed in Turkey. *Environmental Monitoring and Assessment*,Vol. 149, No. 1–4, p. 283–289.
8. Ergun R., Lietha R., Hartel R. W. (2010) Moisture and Shelf Life in Sugar Confections. *Critical Reviews in Food Science and Nutrition*, Vol. 50, Issue 2, p. 162–192.
9. Gibis D., Rieblinger K. (2011) Oxygen scavenging films for food application. *Proceding Food Science*, Vol. 1, p. 229–234.
10. Labuza T., Roe K., Payne C., Panda F. (2004) Storage Stability of Food Systems: Influence of State Changes during Drying and Storage. In: *14th International Drying Symposium*, IDS 2004, SäoPaulo, Brazil, Vol. A, p. 48–68.
11. Lagaron J.M., López-Rubio A. (2010) Latest developments and future trends in food packaging and biopackaging. **In:** *Innovation in Food Engineering New Techniques and Products.* M.L. Passos, C.P. Ribeiro (eds). USA: CRC Press Taylor & Francis Groupp, p. 485–503.
12. Londhe G., Pal D., Raju N. (2012) Effect of packaging techniques on shelf life of brown peda, a milk-based confections. *LWT - Food Science and Technology*, Vol. 47, Issue 1, p. 117–125.
13. Robertson G.L. (2006) *Foodpackaging: principles and practice*. London: CRC Press Taylor & Francis Group, 330 p.
14. RomeoF.V., Luca S.De, Piscopo A., Santisi V., Poiana M. (2010) Shelf-life of almond pastry cookies with different types of packaging and levels of temperature. *Food Science and Technology International*, Vol. 16, Issue 3, p. 233–240.
15. *SanPin, Enactment date of sanitary and epidemiological rules and regulations „Hygienic Requirements for Safety and Nutrition Value of Food Products. Sanitary Rules and Regulations 2.3.2.1078-01"* [Accessed on 12.03.2014]. Available:http://ec.europa. eu/food/international/trade/docs/SanPin2.3.2-1078-01_consolidated
16. Subramaniam P.J. (2000) Confectionery products. **In:** *The Stability and Shelf-Life of Food.* D. Kilcast; P.Subramaniam (eds). Cambridge, England: CRC Press LLC, p. 221–246.
17. Sucharzewska D., Stochmal A., Oleszek W. (2003) The effect of *Yucca schidigera* extract on the physical structure and on the oxidative stability of sugar-candy foam products. *Lebensmittel Wissenschaft und Technologie*, Vol. 36, Iss. 3, p. 347–351.
18. Willis D. (1998) Water activity and its importance in making candy. *Manufacturing Confectioner*, Vol. 78, p. 71–74.

TECHNOLOGICAL ASPECTS OF THE RESULTS ON RHEOLOGICAL STUDIES OF CANDY MASS

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Abstract

The aim of the research was to study the rheological behavior of candy mass depending on recipes in order to optimize technological regimes for processes of tempering and molding products with specified structural and mechanical properties. The objects of the study were fondant, jelly and whipped semi-finished products made according to the classical recipes and with application of vegetable powders and phytonutrients. According to the research results, the effect of dosing and dispersion of phytonutrients and vegetable powders on candy mass viscosity at different temperatures and strain rates allowed to develop recommendations for selecting modes of thermomechanical processing of fondant and jelly masses at the stage of tempering and molding.

It was found that the nature of changes in plastic strength of semi-finished products depends on regimes of structure formation and influence of plant additives on this process. The research showed that powder additives contribute to significant reduction in the duration of structure formation.

The paper also studies the effect of phytonutrients and vegetable powders on the strength of adhesive contacts between the layers of candy mass in forming multilayered confectionery products. Defined interval of strength values of adhesive contacts allows to prevent delamination and to formulate the main requirements for the conditions and methods for molding candies with a combined body.

The obtained results of complex rheological studies make it possible to implement the evidence-based approach to the management of technological processes of confectionery products manufacture and ensure the achievement of specified technological and consumer characteristics.

Keywords: rheology, adhesion, candy mass, phytonutrients.

Introduction

Rheological and structure-mechanical characteristics are important indicators of the properties of the candy mass as a semi-finished product coming to further process steps, as well as determine the structural and mechanical characteristics of the finished product. Investigation of adhesion properties allows to evaluate the possibility of using various molding methods or fusion of different physico-chemical and rheological properties of the candy mass for making combined products.

Currently the enrichment of confectionery products with various additives increasing their quality, improving organoleptic and structure-mechanical characteristics of the finished product has become very common, as it allows meeting the human need for biologically active substances. Great contribution to the development of scientific bases of confectionery production using herbal additives were made by famous scientists in this scientific area: Aksenov, Koryachkina, Magomedov, Obolkina, Savenkov, Skobelskaya, etc. In this regard, it is necessary to conduct a thorough rheological study of semi-finished products with the addition of non-traditional raw materials in candy recipes and structure-mechanical characteristics of candies produced from them. The aim of the research is to validate the modes of candy production using the rheological and structural and mechanical properties of semi-finished products.

Materials and Methods

The objects of the study were samples of fondant, jelly and whipped mass and candies made on their basis according to classic recipes, as well as with the addition of vegetable powders (pumpkin, carrot) and medicinal-technical raw materials (nettle leaves, lemon balm, raspberry) of various concentration and dispersion. Herbal additives used in the form of powder, hydrated powders (puree) and water-alcoholic extracts.

The study of rheological properties of semi-finished products and structure-mechanical characteristics of candy bodies were performed on viscotester HAAKE VT6R plus (Thermo Fisher Scientific, Germany) and texture analyzer Brookfield CT-3 (Brookfield Engineering laboratories, inc., USA) equipped with a wide range of sensors, devices and accessories to meet the challenges associated with the analysis and measurement of texture. Detailed description of methods for analysis of raw materials, semi-finished and finished products is given in (Смолихина, 2013).

Results and Discussion

Studying rheological properties of investigated candy mass has shown that, irrespective of the recipe, they belong to pseudoplastic materials. In a wide range of shear rates 0–100 s^{-1} the flow of the fondant mass is well approximated by the equation of Herschel-Bulkley, while the jelly and whipped mass satisfies the equation of Ostwald de Waele. In all cases, the rheological curves are well approximated by a linear function over a fairly wide range of shear rates (by a factor of no less than 0.98 approximation), which explains the choice and use of these rheological equations.

The curves of changes in viscosity versus shear rate have a form that is characteristic of structured systems. The viscosity decreases when the shear rate is increasing and especially fast in the range of relatively low shear rates, while the further increase in rate varies viscosity slightly. This rheological property is

explained by the fact that in a stationary environment the arrangement of the particles is characterized by strong randomness, and under the influence of increasing shear forces the orientation of the particles in the flow direction is raising. Increasing the rate leads to decreasing the interaction between particles. Tests were conducted at temperatures typical of molding for this candy mass (Fig. 1).

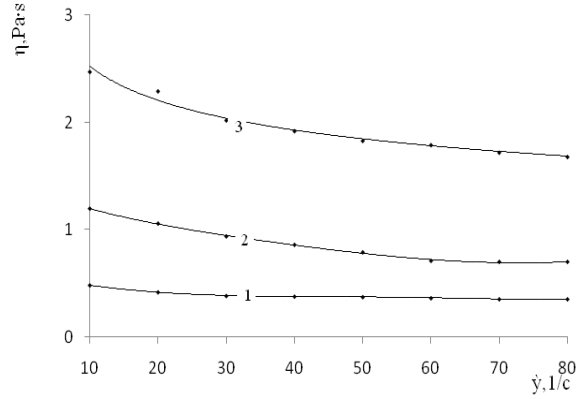


Figure 1. Dependence of the candy mass viscosity on shear rate

1 – jelly mass (85 °C), 2 – fondant mass (70 °C), 3 – whipped mass (50 °C)

The viscosity of the whipped mass is five times higher than jelly mass and twice than fondant mass that is due to the presence in the system of a surfactant adsorbed and coagulated to egg protein films forming strong bonds and large amounts of air phase.

Thus, adding dry powder from 2 to 10% (dispersity of 0.14–0.25 mm) into the whipped mass increases the viscosity in 3–10 times, hydrated – in 3.5–11.0 times. The used additives have high adsorption and water-holding capacity, therefore when mixed with the candy mass they swell and form a spatial grid with strong intermolecular bonds. This leads to the "expansion" of the system which causes the reduction in thickness of dispersion environment layers and increase of resistance force, and, accordingly, significant increase in viscosity (Fig. 2). A stronger effect of the hydrated powders, besides the phenomena described above, may be connected with the restoration of powder polymeric structures during its hydration and the dissociation of organic amino acid capable of participating in the jelly forming process which leads to hardening of the mass structure and a high viscosity, respectively.

Adding powdered vegetable semi-finished products of different dispersion into the candy mass significantly changes the rheology of semi-finished products.

Decreasing the size of nettle powder particles results in increasing the viscosity of candy mass (Fig. 3).

According to the results of studies on rheological properties temperature modes for specific stages of candy production were set (Table 1) (Муратова et al., 2008, 2009).

Plastic strength of candy bodies is a key indicator for characterizing the form retention quality. Changes in strength can show the process of structure formation the rate of which is of great importance for selecting regimes for structure formation process of candy mass (Muratowa, Smolikhina, 2013; Смолихина, Муратова, 2013).

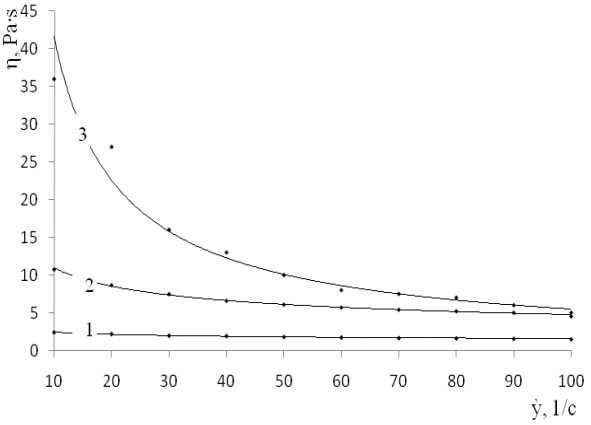


Figure 2. Dependence of the whipped mass viscosity on deformation rate

1 – control; *2*, *3* – with the addition of 5% dry and 5% hydrated vegetable powder respectively

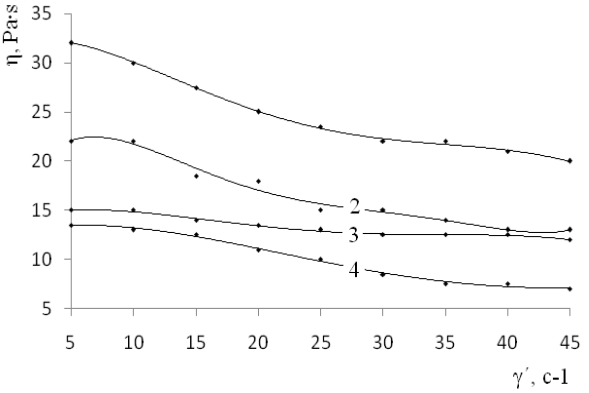


Figure 3. Dependence of the fondant mass viscosity on the shear rate for different dispersion of the nettle powder

1 – 0.08–0.14 mm; 2 – 0.14–0.2 mm; 3 – 0.2–0.25 mm; 4 – 0.25–0.5 mm

Table 1

Temperature modes for specific stages of candy production

Candy mass	Production stages		
	Whipping	Tempering	Forming
	Control		
Fondant	65–75 °C	70–75 °C	65–70 °C
Jelly	–	85–95 °C	75–90 °C
Whipped	55–65 °C	–	45–55 °C
	New recipe		
Fondant	75–85 °C	85–95 °C	80–85 °C
Jelly with the			
extract	–	80–85 °C	75–80 °C
powder	–	85–95 °C	95–105 °C
Whipped		–	

The analysis of experimental studies about the effect of functional ingredients on the process of jelly mass formation showed that the use of powder reduces the plastic strength of jelly by 22% (Fig. 4).
Introduction of vegetable powders at the tempering stage of the jelly mass leads to destruction of the jelly monolithic and formation of uneven structure. Polysaccharides of powders that have high sorption properties violate hydrostatic equilibrium while absorbing moisture from the system the result of which becomes an irregular jelly body.

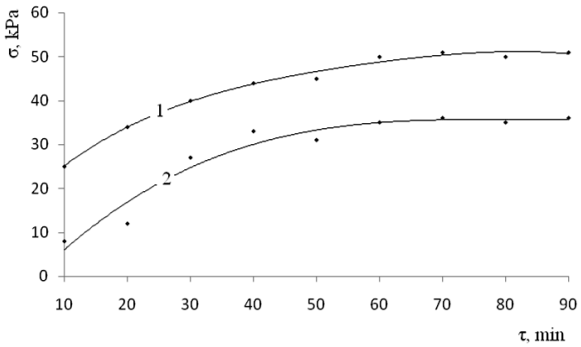
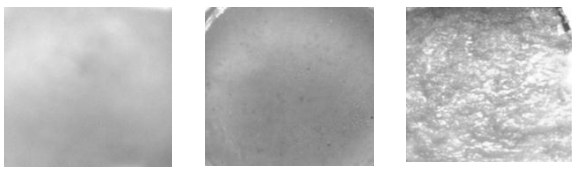


Figure 4. Changes in the jelly plastic strength during structure formation process

1 – control, 2 – with the addition of 0.5% pumpkin powder

Introduction of vegetable powders at the tempering stage of the jelly mass leads to destruction of the jelly monolithic and formation of uneven structure. Polysaccharides of powders that have high sorption properties violate hydrostatic equilibrium while absorbing moisture from the system the result of which becomes an irregular jelly body.
When introducing the functional additives with pectin at the syrup preparation stage we can see the maximum dissolving and swelling of the polysaccharide powder. The formed jelly surface is smooth, on the fracture it is glassy with even distribution of insoluble fiber parts (Fig. 5).



a) b) c)

Figure 5. Jelly mass with the addition of vegetable powders 3%

a – control, b – at the stage of syrup production,
c – at the tempering stage

The strength of whipped jellies with the use of powdered semi-finished products, on the contrary, increases due to the combined action of agar molecules and pectin substances presented in vegetable powders in large quantities. Thus, the hydrated powder increases the strength 2.0 fold, the dry powder – 8.0 fold. When using hydrated powder the whipped mass strength increases owing to additional filling of the space frame surrounding the bubbles with swollen fibers of the vegetable powder. Gelation occurs within 40 minutes after casting at a temperature of 20–22 °C, but a large amount of swollen polysaccharides makes the mass aqueous and prone to syneresis.
In samples containing dry powder syneresis is avoided by narrowing the channel, increasing the roughness of the walls and forming local „gates" from the particles not adhered to the bubbles (Зубченко, 2001). However, the presence of solid particles may have the opposite effect: they may undergo the adsorption of surfactants and the concentration decrease of surfactants in the solution leads to the increase of the surface tension and decrease of the foam dispersion, whereby the syneresis speed can be boosted.
Factors affecting the rate of fondant mass structuring are the ratio of solid and liquid phases, the presence of large crystals, the concentration and dispersion of functional additives, and body temperizing modes. The rate of fondant mass structuring can be judged by the increase in the limit shear stress (Горбатов et al., 1982).
For classical fondant mass at low temperatures (70–75 °C) the limiting shear stress raises dramatically in a short period of time which indicates a high rate of sucrose crystallization. A high degree of supersaturation of the solution leads to intensive crystallization of sucrose not only on the surface but also in internal layers of the body. Structuring process in the mass casting with temperature of 95 °C is slower and the mass cast at temperature of 100 °C reaches normal consistency (critical shear stress of 30–40·103 N m^{-2}) after 3 hours of structure formation process (Зубченко, 1986).
The structure formation process of the fondant mass can be traced according to increase in the strength of the structure of candy body. Figure 6 shows the dependence of the strength of the fonadant sample on the depth of the indenter.

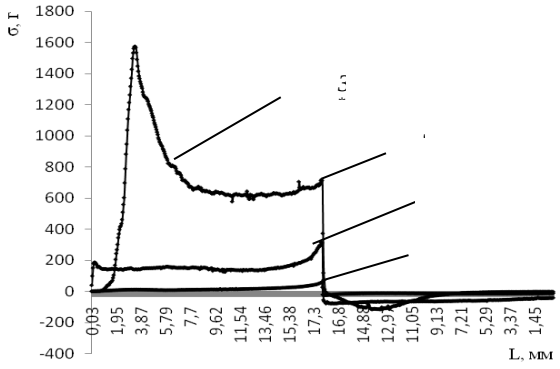


Figure 6. Changes in the consistency of the fondant mass in the process of structuring after casting in

1 – 15 min, 2 – 35 min, 3 – 60 min.

On the surface of the semi-finished product there is a dense crystalline crust formation the hardness of which increases during the first hour (up to 1600 g), and after two hours of temporizing the thickness reaches 2.5 mm. Inside the formed body there is thick mass

with large crystals of sucrose (the presence of crystals characterizes the presence of peaks within curve) (section 3*). In the crystallization process the adhesion of samples decreases to stainless steel.

In 2.5–3 hours of structure formation process at ambient temperature of 23–25 °C the candy body has a solid crystalline structure with the strength $4 \cdot 10^3$ g (Fig. 7).

The study on adhesive properties of the candy mass helps to evaluate the possibility of using various molding techniques for the manufacture of candy bodies.

The violation of production modes, moisture migration between the layers and syneresis during the storage leads to weakening of the adhesive interactions and changing in structural and mechanical characteristics of the products.

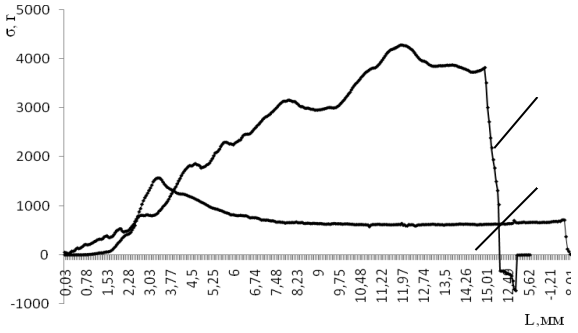


Figure 7. Changes in the consistency of fondant mass in the process of structuring in
1 – 15 min, 2 – 60 min

While forming the adhesive bonding through the combination of jelly and whipped semi-finished products in the combined body, highly viscous masses come into contact. In this case, for describing the process of contact formation rheological characteristics of the adhesive and the terms of the contact become important depending on the method of molding semi-finished products.

The molding of combined jelly-whipped candies can be produced with methods of co-extrusion, smearing followed by cutting, and casting.

The practice of molding by co-extrusion method showed that for each production it is necessary to make corrections of technological modes taking into account the constructive features of the molding equipment, or using them for combining confectionery masses close by rheological characteristics and having thixotropic properties (Оболкина et al., 2008).

The formation of candies by casting and smearing minimizes mechanical effect on the formable candy mass which does not destroy their structure and reduces the residual stress at the phase boundary. The smearing method allows to make multilayer products, but in the process of cutting there may be possible violations of structures, displacement of the layers due to their deformation (Fig. 8).

Figure 8. Adhesion failure in forming combined bodies by smearing and cutting methods

The formation of jelly-whipped candies was performed by the smearing method followed by cutting and casting. The ability to control rheological properties of the jelly mass by changing the temperature at the molding stage is a determining factor in the choice of jelly layer as the top. When the temperature is increased, the viscosity of the jelly mass decreases non-linearly with average increase of temperature by 1 °C per 0.01 Pa·s (Леонов, Муратова, 2011). At low viscosity the liquid adhesive wets the surface of the substrate providing a flawless full contact with the whipped mass with the maximum filling of micropores on the surface, but there is a formation of adhesive interaction and hardening of adhesive contact. However, even in this case, the adhesive strength of the contact is insufficient to prevent full separation of the structure by mechanical action on the semi-finished product during cutting the layer obtained by smearing or during removing candy bodies made by the method of casting to the starch forms (Fig. 9).

Figure 9. Mixed destruction of adhesive compound in forming combined candy bodies by smearing and cutting methods

When adding vegetable powders in the whipped layer in the amount of less than 2%, the adhesive strength between the layers of the body does not differ significantly from indicators obtained for the combined bodies without additives. Introducing powders into the whipped layer in the amount more than 10 wt. % leads to excessive development of microrelief which adversely affects the achievement of maximum contact area: a large number of connections reduces the mobility of macromolecules in the boundary layer, increases internal pressure, changes the structure of the surface layer, which results in defective areas that serve

as centers where the destruction of adhesive compounds begins (Зимон, Евтушенко, 2008; Смолихина, Муратова, 2012). The maximum adhesion between the layers of candy mass is attained by adding vegetable powders in the whipped layer in the amount of 5–10 wt.%, in the jelly layer – less than 3%, while the strength of adhesion contacts increases by more than 30% compared to control samples (Fig. 10 and 11).

Figure 10. Dependence of adhesive contacts strength on the depth of separation with the powder in the whipped mass and molding temperature of the jelly layer

1 – 5%, 105 °C; 2 – 5%, 95 °C; 3 – without powders, 95 °C; 4 – without powders, 105 °C

Figure 11. Cutting of combined candy bodies with 5% carrot powder in the whipped layer; 3% powder pumpkin in the jelly layer

Based on the above, it can be concluded that adding the carrot powder into the whipped mass allows to improve the contact area of the adhesive-substrate and to increase the adhesive connection strength due to the formation of a rough surface of the whipped mass and maximum filling of microdefects in the jelly mass. Moreover, the persistence of structural and mechanical properties of the whipped mass in the result of enhancing foam frame by rough fibers of the carrot powder provides the structuring capacity of the whipped layer and allows the operation of casting the jelly mass with density of 1350 kg m^{-3} at 45–60 minutes after the formation. Vegetable powders having high water-binding capacity absorb moisture from the candy mass surface to improve their adhesion to the combinable layer. Adding powders into the jelly mass reduces jelly strength and allows to change the adhesive tense on its surface which has a positive impact on the quality of the adhesive contact with the whipped mass (Смолихина, Муратова, 2013).

The studies have shown that during tests of control samples there is usually a mixed adhesive breaking, and that during tests of samples with the addition of vegetable powders the breaking is predominantly cohesive.

Based on the influence of phytonutrients dependencies on structural and mechanical characteristics of the fondant and whipped masses, the recommendations were made for the conditions and methods of molding (Table 2).

Table 2

Recommended methods of molding candy mass at different temperatures

Molding temperature, °C	**Candy mass**	
	Control	**New recipe**
Fondant mass		
>70	Casting followed by prolonged structure formation process	
40–70	Casting, smearing	Smearing, pressing
<25–40		
Whipped mass		
>60	Casting	
45–60	Casting, smearing	Smearing
30–45	Smearing	Smearing, pressing
25–30	Smearing	Settling, pressing
<25	Not recommended	

When the molding temperature is above 70 °C, the fondant structuring occurs after casting the bodies, thus the shear rate in the molding can be set arbitrarily. Temporizing of the bodies proceeds to a temperature of 20–22 °C for 35–40 minutes. Structuring process is characterized by the formation of crystallization centers with the addition of new molecules to the grid and proceeds spontaneously as it is accompanied by decreasing free energy of the system. When using the fondant mass as fillings at the temperature range of 40–70 °C, their supply to product bodies may be performed at any shear rate. While molding the fondant mass at temperatures below 40 °C in order to avoid destroying the structure of the mass, the shear rate should be less than the lowest critical rate, i.e. 25 s^{-1}.

Structuring occurs after casting of the whipped mass at high temperatures, so the shear rate during the formation process can be set arbitrarily. At temperatures below 60 °C the process of jelly formation begins, therefore the impact on the structure should be minimal. This requirement corresponds to the smearing method. For the temperature range of 30–45 °C the whipped mass made according to traditional recipes should be formed by smearing,

while for the whipped mass with the addition of vegetable powders the pressing method can be used because the moulded products retain their shape well in the form of a slice. If the temperature is 25–30 °C the whipped mass with the addition of vegetable powders can be molded by settling. Thus molded product retains their shape well as the process of structure formation by the time of molding has largely been completed. To prevent the destruction of the mass structure during the molding the shear rate should be below the lowest critical rate, i.e. 30 s^{-1}.

Conclusions

Research results testify to operational parameters for candy mass forming which can be recommended for practical use in the candies production according to traditional recipes and adding powders and herbal extracts. It is found that rheological behavior in the range of shift rates studied is similar to mixture composition which contains powders of medicative, technical and vegetable raw materials characterized by similar parameters of water-retaining and adsorption capacities. Thus, the results gained could be recommended not only for candies production with above mentioned additives but also for using different herbal additives with the same physical and chemical properties.

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References

1. Muratowa E.I., Smolikhina P.M. (2013) The use of regional raw materials for creating confectionery products for functional purposes **In:** *Proceeding of international scientific and technical Conference after Leonardo da Vinci. Vol. 1*. – Berlin: Wissenschaftliche Welte. V., 2013. – p. 177–185.
2. Горбатов А.В., Маслов А.М., Мачихин Ю.А. (1982) Структурно-механические характеристики пищевых продуктов. *Москва: Легкая и пищевая промышленность*. 296 с.
3. Зимон А.Д., Евтушенко А.М. (2008) Адгезия пищевых масс. *Москва: ДеЛи принт*. 398 с.
4. Зубченко А.В. (1986) Влияние физико-химических процессов на качество кондитерских изделий. *Москва: Агропромиздат.* 296 с.
5. Зубченко А.В. (2001) Физико-химические основы технологии кондитерских: учебник. *Воронеж: Воронеж. гос. технол. акад*. 389 с.
6. Леонов Д.В., Муратова Е.И. (2011) Использование результатов реологических исследований при разработке новых видов желейных конфет *Известия ВУЗов. Пищевая технология.* Vol. 4(322). с. 47–50.
7. Муратова Е.И., Смолихина П.М. (2013) Реология кондитерских масс: монография. *Тамбов: Изд-во ФГБОУ ВПО «ТГТУ»*. 188 с.
8. Муратова Е.И., Смолихина П.М., Леонов Д.В. (2008) Определение температурных режимов при производстве новых видов конфет. *Вестник Тамбовского государственного технического университета*. Тамбов: ТГТУ.Т.14, Vol. 3, с.667–669.
9. Муратова Е.И., Смолихина П.М., Донских Н.В. (2009) Обоснование режимов производства новых видов конфет по результатам исследований реологических свойств конфетных масс. *Вопросы современной науки и практики. Университет им. В.И. Вернадского.* Vol. 1 (15). с. 117–121.
10. Оболкина В.И., Лебедева Л.Н., Залевская Н.О. (2008) Формование комбинированных кондитерских изделий. *Продукты & Ингредиенты*. Vol. 8(50). с. 36–37.
11. Смолихина П.М., Муратова Е.И. (2012) Изучение адгезионных свойств конфетных масс для обоснования способов формования конфет с комбинированными корпусами. *Вестник Воронежского государственного университета инженерных технологий*. Vol. 3, с. 82–86.
12. Смолихина П.М. (2013) Разработка технологий желейно-сбивных конфет повышенной пищевой ценности с использованием овощных порошков. Диссертация на соискание ученой степени кандидата технических наук. *Воронеж*. 160 с.
13. Смолихина П.М., Муратова Е.И. (2013) Способы повышения прочности адгезионных контактов при послойном формовании корпусов конфет **In:** *XI Международная научная конференция Инновации в науке, образовании и бизнесе – 2013*. Калининград: Изд-во ФГБОУ ВПО «КГТУ», 2013. с. 171–174.

DETERMINATION OF ACRYLAMIDE LEVELS IN SELECTED FOODS IN LATVIA AND ASSESSMENT OF THE POPULATION INTAKE

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Abstract

The aim of this study was to investigate acrylamide levels in foods obtained from the Latvian market. Eight sample groups including traditional Latvian food products (rye bread, rye bread enriched with seeds, dried fruits or vegetables, fine rye bread (sweet-and-sour bread), wheat bread, potato chips, coffee, pastry products, dried bread products and gingerbread) were analyzed for their acrylamide content. A total of 435 samples were analyzed for acrylamide concentration using the ultra high performance liquid chromatography with tandem mass spectrometric detector and QuEChERS sample preparation methodology. An appropriate average recovery (106%), within-laboratory repeatability (3–10%) and limit of quantification (10 μg kg^{-1}) was specific to the applied UPLC-MS/MS methodology. Results revealed that the acrylamide content of processed foods shows a great variation between different food groups as well as between brands and within brands. Highest concentration of acrylamide was revealed in potato chips and chicory coffee with average concentration 564 μg kg^{-1} and 2790 μg kg^{-1} respectively. No single bread sample has exceeded the European Commission Recommendation 2013/647/EU although the addition of seeds, fruits or vegetables clearly had an increasing effect on acrylamide concentration in bread. The dietary exposure assessment was performed, using analytical results on acrylamide levels in certain food groups and the relevant food consumption data. The calculated data indicates high dietary intake of acrylamide by certain consumers that may potentially cause adverse health effects. Therefore, adequate efforts should be made to diminish acrylamide levels in processed foods in order to reduce the dietary risk to the human health for Latvian population.

Keywords: acrylamide, Latvian food, population intake.

Introduction

Acrylamide, a thermal process-induced contaminant in food, has attracted worldwide researchers to study the mechanism of its formation in foods, the risks associated for consumers and possible strategies to lower acrylamide levels in foodstuffs (Lui et al., 2013).

The International Agency for Research on Cancer, taking into consideration an option of industrial exposure to this compound and its intake from drinking water and tobacco smoke, classified acrylamide already in 1994 as a compound „probably carcinogenic for humans" (Group 2A) (IARC, 1994). Experimental studies in animals have shown genotoxic and carcinogenic effects of acrylamide. The genotoxicity of acrylamide could be partly mediated by its main metabolite, glycidamide (Gamboa da Costa et al., 2003; Baum et al., 2005) which is mutagenic (Blasiak et al., 2004; Ghanayem et al., 2005).

Since April 2002, when the Swedish National Food Administration published for the first data set about high content of acrylamide in food, several studies in different countries were performed dedicated to the assessment of acrylamide content in foods. The presence of acrylamide in different foodstuffs and its toxic impact, stirred up interest on a global scale in a possible risk to human health. Consequently, many countries have started to estimate acrylamide dietary intake (Mojska et al., 2012).

Currently it is well known that acrylamide is mainly formed as a result of reaction between amino acids and reducing sugars (particularly glucose and fructose) as a part of Maillard reaction (Mottram et al., 2002; Stadler, Scholz, 2004). Studies also have shown that acrylamide formation is affected by several processing parameters, such as heating temperatures, duration of heating, reducing sugar content, addition of components that bind water, and surface to volume ration (Boon et al., 2005)

The main source of acrylamide in the diet are primarily products made of potato such as French fries and potato crisps, and also cereal products such as bread, breakfast cereals, cookies and biscuits. Acrylamide is also formed during the coffee roasting process (Mojska et al., 2012)

Due to multiple sources of dietary exposure to acrylamide, a consultation was held in June 2002 by the Food and Agriculture Organization/World Health Organization (FAO/WHO) to discuss possible health risks due to acrylamide (WHO, 2002). Based on the expert committee evaluation, 1 μg kg^{-1} body weight (bw) day^{-1} is considered as an average exposure to acrylamide while 4 μg kg^{-1} bw day^{-1} is considered as high exposure to acrylamide (JECFA, 2006). The mean dietary exposure range to acrylamide is 0.2–1.0 μg kg^{-1} bw day^{-1} for the general adult population while 95th percentile range is 0.6–1.8 μg kg^{-1} bw day^{-1} (JECFA, 2011)

Currently, acrylamide is monitored in the European Union according to the European Commission Recommendation (2010/307/EU). Indicative acrylamide values based on the EFSA (European Food Safety Authority) monitoring data from 2011 to 2012 were established within the European Commission recommendation released on November 8, 2013 ("On investigations into the levels of acrylamide in food"). The indicative values are not safety thresholds, but, if exceeded, subsequent investigation of the reasons should be conducted.

To sum up significant variation of acrylamide content within the Latvian food products was obtained mainly as a result of the variable critical process parameters (critical temperature threshold, processing time, etc.) which are characteristic for each type of food,

manufacturer, product brand, even the batch. The content of acrylamide in traditional Latvian origin products (rye bread and rye bread with addition of seeds and fruit) is in line with the EU recommendation 2010/307/EU. The data presented here for other Latvian foods are in the same range as published in other countries.

The aim of this study was to investigate acrylamide levels in foods obtained from the Latvian market and to assess the average dietary acrylamide exposure of the Latvian population.

Materials and Methods

Food sampling

Given that acrylamide is formed in heat-treated potato and cereal products such e.g. bread, breakfast cereals, cookies and biscuits and during the coffee roasting process, the food products produced in Latvia were selected for testing purposes that might be a prospective source of dietary acrylamide intake for the Latvian population.

Eight product groups were selected for the study:

1) rye bread, rye bread enriched with seeds, dried fruits or vegetables;
2) sweet-and-sour bread;
3) wheat bread;
4) potato chips;
5) coffee, including instant;
6) confectionery (cookies, crackers);
7) gingerbread;
8) bread products (bagels, crackers, etc.).

A total of 435 samples obtained from all Latvian regions were analyzed for the acrylamide content.

Chemicals

Acrylamide (99%) and acrylamide-d_3 standards were obtained from Sigma (St. Louis, MO, USA). Stock and working standard solutions of acrylamide and acrylamide-d_3 were prepared in acetonitrile. Methanol, hexane and acetonitrile were a gradient grade for HPLC and formic acid (98%) from Sigma (St. Louis, MO, USA). Deionized water was prepared by a Milli-Q (Millipore, Billerica, MA, USA) water purification system. Sodium chloride and anhydrous magnesium sulfate were purchased from Scharlau (Barcelona, Spain). Primary secondary amine (PSA) sorbent was purchased as a bulk sorbents from UCT (Brockville, ON, Canada).

Analysis by LC-MS/MS of acrylamide in foodstuffs

Sample (2 g), the internal standard (volume, corresponding to the concentration in sample 100 ng g^{-1}) and 5 mL of hexane were added into a 50 mL centrifuge tube, then the tube was vortexed. Distilled water (10 mL) and acetonitrile (10 mL) were added followed by the the QuEChERS extraction salt mixture (4 g anhydrous $MgSO_4$ and 0.5 g NaCl). The sample tube was shaken for 1 min vigorously and centrifuged at 4500 g for 5 min. The hexane layer was discarded, and 1 mL of the acetonitrile extract was transferred to a tube containing 50 mg of PSA-sorbent and 150 mg of anhydrous $MgSO_4$. The tubes were vortexed for 30 s and then the purified extract was analyzed by the ultra performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

The quantitative analysis of acrylamide was performed by LC-MS/MS using Acquity Ultra Performance LC system (Waters, Milford, MA, USA) coupled to a QTrap 5500 (AB Sciex, MA, USA). The separation of acrylamide was achieved with Luna 3u HILIC column (100×2.00 mm; Phenomenex, Torrance, USA). Methanol (6%) solution in acetonitrile acidified with 0.1% formic acid was used as a mobile phase (flow rate 0.25 mL min^{-1}, column temperature 40 °C and injection volume 10 µL). The conditions for the detection by MS/MS were as follows: 30 psi curtain gas (CUR), 5500 V ion spray voltage, 400 °C temperature, 40 psi ion source gas (GS1), 50 psi ion source gas (GS2), 60 V declustering potential (DP).

Acrylamide was quantified in food samples using the product ion *m/z* 55 (precursor ion *m/z* 72) for the analyte and *m/z* 58 (precursor ion *m/z* 75) for the internal standard (acrylamide-d3). The quantification was based on the peak area of analyte compared to that of the deuterated internal standard.

The limit of quantification for acrylamide was 10 µg kg^{-1}.

Quality control

The LC-MS/MS method for determination of acrylamide in food was validated. The following parameters were defined and tested: limit of quantification (LOQ), within-day precision, between-day precision and recovery. The laboratory has participated in the inter-laboratory proficiency testing organized in 2011 by FAPAS analyzing a biscuit test material and has received a Z-score -0.3. Furthermore, the analytical quality control sample was also used in each series of analysis to determine recovery of acrylamide during the sample preparation procedure.

Food consumption data collection

The comprehensive National Food Consumption database was developed in 2007-2009 and is updated from 2012-2013 to facilitate:

- risk assessment in relation to chemical and biological hazards in food,
- estimation of nutrient intakes by target population,
- development of scientifically based food policy documents.

During the first stage of the fieldwork in total 2000 individuals aged 19 to 64 years old and living in private households in Latvia were interviewed. Three age groups (19–35, 36–50, 51–64) were created. Information on food intake for each age group was collected using two non-consecutive 24 h recalls in combination with food frequency questionnaire. Additional data on sociodemographic and lifestyle characteristics were obtained in face-to-face interviewing process. Nutritional data were processed with the help of software for the storage and analysis of food consumption and composition data that was developed in frame of this project.

Design and the methodology of the food consumption survey was based on „European Food Consumption Survey Method (EFCOSUM)" recommendations (EFCOSUM, 2002), developed within the framework of the EU Programme on Health Monitoring to provide the common method for monitoring of food consumption in Europe in a comparable way.
Strictly standardized procedures were used in order to harmonise research methodology (including sample size, data analysis and data presentation) and to obtain data comparable with the data from other EU countries. For the quantification of portion sizes the food picture book was used. For presentation of the data, parameters of interest were mean, median, quartiles, P5 and P95.
The exposure assessment on the base of National consumption data has been conducted concerning chemical contamination risk and related nutrition risk to develop scientifically based conclusions and to ensure relevant risk management and communication activities.
At present very limited information about the content of acrylamide in Latvian origin food is available, therefore, the main objective of our study was to determine the content of this compound in the Latvian foods and to assess the average dietary acrylamide exposure of the Latvian population.

Results and Discussion

The acrylamide content in 435 samples of food manufactured in Latvia ranged from 8 to 2790 μg kg^{-1} of foodstuff. High standard deviation indicates significant variation of results obtained for individual product groups.
All food products samples were divided into three groups based on the mean value of acrylamide (<100 μg kg^{-1}, from 100 to 200 μg kg^{-1} and from 230 to 900 μg kg^{-1}). The highest mean acrylamide content falling into the range from 200 to 900 μg kg^{-1} was obtained for the following product groups: crisps, grain biscuits, gingerbread and coffee. The highest variations were observed in the group of crisps – from 42 to 1570 μg kg^{-1}. Relatively higher acrylamide content was determined in crisps with added flavour combinations and crisps produced, using potato varieties with high sugar content. The studies on mechanisms of the acrylamide formation (Taeymans et al, 2004) show that the increased presence of reducing sugars and fats significantly increases the acrylamide formation.
Our results were similar to those obtained by Eerola et al. (2007) in Finland (539 μg kg^{-1}), Murkovic (2004) in Austria (627 μg kg^{-1}) and EFSA (2011) (635 μg kg^{-1}). Slightly higher mean content of acrylamide in crisps was observed by Mojska et al. (2010) in Poland (904 μg kg^{-1}) and Sirot et al. (2012) in France (954 μg kg^{-1}). In all quoted studies the high acrylamide content variation within product groups was observed.
Mean acrylamide value in the range from 100 to 200 μg kg^{-1} was obtained for five product groups: bagels, biscuits, chocolate biscuits, savoury biscuits and puff pastry.

Table 1

Acrylamide amounts of various foods

Product description	N	Mean	S.D.	Median	Range, μg kg^{-1}
Bread					
Bread wheat	48	14	6	12	<10–36
Bread wheat with additives	11	29	16	31	10–54
Bread rye	77	48	16	47	14–87
Bread sweet sour	47	28	20	25	<10–133
Bread rye with additives	33	54	30	52	14–152
Rusk	16	65	27	57	27–93
Toast, rye bread	12	40	17	37	20–79
Snacks					
Crisps	55	564	517	303	42–1570
Bagels	22	162	128	119	39–588
Coffee					
Roasted	13	450	120	450	300–600
Soluble	4	900	110	920	790–980
Chicory	1	2790			2790
Pastry					
Biscuit	53	187	118	91	<10–1060
Biscuit, nuts	7	97	90	68	<10–279
Biscuits, grain	7	400	165	393	147–606
Biscuit, chocolate	6	141	122	115	23–341
Biscuits, savoury	8	125	123	66	<10–322
Butter biscuit, toffee	4	28	9	26	19–41
Gingerbread	2	238	59	238	196–280
Puff pastry	9	102	62	82	20–200

This group also showed rather high variation of the acrylamide content. Similar values have been reported by other authors (Murkovic, 2004; Rufian-Henares, 2007) with revealed acrylamide levels up to 2085 μg kg^{-1}. Biscuits and bagels produced with addition of different components, e.g. sesame, bran, poppies, and onions, contained higher levels of acrylamide.
The lowest acrylamide content was found in bread, bread products, biscuits with nuts and toffee. It should be noted that the acrylamide content in bread with addition of seed and dried fruit was significantly higher in comparison with samples of bread without any additives and this conclusion complies with the study of Taeymans, Wood et al, 2004 about the acrylamide content in lipid rich foods.

Table 2

Estimation of the acrylamide exposure for adults aged 19–35 years

Adults 19–35 years	Consumption, g per person per day			Acrylamide content, µg g^{-1}			Exposure assessment, µg per person per day		
Food products	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
Rye bread	54.51	0.16	900.00	0.05	0.01	0.09	2.62	0.00	78.30
Rye bread with dried fruits	54.51	0.16	900.00	0.07	0.01	0.17	3.76	0.00	153.00
Sweet and sour bread	27.14	0.03	375.00	0.03	0.01	0.13	0.76	0.00	49.88
Wheat bread	52.57	0.05	1500.00	0.02	0.01	0.05	0.89	0.00	81.00
Cookies	11.29	0.03	213.70	0.16	0.01	0.61	1.76	0.00	129.50
Potatoes chips	9.90	0.01	136.88	0.56	0.04	1.57	5.58	0.00	214.90
Coffee	6.61	0.00	39.42	0.90	0.45	2.79	5.95	0.00	109.98

Table 3

Estimation of the acrylamide exposure for adults aged 36–50 years

Adults 36–50 years	Consumption, g per person per day			Acrylamide content, µg g^{-1}			Exposure assessment, µg per person per day		
Food products	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
Rye bread	57.37	0.14	1600.00	0.05	0.01	0.09	2.75	0.00	139.20
Rye bread with dried fruits	57.37	0.14	1600.00	0.07	0.01	0.17	3.96	0.00	272.00
Sweet and sour bread	34.93	0.07	625.00	0.03	0.01	0.13	0.98	0.00	83.13
Wheat bread	58.31	0.07	750.00	0.02	0.01	0.05	0.99	0.00	40.50
Cookies	11.56	0.05	300.00	0.16	0.01	0.61	1.80	0.00	181.40
Potatoes chips	2.79	0.05	78.36	0.56	0.04	1.57	1.57	0.00	123.03
Coffee	9.06	0.01	231.73	0.90	0.45	2.79	8.15	0.00	646.53

Table 4

Estimation of the acrylamide exposure for adults aged 51–64 years

Adults 51–64 years	Consumption, g per person per day)			Acrylamide content, µg g^{-1}			Exposure assessment, µg per person per day		
Food products	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
Rye bread	86.93	0.16	810.00	0.05	0.01	0.09	4.17	0.00	70.47
Rye bread with dried fruits	86.93	0.16	810.00	0.07	0.01	0.17	6.00	0.00	137.70
Sweet and sour bread	41.48	0.08	750.00	0.03	0.01	0.13	1.16	0.00	99.75
Wheat bread	51.41	0.05	470.14	0.02	0.01	0.05	0.87	0.00	25.39
Cookies	11.84	0.05	225.01	0.16	0.01	0.61	1.85	0.00	136.36
Potatoes chips	2.11	0.03	58.77	0.56	0.04	1.57	1.19	0.00	92.27
Coffee	8.49	0.01	70.52	0.90	0.45	2.79	7.64	0.00	196.75

Assessment of Latvian population exposure to acrylamide intake from food products

The mean daily consumption of seven product groups in which acrylamide intake was determined analytically and estimated exposure to dietary acrylamide intake for three Latvian individual age groups (adults 19–35 years, 36–50 years and 51–64 years) is presented in Table 2, 3 and 4. Based on

the results of the actual daily consumption, out of all in the highest quantity. In the oldest population group (51–64 years) the consumption of bread was 179 g per person per day. The highest consumption of potato chips is observed for adults group 19–35 years (9.9 g per person per day). Mean consumption of coffee was equal for all groups of population with the high variability between individuals. The mean daily dietary acrylamide intake ranged from 17 μg per day in the adults group aged 19–35 years and 51–64 years to 18.7 μg per day in the adults group aged 36–50 years.

The acrylamide intake from food originated mainly in three product groups. In the Latvian population bread supplied 31% of the total dietary acrylamide intake in the group of adults aged 19–35 years, 33% in the group of adults aged 36–50 years and even up to 46% in the group of adults 51–64 years.

Potato chips on average supplied 16% of acrylamide in the adult population (19–64 years) with significant difference between age groups (from 32% in the group of adults aged 19–35 years to only 7% in the group of aged 51–64 years). The significant source of dietary acrylamide in the Latvian population was also coffee that supplied 40% of total dietary acrylamide intake in the adult population.

The calculated average dietary acrylamide exposure for the Latvian population aged 19–64 years is 0.26 μg kg^{-1} of body weight per day and this estimation is in good agreement with the WHO data showing that the average dietary acrylamide exposure for the general population ranges between 0.3 and 0.8 μg kg^{-1} of body weight per day (WHO, 2002). However it should be taken into account that not all foodstuffs that might contain acrylamide were regarded in our study including home-made products that might represent a major source of dietary acrylamide intake. Therefore the estimated acrylamide intake is probably lower than actual and further research is necessary. Besides it is disturbing to note that for the high consumer group the estimated exposure to dietary acrylamide intake exceeds even ten fold the estimate mean exposure for the total population (up to 3 μg kg^{-1} of body weight per day). This level coincide with the tolerable daily intake (TDI) for cancer estimated in other study (Tardiff et al., 2012) and therefore high dietary intake of acrylamide by certain consumers may potentially cause adverse health effects and certain activities should be made to diminish acrylamide levels in processed foods with elevated content of this compound in order to reduce the dietary risk to the human health for Latvian population.

Conclusions

A survey of the food products produced in Latvia was performed for determining the acrylamide contents in these products. The obtained data on content of acrylamide in foodstuffs was used to evaluate the dietary exposure estimates of the Latvian population. The highest level of acrylamide was detected in potato crisps and chicory coffee with average concentration 546 μg kg^{-1} and 2790 μg kg^{-1} respectively. In general, analysed product groups, bread was consumed crisps, biscuits and coffee were among the food products with the high level of acrylamide. Significant differences were observed in the acrylamide contents between different brands and within brands of the snacks and biscuits. The addition of seeds, fruits or vegetables in bread had an increasing effect on acrylamide content.

References

1. Baum M., Fauth E., Fritzen S., Herrmann A., Metres P., Merz K., Rudolphi M., Zankl H., Eisenbrand G. (2005) Acrylamide and glycidamide: genotoxic effects in V79-cells and human blood. *Mutation Research*, Vol. 580, p. 61–69.
2. Blasiak J., Gloc E., Wozniak K., Czechowska A. (2004) Genotoxicity of acrylamide in human lymphocytes. *Chemico-Biological Interaction*, Vol. 149, p. 137–149.
3. Boon P. E., Mul A., Voet H., Donkersgoed G., Brette M., Klaveren J. D. (2005) Calculation of dietary exposure to acrylamide. *Mutation Research*, Vol. 580, p.143–155.
4. EFCOSUM – *European food consumption survey method*, Annex D „Overview nation – wide food consumption surveys with nutrient intake data on an individual level". Available: http://www.public-health.tu-dresden.de/dotnetnuke3/eu/Projects/PastProjects/EFCOSUM/tabid/338/Default.aspx
5. EFSA (2011) Results on acrylamide levels in food from monitoring years 2007–2009 and exposure assessment. *EFSA Journal*, Vol. 9, Iss. 4, p. 2133–2181.
6. Erola S., Hollebekkers K., Hallikainen A., Peltonen K. (2007) Acrylamide levels in Finnish foodstuffs analysed with liquid chromatography tandem mass spectrometry. *Molecular Nutrition and Food Research*, Vol. 51, p. 239–247.
7. Gamboa da Costa G., Churchwell M. I., Hamilton L. P., Von Tungeln L. S., Beland F. A., Marques M. M., Doerge D. R. (2003) DNA adduct formation from acrylamide via conversion to glycidamide in adult and neonatal mice. *Chemical Research in Toxicology*, Vol. 16, p. 1328–1337.
8. Ghanayem B. I., Witt K. L., El-Hardi L., Hoffler U., Kissling G. E., Shelby M. D., Bishop J. B. (2005) Comparison of germ cell mutagenicity in male CYP2E1-null and wild-type mice treated with acrylamide: evidence supporting a glycidamide-mediated effect. *Biology of Reproduction*, Vol.72, p. 157–163.
9. International Agency for Research on cancer (IARC) (1994) Acrylamide, IARC monographs on the evaluation of carcinogenic risks to humans. *Some industrial chemicals*, Vol. 60, p. 389–433.
10. Liu J., Man Y., Zhu Y., Hu X., Chen F. (2013) Simultaneous analysis of acrylamide and its key precursors, intermediates, and products in model systems by liquid chromatography – triple quadrupole mass spectrometry. *Analytical Chemistry*, Vol. 85(19), p. 9262–9271.
11. Mojska H., Gielecinska I., Stos K. (2012) Determination of acrylamide level in commercial baby foods and an assessment of infant dietary exposure. *Food and Chemical Toxicology*, Vol. 50, p. 2722–2728.
12. Mojska H., Gielecinska I., Szponar L., Oltarzewski M. (2010) Estimation of the dietary acrylamide exposure of the Polish population. *Food and Chemical Toxicology*, Vol 48, p. 2090–2096.

13. Mottram D. S., Wedzicha B. L., Dodson A. T. (2002) Acrylamide is formed in Maillard reaction. *Nature*, Vol. 419, p. 419–448.
14. Murkovic M. (2004) Acrylamide in Austrian foods. *Journal of Biochemical and Biophysical Methods*, Vol. 61, p. 161–167.
15. Rufian–Henares J. A., Arribas-Lorenzo G., Morales F. J. (2007) Acrylamide content of selected Spanish foods: survey of biscuits and bread derivatives. *Food Additives and Contaminants*, Vol. 24, Iss. 4, p. 343–350.
16. Sirot V., Hommet F., Tard A., Leblanc J. C. (2012) Dietary acrylamide exposure of the French population: Results of the second French Total Diet Study. *Food and Chemical Toxicology*, Vol. 50, Iss. 3–4, p. 889–894.
17. Stadler R. H., Scholz G. (2004) Acrylamide: an update on current knowledge in analysis, level in food, mechanism of formation, and potential strategies of control. *Nutrition Reviews*, Vol. 62, p. 449–467.
18. Taeymans D., Wood J., Ashby P., Blank I., Studer A., Stadler R. H., Gonde P., Van Eijck P., Lalljie S., Lingnert H., Lindblom M., Matissek R., Muller D., Tallmadge D., O'Brien J., Thompson S., Silvani D., Whitmore T. (2014) A Review of acrylamide: An industry perspective of research, analysis, formation, and control. *Critical Reviews in Food Science and Nutrition*, Vol. 44, Iss. 5, p. 323–347.
19. Tardiff R. G., Gargas M. L., Kirman C. R., Carson M. L., Sweeney L. M. (2012) Estimation of safe dietary intake levels of acrylamide for humans. *Food and Chemical Toxicology*, Vol. 48, Iss. 2, p. 658–667.
20. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2006) Evaluation of certain food contaminants: sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO*, Geneva.
21. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2011) Evaluation of certain contaminants in food: seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO*, Geneva.
22. WHO (2002) Health implications of acrylamide in food: Report of a Joint FAO/WHO Consultation. WHO, Geneva. Available: http://www.who.int/foodsafety/publications/chem/en/acrylamide_full.pdf?ua=1

ACRYLAMIDE REDUCTION OPTIONS IN RYE BREAD

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Abstract

Acrylamide is a food contaminant can be formed in foods when heated above 120 °C, if carbohydrates, especially reducing sugars, and asparagine are present. Bread is among the products that can contain high levels of acrylamide. The aim of this study is to evaluate acrylamide content in bread samples from different varieties of rye grains from different soils and to analyse acrylamide content reduction possibilities. Within the framework of the research, soil sulphur sufficiency in experimental fields of rye was analysed, and the content of amino acids was determined in rye grains, dough and bread by using standard methods. The samples were obtained from rye grain, flour and bread, then the impact of enzyme asparaginase, rosemary extract, and citric acid on the acrylamide content in the bread were analysed. The rye bread baking tests were carried out in an industrial bakery. The results of the soil analysis indicated a deficiency of sulphur containing compounds. The usage of enzyme asparaginase in rye bread production caused no significant reduction ($p>0.05$) of acrylamide content in rye bread. Analysis of the economic aspects of asparaginase utilization concluded that its utilization in production is not cost effective. The addition of rosemary extract to rye dough did not reduce the acrylamide content in bread. The addition of citric acid to rye dough reduced the content of acrylamide in rye bread by 66%, but had a negative effect on taste. Therefore, it is necessary to find the optimum quantity of citric acid that can be added to bread without changing the sensory properties of acidity and flavour.

Keywords: rye bread, acrylamide, asparaginase, citric acid, rosemary extract.

Introduction

Swedish scientists found acrylamide in food in 2002 (Yuan, 2011; Claus et al., 2008a). At present, a lot of research has been carried out in the world to understand its formation mechanism and to reduce the content of acrylamide in foodstuffs. Investigations made by Maastricht university (Hogervorst et al., 2007), indicate close correlation between acrylamide quantity in food and cause of separate cancerous disease. The International Agency for Research on Cancer is classified acrylamide as probably carcinogenic (2A group) to humans (IARC, 1994), however indistinctness still exist in that matter, because several investigations deny it (Laroque et al., 2008).

Acrylamide is substance, which may be form in starch containing products processed in high temperature (above 120 °C) – by fritting, frying, toasting. The most concentration of acrylamide is in bread`s crust (Brathen, Knutsen, 2005).

Several scientists concluded that acrylamide forms during Maillard reaction (Zyzak et al., 2003; Stadler et al., 2002; Becalski et al., 2003). Investigations showed that acrylamide does not form in boiled starch containing products. It is possible decomposing of acrylamide at protractedly frying at high (200 °C) temperature (Rydberg at al., 2003, Ahrne at al., 2007).

Factors what promote formation of acrylamide in foodstuffs are different, and strengthening each other. The most important of these is presence of amino acid asparagine, reducing sugar fructose as well as temperature, treatment time, water activity. Asparagine (Asn) is neutral amino acid and results of scientific investigations show that it is one of the main causes of acrylamide in bread and bakery products (Zhang et al., 2009).

Asparagine is soluble in alkalis, acids, and forms white monohydrate crystals with water.

The ratio of N : C in asparagin molecule is 2 : 4 which provide reserve and transportation of nitrogen in plants (Lea, 2007). Asparagine accumulates in plants under stress conditions. The growth of asparagine by 50% in wheat is observed under sulphur deficiency conditions. Cereals have been little studied in this area but existing investigations show that genetic as well as environmental factors dramatically affect asparagine in cereals (Zhang et al., 2009, Halford et al., 2007). Latvia no data about asparagine content in grain products, up to now in amino acids analysis was determined acidic amino acid - aspartic acid (Asp).

Swedish researchers found that the addition of asparagine dough stated notable acrylamide growth in bread from 80 to 6000 ppm, 99% of this amount was in crust (Yaylayan et al., 2003; Mottram et al., 2002).

European Food Safety Authority (EFSA) Scientific Panel on 19 April 2005 adopted statement on acrylamide in food where confirmed the need for the risk assessment. The EU has not imposed restrictions on the acrylamide concentration in food but has developed recommendations for its reduction, as well as EFSA incorporated data from manufacturers about its content in food. EFSA acrylamide indicative figures mentioned that fresh bread should not exceed 150 $\mu g\ kg^{-1}$, crisp-bread – 500 $\mu g\ kg^{-1}$ of acrylamide (EFSA, 2009).

The Scientific literature (Claus et al., 2008a) data on acrylamide content in bread and bakery products has wide range due not only to different bread recipes and dough making technology but also to the kind of oven used and the kind of baking process.

Agronomical measures are recommended carry out in order to reduce acrylamide content in bread as well as make changes in the recipe and technology. Scientists recommended controlling sulphur level in the soil during cereals breeding as agronomical measure. This is reflected in the study where sulphate deficiency in

cereals during breeding period called dramatic increases in the concentration of asparagine in grains and unexpectedly high acrylamide content in bread (Muttucumaru et al., 2006; Claus et al., 2008b).

German scientists investigating asparagine in rye have found that it is 319–791 mg kg^{-1} (Springer et al., 2003). There no investigations in Latvia about asparagine content in rye and bread but there are data about aspartic acid content in grain samples of five rye varieties as 0.55–0.70 g 100 g^{-1} (Straumite, 2006, Ozolina, 2012).

Following the guidelines of CIAA, would be useful to develop cooperation between farmers, manufacturers and scientists on sulphur containing fertilizers utilization on cereals cultivation, as well as on new cereals varieties containing as low as possible amount of asparagines (CIAA, 2009).

The aim of study is to evaluate acrylamide content in bread samples from different varieties of rye grains and to analyse acrylamide content reduction possibilities.

Within the framework of the research, soil sulphur sufficiency in experimental fields of rye was analysed, and the content of amino acids was determined in rye grains, dough and bread. The samples were obtained from rye bread baking tests, then the impact of enzyme asparaginase, rosemary extract, and citric acid on the acrylamide content in the bread were analysed. The rye bread baking tests were carried out in an industrial bakery. The content of amino acids in dough and bread was determined, and aspartic acid relevance to acrylamide formation was evaluated.

Materials and Methods

The Influence of Meteorogical Conditions

Winter rye was sown in late September in weather conditions favourable to winter crops.

Snow cover formed in the last ten days of October yet did not cause significant damage and winter rye growth continued until the second ten days of November. At the end of the active growth period, winter crops had begun their tillering phase. In total, the winter months were colder and richer in precipitation compared with long -term mean data.

As the ground under the snow was not frozen, it delayed the plants overheating under a thick snow blanket ensuring their survival. In the spring of 2013 winter receded slowly, in the first ten days of April the average temperature was negative, the winter crops recrudesced in the second ten day period of April which is about a week later than usual. April was cool, the warm weather arrived in the first ten days of May, the second and third ten-day periods of May were rainy. The summer of 2013 was warmer and drier than usual. Consequently, winter crop development was faster; they even ripened earlier than usual, at the end of July. Due to this rapid development yield was lower than in 2012.

Materials and methods used in bread baking tests

A hybrid variety of rye ‘Agronom’ and population variety of rye ‘Kaupo’ cultivated at the State Plant Breeding Institute Priekuli and State Cereals Breeding Institute Stende trial fields, were used in the research. Cleaned and prepared for processing, the grain samples were ground in a *Hawos Queen2* mill at the Bread Manufacturing Laboratory of Latvia University of Agriculture, Faculty of Food Technology and whole meal rye flour with moisture content of 11.2 to 12% was obtained for bread baking

The enzyme asparaginase (*Megazyme)* with activity: 15 U/mg (25 °C, pH 8.0), the natural antioxidant rosemary extract „Oleoresin Rosemary 41-19-25” (recommended amount of addition 0.08% to dough mass), and citric acid (E330) were used in the baking tests.

Whole meal rye bread bakery tests

Bread bakery tests were carried out to assess enzyme asparaginase, rosemary extract and citric acid impact on acrylamide formation intensity in rye bread samples, which were prepared in an industrial module system.

Hybrid variety ‘Agronom’ and population variety ‘Kaupo’ whole meal rye flour, sugar, salt and water were used for the dough preparation. The dough recipe is shown in Table 1. The dough was prepared in accordance with bread making technology, including starter preparation, dough kneading and fermentation, dough dividing, loaf formation and baking.

Table 1

Whole meal rye bread basic recipe

Semi-finished product	Raw materials	Amount of product, g
Yeast bread	Rye whole meal flour	250
	Water	250
Dough	Yeast bread	500
	Rye whole meal	250
	Salt	10
	Sugar	30
	Water	150
Total		940

The starter was made using German *Böcker* pure rye bread starter culture. The starter was prepared separately for each flour sample, the necessary temperature (28–30 °C) and fermentation time (2×24 hours) were provided. 50% of the total quantity of rye flour was used for the starter. The starter acidity ranged from pH 4.0 to 3.9, which corresponds to the required acidity for rye bread starter.

The dough was mixed for 10 minutes until all the ingredients were evenly blended and a homogeneous mass resulted. Enzyme asparaginase (500 U) was added to four samples at the end of dough mixing. The dough fermented for 30 minutes, then was divided into 400 gram loaves, then put into forms and fermented for 60 minutes in the post-fermentation chamber at a temperature of 35 °C and 70% relative air humidity.

Fermented semi-finished dough was baked in a *Sweba Dahlen* convection oven at a temperature of 200 °C for 40 minutes. After baking, the bread samples were cooled and their taste was evaluated. The bread samples were baked for 50 minutes in the clay oven at a falling temperature regime (initial temperature 260 °C, final temperature 210 °C). The added rosemary extract Oleoresin Rosemary 41-19-25 proportion was 0.1% and 0.5%, and citric acid proportion was 1%, following the manufacturer's recommendations. The baking tests for rye bread with dried fruits, with and without the addition of rosemary extract (0. 1%), were conducted at the bakery.

Amino acids in flour, dough and rye bread samples
Amino acids were analysed according to AOAC standard method N. 994.12, by using ion change after sample hydrolysis by 6M HCl in an inert atmosphere. The content of amino acids was determined by using an Automatic Amino Acids Analyzer (Microtechna Praha), and as the standard, the „Amino acid Standard solution for protein hydrolysates 0.5 μmoles per mL" (Sigma).

Acrylamide Acrylamide separation from the bread samples was achieved with solid phase extraction, but the acrylamide content was analysed with liquid chromatograph-tandem mass spectrometry (LC-MS/MS). For analysis 2±0.0001 g of dried and homogeneous sample was weighed into a 50 mL centrifuge tube. The internal standard was added (d3-acrylamide) at 100 μg kg^{-1}. Then 5 mL of hexane was added for sample degreasing, and then the tube was vortexed. After 10 mL of water, 10 mL of acetonitrile, 4.0 g $MgSO_4$ and 0.5 g NaCl were added the tube was vigorously shaken for 1 min, then centrifuged at 4000 rpm for 5 min. The hexane layer was discarded and 1 mL of the acetonitrile extract was transferred to a tube containing 50 mg of primary and secondary amines (PSA) and 150 mg $MgSO_4$. The tube was vortexed for 30 sec and then placed in an autosampler vial, and analysed with LC-MS/MS (Waters 2695, Quattro Premire XE). The determination of acrylamide was performed at the Latvia Institute of Food Safety, Animal Health and Environment "BIOR"

Statistical analysis. Experimental data evaluation was done using two–factor analysis of variance by Fisher's criteria and least significant difference ($LSD_{0.05}$). Components of variance ANOVA for each quality characteristic were expressed as percentages to illustrate the relative impact of each source to the total variance.

Results and Discussion

Field trials and rye cultivation
Hybrid rye variety 'Agronom' and population rye variety 'Kaupo' were grown in field trials. Scientific literature indicates that rye hybrid varieties generally are higher yield than rye population varieties (Petr, 2006). In this 2013 trial, such a difference was not observed. This may be due to the relatively rapid development of the plants in the summer of 2013, as a result hybrid rye have not realizing its potential for yield. The 2013 yield was influenced significantly by fungicide usage at sowing – both varieties have significantly higher yield with fungicide usage – more than one tonne per hectare (Table 2).

Table 2

Yield of hybrid and population rye varieties, t h^{-1}

Variety	NPK+R+H	NPK+R+H+F	Average
'Agronom' F1 (hybrid)	5.62	6.65	6.13
'Kaupo' (population)	5.73	6.48	6.11

In 2013 there was no significant difference between the tested populations of hybrid varieties of rye and yields. The yield of both varieties of rye in the presence of fungicide increased. Samples were submitted from both varieties harvested and submitted for further analysis. The study locations were the State Plant Breeding Institute Priekuli (hereinafter referred to as Priekuli) and State Cereals Breeding Institute Stende (hereinafter referred to as Stende).

Table 3

The soil agrochemical parameters

Sample of soil	Reac-tion	Organic matter	P_2O_5	K_2O	S-SO_4
	pH, HCl	%	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Stende 1	5.4	1.7	63.0	121.0	4.0
Stende 2	4.0	4.0	271.0	145.0	1.2
Stende 3	4.0	2.1	256.0	140.0	1.2
Priekuli 1	4.8	1.9	95.0	106.0	0.6
Priekuli 2	6.0	2.5	101.0	88.0	0.6
Priekuli 3	4.9	2.1	100.0	127.0	0.6

In both study sites the research was carried out on the soil of relatively poor sod-podzoloic and sod-podzoloic sandy loam. The agro-chemical characteristics of the soil were assessed in accordance with the March 15th, 2007, Ministry of Agriculture (Latvia's) guideline No. 12 "Methodological procedures for agro-chemical soil exploration and evaluation of the results", as outlined in Table 3. Stendes soil is characterized by medium acid to very acid reaction, from insufficient to elevated organic matter content, from low to very high phosphorus content, and medium calcium content. Priekuli soil is characterized by very to normal acid reaction, insufficient to optimal organic matter content, and low to medium calcium content.
In all of the soil samples found in both Stende and Priekuli, sulphur content was very low. According to research results, the optimal (sulphate) sulphur content

of soil could be from 10 to 60 mg kg^{-1}, depending on organic matter in the soil.

However, soils poor in organic matter contain sulphur of less than 5 mg kg^{-1}. One explanation might be the soil fertilization trend of recent years: natural manure appears to have been replaced with manufactured phosphorous fertilizers as the European Union and other countries try to enforce measures aimed at the reduction of sulphur emissions from industrial production processes (Smit et al., 2009). It should be noted that sulphate sulphur leaches out of soil.

Results of the soil analysis show that there is a need to further study asparagine and aspartic acid content changes in rye which occur as a result of cereal growing technologies used, taking into account both fertilization systems and the agrochemical properties of agricultural lands.

Amino acid content of rye flour

Aspartic acid is an amino acid, its structure has one amino group and two carboxyl groups, but in reaction with free NH_3 forms amide of aspartic acid, or asparagine, it is known as a neutral polar amino acid. The study identified 16 amino acids in rye flour.

Table 4

Amino acids of whole grain rye flour, g 100 g^{-1}

Amino acids	Kaupo 1	Kaupo 2	Agronom 2
Valine	0.41	0.50	0.58
Leucine	0.66	0.75	0.82
Isoleucine	0.37	0.41	0.46
Phenylalanine	0.98	1.08	1.22
Lysine	2.92	2.53	2.68
Arginine	0.49	–	0.52
Aspartic acid	0.65	0.79	0.90
Serine	0.43	0.46	0.53
Glutamic acid	2.29	2.65	3.02
Glycine	0.49	0.54	0.58
Threonine	0.31	0.35	0.39
Alanine	0.36	0.54	0.62
Proline	1.00	1.13	1.31
Tyrosine	0.19	0.22	0.26
Methionine	0.11	0.10	0.10
Total:	11.65	12.05	13.97

The amino acids content of rye flour is given in Table 4. The total content in 'Kaupo' variety rye whole meal flour was from 11.65 to 12.05 g 100 g^{-1} but in hybrid variety 'Agronom' was 13.97 g 100 g^{-1}. The aspartic acid content of rye whole meal flour was from 0.65 to 0.9 g 100 g^{-1}. Literature data shows that the aspartic acid content of whole grain rye is 503 µg kg^{-1} (Mustafa et al., 2007). This indicates that the flour used in the study has higher aspartic acid content.

Rye bread baking tests for evaluating enzyme asparaginase, rosemary extract and citric acid influence on acrylamide formation. To evaluate the impact of recipes and bread baking technology on acrylamide formation, the amino acid content was measured in dough and bread, as well as acrylamide content in the bread samples**.** Obtained results are given in Table 5.

Table 5

Acrilamide content in samples of different types of rye bread

No	Description of rye bread (rye grain variety, growing place)	Acrilamide content, µg kg^{-1}
1	Rye bread baked in form ('Kaupo', Priekuli)	10.9
2	Rye bread baked in form ('Kaupo', Priekuli) with enzyme	14.9
3	Rye bread baked in form (Agronom, Priekuli)	17.4
4	Rye bread baked in form ('Agronom', Priekuli) with enzyme	16.4
5	Rye bread baked in form ('Kaupo', Stende)	12.5
6	Rye bread baked in form ('Kaupo', Stende) with enzyme	15.6
7	Rye bread baked in form ('Agronom', Stende)	16.8
8	Rye bread baked in form ('Agronom', Stende) with enzyme	18.2
9	Rye bread ('Kaupo', Priekuli) control	98.6
10	Rye bread ('Kaupo', Priekuli) with Rosemarie extract 0.1%	100.7
11	Rye bread ('Kaupo', Priekuli) with citric acid 1%	34.3
12	Rye bread ('Kaupo', Priekuli) with Rosemarie extract 0.5%	106.6
13	Rye bread with fruits (at production company) control	177.1
14	Rye bread with fruits (at production company) with Rosemarie extract 0.1%	238.6

In analysing amino acid content in rye dough and bread, the most attention was heeded to aspartic acid content and its changes during bread production. The aspartic acid in rye dough samples was from 0.38 to 0.48 g 100 g^{-1}. Significant distinctions between different samples and samples with or without adding enzyme asparaginase were not observed. The aspartic content of bread samples ranged from 0.32 to 0.84 g 100 g^{-1}. However, it should be noted, the relation between different samples was not observed.

The enzyme asparaginase was added to bread samples, 500 U kg^{-1} in relation to flour weight. Scientific literature indicates that the amount of added enzyme can be from 200 to 2000 U kg^{-1}, and can reduce acrylamide content up to 70% (Capuano, 2009). The bread samples baked in forms (Fig. 1) do not have dark and thick crusts, and the acrylamide content is from 10.9 to 18.2 µg·kg^{-1}. Such acrylamide content is

insignificant, according to the Joint Food and Drug Administration (FDA) and World Health Organization (WHO) Expert Committee on Food and Additives (JECFA). Their recommendation is that the acrylamide content in bread should not exceed 150 $\mu g \cdot kg^{-1}$ (EFSA, 2009). The impact of enzyme action on changes in acrylamide content was not observed. Admittedly, the enzyme asparaginase is expensive, and its wider utilization in bread manufacturing is not cost effective. The evaluation of the bread quality and physical chemical indicators between different varieties of rye grains and different cultivation regions did not show significant differences. The moisture of all dough samples was similar, ranging from 48.5 to 50%. The moisture of the bread samples was from 40.2 to 41.6%.

The study evaluated the impact of a natural antioxidant and citric acid on acrylamide content and rye bread quality.

The bread samples were baked on clay, resulting in a darker and thicker crust than in samples baked in forms.

Analysis of acrylamide content in bread samples baked on clay (Table 5) show that it has increased – from 100.7 to 106.6 $\mu g\ kg^{-1}$ in samples with added rosemary extract, and 98.6 $\mu g\ kg^{-1}$ in the control sample. From this it can be concluded that rosemary extract added to rye bread dough, in amounts of 0.1 and 0.5% does not have significant impact on acrylamide content.

The rye bread with fruits, baked in an industrial bakery, has an acrylamide content of 177.1 $\mu g\ kg^{-1}$, which exceeds the recommended quantity, but the addition of 0.1% rosemary extract raises acrylamide content up to 238.6 $\mu g\ kg^{-1}$.

The research carried out by Ozolina (2011) concluded that bread type, baking oven, and sugar content in dough do not have significant impact on acrylamide content in bread, but that the addition of dried fruits significantly impacts the content of acrylamide in the bread ($p=0.025$). The sugar content is not the main reason for the presence of acrylamide in bread, as also confirmed by a similar study (Springer et al., 2003). The scientific literature has no information about the possible influence of dried fruit additives on the acrylamide content in bread and other products.

The bread samples with 1% citric acid showed an acrylamide decrease of 66%, and in the bread it is 34.3 $\mu g\ kg^{-1}$.

It should be noted that the sensory qualities of bread with such a large citric acid presence were lower than that of control samples because the bread taste was markedly sour. These results suggest that the presence of citric acid can reduce acrylamide content in bread, only the quantity to be added must be correctly chosen, in order to not decrease the product sensory characteristics.

Based on the acrylamide content in foodstuff, it is possible to calculate contamination intake level in the human body. If daily ingestion of whole grain rye bread is 200 g, then acrylamide intake is 9 to 10 µg. Thus the calculation for a person weighing 70 kg, is approximately 0.13 µg acrylamide per kg body weight.

Figure 1. Samples of rye bread baked in forms

According to World Health Organization information, the level of acrylamide intake from the Latvian bread used in this case is medial. Although this indicator is not high, the issue of acrylamide content and the means to reduce it are actually urgent.

Conclusions

The results of soil composition analysis indicated insufficient content of sulphur containing compounds, which significantly impacted aspartic acid and asparagine content in grains as well as on bread quality.

The results of soil analysis give evidence for further investigations necessary on asparagines and aspartic acid changes in rye in connection with technology used in cereals cultivation in Latvia, with evaluation on fertilizer systems as well as agricultural land agrochemical properties.

Baking time significantly influenced the formation of acrylamide, therefore it is critical that the optimum baking time for specific bread types is not exceeded.

The results confirm the studies of other researchers who have observed that acrylamide content in bread increases with prolonged baking time and increased temperature.

The use of the enzyme asparaginase in the production of rye bread does not significantly reduce acrylamide content.

The addition of rosemary extract to rye dough up to 0.5% does not reduce the content of acrylamide in rye bread.

The addition of citric acid (1%) to rye dough reduced the acrylamide content in bread by 66%. It is necessary to evaluate the admissible amount of citric acid in order to not change the bread sensory qualities of acidity and aroma.

Acrylamide content in rye bread significantly increases with the introduction of dried fruit into the bread.

The reduction of acrylamide content in bread should be treated as a complex system of interlinked factors: soil, grains, technological processing and bread.

References

1. Ahrne L., Andersson C-G., Floberg P., Rosen J., Lingnert H. (2007) Effect of crust temperature and water content on acrylamide formation during baking of white

bread: Steam and falling temperature baking. *LWT-Food Science and Technology*, Vol. 40, p. 1708–1715.
2. Becalski A., Benjamin P.-Lau Y., Lewis D., Seaman S. W. (2003) Acrylamide in Foods: Occurrence, Sources, and Modelling. *Journal of Agricultural and Food Chemistry*, Vol. 51, p. 802–808.
3. Brathen E., Knutsen S. H. (2005) Effect of temperature and time on the formation of acrylamide in starch-based and cereal model system, flat breads and bread. *Food Chemistry*, Vol. 92, p. 693–700.
4. Capuano E., Ferrigno A., Acampa I., Serpen A., Ç., Açar Ö., Gökmen V., Fogliano V. (2009) Effect of flour type on Maillard reaction and acrylamide formation during toasting of bread crisp model systems and mitigation strategies. *Food research International*, No. 42, p. 1299–1302.
5. CIAA. (2009) The CIAA Acrylamide Toolbox. [accessed on 16.02.2012.] Available at: http://www.ciaa.be/ documents/brochures/ac_toolbox_20090216.pdf.
6. Claus A., Mongili M., Weisz G., Schieber A., Care R. (2008a) Impact of formulation and technological factors on the acrylamide content of wheat bread and bread rolls. *Journal of Cereal Science*, Vol. 47, p. 546–554.
7. Claus A., Carle R., Schieber A. (2008b). Acrylamide in cereal products: A review. *Journal of Cereal Science*, Vol. 47, p. 118-133.
8. EFSA (2009) Technical report of EFSA prepared by Data Collection and Exposure Unit (DATEX) on "Monitoring of furan levels in food". The EFSA Scientific Report, No. 304, p. 1–23.
9. Halford N. G., Muttucumaru N., Curtis T. Y. and Parry M. A. (2007) Genetic and agronomic approaches to decreasing acrylamide precursors in crop plants. *Food Additives and Contaminants*, Vol. 24, p. 26–36.
10. Hogervorst J. G., Schouten L. J., Konings E. J., Goldbohm R. A., and Brandt P. A. (2007) A prospective Study of Dietary Acrylamide Intake and the Risk of Endometrial, Ovarian and Breast Cancer. *Cancer Epidemiology Biomarkers and Prevention*, Vol. 16, p. 2304–2313.
11. IARC. International Agency on Research on Cancer. (1994) Acrylamid. **In:** *IARC monographs on the evaluation of carcinogenic risks to humans in some industrial chemicals*, Lyon, France, Vol. 60, p. 389–433.
12. Laroque D., Inisan C., Berger C., Vouland E., Dufosse L., Guerard F. (2008) Kinetic study on the Maillard reaction. Consideration of sugar reactivity. *Food Chemistry*, Vol. 111, p. 1032–1042.
13. Lea P.J., Sodek L., Parry M. A. J.,Shewry P. R., Halford N.G. (2007) Asparagine in plants. *Annals of Applied Biology*, No. 150, p. 1–26.
14. Muttucumaru N., Halford N. G., Elmore S. L., Dodson A. T., Parry M., Shewry P. P., Mottram D. S. (2006). Formation of high levels of acrylamide during the processing of flour derived from sulphate-deprived wheat. *Journal of Agricultural and Food Chemistry*, Vol. 54, p. 8951–8955.
15. Mottram D.S., Wedzicha B.L., Dodson A.T. (2002) Acrylamide is formed in the Maillard reaction. *Nature*, No. 419, p. 448–449.
16. Mustafa A., Aman P., Andersson R., Kamal-Eldin A. (2007) Analysis of free amino acids in cereal products. *Food Chemistry*, Vol. 105, p. 317–324.
17. Ozolina V., Kunkulberga D., Dimins F. (2011) *Formation of 5-hydroxymethyl-furfuraldehyde in Latvian whole meal rye bread during baking*. Foodbalt-2011: 6th Baltic Conference on Food Science and Technology "Innovations for Food Science and Production", Conference proceedings, - Jelgava: LLU, p. 61–66.
18. Ozolina V. (2012) *Changes in biologically active compounds during rye bread production process.* Promotion work for acquiring the Doctor`s degree of Engineering Sciences in sector of Food Sciences. LLU, Jelgava. p. 96.
19. Petr J., v. Mikšft V. (2006) Rye Quality of Hybrid and Population Varieties from Intensive and Ecological Conditions. *Scientia Agriculturae Bohemica*, No. 37. p. 1–8.
20. Rydberg P., Eriksson S., Tareke E., Karlsson P., Ehrenberg L. and Tornqvist M. (2003) Investigations of factors that influence the acrylamide content of heated foodstuffs. *Journal of Agricultural and Food Chemistry*, Vol. 51, p. 7012–7018.
21. Smit A.L., Bindraban P.S., Schröder J.J., Conijn H.G., van der Meer J.G. (2009) *Phosphorus in agriculture: global resources, trends and developments*. Plant Research International B.V., Wageningen, Report No. 282. [accessed on 3.02.2014.] Available at: http://edepot.wur.nl/1257.
22. Springer M., Fischer T., Lehrack A., Freund W. (2003) Acrylamidbildung in Backwaren. *Getreide, Mehl und Brot*, Vol. 57, S. 274–278.
23. Stadler R. H., Blank I., Varga N., Robert F., Hau J., Guy P. A., Robert M. C. and Riediker S. (2002) Acrylamide from Maillard reaction products. *Nature*, Vol. 419, p. 449–450.
24. Straumīte E. (2006) Research of rye flour baking properties. Promotion work for acquiring the Doctor`s degree of Engineering Sciences in sector of Food Sciences. LLU, Jelgava. 97 p.
25. Yuan Y., Shu C., Zhou B., Qi X., Xiang J. (2011). Impact of selected additives on acrylamide formation in asparagine/sugar Maillard model systems. *Food Research International*, Vol. 44, p. 449–455.
26. Yaylayan V. A., Wnorowski A., and Locas Perez C. (2003) Why asparigine needs carbohydrates to generate acrylamide. *Journal of Agricultural and Foods Chemistry*, Vol. 51, p. 1753–1757.
27. Zhang Y., Ren Y., Zhang Y. (2009) New Research on Acrylamide: Analytical Chemistry, formation mechanism, and mitigation recipes. **In:** *Chemial Rewiews*, Vol. 109, p. 4375–4397.
28. Zyzak D. V., Sanders R. A., Stojanovic M. (2003) Acrylamide formation mechanism in heated foods. *Journal of Agricultural and Food Chemistry*, Vol. 51, p. 4782–4787.

QUALITY CHANGES OF CEREAL MUESLI WITH SEEDS DURING STORAGE

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Abstract

Cereals play a vital role in diet because they not only provide humans with essential macronutrients such as protein, fat and carbohydrate for growth and maintenance but also supply with vitamins, minerals, and micronutrients for optimal health. In recent years the activities of human consumption "clean" products, with no extra added sugars, food additives and preservatives increases. The aim of this study was to evaluate cereal muesli with seeds quality changes during storage. Samples of muesli with seeds (linseeds, pumpkins seeds, almond slices) were packaged in 3 different packages –paper bag, paper tubes and *Doypack* (standup pouches) and stored for 6 months (at 20±2 °C temperature and air humidity – 50±5%). During the storage moisture content, water activity, and microbiological safety were evaluated.

After 6 months storage of muesli with seeds, the moisture content in samples packaged in the paper tube decreases by 14.3%, in *Doypack*–20.5% and in paper bag by 34.9%. The lowest moisture content was determined for muesli samples storage in the paper package – 6.00%.Water activity compared to the fresh prepared muesli (a_w=0.56) decreased, in paper tube it was 0.33, in paper bag – 0.16 and in *Doypack* – 0.47. As more unusable for cereal muesli with seeds packaging paper bags was detected, because of essential quality changes of samples during storage. After 6 months storage total count of microorganisms and moulds increase, but remained within the normal range. Therefore, the shelf-life of cereal muesli with seeds packaged in paper tube or *Doypack* for 6 months could be recommendable.

Keywords: cereals, muesli, packaging, storage time.

Introduction

Muesli is a mixture of grain flakes and dried fruits, where can be also added seeds and nuts. Thanks to its easy and quick preparation with milk, yogurt or hot water, muesli is often called as a breakfast flakes. Cereals play a vital role in diet because they are not only providing humans with essential macronutrients such as protein, fat and carbohydrate for growth and maintenance but also supply other important vitamins, minerals, and micronutrients for optimal health (Collins et al., 2010). The breakfast cereals are generally high in carbohydrate are low in fat; some have high bran contents, and many contain appreciable amounts of certain vitamins and minerals, partially satisfying the mineral and vitamin requirements (Albertson et al., 2008; Bertais et al., 2000). Eating cereal at breakfast was associated with increased consumption of fibre and carbohydrates and decreased consumption of fats throughout the day (Gibson, Gunn, 2011; Albertson et al. 2008; Williams, 2007; Wilson et al., 2006), and improved mental and greater physical performance (Albertson et al. 2008; Rampersaud et al., 2005). This may be because they are a good source of whole grains and commonly eaten with milk. Whole grains are rich in dietary fibre and both grains and milk will improve micronutrient intake (Coudray, 2011; Drewnowski, 2011; Vissers et al., 2011; Williamson, 2010). Breakfast cereals are often now also fortified with vitamins such as folate and thiamine (Loui et al., 2012; Hannon et al., 2007; Berner et al., 2001). Cereal consumption as part of a healthy lifestyle may play a role in maintaining adequate nutrient intake and physical activity (Albertson et al., 2008).

The earliest breakfast cereal manufacture was based on boiling then drying, extrusion has become a well-established industrial technology with beneficial effects on the nutritional properties and texture of the raw material. The pleasant flavours and colours of breakfast cereals are produced in the drying and toasting steps (Delgado-Andrade et al., 2007; Rufian-Henares et al., 2006; Rada-Mendoza et al., 2004).

The majority of cereal-associated bacteria are located in the outer layers of the kernel and are thus concentrated in bran (ICMFS (International Commission on Microbiological Specifications for Foods), 2005; Laca et al., 2006). Contamination of the grains during storage, transport, and processing further affects the microflora.

Texture in ready-to-eat cereals is fundamental for product acceptance by consumers. Textural properties of food are used by consumers as key quality indicators that contribute to product acceptability. Crispness is one such key indicator and is considered a primary textural attribute of breakfast cereal. Cereal hardness is another force claimed that crispness is the most important attribute affecting consumer acceptability (Pathare et al., 2012; Burrington, 2001). Moisture content for breakfast cereal is as the critical quality parameter and relative humidity as the most influential environmental factor (Macedo et al., 2011).

The use of packaging in the food supply chain is very important and is an essential part of food processing. It can be used for various purposes such as the assurance of protection of food from external infections (microorganisms), surrounding conditions (like atmosphere gases, water activity, etc.), and the appropriate labelling of foods (La Storia et al., 2008). Flexible pouch used to package breakfast cereal in air is not integral to the product. The cereal could be removed from the package and poured into a plastic dispenser and not suffer any loss in shelf life. Cereal products are usually packaged in paper/polyethylene

packaging (Galic et al., 2009), but there is an interest in replacing its packaging and potentially use different and more environmentally friendly packaging materials. Paper can be combined with layers of LDPE (low density polyethylene) and aluminium to make sealed bags and pouches that provide a better barrier to moisture and oxygen for the food product (Krochta, 2006). Similar products such as granola muesli, the recommended period of validity is 270 days (Macedo et al., 2013).

The aim of this study was to evaluate cereal muesli with seeds quality changes during storage.

Materials and Methods

Characterisation of muesli with seeds

Muesli with seeds contains:

- 40% roasted whole grain triticale flakes (200 °C for 10 min),
- 25% roasted whole grain rye flakes (200 °C for 10 min),
- 15% linseeds,
- 10% almond pieces,
- 10% pumpkin seeds.

Characterisation of packaging materials

Samples of muesli with seeds (linseeds, pumpkins seeds, almond slices) were packaged in 3 different packaging materials:

- paper tubes – tubes with aluminium layer inside and LDPE (low density polyethylene) black cover ;
- paper bag –brown kraft paper 90–100 g m^{-2} with PP (polypropylene) window;
- *Doypack* (standup pouches) – Pap50g/Alu7/Pe60.

Amount of sample in package – 250 g, storage time 6 months at 20±2 °C temperature and air humidity – 50±5%.

Moisture content

Moisture content of muesli with seeds was determined using heating oven Memmert (GmbH) Memmert, Germany) – 5.00±0.03 g sample for 1 hour was dried at 110±1 °C temperature (LVS EN ISO 712:2010A). In the analysis on moisture content was run in duplicate and averaged.

Water activity (a_w)

Water activity (a_w) was determined using LabSwift-aw (AG Novasina, Switzerland) equipment. In the analysis on water activity analysis was run in triplicate and averaged.

Microbiological parameters

Total plate count of mesophylic aerobic and facultative anaerobic microorganisms (MAFAm) were determined in conformity with the standard LVS EN 4833:2003;yeasts and moulds – with the standard ISO 21527-2:2008.

Statistical analysis

The mean and standard deviation were processed by mathematical and statistical methods.

Results and Discussion

Moisture content

During 6 months of storage, muesli moisture content in paper tube decreased by 14.3%, in paper bag with window – by 34.9% and in *Doypack* by 20.5% (Figure 1.). Muesli moisture varied from 9.2% at first to 6.0% at the end.

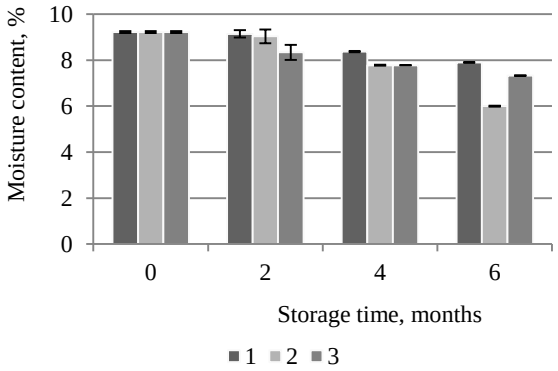

Figure 1. Changes in moisture content of muesli with seeds during storage
1 – paper tube, 2– paper bag, 3– *Doypack*

The lowest moisture content was determined in the paper bag with PP window after 6 month storage – 6.00%. Legislation lay down that moisture content for flakes has to be in the range between 10–14% (Prasības..., 2008). Comparing obtained results with other researches, results shows, that oat flakes moisture content is between 6.0–8.7% (Gates et. al., 2007).

In the paper bag with PP window it has decreased more than in the paper tube and *Doypack*. This difference can be due to the quality of the packaging material. High moisture content may be caused to the additives and their interaction with each other by connecting a number of components. Each additive moisture content is variable and storing the environment constitute the final product which resulted on the product quality. During storage, the product has seen a steady, slow decline of moisture, which can be related to the raw materials used in the product, which has the ability to bind moisture from one to another by connecting to creating a favourable environment for microbial growth.

Water activity (a_w)

Water activity was used to determine stable shelf-life for foods. During storage of the muesli a decrease in water activity was observed (Fig. 2.).

Water activity for muesli with seeds ranged between 0.56 at first to 0.16 at the end. These differences affected packaging materials – the lowest water activity was determined in the paper bag – 0.16. In the paper tube water activity (a_w) decreased for 41.1% and in *Doypack* – for 16.1%. Water activity for muesli products is not defined in legislation, but there are some researches, where water activity for dried foods is

defined like no microbial proliferation below 0.6 (Beuchat, 1981).

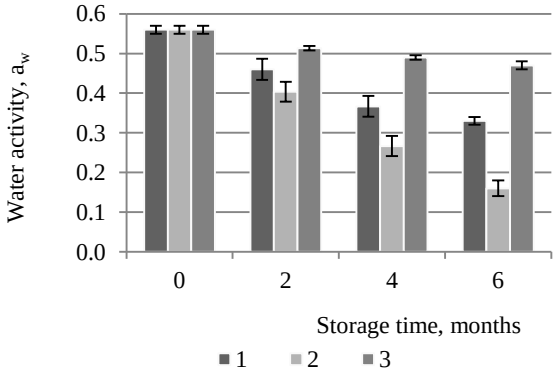

Figure 2. Changes in water activity *(a_w)* of muesli with seeds during storage

1 – paper tube, 2 – paper bag, 3 – *Doypack*

Reduced water activity does not provide a complete inactivation of microorganisms, but partially restrict their activities in the product, which helps to ensure a longer shelf-life. Having a water activity below 0.6 products are stable against microbial growth, but chemical and enzymatic reactions can occur which results in deterioration. Comparing data with other research, it can be concluded that the muesli stored up to 12 months, the water activity is 0.22 (Labuza, 1980).

Microbiological parameters

Taking into account that the grain seed mixture contains more different components that are re-cooked as they are received from a supplier, the product is increased microbial risk.

Table 1

Changes of microbiological parameters during muesli storage

Parame-ters / Samples	Storage time, months			
	0	**2**	**4**	**6**
MAFAm CFU g^{-1}				
paper tube	1.8×10^3	2.3×10^4	3.4×10^4	5.6×10^4
paper bag	1.8×10^3	2.9×10^4	4.2×10^4	1.3×10^5
Doypack	1.8×10^3	2.4×10^4	3.8×10^4	5.2×10^4
Yeasts CFU g^{-1}				
paper tube	1.3×10^2	2.3×10^3	2.8×10^3	3.3×10^3
paper bag	1.3×10^2	3.4×10^3	4.2×10^3	5.6×10^3
Doypack	1.3×10^2	2.1×10^3	3.1×10^3	3.7×10^3
Moulds CFU g^{-1}				
paper tube	1.1×10^2	1.7×10^3	2.9×10^3	4.8×10^3
paper bag	1.1×10^2	2.8×10^3	3.6×10^3	5.3×10^3
Doypack	1.1×10^2	2.2×10^3	2.7×10^3	4.2×10^3

Known to increase the risk of storing the product packaging that is air, moisture, aroma and light permeable. It is important to judge the preparation to avoid pollution in the early stages of some raw materials.

Microbiological parameters (mesophylic aerobic and facultative anaerobic microorganisms (MAFAm), yeasts and moulds) were analysed during 6 months storage (Table 1). The total count of mesophylic aerobic and facultative anaerobic microorganisms (MAFAm), increased during storage. The lowest total mesophylic aerobic and facultative anaerobic microorganisms'count during storage is 5.2×10^4 CFU g^{-1} in *Doypack*, after 6 months. These parameters are included in within acceptable limits for total plate count of mesophylic aerobic and facultative anaerobic microorganisms are from 10^4 to 10^5 CFU g^{-1} (Nerbrink, 2007). The most rapid growth was observed for yeasts in the paper bag with windows – from 1.3×10^2 to 5.6×10^5 CFU g^{-1} after 6 months storage. Comparing the count of moulds during storage, they are from 1.1×10^2 to 5.3×10^3 CFU g^{-1}, an acceptable range is from 10^2 to 10^4 CFU g^{-1} ICMFS (International Commission on Microbiological Specifications for Foods, 2005). Microorganism increase can be attributed with possible flakes environmental pollution. Flakes, which are made from the outside of the grain layer, there has been an increased moulds contamination products. Flaking process cannot be observed all HACCP (Hazard Analyses and Critical Control Points) conditions as a result of a dry product with a reduced water activity can develop pollution and increase microorganisms.

To minimize the risk of microorganisms' multiplication, the product must be stored in containers able to maintain an inert environment for the entire storage period. Evaluating product packaging materials, the most appropriate of these is *Doypack* packaging, which is able to keep the product during storage of a positive effect on the safety of the product. The products are suitable for the storage of selected packages specified period of time, but extending the shelf-life should be evaluated in addition to the quality indicators.

Conclusions

The shelf-life of cereal muesli with seeds packaged in paper tube with aluminium layer inside and LDPE black cover or *Doypack* (stand-up pouches) Pap50g/Alu7/Pe60 for 6 months could be recommendable.

The shelf-life of cereal muesli with seeds packaged in paper bag with PP window for 4 months could be recommendable.

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References

1. Albertson A. M., Thompson D., Franko D. L., Kleinman R. E., Barton B. A., Crockett S. J. (2008)

Consumption of breakfast cereal is associated with positive health outcomes. Evidence from the National Heart, Lung, and Blood Institute Growth and Health Study. *Nutrition Research*, Vol. 28, p. 744–752.
2. Berner L. A., Clydesdale F. M., Douglass J. S. (2001) Fortification contributed greatly to vitamin and mineral intakes in the United States, *Journal of Nutrition*, Vol. 131, p. 2177–2183.
3. Bertais S., Luque M. L. P., Preziosi P., Fieux B., Torra de Flot M., Galan P. (2000) Contribution of ready-to-eat cereals to nutrition intakes in French adults and relations with corpulence. *Annals of Nutrition and Metabolism*, Vol. 44, p. 249–255.
4. Beuchat L.R. (1981) Cereal Foods World. Vol. 26, p.345–349.
5. Burrington K. (2001) Keeping the crunch in breakfast cereals. *Food Product Design*, p. 63.
6. Collins H. M., Burton R. A., Topping D. L., Liao M. L., Bacic A., Fincher G. B. (2010) Variability in fine structures of non-cellulosic cell wall polysaccharides from cereal grains; potential importance in human health and nutrition. *Cereal Chemistry*, Vol. 87, p. 272–282.
7. Coudray B. (2011) The contribution of dairy products to micronutrient intakes in France. *Journal of the American College of Nutrition*, Vol. 30, p. 410–414.
8. Delgado-Andrade C., Rufian-Henares J.A., Morales F. J. (2007) Lysine availability is diminished in commercial fibre-enriched breakfast cereals. *Food Chemistry*, Vol. 100, p. 725–731.
9. Drewnowski A. (2011) The contribution of milk and milk products to micronutrient density and affordability of the U.S. diet. *Journal of the American College of Nutrition*, Vol. 30, p. 422–428.
10. Galic K., Curic D., Gabric D. (2009) Shelf-life of packaged bakery goods. *A review Food Science and Nutrition*. Vol.49, p. 405–426.
11. Gibson S. A., Gunn P. (2011) What's for breakfast? Nutritional implications of breakfast habits. Insights from the NDNS dietary records. *Nutrition Bulletin*, Vol. 36, p. 78–86.
12. ICMFS (International Commission on Microbiological Specifications for Foods) (2005) Cereals and cereal products. Micro-Organisms in Foods 6; Microbial Ecology of Food Commodities. Kluwer Academic & Plenum Publishers, New York, p. 392–439.
13. Hannon E. M., Kiely M., Flynn A. (2007) The impact of voluntary fortification of foods on micronutrient intakes in Irish adults. *British Journal of Nutrition*, Vol. 97, p. 1177–1186.
14. John M. Krochta (2006) *Handbook of Food Engineering 2rd ed.* p. 847–927.
15. Nerbrink E. (2007) Guidelines for Product safety; Analyzes and maximum levels. Version 6.
16. Labuza T.P. (1980) The effect of water activity on reaction kinetics of food deterioration. *Food Technology* Vol. 34, p. 36–41.
17. Laca, A., Mousia, Z., Diaz, M., Webb, C., Pandiella, S.S. (2006). Distribution of microbial contamination within cereal grains. Vol. 72, p. 332–338.
18. La Storia A. D., Mauriello P. G., Musso S. S. (2008). Development and application of antimicrobial food packaging. **In**: *TesisiDottorato di Ricerca in Scienze e TecnologiedelleProduzioni Agro-Alimentari XX ciclo*, Italy, Naples, p. 1–113.
19. Louie J. C. Y., Dunford E. K., Walker K. Z., Gill T. P. (2012) Nutritional quality of Australian breakfast cereals. Are they improving? *Appetite*, Vol. 59, p 464–470.
20. Macedo I. S. M., Sousa-Gallagher M. J., Byrne E. P. (2011) Moisture sorption isotherms of Granola breakfast product. **In**: *40th annual UCC food research conference*, Ireland, Cork, p. 50.
21. Macedo I. S. M., Sousa-Gallagher M. J., Oliveira J.C., Byrne E. P. (2013) Quality by design for packaging of granola breakfast product **In**: *Food control*. Vol. 29, p. 438–443.
22. Pathare P. B., Bas N., Byrne E. P.(2012) Comparison of quality parameters of granola produced by wet granulation with commercially available product. *Food and Bioproducts Processing*, Vol. 90, p. 729–736.
23. Rada-Mendoza M., Sanz M. L., Olano A., Villamiel M. (2004) Study on nonenzymatic browning in cookies, crackers and breakfast cereals by maltulose and furosine determination. *Food Chemistry*, Vol. 85, p. 605–609.
24. Rampersaud G. C., Pereira M. A., Girard B. L., Adams J., Metzl J. D. (2005) Breakfast habits, nutritional status, body weight, and academic performance in children and adolescents. *Journal of the American Dietetic Association*, Vol. 105, p.743–760.
25. Rufian-Henares J. A., Delgado-Andrade C., Morales F. J. (2006) Analysis of heat-damage indices in breakfast cereals: Influence of composition. *Journal of Cereal Science*, Vol. 43, p. 63–69.
26. Vissers P. A., Streppel M. T., Feskens E. J., de Groot L. C. (2011) The contribution of dairy products to micronutrient intake in the Netherlands. *Journal of the American College of Nutrition*, Vol. 30, p. 415–421.
27. Williams P. (2007). Breakfast and the diets of Australian children and adolescents. An analysis of data from the 1995 National Nutrition Survey. *International Journal of Food Sciences and Nutrition*, Vol. 58, p. 201–216.
28. Williamson C. S. (2010) Breakfast cereals. Why all the bad press? *Nutrition Bulletin*, Vol. 35, p. 30–33.
29. Wilson N. C., Parnell W. R., Wohlers M., Shirley P. M. (2006) Eating breakfast and its impact on children's daily diet. *Nutrition and Dietetics*, Vol. 63, p. 15–20.
30. *Prasības pārtikas kvalitātes shēmām, to ieviešanas, darbības, uzraudzības un kontroles kārtība* [accessed on 20.04.2014.]. Available at: http://m.likumi.lv/doc.php?id=180014

APPLICATION OF ENZYMATIC TREATMENT TO IMPROVE THE CONCENTRATION OF BIOACTIVE COMPOUNDS AND ANTIOXIDANT POTENTIAL OF WHEAT AND RYE BRAN

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Abstract

The present study was undertaken to establish the effect of enzymatic treatment on the content of total phenolic compounds and antioxidant activity in enzymatically treated bran. Enzymatic hydrolysis of bran was carried out by α-amylase from *Bacillus amyloliquefaciens* (Sigma Aldrich) for breakdown the bonds between glucose monomers in starch. Multi enzyme complex (Viscozyme L) containing a wide range of carbohydrases were used for depolymerisation of cellulose and hemicelluloses molecules. The 80% ethanol was used to extract the antioxidant compounds from bran. Free radical scavenging activity of samples was measured using 2.2-diphenyl-1-picrylhydrazyl (DPPH). Assay and the data were expressed in Trolox equivalents (TE) per 100 g^{-1} of sample, as well the reducing power was determined using ferric reducing antioxidant power (FRAP) assay and the data were expressed in the same indices. The obtained results showed that the enzymatically treated bran samples had the highest concentration of total phenolic compounds, on the other hand the enzymatically treated bran showed higher antioxidant potential than non-enzymatically treated bran samples. Extract from enzymatically treated rye bran had the highest concentration of phenolic compounds, 1230±42.57 mg GAE 100 g^{-1} DW. The lowest concentration of phenolic compounds was found in untreated wheat bran samples and this amount was equal to 377±9.78 mg GAE 100 g^{-1} DW. Two different methods of evaluation of the bran antioxidant activity showed potential usefulness of enzymatic treatment.

***Keywords*:** phenolic compounds, antioxidant activity, bran, enzymatic hydrolysis.

Introduction

Bran of wheat and rye is a by-product from the milling process of flour and is a composite material formed from different histological layers, and three different strips. The outer strip corresponds to outer pericarp (epidermis and hypodermis), the inner one corresponds to the aleurone layers, and the intermediate one remains a composite of several tissues (inner pericarp, testa, and nuclear tissue (Hemery et al., 2010). Phenolic compounds derived from whole grain as well as from grain fractions have health-promoting effect. In the plant kingdom, phenolic compounds are essential molecules against oxidative damage, as they have UV-absorption properties and radical-scavenging activities. Therefore, the majority of the phenolic compounds are located in the most external tissues of the plant (Liu et al., 1995). In wheat grain, most of the phenolic compounds are located in the bran, which constitutes the outermost parts of the grain. One of the milling functions is cereal gain dehulling and debranning with the aim to obtain the white flour without any impurities.

Cereal grains and especially outer parts of the cereal are good source of phenolic compounds. In cereal grains located considerable amount of bioactive compounds e.g. phenolic acids, saponins, while flavonoids and phytoestrogens are presented in small quantities (Dordević et al., 2010). Scientific works imply that phenolic compounds have relatively high antioxidant activity, which may promote to their health benefits. The most predominant phenolic compound in cereals is ferulic acid, which forms up to 90% of total polyphenols. Other phenolic acids like p-coumaric, m-coumaric, syringic and vanilic acid have also been reported in cereals (Hosseinian, Mazza 2009).

Grain as well as bran chemical composition including phenolic compounds mostly depends from grain genetic / agricultural backgrounds, growing conditions and storage. On the other hand strong effect on chemical composition renders the milling procedure (Adom et al., 2005).

Liyana-Pathirana and Shahidi (2006) reported that the contribution of bound phenolics to the total phenolic content in wheat was significantly higher than free and esterified fractions, and the bound phenolic fraction demonstrated a significantly higher antioxidant capacity than free and esterified phenolics. Li et al. (2010) have reported that the phenolic compounds in cereals were mostly found in three forms: insoluble (66–80%), soluble conjugated (17–30%) and free phenolics (6%). The covalently bound ferulic acids during the fermentation of wheat bran fiber in a human model colon were released (Kroon et al., 1997). Although the solvent extraction is the major method to extract bioactive compounds from plant materials, or to obtain plant extracts rich in bioactive compounds. In the world science there have been several contentious moments e.g. after the extraction of bioactive compounds by using different types of solvents the components have low recovery and strict regulations for the use of these kind of products in the food industry. Enzymatic hydrolysis is one of the extraction techniques without any organic solvents and toxic chemicals that gave positive result and advantages among other conventional procedures. Main mechanisms of enzymatic hydrolysis are convert water-insoluble components into water soluble materials (Athukorala et al., 2006). For example, Heo et al. (2005) reported that enzymatic hydrolysis of brown seaweeds gained high bioactive compound yield and showed enhanced biological activity compared

with water and organic extract counterparts. Alrahmany et al. (2013) was reported that the enzymatic hydrolysis of oat bran give the possibility to increase the concentration of total phenolic acids upon treatment with carbohydrases. The purpose of this study was to investigate the content of total phenolic compounds and antioxidant activity in enzymatically treated bran in order to evaluate the effect of enzymatic treatment on these properties.

Materials and Methods

Experiments were done at the Latvia State Institute of Fruit – Growing collaboration with Riga Technical University.

Chemicals

Ethanol (96%) was received from SIA Jaunpagasts Plus (Company Jaunpagasts Plus Ltd., Latvia). Methanol, ethanol, ethyl acetate, asodium chloride (NaCl), hydrochloric acid (HCl), Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (Steinheim, Germany).

Bran samples

Summer wheat (*Triticum aestivum*) and rye *(Secale cereale)* bran samples were collected from industrial mills in Latvia:

1) SC Dobeles dzirnavnieks – small particle size wheat bran (WSSD);
2) SC Dobeles dzirnavnieks – wheat bran with large particle size bran (WLSD);
3) SC Rigas dzirnavnieks – large particle size wheat bran (WLSR);
4) SC Jelgavas dzirnavnieks – small particle size rye bran (RSSJ).

Enzymes

Industrial enzymes preparations produced by "Novozyme Corporation" (Bagsvaerd, Denmark) and purchased from Sigma-Aldrich. Two commercial preparations of enzymes: α-amylase from *Bacillus amyloliquefaciens* and Viscozyme L from *Aspergillus spp.*, were used to hydrolyze carbohydrates. α-amylase has a declared activity ≥250 units g^{-1}, optimum conditions of enzymatic pretreatment is pH 5.0–8.0, temperature 55±1 °C and incubation time 0.5 h (Demirkan et al., 2004) form Viscozyme L declared activity is 100 FBG g^{-1}, optimum conditions are pH 4.6, temperature 44±1 °C and incubation time 3.2 h. In this scientific work enzymes were tested both independently and in combination for establishing the synergetic interaction

Enzymatic Hydrolysis

For α-amylases treatments, wheat bran (10 g) was mixed with 90 mL of distillated water in 1000 mL Reagent bottle with screw cap with dilutions 1 : 9, and then 500 µL of α-amylase was added. Hydrolysis was carried out in a water bath at temperature 55±1 °C, incubation time 0.5 h and shaking intensity 60 rpm. After starch hydrolysis and enzyme inactivation (10 min temperature 100±1 °C) wheat bran mash was 3 minutes homogenized, the pH of the suspension was adjusted to pH 4.6 with 0.2 mL 50% citric acid in each dilutes and Viscozyme L 400 µL was added. Incubation time is 3.2 h, temperature 44±1 °C, and shaking intensity 60 min^{-1}.

Extraction of Phenolic acids

The free phenolic acids were isolated using the procedure explained by Wang et al. (2006) with slight modification, and soluble conjugated phenolic compounds were isolated using procedure described by Robbins (2003) and is depicted Figure 1.

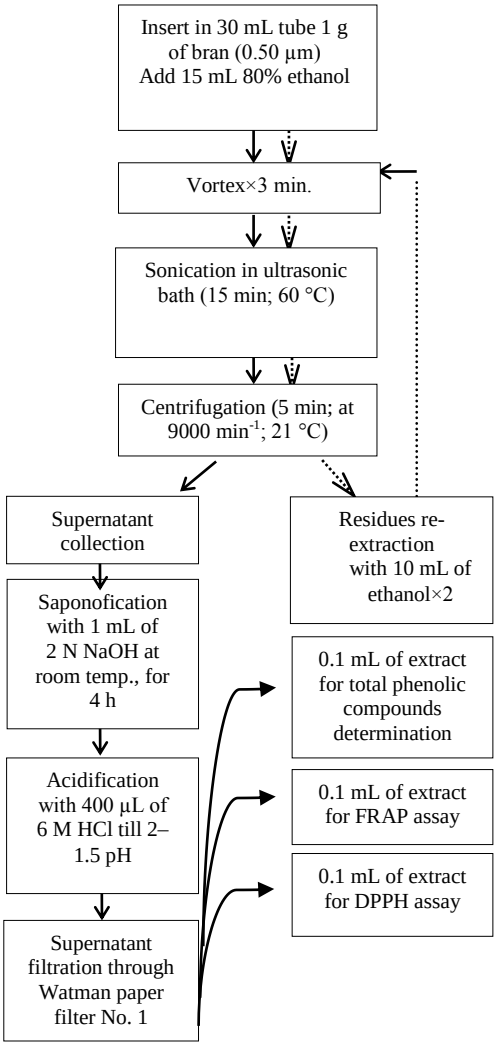

Figure 1. Flow diagram of extraction procedure of phenolic compounds

After free phenolic compounds extraction (×3 times) with 80% ethanol, after supernatant collection and the alkaline hydrolysis with 2 N NaOH was done. Alkaline hydrolysis was carried out at room temperature for 4 h, and after the hydrolysis the alkaline extract was neutralised with 6 M HCl. For elimination of precipitation the filtration through Watman paper filter No. 1 was done. The antioxidant activity and total phenolic compounds was immediately analyzed.

Determination of total phenolic compounds

Determination of total antioxidants reducing capacity by using Folin-Ciocalteu reagent was carried out as described by Sacchetti et al. (2009) with minor modifications. To volumetric flasks (10 mL) were pipette 100 μL of analyzed sample, 5 mL of deionized water and 0.5 mL of Folin reagent. After 3 minutes was added 1 mL of saturated sodium carbonate solution and supplemented with dionized water to 10 mL. The mixture was incubated for one hour at 23±2 °C, under dark, and then measured the absorption on wavelength λ=765 nm. The results obtained were expressed as mg gallic acid equivalent (GAE) per g dry weight (DW).

Free Radical Scavenging Activity

Free radical scavenging activity of samples was measured using the 2.2-difenyl-1-picrylhydrazyl (DPPH) according to the procedures described by Yen, Chen (1995) with slight modification. The extracts (100 μL) were reacted with 2.9 mL of DPPH solution (0.0039 g DPPH in 100 mL methanol). Absorbance of the cereal extracts was determined using UV – Visible Spectrophotometer SHIMADZU at 515 nm. Free radical scavenging activity of the samples was expressed as mg Trolox equivalent antioxidant capacity per 100 g^{-1} dry weight (mg TEAC g DW).

Free reducing antioxidant power (FRAP)

Free reducing antioxidant power (FRAP) was determined by its ability to reduce ferric to ferrous ions. When iron is complexed with 2, 4, 6-tripyridyl-s-trizine (TPTZ) in sodium acetate solution at an acidic pH, its reduction results in a color change of the solution, from pale rust to blue. The absorbance of the solution at 593 nm reflects the extent of reduction. The reduction power was expressed as mg Trolox equivalent. The extracts (100 μL) were reacted with 3.6 mL FRAP reagent and after vortex the absorption was spectrophotometrically detected.

Statistical analysis

Data was processed by SPSS software version 17.0. Data was analysed using descriptive statistics and processed by one-way analysis of variance ANOVA (one way ANOVA), as well as for comparing all bran samples depending from pre-treatment were used (two way ANOVA). Microsoft office software version 2007 was used to determine significant differences between the samples.

Results and Discussion

The plant cell wall is a complex design of polysaccharides. For the complete hydrolysis of these polysaccharides, a battery of enzymes is needed. By specific enzymatic treatments, cell wall polymer properties can be altered which can be utilised in food processing. Many plant cell walls contain phenolic acids residues that are ester-linked to the polysaccharide network. In grasses and cereals, these phenolic compounds (hydroxycinnamic acids) are mainly found esterified to arabinoxylans (5-*O*-feruloyl group). In decotyledons, such as spinach and sugar beet, ferulic acid is esterified to *O*-2 or *O*-3 position of arabinose and to *O*-6 position of galactose residues in pectin (Ralet et al., 1994; Fry, 1982).

The main activity of the Viscozyme L enzyme preparation is endo- (EK 3.2.1.4), exo-xylanases (EK 3.2.1.74), endo- (EC 3.2.1.4), exo- glucanase (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21). Endo-glucanase and exo- glucanase are known to act synergistically in cellulose hydrolysis (Wood, McRae, 1978) while β-glucosidase is needed for hydrolysing cellobiose (Woodward, Wiseman, 1982). Feruloyl esterase (FAE; E.C. 3.1.1.72), are sometimes called hemicellulase accessory enzymes, subclass of the carboxylic acid esterases (E.C. 3.1.1.1). They splits the ester bond between the glycosides and hydroxycinnamic acids, which are predented in the plant cell wall (Williamson et al., 1998). The esterases act to enable and facilitate the access of glycosyl hydrolases to the backbone wall polymers. Most feruloyl esterases act synergistically with xylanases, cellulases or pectinases to breakdown complex plant cell wall carbohydrates (Faulds, Williamson, 1995; Kroon, Williamson, 1996). Investigation into the effect of enzymatic treatments, on the content of bioactive compounds of the wheat and rye bran, revealed that treatments had a significant effect on the content of phenolic compounds, anthocyanins, as well as antioxidant potential. The influence of enzymatic treatments on the chemical compositions of the wheat and rye bran products indicated that the use of enzymes yielded a higher concentration of bioactive compound, than the untreated bran (Figure 2).

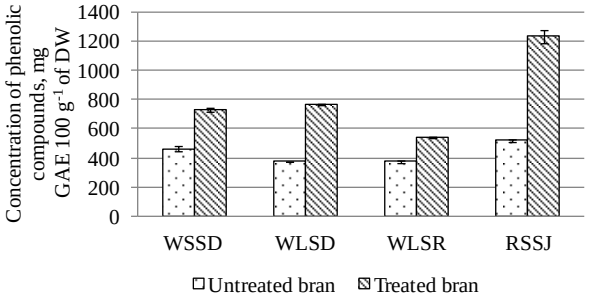

Figure 2. Concentration of total phenolic compounds in wheat and rye bran

The highest concentration of total phenolic compounds were found in enzymatically treated rye bran sample (RSSJ), and this amount was equal to 1230±42.57 mg GAE 100 g^{-1} DW, followed by (WLSD) – 765.2±1.65 mg GAE 100 g^{-1} DW, (WSSD) – 730.8±13.05 mg GAE 100 g^{-1} DW, and (WLSR) – 541.9±3.41 mg GAE 100 g^{-1} DW respectively. While the concentration of total phenolic compounds in untreated rye bran sample (RSSJ) were 520.7±7.17 mg GAE 100 g^{-1} DW, followed by (WSSD), (WLSD), (WLSR), 461.3±17.16, 377.4±0.41, 377.0±9.78 mg GAE 100 g^{-1} DW, respectively. The similar data was reported by Sungsopha (2009), which implies that after enzymatic treatments of rice bran the total phenolic compounds was increased by

476%, from 223.16 mg GAE 100 g^{-1} DW to 836.21 mg GAE 100 g^{-1} DW, and after author was made some conclusions, that it's due to the effect that of enzymatic hydrolysis, liberates and frees phenolic components and increases the level of total phenolic compounds. Khoddami et al. (2013) was reported that bran treated with carbohydrases is significantly higher compared to untreated bran. Highest increase in vanillic and caffeic acids relative to the untreated bran was achieved by cellulase (3.7-fold) and Viscozyme (4.4-fold). The total content of free and bound phenolic acids was 668.5 μg g^{-1} for untreated oat bran and after enzymatic treatments with Viscozyme L this amount has increased to 1116.0 μg g^{-1}.

Microstructure of bran

Wheat and rye bran is a multilayered composite, comprising a range of tissues, including the pericarp (epidermis, hypodermis, cross and tube cells) with the attached seed coat, the nucellar epidermis, the aleurone layer, and remnants of the starchy endosperm. All of these tissues are dietary fiber with very low bioactive compounds and antioxidant bioaccessibility and bioavailability. Research concerning the bioaccessibility of phenolic compounds and other antioxidants from solid matrices are important, since only the compounds released from the food matrix and/or absorbed in the small intestine are potentially bioavailable and in a condition to exert their beneficial effects (Tagliazucchi et al., 2009). Phenolic compounds bound to dietary fiber need to be hydrolyzed by specific enzymes in the upper area of the intestine; otherwise, these compounds will not be bioaccessible for absorption in the human intestine but will be susceptible to degradation by the colonic microflora in the large intestine (Perez et al., 2009). Our study imply that the using of enzymes gives the possibilities release the bound form of phenolic compounds which increase the bioavailability of these material.

Microscopy of the bran samples showed that degradation of cell walls was initiated in the pericarp layer (Figure 3B), as well in the starch/protein matrix (Figure 3C).

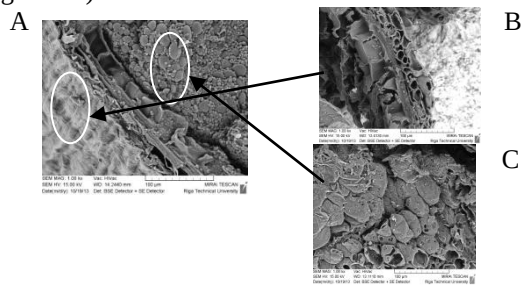

Figure 3. Microstruture of bran obtained by SEM
A– untreated wheat bran; B–enzymatically treated wheat bran (pericarp degradation), C–enzymatically treated wheat bran (starch/protein matrix degradation)

During the enzymatic hydrolysis the degradation of cell walls were observed by scanning electron microscope (Figure 3). Enzyme aided hydrolysation had large effects on wheat and rye bran characteristics, and partial hydrolysis of cell wall components was reflected in altered bran microstructure.

Free Radical Scavenging Activity

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reductions capability of DPPH is determined by the decrease in its absorbance induced by antioxidant. In this study, the total antioxidant capacity of wheat and rye bran treated by using enzymes significantly increased ($p<0.05$) the number of equivalents of all the antioxidant standards. Analyzing data of free radical scavenging activity was detected only one exception for WLSR bran sample. After enzymatic treatment antioxidant capacity of this sample decreased from 18.35±0.70 to 15.84±0.59 mmol Trolox 100 g^{-1} (Figure 4). It was useful to know that Folin-Ciocalteau assay give the possibility estimate not only phenolic compounds but also amino acids, carbohydrates, ascorbic acid, and other components which may increase the antioxidant activity of the samples. How it was reported in Kim and Wampler work that determination of total phenolic compounds by chemical method Folin-Ciocalteau assay gives the higher value than using instrumental HPLC method. There was reported that two methods are different, with different advantages and disadvantages (Kim, Wampler, 2011). Another author was reported that Folin-Ciocalteau assay gives a crude estimate of the total phenolic compounds present in an extract, whereas the free radical scavenging assay is not only specific to polyphenols (Prior et al., 2005). Our other work which at the moment not published suggest that during the extraction using different types of solvents as well as different techniques give opportunity extract from plant materials not only phenolic compounds but also some another components, which can interact with Folin-Ciocalteau reagent and simultaneously providing incorrect results. The highest scavenging effect of bran extracts on DPPH radical was observed in enzymatically treated bran (Figure 4).

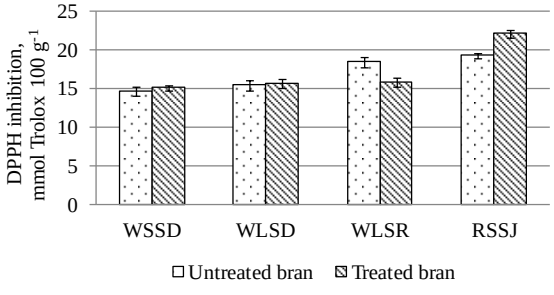

Figure 4. Free radical scavenging activity of bran extracts expressed as mg Trolox equivalent antioxidant capacity per 100 g^{-1} of dry weight

The highest scavenging effect was recorded in RSSJ bran sample (22.03±0.49 mmol Trolox 100 g^{-1}) followed by WLSR (15.84±0.59 mmol Trolox 100 g^{-1}), WLSD (15.63±0.53 mmol Trolox 100 g^{-1}), and WSSD

(15.06±0.32 mmol Trolox 100 g^{-1}). The Sungsopha was reported similar results obtained during enzymatic hydrolysis of rice bran. After enzymatic treatments the antioxidant capacity was significantly increased (Sungsopha et al., 2009).

The antioxidant power of bran extracts was evaluated by FRAP assay in Figure 5.

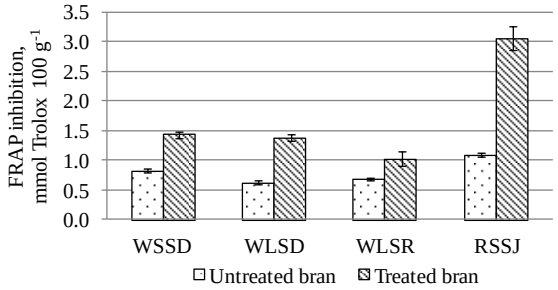

Figure 5. Free reducing antioxidant power of bran extracts expressed as mg Trolox equivalent antioxidant capacity per 100 g^{-1} of dry weight (mmol Trolox 100 g^{-1} DW)

Our obtained results showed that enzymatically treated rye bran has a greater ability of antioxidant properties than other bran samples RSSJ (3.05±0.20 mmol Trolox 100 g^{-1} DW), followed by WSSD (1.43±0.06 mmol Trolox 100 g^{-1} DW), WLSR (1.03±0.12 mmol Trolox 100 g^{-1} DW). Comparing the data it's possible to conclude that the antioxidant power of enzymatically treated bran compared to untreated was increased by 2.8 fold for RSSJ, 2.2 fold for WLSD, 1.7 folds for WSSD and 1.5 fold for WLSR respectively. On the other hand the significantly positive correlation was observed using the FRAP assay (R^2=0.970). Literature data from McCarthy and other authors shows that they got similar significantly strong correlation between total phenolic compounds and FRAP activity (McCarthy et al., 2012).

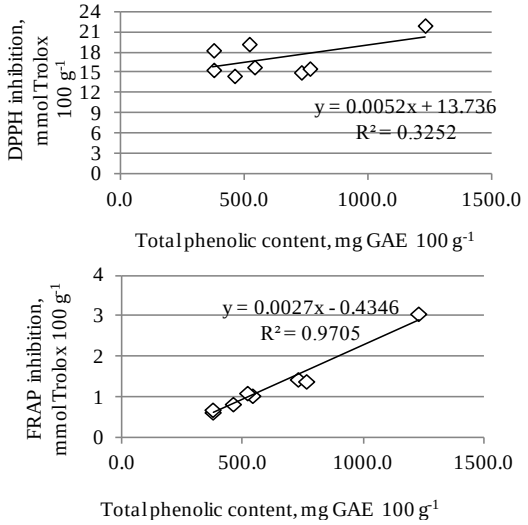

Figure 6. Correlation graphs for DPPH (A) and FRAP (B) mmol Trolox 100 g^{-1} values and total phenolic contents

In the present study, correlation graphs were plotted between IC50 values (including of DPPH, FRAP) and total phenolic contents. Two typical correlation graphs (*i.e.*, DPPH *vs* total phenolic, FRAP *vs* total phenolic) are shown in Figure 6.

Several studies have attempted to correlate the DPPH scavenging activity of molecules to total phenolic content and to individual phenolic acids. In that respect Li, Wu, and Huang (2009) found that there was no direct correlation between DPPH inhibitory activity and total phenols, ferulic or caffeic acid contents of Radix angelicae sinensis, although they reported a correlation with 1/IC50 values (Li et al., 2009).

Another authors Gamel, Abdel-Aal (2012) were reported that total phenolic contents of barley samples correlated with DPPH inhibitory capacity. Verardo et al. (2011) found a strong correlation (R^2=0.93) of DPPH result of five oat cultivars to total free phenolic compounds. In this study the correlation (R^2=0.325) between DPPH and total phenolic compounds for the enzymatically treated wheat and rye bran is much weaker. The obtained results are incomparable due to fact that during the extraction of phenolic compounds was used different extraction techniques, which can effect on the extraction capacity.

Conclusions

This study suggest, that enzymatic treatment of wheat and rye bran are effective methods to improve the concentration of bioactive compounds and antioxidant activity. Therefore, these bran products may be exploited as a potent source of bioactive compounds and antioxidants, for nutraceutical and functional food products.

References

1. Adom K. K., Sorrells M. E., Rui H. L. (2005) Phytochemicals and antioxidant activity of milled fractions different wheat varieties. *Journal of Agricultural and Food Chemistry*, No. 53, p. 2297–2306.
2. Alrahmany R., Avis T. J., Tsopmo A. (2013) Treatment of oat bran with carbohydrases increases soluble phenolic acid content and influences antioxidant and antimicrobial activities. *Food Research International*, No. 52, p. 568–574
3. Athukorala Y., Kim K. N., Jeon Y. J. (2006) Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga *Ecklonia cava*. *Food and Chemical Toxicology*, No. 44, p. 1065–1074.
4. Demirkan E. S., Mikami B., Adachi M., Higasa T., Utsumi S. (2004) α- Amylase from *B. Amyloliquefaciens*: purification, characterization, raw starch degradation and expression in *E. coli*. *Process Biochemistry*, No. 40, p. 2629–2636.
5. Dordević T. M., Siler-Marinkovis S. S., Dimitrijevic-Brankovic S. I. (2010) Effect of fermentation on antioxidant properties of some cereals and pseudo cereals. *Food Chemistry*, No. 119, p. 957–963.
6. Faulds C. B., Williamson G. (1995) Release of ferulic acid from wheat bran by a ferulic acid esterase (FAE-III) from *Aspergillus niger*. *Applied Microbiology and Biotechnology*, No. 43, p. 1082–1087.

7. Fry S. C. (1982) Phenolic components of the primary cell wall: feruloylated disaccharide of D-galactose and L-arabinose from spinach polysaccharide. *Biochemical Journal*, No. 203, p. 493–504.
8. Gamel T. H., Abdel-Aal E. M. (2012) Phenolic acids and antioxidant properties of barley wholegrain and pearling fractions. *Agricultural and Food Science*, No. 21, p. 118–131.
9. Hemery M., Mabille F., Milena R., Rouau M. X. (2010) Influence of water content and negative temperatures on the mechanical properties of wheat bran and its constitutive layers. *Journal of Food Engineering*, No. 98, p. 360–369.
10. Heo S. J., Park E. J, Lee K. W., Jeon Y. J. (2005) Antioxidant activities of enzymatic extracts from brown seaweeds, *Bioresource Technology*, No. 96, p. 1613–1623.
11. Hosseinian F. S., Mazza G. (2009) *Triticale* bran and straw: Potential new sources of phenolic acids, proanthocyanidins, and lignans. *Journal of Functional Foods*, No.1, p. 57–64.
12. Khoddami A., Wilkes M. A., Roberts T. H. (2013) Techniques for Analysis of Plant Phenolic Compounds. *Molecules*, No. 18, p. 2328–2375.
13. Kim Y., Wampler D. J. (2011) Total polyphenol content in ready to drink teas determined by the Folin-Ciocalteu method and HPLC analysis. *Sensus Technical Note*, No. 0034, p. 1–3.
14. Kroon A. P. Williamson G. (1996) Release of ferulic acid from sugar-beet pulp by using arabinase, arabinofuranosidase and esterase from *Aspergillus niger*. *Biotechnology and Applied Biochemistry*, No. 23, p. 263–267.
15. Kroon P. A., Faulds C. B., Ryden P., Robertson J. A., Williamson G. (1997) Release of covalently bound ferulic acid from fiber in the human colon. *Journal of Agricultural and Food Chemistry*, No. 45, p. 661–667.
16. Li L., Harflet C., Beale M. H., Ward L. L. (2010) Phenolic acids. **In**: *Healthgrain Methods. Analysis of bioactive components in small grain cereals.* P. R. Shewry, J. L. Ward (eds). St.Paul Minesota USA: AACC International, p. 41–52.
17. Li X., Wu X., Huang L. (2009) Correlation between antioxidant activities and phenolic contents of Radix angelicae sinensis (Danggui). *Molecules*, No. 14, p. 5349–5361.
18. Liu L., Gitz D. C., McClure J. W. (1995) Effects of UV-B on flavonoids, ferulic acid, growth and photosynthesis in barley primary leaves. *Plant Physiology*, No. 93, p. 725–733.
19. McCarthy A. L., O'Callaghan Y. C., Connolly A., Piggott C. O., FitzGerald R. J., O'Brien N. M. (2012) Phenolic extracts of brewers' spent grain (BSG) as functional ingredients – Assessment of their DNA protective effect against oxidant-induced DNA single strand breaks in U937 cells. *Food Chemistry*, No. 134, p. 641–646.
20. Perez J., Serrano J., Tabernero M., Arranz S., Dıaz M.E., Garcıa L., Goni I., Saura F. (2009) Bioavailability of phenolic antioxidants associated with dietary fiber: plasma antioxidant capacity after acute and long-term intake in humans. *Plant Foods for Human Nutrition*, No. 64, p. 102–107.
21. Pathirana L.C.M., Shahidi F. (2006) Importance of insoluble-bound phenolics to antioxidant properties of wheat. *Jornal of. Agricultural and Food Chemistry*, No. 54, p. 1256–1264.
22. Prior R.L., Wu X. Schaich K. (2005) Standardised methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, No. 53, p. 4290–4302.
23. Ralet M. C., Faulds C. B., Williamson G., Thibaults J. F. (1994) *Carbohydrate Research*, No. 263, p. 257-269.
24. Robbins R. J. (2003) Phenolic acids in foods: an overview of analytical methodology. *Journal of Agrucultural and Food Chemistry*, No. 51, p. 2866–2887.
25. Sacchetti G., Mattia C. D., Pittia P. D. (2009) Effect of roasting degree, equivalent thermal effect and coffee type on the radical scavenging activity of coffee brews and their phenolic fraction. *Journal of Food Engineering*, No. 90, p. 74–80.
26. Sungsopha J., Moongngarm A., Kanesakoo R. (2009) Application of Germination and Enzymatic Treatment to Improve the Concentration of Bioactive Compounds and Antioxidant Activity of Rice Bran. *Australian Journal of Basic and Applied Science*s, No. 3, p. 3653–3662.
27. Tagliazucchi D., Verzelloni E., Bertolini D., Conte A. (2009) In vitro bio-accessibility and antiox-idant activity of grape polyphenols. *Food Chemistry*, No. 120, p. 599–606.
28. Verardo V., Serea C., Segal R., Caboni M. F. (2011) Free and bound minor polar compounds in oats: Different extraction methods and analytical determinations. *Journal of Cereal Science*, No. 54, p. 211–217.
29. Wang J., Yuan X., Sun B., Tian Y., Cao Y. (2006) Scavenging Activity of Enzymatic Hydrolysates from Wheat Bran. *Food Technology and Biotechnology*, No. 47, p. 39–46.
30. Williamson G., Kroon P. A., Faulds C. B. (1998) Hairy plant polysaccharides: a close shave with microbial esterases. *Microbiology*, No. 144, p. 2011–2023.
31. Wood T. M., McCrae S. I. (1978) The cellulase of *Trichoderma koningii*. Purification and properties of some endoglucanase components with special reference to their action on cellulose when acting alone and in synergism with the cellobiohydrolase. *Biochemical Journal*, No. 171, p. 6–72.
32. Woodward J., Wiseman A. (1982) Fungal and other *β*-d-glucosidases-their properties and applications. *Enzyme and Microbial Technology*, No. 4, p. 7–79.
33. Yen G. C., Chen H. Y. (1995) Antioxidant activity of various tea extracts in relation to their Antimut agenicity. *Journal of Agricultural and Food Chemistry*, No. 43, p. 27–32.

INVESTIGATION OF TOTAL DIETARY FIBER, B_1 AND B_2 VITAMIN CONTENT OF FLOUR BLEND FOR PASTA PRODUCTION

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Abstract

The main purpose of the current research was to investigate total dietary fiber and vitamin B_1 and B_2 content in flour blends made from several types' whole grain flour for pasta production. In 2012 harvested convectional rye 'Kaupo', wheat 'Zentos', hull-less barley line 'PR 5099' and triticale line '9405-23' grain and, for flour blends obtaining, wheat flour type 550 was used in the experiments. Following quality parameters of flours using standard methods was evaluated: total dietary fiber content (ISO 5498) and vitamins B_1 and B_2 – by AOAC 986.27 and 970.65 respectively. In the present research significant differences in total dietary fiber of analysed whole grain rye, hull-less barley, triticale and wheat flour samples were established. Higher dietary fiber content was found in whole grain hull-less barley and rye flour, the lowest in the wheat flour type 550. Lower dietary fiber content was obtained for whole wheat and triticale flour blends. However, the higher, for whole rye and hull-less barley flour blend. B_1 vitamin content in whole grain rye and wheat flour was significantly higher than in hull-less barley and triticale. Lowest vitamin B_2 content was obtained in wheat flour type 550. Lower B_1 vitamin content, as 2.70±0.14 mg kg^{-1} was obtained for whole hull-less barley flour blend, the higher as 3.19±0.28 mg kg^{-1} for whole wheat flour blend. Lower B_2 vitamin content, as 0.91±0.06 mg kg^{-1}, was obtained for whole hull-less barley flour blend, however, the higher B_2, as 0.81±0.28 mg kg^{-1} in whole wheat flour blend.

Keywords: grain, vitamins, dietary fibre, pasta, flour blends.

Introduction

Cereals and their products constitute an important part of the human diet, providing a high proportion of carbohydrates, proteins, fats, dietary fibres, B group vitamins and minerals (Okarter et al., 2010).

Whole grains include cereal grains that consist of the intact, or ground, cracked or flaked fruit of the grains whose principal components are present in the same proportions as they exist in the intact grain containing a higher fiber, minerals and antioxidants (Slavin, 2004; Franz et al., 2006; Liu, 2007). Consumption of whole grain foods has been associated with decreased risk of cardiovascular disease and certain cancers, favourable effects on blood lipids and glucose, improved insulin resistance, and higher intakes of dietary fiber and micronutrients (McKeown et al., 2002). However, an understanding of optimal whole grain consumption levels, as well as the mechanisms by which whole grain foods exert their favourable effect's is unclear (Lang et al., 2003; Slavin, 2003). Health benefits are associated with an increased intake of dietary fiber, including a reduced risk of coronary heart disease, diabetes, obesity, and some forms of cancer, as well as a reduction in cholesterol and fat (Anderson et al., 2009a; Brownlee, 2011).

Dietary fiber has an outstanding application as one of the key nutritional factors in the frame of a healthy diet. Dietary fiber remains a nutritional concept and comprises the edible part that escapes digestion in the small intestine and passes into the large intestine intact (Anderson et al., 2009a,b; Brownlee, 2011). Dietary fiber consists of non-starch polysaccharides such as arabinoxylans, cellulose, and many other plant components such as resistant starch, resistant dextrins, inulin, lignin, waxes, chitins, pectins, β-glucans, and oligosaccharides (Gaoshuang et al., 2012).

Thiamine is known to occur in the outer integuments and germ of cereal grain. In wheat grain, the endosperm represents 80–85% of grains dry mass (DM), but contains only 3% of the total thiamine. The highest proportion (80%) of thiamine is found in the external layers of wheat grain, but these are missing in white flour (Batifloulier et al., 2006). In the scientific literature are mentioned study reports on the thiamine and riboflavin contents of some Finnish cereal products including rye flour, crispbreads, and breakfast cereals, as well as their contents in some imported crispbreads and breakfast cereals sold in Finland. The mean thiamine contents of Finnish rye meal and flour (ash content 0.9–1.2%) were 0.28±0.01 and 0.27±0.04 mg 100 g^{-1} fresh weight (FW), and their respective riboflavin contents were 0.10±0.01 and 0.08±0.02 mg 100 g^{-1} FW. The thiamine contents of domestic crispbreads were 0.27±0.03 mg 100 g^{-1} FW, and the riboflavin contents were 0.16±0.05 mg 100 g^{-1} FW, which is significantly higher than levels in imported products. The thiamine contents of muesli and other breakfast cereals averaged 0.29±0.07 and 0.40±0.15 mg 100 g^{-1} FW, respectively, and their riboflavin contents averaged 0.19±0.23 and 0.21±0.50 mg 100 g^{-1} FW, respectively (Hägg et al., 1993).

Whole wheat flour, one of the most common and important whole grains, retains wheat bran and germ and acts as a rich source of dietary fibre, vitamins, minerals and antioxidants (Wang et al., 2014). Rye is second to wheat, the most commonly used grain in the food. Among the grain, rye is the only one with a wholegrain culture, and the consumption of rye should be increased in light of this benefit. In general, rye could be exploited more efficiently in new types of cereal products due to its positive health effects. Nowadays, its use is limited mainly as a result of the problems arising from its flavour; not all European consumers are familiar with the somewhat foreign, rye-like flavour, perceived as bitter and intense.

However, rye consumption in Europe might increase if ingredients were produced with the specific rye-like flavour modified to a slightly milder one, without significantly decreasing the contents of fibre and bioactive compounds in the rye. In general, volatile compounds that influence the cereal flavour have been extensively studied, but the sensory perception and, in particular, the methods of modifying the flavour of rye are not well known. With regard to the different flavour of the fractions of a rye grain, milling fractionation could be a valuable processing technique for modifying the rye-like flavour in the desired direction (Heiniö et al., 2003).

Triticale is a type of small grain created by genetically combining wheat and rye. Triticale grain, flours, and prepared products are available through both health food and commercial outlets on a limited basis. The data indicate that while the nutritional quality of triticale is considered superior to wheat, the higher ash content, lower milling yields of flour, and inferior loaf volume and texture distract from commercial baking use of triticale. In comparison with bread wheat, triticale has low gluten content, efficient gluten viscoelasticity and, therefore, inferior bread-making quality (Doxastakis et al., 2002).

Hull-less barley has been intensively investigated in respect to its food, feed and industrial applications. The advantage of hull-less barley compared to hulled barley in food uses is that pearling is not needed, so that the outer part of the endosperm, the aleurone, which contains proteins with essential amino acids and vitamins, is retained, as well as other bioactive compounds. It can be milled using conventional equipment available for wheat milling, with extraction yields of 73%. Hull-less barley flour has been successfully used in chemically leavened products such as muffins, pancakes, biscuits and cookies (Andersson et al., 2009a,b).

Traditionally, pasta is manufactured solely from durum wheat, which results in a product considered to be of superior quality to pasta made from cheaper common wheat or a blend of the two species. The manufacture of pastas from mixtures of durum and common wheat without adequate labelling is usually considered as adulteration. Durum wheat pasta for export outside the European Union may contain a maximum of 3% common wheat from unavoidable adventitious contamination during agricultural processing. Pasta is consumed in large quantities throughout the world. Scientific research has been undertaken to understand the parameters influencing pasta processing and the final product quality. Ideally, cooked pasta is of al dente quality. It is firm and resilient with no surface stickiness and little if any cooking losses (Sissons et al., 2005; Troccoli et al., 2000).

In the scientific literature practically was not found data about whole grain flour made form hull-less barley, rye, triticale and wheat application in pasta production, as well as dietary fibre and B_1 and B_2 vitamins content of possible whole flour blends for pasta making.

The main purpose of the current research was established to investigate total dietary fiber and vitamin B_1 and B_2 content in flour blends made from several types' whole grain flour for pasta production.

Materials and Methods

The study was realised at the scientific laboratories of Faculty of Food Technology at Latvia University of Agriculture (LLU) and at the laboratory of the JSC Jelgavas dzirnavas (Latvia), at the laboratories of Latvia University Institute of Biology (Latvia).

Characterisation of raw materials

In 2012 harvested from State Priekuli Plant Breeding Institute (Latvia) convectional rye ('Kaupo'), hull-less barley (line 'PR 5099') and triticale (line '9405-23'), as well as form LLU research station „Peterlauki" (Latvia) wheat ('Zentos') grain was used in the experiments. For the flour blend obtaining wheat flour 550 type (Latvia) was used.

Before experiments grain were grounded in laboratory mill Hawos (Hawos Kornmuhle GmbH, Germany) obtaining fine whole grain flour.

Preparation of flour blend samples

Whole grain blend proportions was pre-selected for the analysis of finished products − pasta. At the previous experiments optimal wheat flour 550 type and whole grain flours ratio was obtained as follow: 70% wheat flour 550 type and 30% whole wheat flour (W/W); 80% wheat flour 550 type and 20% whole rye flour (W/R); 70% wheat flour 550 type and 30% whole triticale flour (W/T); 80% wheat flour type and 20% whole hull-less barley flour (W/H). As a control sample wheat flour 550 type was analysed.

Analysed parameters

Total dietary fiber content was measured by Fibertec system 1010 Heat Extractor corresponding to ISO 5498. Flour blend added 50 mL Phosphate buffer solution, pH 6.0, to each flask. The testing procedure was as follow: check pH and adjust if necessary to pH 6.0±0.2 by adding 0.275N NaOH or 0.325N HCl. Added 100 µL of Alpha amylase. Cover flasks with Aluminium foil and incubate in boiling water bath for 30 minutes. Adjust to pH 7.5±0.2 by adding 10 mL 0.275N NaOH than add 100 µL Protease solutions to each flask. Cover flasks with Aluminium foil and incubate in boiling water bath for 30 minutes at 60±1 °C. Dispense 10 mL 0.325N HCl into flasks while stirring. Adjust to pH 4.0-4.6. Add 200 µL Amyloglucosidase solutions while stirring. Cover flasks with Aluminium foil and incubate in boiling water bath for 30 minutes at 60±1 °C. To each digested sample added 280 mL 60 °C hot 95% Ethanol OH. Measure the volume of Ethanol OH prior to heating. Remove from bath and cover flasks with Aluminium foil. Let precipitate for 1h at room temperature. Wet and redistribute Celite bed in previously tarred crucible using a few mi 78% Ethanol OH from wash bottle.

Apply suction to draw Celite onto fritted glass as an even mat. Attach the crucibles and the Incubation flasks to the Fibertec E, Filtration Module according to instructions under section Filtration in AN 302. Filter alcohol-treated enzyme digested through crucible. Using wash bottle with 78% EtOH and rubber spatula transfer all remaining particles to crucible.
Vitamins B_1 and B_2 content was measured by AOAC Official methods 986.27 and 970.65 respectively.

Mathematical data processing

Data are expressed as mean ± standard deviation; for the mathematical data processing ($p \leq 0.05$) (ANOVA) was calculated.

Results and Discussion

The consumption of whole grains, which are rich in dietary fiber, resistant starch, vitamins, minerals and micro constituents, was reported to have many physiological benefits related to "western diseases" such as coronary heart disease, colon cancer and diabetes. However, the whole grain foods have not been attractive to consumers because the higher bran and germ in whole grain flour reduced the quality and sensory value of the end-use products (Hung et al., 2007).

Dietary fiber

Over the last decades, consumer demands in the field of industrially food production have changed considerably. For this reason, foods today are not intended only to satisfy hunger and to provide necessary nutrients, but also to prevent nutrition-related diseases and enhance physical and mental well-being of consumers. In this regard, functional foods offer an outstanding opportunity to improve the quality of products. Early functional foods were characterised by fortification with vitamins. Subsequently, the focus has shifted to foods enriched with dietary fibre (Foschia et al., 2013).
In the present research significant differences ($p=0.007$) in total dietary fiber content of analysed flour samples were established (Table 1).

Table 1

Total dietary fiber, vitamin B_1 in B_2 content in flour samples

Flour sample	Dietary fiber, g 100 g^{-1}	Vitamin, mg kg^{-1}	
		B_1	B_2
Hull-less barley*	16.68±0.13	3.5±0.2	0.91±0.01
Rye*	16.71±0.12	4.2±0.1	1.28±0.02
Triticale*	12.80±0.14	3.6±0.2	1.01±0.02
Wheat*	11.66±0.11	4.8±0.1	1.28±0.04
Wheat 550 type	3.90±0.10	2.5±0.1	0.62±0.02

*whole grain flour

Higher dietary fiber content was found in whole grain hull-less barley and rye flour (Table 1); it was by approximately − 77% higher ($p=0.008$) comparing with wheat flour 550 type, by approximately − 23% higher ($p=0.001$) comparing with whole grain triticale flour and by approximately − 30% higher ($p=0.001$) comparing with whole grain wheat flour samples. However there are not found significant differences ($p=0.445$) in dietary fiber content in analysed whole grain hull-less barley and rye flour samples (Table 1).
According to the scientific literature, the total dietary fiber content of whole grain rye flour could be in the range from 18.7 to 22.2% (Andersson et al., 2009a), whole grain wheat flour – from 12.7 to 22.1% (Gebruers et al., 2008), whole grain triticale – from 13.2 to 16.0% (Rakha et al., 2011), hull-lessbarley – of 10.1 to 21.6% (Yalcin et al., 2007), what mainly corresponds to present results in this research.

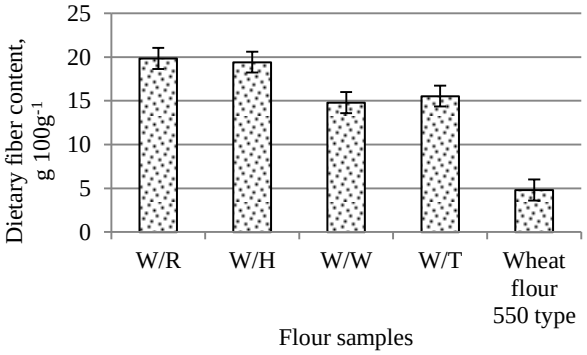

Figure 1. Total dietary fiber content of whole grain flour blends

W/R – 80% wheat flour 550 type and 20% whole rye flour; W/H – 80% wheat flour 550 type and 20% whole hull-less barley flour; W/W – 70% wheat flour 550 type and 30% whole wheat flour; W/T – 70% wheat flour 550 type and 30% whole triticale flour

As results of our experiments show, the lowest total dietary fiber contend was obtained in wheat flour 550 type (Figure 1, Table 1), what mainly could be explained with only grain middle part milling. Lower analysed parameter content, as 14.786±0.148 g 100 g^{-1} and 15.533±0.005 g 100 g^{-1} (Figure 1), was obtained for whole wheat and triticale flour blend. However, the higher total dietary fiber content, as 19.833±0.175 g 100 g^{-1} and 19.412±0.352 g 100 g^{-1} (Figure 1) was obtained in whole rye and hull-less barley flour blend.

Vitamins

B vitamins all function as coenzymes in the central pathways by which fat and carbohydrate are metabolized within the cell. The vitamins are concentrated in the aleurone layer of cereal seeds and milling and refining of cereals can lead to large losses (Sanders et al., 2003).
According to the literature, in the present research higher B_1 and B_2 vitamin content was obtained in whole grain flour (Table 1) comparing with wheat flour 550 type, because of milling of grain all parts including aleurone layer.
Similar vitamin B_1 and B_2 content was found in whole grain rye and wheat flour samples. However lower vitamins content was found in wheat flour type 550.

During mathematical data processing it was detected, that B_1 vitamin content in whole grain rye and wheat flour was significantly (p=0.114) higher than in hull-less barley and triticale – by 25%; and by 46% higher (p=0.744) comparing with wheat flour type 550. Similar results were detected during analysing of vitamin B_2 content in flour samples. As a result lowest vitamin B_2 content was obtained in wheat flour type 550; it was by 52% lower comparing with vitamin content in whole grain rye and wheat flour, by 32% lower comparing with whole grain hull-less barley flour and by 37% lower comparing with whole grain triticale flour. Obtained results mainly could be explained with grain individuality and vitamin forming intensity during grain growing.

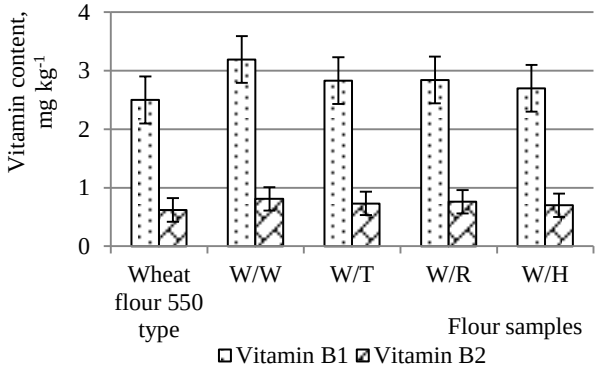

Figure 2. Vitamins content in whole grain flour blends

W/R – 80% wheat flour 550 type and 20% whole rye flour; W/H – 80% wheat flour 550 type and 20% whole hull-less barley flour; W/W – 70% wheat flour 550 type and 30% whole wheat flour; W/T – 70% wheat flour 550 type and 30% whole triticale flour

Lower B_1 vitamin content, as 2.7±0.14 mg kg^{-1} was obtained for blend with whole hull-less barley flour (W/H). However, the higher, as 3.19±0.28 mg kg^{-1}, was obtained for blend with whole wheat flour (W/W) (Figure 2). Differences mainly could be explained with grain individuality. In the scientific literature it is mentioned – grains are rich in thiamine, especially barley 0.356, mg 100 g^{-1} (Lebiedzinska et al., 2006). Lower B_2 vitamin content was obtained for flour blend with whole hull-less barley flour (W/H), whole rye (W/R) and whole triticale grain (W/T) flour. However, the higher B_2 vitamin content, as 0.81±0.28 mg kg^{-1} was obtained in flour blend with whole wheat flour (W/W) (Figure 2).

As a result it could be possible to increase the dietary fiber and B_1 and B_2 vitamins content in pasta by using of the whole-grain flour blends. Because in the scientific literature is mentioned, that, for example, wheat whole grain is a source of health-promoting phytochemicals in addition to traditional nutrients, including carbohydrates, proteins, lipids, vitamins, and minerals. Removal of wheat bran outer layers and wheat germ to produce semolina for pasta production significantly decreases phytochemicals and nutrients responsible for the health benefits of whole wheat grain. Whole wheat pasta is an ideal product for retention of the natural substances present in the durum wheat kernel. This chapter provides an overview of the relationship between whole grains and current health conditions, use of whole grain in pasta products, bioactive phytochemicals in whole grain and pasta, and, lastly, the major and minor nutrients in whole wheat pasta (Hirawan and Beta, 2014).

Conclusions

In the present research significant differences in total dietary fiber of analysed whole grain rye, hull-less barley, triticale and wheat flour samples were established. Higher dietary fiber content was found in whole grain hull-less barley and rye flour, lower − in whole wheat and triticale flour blend.

B_1 and B_2 vitamin content in whole grain rye and wheat flour was significantly higher than in hull-less barley and triticale. Lower B_1 vitamin content, as 2.7±0.14 mg kg^{-1} was obtained for hull-less barley flour blend, the higher as 3.19±0.28 mg kg^{-1} for wheat flour blend. Lower B_2 vitamin content was obtained for flour blends made with whole hull-less barley, triticale and rye flour, however, the higher B_2, as 0.81±0.28 mg kg^{-1} in blend with whole wheat flour.

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References

1. Anderson J.W., Baird P., Davis J.R.H., Ferreri S., Knudtson M., Koraym A. (2009a) Health benefits of dietary fiber. *Nutrition Reviews*, No. 67, p.188–205.
2. Andersson R., Fransson G., Tietjen M., Aman P. (2009b) Content and molecular-weight distribution of dietary fiber components in whole-grain rye flour and bread. *Agriculture and Food Chemistry*, Vol. 57, p. 2004–2008.
3. Batifloulier F., Verny M.A., Chanliaud E., Demigne C. (2006) Variability of B vitamin concentrations in wheat grain, milling fractions and bread products. European *Agronomy*, Vol.25, p. 163–169.
4. Brownlee I.A. (2011) The physiological roles of dietary fibre. *Food Hydrocolloids*, Vol. 25, p.238–250.
5. Doxastakis G., Zafiriadis I., Irakli M., Marlani H., Tananaki C. (2002) Lupin, soya and triticale addition to wheat flour doughs and their effect on rheological properties. *Food Chemistry*, Vol. 77, Iss. 2, p. 219–227.
6. Foschia M., Peressini D., Sensidoni A., Brennan C.-S. (2013) The effects of dietary fibre addition on the quality of common cereal products. *Cereal Science*, Vol. 58, Iss. 2, p. 216–227.
7. Franz M., Sampson L. (2006) Challenges in developing a whole grain database: definitions, methods, and quantification. *Food Composition and Analysis*, Vol. 19, p. 38–44.

8. Gaoshuang L., Chen H., Chen S., Tian J. (2012) Chemical composition and physicochemical properties of dietary fiber from *Polygonatum odoratum* as affected by different processing methods. *Food Research International*, Vol. 46, p. 406–410.
9. Gebruers K., Dornez E., Boros D., Fras A., Dynkowska W., Bedo Z. (2008) Variation in the content of dietary fiber and components thereof in wheat's in the health rain diversity screen. *Agriculture and Food Chemistry*, Vol. 56, p. 9740–9749.
10. Hägg M., Kumpulainen J. (1993) Thiamine and riboflavin contents in domestic and imported cereal products in Finland. *Food Composition and Analysis*, Vol. 6, Iss. 4, p. 299–306.
11. Heiniö R.-L., Liukkonen K.-H., Katina K., Myllymäki O., Poutanen K. (2003) Milling fractionation of rye produces different sensory profiles of both flour and bread. *LWT – Food Science and Technology*, Vol. 36, Iss. 6, p. 577–583.
12. Hirawan R., Beta T. (2014) *Whole wheat pasta and health.* **In**: Wheat and Rice in Disease Prevention and Health, Academic Press, p. 5−16.
13. Hung P.V., Maeda T., Morita N. (2007) Dough and bread qualities of flours with whole waxy wheat flour substitution. *Food Research International*, Vol. 40, Iss. 2, p.273−279.
14. Lang R., Jebb S.A. (2003) Who consumes whole grains, and how much? *Proceedings of the Nutrition Society*, Vol.62, p. 123–127.
15. Lebiedzinska A., Szefer P. (2006) Vitamins B in grain and cereal–grain food, soy-products and seeds. *Food Chemistry*, Vol. 95, p. 116–122.
16. Liu R.H. (2007) Whole grain phytochemicals and health. *Journal of Cereal Science*, Vol. 46, p. 207.
17. McKeown N.M., Meigs J.B., Liu S., Wilson P.W.F., Jacques P.F. (2002) Whole-grain intake is favourably associated with metabolic risk factors for type 2 diabetes and cardio vascular disease in the Framingham Offspring Study. *American Journal of Clinical Nutrition*, Vol. 76, p. 390–398.
18. Okarter N., Liu R.H. (2010) Health benefits of whole grain phytochemicals. *Food Science and Nutrition*, Vol. 50, p.193–208.
19. Rakha A., Aman P., Anderson R. (2011) Dietary fiber in triticale grain: variation in content, composition, and molecular weight distribution of extractable components. *Cereal Science*, Vol. 54, p. 324–331.
20. Sanders T., Emery P. (2003) *Molecular basis of human nutrition.* CRC Press, p. 94–96.
21. Sissons M.J., Ames N.P., Hare R.A., Clarke J.M. (2005) Relationship between glutenin subunit composition and gluten strength measurements in durum wheat. *Science of Food and Agriculture*, Vol. 85, p. 2445–2452.
22. Slavin J. (2003) Why whole grains are protective: biological mechanisms. *Proceedings of the Nutrition Society*, Vol. 62, p. 129.
23. Slavin J. (2004) Whole grains and human health. *Nutrition Research Reviews*, Vol. 17, p. 99–110.
24. Troccoli A., Borelli G.M., Vita De P., Fares C., Fronzo Di N. (2000) Durum wheat quality: a multidisciplinary concept. *Cereal Science*, Vol. 32, p. 99–113.
25. Wang L., Deng L., Wang Y., Zhang Y., Qian H., Zhang H., Qi X. (2014) Effect of whole wheat flour on the quality of traditional *Chinese Sachima*. *Food Chemistry*, Vol. 152, Iss. 1, p. 184–189.
26. Yalcin E., Celik S., Akar T., Savim H., Koksel H. (2007) Effects of genotype and environment on β-glucan and dietary fiber contents of hull-less barleys grown in Turkey. *Food Chemistry*, Vol. 101, p. 171–176.

NUTRITIONAL EVALUATION OF PULSE SPREADS IN COMPARISON TO NUTRIENT RECOMMENDATIONS

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Abstract

Non-dairy and reduced fat/calorie spreads are becoming important for health conscious people; as well as increasing consumer choice, animal product alternatives have the potential to contribute to overall public health. Pulses and pulse products are primarily popular among vegetarian and health conscious consumers and could benefit people struggling with dietary changes.

Therefore, the aim of this research was to analyse and collate nutritional values of four commercially available pulse (chickpeas or soy) spreads and one newly developed pulse (bean) spread in comparison to recommended daily allowance (RDA) of nutrients for adolescents and adults.

Bean spread was made of ground re-hydrated cooked seeds of beans, to which salt, spices and other ingredients were added. Macro-nutrients were determined according to standard methods. Nutritional values of commercially available pulse spreads were given according to product label information.

The results show that there are significant nutritional differences among the tested pulse spreads ($p<0.05$). A serving (100 g) of pulse spreads covers 5.2–9.8% protein, 3.8–32.2% fat, 1.5–3.8% carbohydrates and 3.4–14.0% energy of RDA for adolescents while 6.9–12.0% protein, 5.7–46.8% fat, 1.7–4.1% carbohydrates and 4.2–16.8% energy of RDA is covered for adults.

A serving of pulse spreads covers the least of RDA for male adolescents and the most for female adults out of the four groups. Even though pulses are considered a good source of B vitamins, pulse spreads are low in all but folic acid. Solely the new bean spread also contains significant amount of thiamine.

Keywords: pulse spreads, nutritional value, recommended daily allowance.

Introduction

Non-dairy and reduced fat/calorie spreads are becoming important for health conscious people who are seeking attractive products from different sources. As well as increasing consumer choice, animal product alternatives have the potential to contribute to overall public health. Pulses can serve as the base raw materials with added advantages of lower price of products and increased protein content (Veena, Bhattacharya, 2012). Pulse products are primarily popular among vegetarian and health conscious consumers and could benefit people struggling with dietary changes.

Pulses (grain legumes) are dry seeds of leguminous plants which are distinguished from leguminous oil seeds (soy, peanuts) by their low fat content. Pulses also exclude such leguminous vegetables as green peas and snap beans which are immature legume pods and green seeds (Codex Alimentarius Standart 171-1989, Rev. 1, 1995). This must be taken into account since the best known legume spread is peanut butter; pulse seeds, however, are made from dry and then re-hydrated edible variety of bean, pea and lentil seeds.

Pulses (family *Fabaceae*) have been consumed for at least 10 000 years and are among the most extensively used foods in the world. Nutritionally, they are characterised by high protein content (about 20–30%), a very high proportion of carbohydrate (about 50–65%) and a very low fat content (about 1%). They are a significant source of many nutrients, including fibre, protein and iron, as well as B group vitamins (Mudryj et al., 2012).

Pulses contain a mixture of soluble and insoluble fibre, which lowers total serum and low-density lipoprotein (LDL) cholesterol and aids in gastrointestinal function, respectively (Tosh, Yada, 2010); pulse consumption results in higher intakes of fibre, carbohydrate, protein, Ca, K, folate, Zn, Fe and Mg, with lower intakes of saturated as well as total fat (Mitchell et al., 2009). Pulses are also gluten-free, so products made from pulse flours provide alternatives to wheat flour based products (Siddiq et al., 2013).

However, their consumption in the Western world remains quite low at less than 3.5 kg per capita per year. In other parts of the world, annual pulse consumption can range from 10 kg per capita (South America and India) to 40 kg per capita (Burundi) (Mudryj et al., 2012). Among European countries, higher pulse consumption is observed around the Mediterranean, with per capita daily consumption between 8 and 23 g, while in Northern Europe, the daily consumption is less than 5 g per capita (Bouchenak, Lamri-Senhadji, 2013). In 2009, legume (including pulses, soy and nuts) consumption represented only 0.7% of total regularly consumed foods in Latvian food basket, with daily consumption 32±2 g per capita (Joffe et al., 2009). According to the Latvian Central Statistical Bureau (CSB) data, the average pulse consumption was 9 g per capita per day in 2013, with annual consumption 3.28 kg per capita.

Commercially available pulse spreads are a fairly new concept, while the main spread-like vegetable protein product humus can be described as ancient food. Today, humus, a Middle Eastern and Arabic food dip or spread made from cooked, mashed chickpeas blended with tahini, olive oil, lemon juice, salt and garlic, is popular throughout the Middle East, North Africa, Mediterranean and in Middle Eastern cuisine around the globe (Marks, 2010).

There are about 10 different plant-derived spreads commercially available in Latvia varying a lot in

nutritional value and ingredients. About half of them are oil, yeast or seed based and do not qualify as pulse (or legume) spreads. Latvian-produced spreads are more accessible to consumers in terms of price; however, they contain additives to ensure the texture or improve the structure of the product (thickeners, emulsifiers), improve the taste (flavour enhancing agents) and extend shelf life (preservatives).

The main difference in need for nutrients between adolescents and adults is higher total energy intake for adolescents because they experience greater increases in height, weight, and other aspects of body composition than adults; adolescent growth spurt is sensitive to energy and nutrient deprivation which can lead to delayed puberty or growth retardation (Stang, Story, 2005). Adolescents also need higher intakes of healthy fat which is necessary for brain and nerve cell development and growth (Nettleton et al., 2013).

In order to determine the need for nutritionally improved products, one needs to ascertain the existing products on the market. Therefore, the aim of this research was to analyse and collate nutritional values of four commercially available pulse (chickpeas or soy) spreads and one newly developed pulse (bean) spread in comparison to recommended daily allowance (RDA) of nutrients for adolescents and adults.

Materials and Methods

Pulse (bean) spread with sun-dried tomatoes was prepared at the laboratory of Faculty of Food Technology (Latvia University of Agriculture) according to the vegetarian spread preparation technology in RL patent Vegetarian bean spread production method application. Bean spread with sun-dried tomatoes was made of ground re-hydrated cooked seeds of white (navy) beans, to which salt, spices and other ingredients were added (Kirse et al., 2013).

Macro-nutrients were determined according to standard methods: protein content (AACC 46-20), fat content (AOAC 2003.06), total dietary fibre content (AOAC 994.13), ash content (ISO 2171:2010); available carbohydrates (g per 100 g of spreads) were determined by difference (Menezes et al., 2004) according to formula:

$$Available\ carbohydrates = 100 - (weight\ in\ grams\ [protein + fat + water + ash + dietary\ fibre]\ in\ 100\ g\ of\ food) \quad (1)$$

Nutritional values of commercially available pulse spreads are given according to product label information. Dietary fibre (for commercially available spreads) and vitamin content (mg or g per 100 g of spreads) was calculated according to formula:

$$\text{Vitamin or dietary fibre} = n \times \frac{a}{b} \quad (2),$$

where n – the amount of nutrient (vitamin or dietary fibre) in 100 g of cooked pulses/legumes, a – protein content in the given spread (per 100 g), b – protein content in the cooked pulse/legume (per 100 g).

USDA National Nutrient Database for Standard Reference was used for dietary fibre and vitamin content reference in cooked pulses.

The calculation of the amount of nutrients needed (recommended daily intake of nutrients expressed as recommended daily allowance) for adolescents and adults has been done based on the ordinance No 174 Recommended allowance of energy and nutrients for Latvian citizens, issued by Ministry of Health of the Republic of Latvia on October 15, 2008. Calculations were carried out in both groups per gender (female and male) and average values were used for further analysis.

The obtained data processing was performed using mathematical and statistical methods with statistical software *R 3.0.2*; differences among results were considered significant if p-value $<\alpha_{0.05}$. For the interpretation of the results it was assumed that α=0.05 with 95% confidence (Næs et al, 2011). Differences among nutritional value of different pulse spreads were analysed using one way analysis of variance and Tukey's test.

Results and Discussion

Five pulse spreads were compared in this study, four of which are commercially available: *ILO* Hummus by *Silva*, Finland (sample A), *Hum-Hum* Hummus by *Zila Laguna*, Latvia (sample B), *WD fit* soy spread by *W-D*, Poland (sample C), *BioGreno* Curry-Pineapple spread by *Bartels-Langness*, Germany (sample D) and bean spread with sun-dried tomatoes experimentally developed at the laboratory of Faculty of Food Technology in Latvia University of Agriculture, Latvia (sample E). General information about the investigated spreads is given in Table 1.

Table 1

Comparison of pulse spreads

Pulse spreads	Type of pulses used	Approximate shelf life	Price, EUR kg^{-1}
A	Chickpeas *Cicer arietinum* L.	8 weeks*	14.70
B	Chickpeas	4 weeks*	6.20
C	Soy *Glycine max* (L. Merr.)	4 weeks	8.00
D	Chickpeas and soy	8 months	13.80
E	Beans *Phaseolus vulgaris* L.	7 days	5.60**

* preservatives used

** price forecast

Data presented in Table 1 show that chickpeas and soy are the main legumes used in commercially available non-dairy spreads. As mentioned before chickpeas and soy are both legumes but belong to different subtypes of legumes, pulses and leguminous oil seeds, respectively. In this study soy spread has been included because soy is used for the content of protein not oil.

There are significant differences among the shelf life of these spreads, e.g. sample D has the longest shelf life

because curry-pineapple spread is filled in small glass bottles and heat treatment (pasteurisation) has been performed. Spreads A and B (hummus) have the shelf life up to two months because preservatives (sorbic acid E200, lactic acid E270, potassium sorbate E202) have been used. The shelf life of bean spread with sun-dried tomatoes is only one week because no heat treatment or preservatives were added and the proper packaging is currently being researched. The shelf life of bean spread with sun-dried tomatoes is equal to the expiration date of commercially available spreads when kept in the refrigerator after opened.

There are also significant differences among the price of the investigated spreads. The price of three spreads (B, C and E) is less than 10 EUR kg^{-1} and it is comparable to the price of dairy spreads. The ingredients of spread D have been grown in biological agriculture and this could be the reason for a higher price. The price of spread A could be higher because of increased marketing margin and raw materials of higher quality and price.

Nutritional value of pulse spreads is given in Table 2. Nutritional information of samples A-D is given according to product label information; protein, fat and carbohydrate content in bean spread with sun-dried tomatoes has been determined experimentally according to standard methods.

Table 2

Nutritional value of different pulse spreads, 100 g^{-1}

Pulse spreads	Protein, g	Fat, g	Carbohydrates, g	Energy, kcal
A	6.0[a]	10.0[a]	8.0[a]	146.0[a]
B	6.5[a]	29.0[b]	12.0[b]	335.0[b]
C	6.0[a]	22.2[c]	6.0[c]	247.8[c]
D	5.2[b]	4.3[d]	10.6[d]	101.9[d]
E	7.8[c]	8.3[e]	8.4[a]	139.5[a]

* values within a column not sharing a superscript letter are significantly different ($p<0.05$)

Spread E has the highest protein content out of all investigated spread samples ($p=0.012$). Commercially available non-dairy spreads contain significantly less protein and spread D has the least protein. Literature data confirm that white beans can contain significantly more protein than chickpeas and soy (Maskus, 2010).

Fat content in pulse spreads is significantly higher comparing with dry and cooked pulses ($p<0.001$) because of the oil added for a better texture. Spreads A, D and E have the least fat and they qualify as non-dairy, reduced fat spreads. Hummus produced in Latvia (spread B) already has the highest fat content, nevertheless, it is recommended to add more oil before eating it according to product label information.

Carbohydrates available for digestion by human enzymes account to less than 15 g 100 g^{-1} of pulse spreads; even though there are significant differences among five spread samples, pulse spreads are low in carbohydrates.

Bean spread with sun-dried tomatoes (spread E) contains 9.83±0.10 g 100 g^{-1} total dietary fibre; commercially available non-dairy spreads are not labelled as containing fibre.

According to Commission Directive 2008/100/EC and Regulation No 1924/2006, products can be labelled as a source of protein if at least 12% of the energy value of the food is provided by protein. Spreads A, D and E are a source of protein because 16%, 20% and 22% of the energy value is provided by protein, respectively. The same regulations allow labelling bean spread with sun-dried tomatoes (spread E) as high in fibre considering it contains ≥6.0 g fibre 100 g^{-1}.

A claim that a food is energy-reduced may be made if the energy value of the product is reduced by at least 30% with an indication of the characteristic(s) which make(s) the food reduced in its total energy value. Fat content is the characteristic which makes three spreads (A, D and E) energy-reduced as energy value of those spreads is 40.8% to 69.5% lower compared to spreads B and C.

The calculation of the amount of nutrients needed for adolescents and adults has been done based on the ordinance No 174 Recommended allowance of energy and nutrients for Latvian citizens, issued by Ministry of Health of the Republic of Latvia on October 15, 2008.

Reference values of recommended daily allowance (RDA) of nutrients are given in Table 3. The data have been used in subsequent calculations.

Table 3

Recommended daily energy and nutrient intake

Age group	Energy (E), kcal	Protein, E%	Fat, E%	Carbohyd-rates, E%
Adolescent				
– female	2400	10–15*	30–35*	50–55*
– male	3000			
Adult				
– female	2000	10–15*	25–30*	55–60*
– male	2400			

*average values were used for further calculations

Recommended daily energy intake for adolescent females and adult males is 10 042 kJ per day, while the amount is lower for adult females and higher for adolescent males, 8 368 kJ per day and 12 552 kJ per day, respectively. Adolescent males have higher caloric requirements since they experience greater increases in height, weight, and lean body mass than females. Protein needs for adolescents and adults are similar. (Stang, Story, 2005)

Recommended daily intake for fat of total energy intake is higher for adolescents. The human body requires dietary fat and essential fatty acids for normal growth and development; healthy fat (composed of mono- and polyunsaturated fatty acids) is necessary for brain and nerve cell development and growth, which is especially important for growing teens. Fat-soluble

vitamins A, D, E and K cannot function without adequate daily fat intake. (Nettleton et. al., 2013)
The calculation of the amount of nutrients needed for adolescents and adults was completed as the coverage of a nutrient by one serving (100 g) of pulse spreads in comparison to RDA.
The recommended daily intake of protein is 80 g for female adolescents, 100 g for male adolescents, 65 g for adult females and 75 g for adult males. A serving of pulse spreads covers 5.2–9.8% protein for adolescents and 6.9–12.0% protein for adults (Fig. 1).

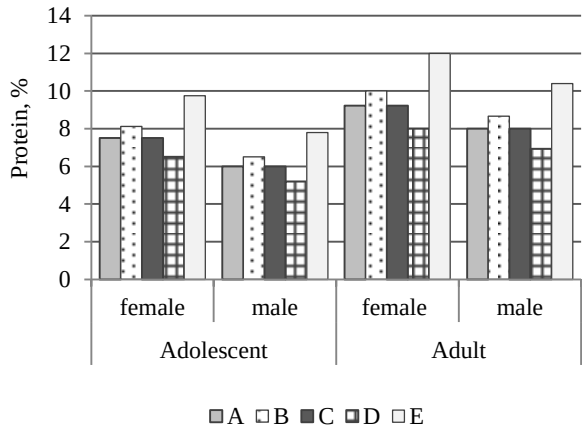


Figure 1. Protein coverage (%) of one serving of pulse spreads in comparison to RDA
A – *ILO* hummus, B – *Hum-Hum* hummus, C – *WD fit* soy spread, D – *BioGreno* Curry-Pineapple spread, E – bean spread with sun-dried tomatoes

As spread E has the highest protein content, protein coverage of bean spread with sun-dried tomatoes is the best. Spread E can be considered a good source of protein for adults. Protein coverage for adolescent males is the lowest because the requirements for protein in this group are the highest.

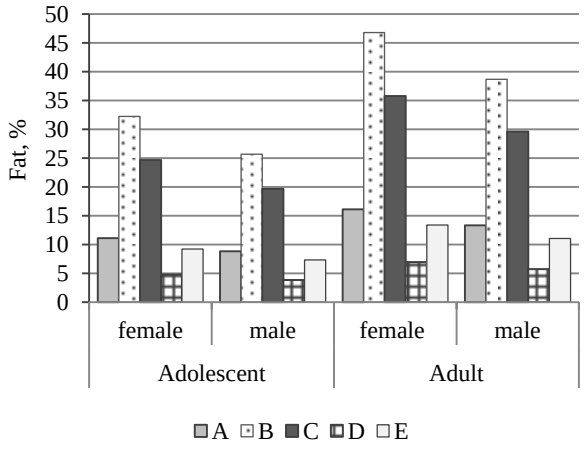


Figure 2. Fat coverage (%) of one serving of pulse spreads in comparison to RDA
A – *ILO* hummus, B – *Hum-Hum* hummus, C – *WD fit* soy spread, D – *BioGreno* Curry-Pineapple spread, E – bean spread with sun-dried tomatoes

The recommended daily intake of fat is 90 g for female adolescents, 113 g for male adolescents, 62 g for adult females and 75 g for adult males. A serving of pulse spreads covers 3.8–32.2% fat for adolescents and 5.7–46.8% fat for adults (Fig. 2). There are significant differences among the coverage of fat in the four groups ($p<0.05$).
Spreads B and C cover the most of fat compared to the other three spreads. Hummus produced in Latvia (spread B) covers almost half of recommended daily intake for adult females.
Contrary to protein, fat coverage as high from one serving cannot be considered a good indicator. A serving of spreads B or C cannot be recommended for daily consumption. Fat coverage for adolescent males is the lowest once more because the requirements for fat in this group are the highest.
The recommended daily intake of carbohydrates is 320 g for female adolescents, 400 g for male adolescents, 295 g for adult females and 355 g for adult males.
A serving of pulse spreads covers 1.5–3.8% carbohydrates for adolescents and 1.7–4.1% carbohydrates for adults (Fig. 3).

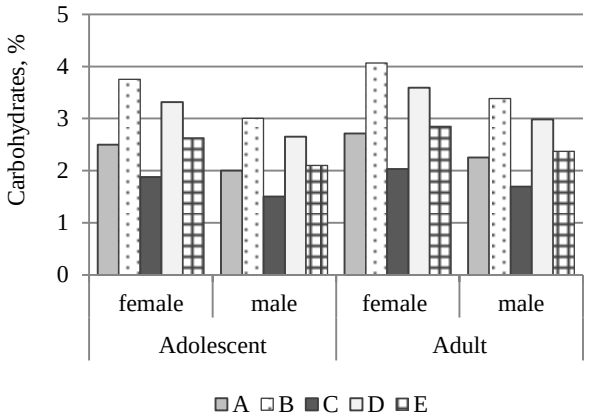


Figure 3. Carbohydrate coverage (%) of one serving of pulse spreads in comparison to RDA
A – *ILO* hummus, B – *Hum-Hum* hummus, C – *WD fit* soy spread, D – *BioGreno* Curry-Pineapple spread, E – bean spread with sun-dried tomatoes

Compared to RDA, carbohydrate coverage is low for all investigated pulse spreads.
As mentioned before, commercially available non-dairy spreads are not labelled as containing fibre, however, after calculations it can be concluded that dietary fibre content in spreads A-C is around 5.2 g 100 g^{-1} and spread D contains at least 4.0 g 100 g^{-1} dietary fibre. Bean spread with sun-dried tomatoes (spread E) contains more total dietary fibre (9.83±0.10 g 100 g^{-1}) than other pulse spreads ($p=0.009$).
According to European Guidelines on cardiovascular disease prevention recommended dietary fibre intake is 30–45 g per day or 3.4 g per 1000 kJ (Perk et al., 2012). While *Guideline Daily Amounts (GDA) Labelling Initiative* (by The Confederation of the Food and Drink Industries of the EU) recommends 25 g

dietary fibre per day for both genders (reference amount for 2000 kcal diet) but no further recommendations for diets in lower or higher calories are given.

Therefore, if European Guidelines on cardiovascular disease prevention are used as reference for optimal dietary fibre intake, a serving of pulse spreads covers 12–21% fibre for adolescent males, 15–26% fibre for adolescent females and adult males and 18–31% fibre for adult females.

A serving of pulse spreads covers 3.4–14.0% energy for adolescents and 4.2–16.8% energy for adults (Fig. 4).

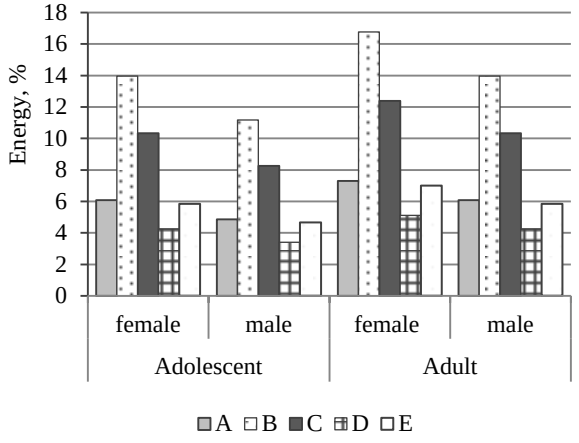
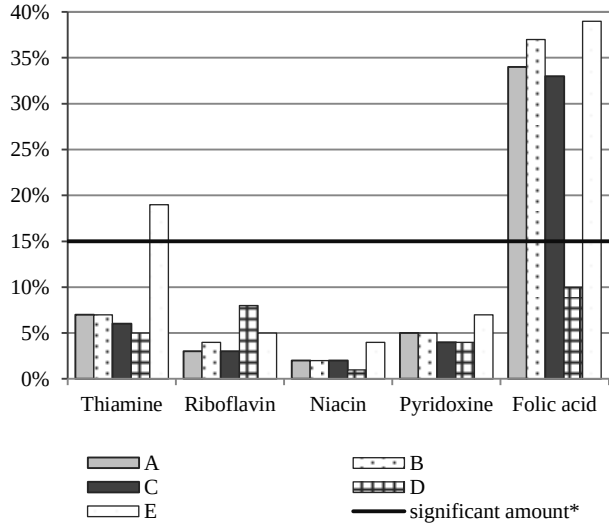


Figure 4. Energy coverage (%) of one serving of pulse spreads in comparison to RDA

A – *ILO* hummus, B – *Hum-Hum* hummus, C – *WD fit* soy spread, D – *BioGreno* Curry-Pineapple spread, E – bean spread with sun-dried tomatoes



*15% of RDA

Figure 5. Vitamin coverage (%) of one serving of pulse spreads in comparison to RDA

A – *ILO* hummus, B – *Hum-Hum* hummus, C – *WD fit* soy spread, D – *BioGreno* Curry-Pineapple spread, E – bean spread with sun-dried tomatoes

Spreads B and C cover as much as twice the amount of calories per one serving of other pulse spreads; most of the calories come from fat. As mentioned before spreads A, D and E are energy-reduced and can be considered a good source of macronutrients for daily consumption.

Pulses are a good source of B vitamins, namely B_1, B_2, niacin, pyridoxine and folic acid (Ofuya et al., 2005); however, pulse spreads are low in most of them (Fig. 5). According to Regulation No 1924/2006 a claim that a food is a *source of vitamins* can be made if the product contains at least a significant amount, i.e., 15% of the recommended daily allowance (which in absolute numbers is equal for adolescents and adults).

All pulse spreads except spread D are a source of folic acid because soy has lower amounts of the vitamin previously classified as B_9. Solely bean spread with sun-dried tomatoes also contains significant amount of thiamine (p=0.008).

Conclusions

A serving of pulse spreads (including soy spread) covers the least of RDA for male adolescents and the most for female adults out of the four groups.

Even though pulses are considered a good source of B vitamins, pulse spreads are low in all but folic acid. Solely the new bean spread also contains significant amount of thiamine.

Nutritional value of pulse spreads is essentially different, depending on the raw materials used for spread production.

References

1. Bouchenak M., Lamri-Senhadji M. (2013) Nutritional quality of legumes, and their role in cardio metabolic risk prevention: A Review. *Journal of Medicinal Food*, No. 16, Vol. 3, p. 185–198.
2. *Codex Alimentarius Standard 171-1989: Standard for Certain Pulses, Rev. 1*. (1995). [accessed on 10.04.2014.]. Available at: http://www. Codexalimentarius.org/input/download/standards/56/CXS_171e.pdf.
3. Commission Directive 2008/100/EC of 28 October 2008 amending Council Directive 90/496/EEC on nutrition labelling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions. Official Journal of the European Union. 2008, L 285, p. 9–12.
4. CSB Statistics Database (2013) *Consumption of food products average per household member per year*. [accessed on 10.04.2014.]. Available at: http://data.csb.gov.lv/.
5. Joffe R., Bartkevics V., Ozoliņš G., Šantare D., Mike L., Briška I. (2009) The National Food Consumption Survey of Latvia, 2007–2009. A Report: National Diagnostic Centre and Food Centre of Food and Veterinary Service of Latvia. [accessed on 10.04.2014.]. Available at: www.pvd.gov.lv/text_files/0_Zinojums.pdf.
6. Kirse A., Karklina D., Strautniece E.V. (2013) Sensory evaluation of new bean spreads for vegetarians. **In**: *Research for Rural Development 2013*; Annual 19th International Scientific Conference Proceedings, Vol. 1, Latvia, Jelgava, p. 79–83.

7. Marks G. (2010) Encyclopedia of Jewish food. New York: John Wiley and Son, 672 p.
8. Maskus H. (2010) P*ulse Processing, Functionality and Application.* Literature Review: Pulse Canada. [accessed on 10.04.2014.]. Available at: http://www.pulsecanada.com/uploads/b1/d6/b1d6e08fdff0a3158ad808fb1510ba86/2010-Pulse-Processing-Functiona lity-and-Application-Litera.pdf.
9. Menezes E.W., de Melo A.T., Lima G.H., Lajolo F.M. (2004) Measurement of carbohydrate components and their impact on energy value of foods. *Journal of Food Composition and Analysis*, Vol. 17, p. 331–338.
10. Mitchell D.C., Lawrence F.R., Hartman T.J., Curram J.M. (2009) Consumption of dry beans, peas, and lentils could improve diet quality in the US population. *Journal of the American Dietetic Association*, No. 109, p. 909–913.
11. Mudryj A.N., Yu N., Hartman T.J., Mitchell D.C., Lawrence F.R., Aukema H.M. (2012) Pulse consumption in Canadian adults influences nutrient intakes. *British Journal of Nutrition*, No. 108, p. 27–36.
12. Næs T., Brockhoff P. and Tomic O. (2011) *Statistics for Sensory and Consumer Science*. West Sussex: John Wiley & Sons, 300 p.
13. Nettleton J.A., Villalpando S., Cassani R.S., Elmadfa I. (2013) Health significance of fat quality in the diet. *Annals of Nutrition and Metabolism*, Vol. 63, No. 1–2, p. 96–102.
14. Perk J., Backer G.D., Gohlke H., Graham I., Reiner Z., Verschuren M., Albus C., Benlian P., Boysen G., Cifkova R., Deaton C., Ebrahim S., Fisher M., Germano G., Hobbs R., Hoes A., Karadeniz S., Mezzani A., Prescott E., Ryden L., Scherer M., Syvänne M., Scholte Op Reimer W. J.M., Vrints C., Wood D., Zamorano J.L., Zannad F. (2012) European Guidelines on cardiovascular disease prevention in clinical practice. The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice. *European Heart Journal*, No. 33, p. 1635–1701.
15. *Recommended allowance of energy and nutrition for Latvian citizens, ordinance No 174*. Ministry of Health of the Republic of Latvia, October 15, 2008. [accessed on 10.04.2014.]. Available: http://www.vm.gov.lv/images/userfiles/ieud.pdf.
16. Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Official Journal of the European Union. 2006, L 404, p. 9–25.
17. Siddiq M., Kelkar S., Harte J.B., Dolan K.D., Nombaire G. (2013) Functional properties of flour from low-temperature extruded navy and pinto beans (*Phaseolus vulgaris* L.), *LWT-Food Science and Technology*, No. 50, p. 215–219.
18. Stang J., Story M. (2005) Nutrition Needs of Adolescents. **In:** *Guidelines for Adolescent Nutrition Services*, J. Stang, M. Story (eds). Minneapolis, MN: University of Minnesota, p. 21–34.
19. The Confederation of the Food and Drink Industries of the EU (2012) *Guideline Daily Amounts Labelling Initiative*. [accessed on 10.04.2014.]. Available: http://gda.fooddrinkeurope.eu/asp2/guideline-daily-amounts.asp.
20. Tosh S., Yada S. (2010) Dietary fibres in pulse seeds and fractions: characterization, functional attributes, and applications. *Food Research International*, No. 43, p. 450–460.
21. *USDA National Nutrient Database for Standard Reference*. Release 26. [accessed on 10.04.2014.]. Available: http://ndb.nal.usda.gov/ndb/search/list.
22. Veena R., Bhattacharya S. (2012) Rheological characterization of raw and roasted green gram pastes. *LWT - Food Science and Technology*, Vol. 46, Iss. 1, p. 260–266.
23. Ofuya Z.M., Akhidue V. (2005) The Role of Pulses in Human Nutrition: A Review. *Journal of Applied Sciences & Environmental Management*, Vol. 9, No. 3, p. 99–104.

EXTRUDED BEAN PRODUCT QUALITY EVALUATION

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Abstract

Aim of study was to develop a new type of bean (*Phaseolus vulgaris* L.) products with various additives using one screw extrusion, define their quality (physical and chemical properties) and ascertain people opinion about such products. Research was carried out at Latvia University of Agriculture Faculty of Food Technology. White beans were used in experiments. Beans were boiled, crushed and extruded through one screw extruder. Different additives were used, as bell peppers, tomatoes, spinach, garlic and red beet. Parameters as crude protein content, ash content, pH and colour were determinate. People at international exhibition "Riga Food 2013" were asked to give their opinion about the experimental product.

Average protein content in beans (*Phaseolus vulgaris* L.) extruded products were 25.5±0.4% of dry matter (DM). Average ash content was 4.99±0.05% of DM and pH 5.77. Dry matter was 91.59±0.03%in average. No more than 5% of lignified protein had been found in product.

Keywords: beans, extrusion, protein, ash.

Introduction

Dietary importance of legumes has been well established. Their high content of protein, carbohydrates, fiber, certain minerals and vitamins make them a good source of nutrients. Nowadays research on nutraceutical properties of beans is a very hot topic (Rocha-Guzma et al., 2006).

Kidney bean (*Phaseolus vulgaris* L.) is the most widely produced and consumed food legume not only in Africa, India, Latin America and Mexico (FAO, 1993) but worldwide. These beans have reported to have numerous health benefits, e.g. heart and renal disease risks (Anderson et al., 1999); lower glycemic index for persons with diabetes (Viswanathan et al., 1989); increased satiation (Leathwood, Pollet, 1988) and cancer prevention (Hangen, Bennink, 2002). Furthermore, kidney beans are regarded as an important source of protein and minerals for livestock feed production, as well as, potential raw materials for processing into human food (Gupta, 1987; Salunkhe, 1982; Kahlon et al., 2005).

The low incidence of diseases related to blood circulation in Asian population is ascribed, in part, to a relatively high consumption of legumes (Kahlon et al., 2005).

However, legumes are an underutilised commodity in most regions of the world due to long cooking time (Deshpande et al., 1984; Nasar-Abbas et al., 2008), and the belief that legumes are of low nutritive value due to the presence of flatulence and anti-nutritional factors (Adsule et al., 1989). However, legumes are potentially valuable dietary components due to a protein content between 20% and 50%, and the presence of complex carbohydrates, especially dietary fibre and water soluble polysaccharides, which give them low glycaemic potency (Ravindran et al., 2011).

The antinutrients (e.g. trypsin inhibitors, phytic acid, saponins, phytoheamagglutinins and tannins) and α-galactosides (e.g. raffinose, stachyose and verbascose) are some of the undesirable components in beans that could limit their protein and carbohydrate utilization (Shimelis, Rakshit, 2007).

Unfortunately, the antinutritional factors reduce biological activity of several chemical compounds or metabolites (Rocha-Guzma et al., 2006).

All processing methods tend to modify the composition and availability of nutrients in raw materials. Among the various technologies, short-time high-temperature extrusion cooking is a well known cost-effective industrial process. Extrusion combines high pressure with a moderately high-temperature and usually high shear for a short period of time. Extrusion processing completely gelatinises the starch and partially or completely destroys antinutritional factors present in many legumes (Melcion, Poel, 1993; Ravindran et al., 2011). Extrusion-cooking technology is a versatile and efficient method of converting raw materials into finished food products. It can replace many conventional processes in food and feed industries. It has been used to develop various types of snack foods, mainly from corn meal, rice, wheat flour, or potato flour, in many shapes and variety of textures. Application of extrusion process for legume flours is a relatively new area, with the exception of soy bean. Several reports show that bean starches have very good expansive and functional properties under extrusion conditions (Gujska, Khan, 1991). According to Gujska, Czarnecki, and Khan (1996), extrusion cooking has good potential for making desirable bean forms that could be economically available in developing countries. Benefits of beans extrusion-cooking are deactivation of heat labile growth inhibitors (Aguilera et al., 1984). Sosulski (1988) claims that legumes flour has not been used widely in foods because of the poor functionality of starch (Rocha-Guzma et al., 2006).

Aim of this research was to develop a new type of bean (*Phaseolus vulgaris* L.) products with various additives using one screw extrusion, define their quality (physical and chemical properties) and ascertain people opinion about such products.

Materials and Methods

White beans (*Phaseolus vulgaris* L.) were boiled, then crushed with hand meat-grinder and extruded by

L Series Göttfert Werkstoff Single screw laboratory extruder Different additives were added before extrusion (Table 1). Additives used in experiment were as shown in Table 1.

Table 1

Amount of additives to samples

No	Additive	Amount of additive,%	Amount of salt,%
1	Control (without additive)	0	0
2	Chia seeds	1	2
3	Nettle powder	3	0.3
4	Caraway-seeds	1	0.3
5	Fresh bell pepper	20	0.5
6	Salt	-	2
7	Beetroot	12	0.3
8	Spinach	12	0.3
9	Almond essence	0.5	0.1
10	Lemon essence	0.5	0.1
11	Thyme powder	0.1	0.3
12	Tomato paste	15	0.2
13	Garlic powder	1	0.3

Sample without additives was used as a control.

Extrusion process was performed at temperature regimes 90–95–105 °C. Before extrusion dry matter of samples was measured using dry matter weights *Precisa XM120*.

After extrusion samples (size of one piece – 15×20 mm) were dried in a rotary type oven *SVEBA DAHLIN S400* for 2 hours at 90 °C temperature. Dry samples were packaged in conventional two layer laminated polymer polyethylene/polyamide (PE/PA) pouches (200×300 mm, material thickness 20/45±2 μm) and then hermetically sealed by chamber type machine *MULTIVAC C300*. Mass of the sample in each package was 100±5 g. Quality of the extruded products was analysed at Faculty of Food Technology and Scientific laboratory of Agronomy Research. Some of samples were tested at the international exhibition "Riga Food 2014", to roughly estimate the consumers' interest in such products.

The total dry matter content was determined according to ISO 6496, 1999. Container with lid for half an hour was dried at 103±3 °C temperature, then cooled to room temperature in desiccators. Weigh was measured with an accuracy of 1 mg. 5 g of sample weight of to the nearest 1 mg were placed in container and put in the drying oven at 103±3 °C for 4.0±0.1 h. After four hours, the container lid were put on, sample was removed from oven and cooled in a desiccators to room temperature. Sample was weighted with container and absolute dry matter was calculated according to method. All chemical parameters were calculated on absolute dry matter.

Crude protein (CP) was determined using Kjeldahl method. Approximately 0.5 g (to the nearest 0.0001 g) of well crushed sample was weighted. The sample was transferred in a temperature resistant glass flask; where copper catalyst was added and 20 mL of concentrated H_2SO_4. Flask was placed in a preheated *BÜCHI* digest automat K-438 (420±5 °C) and gas vacuum suction cap was fixed on. Wet mineralization went for 1 hour until the solution in flask was colourless and clear. Flask and digested sample was removed from the stove and allowed to cool for 15–20 min. Cooled sample was placed in a distillation unit, 50 mL of water, 80 mL of 40% NaOH were added. Ammonium was distilled in 65 mL of 4% H_3BO_3 solution. The steam distillation was carried out for 220 s. Boric acid solution was titrated with 0.2 M HCl (which concentration was checked with 0.1 M NaOH solution) till pH 4.70. 1 g of sucrose was used as a blank sample. For protein calculations coefficient 6.25 was used (LVS EN ISO 5983-2, 2009).

Ash content was determined according ISO 5984:2002/Cor 1:2005. The sample mass 5±0.0001 g was weighted, placed in the porcelain crucible and placed on *Bomann* stove. After 20 min sample was placed in muffle for 4 hours at 550 °C temperature. When muffle has cooled till 100 °C temperature samples are placed in an exsiccator and cooled till room temperature, then weighted on analytic scale.

Lignified protein content was determinate in two steps. First, acid detergent fibre content was determined according to LVS EN ISO 13906, 2008.

Borosilicate crucible is dried for 4 hours at 103±3 °C, and weighted to the nearest 0.1 mg. Sample is taken 1000±2 mg. Crucible is placed in the analyzer. 100 mL of the ADF solution is added and cooling water is switched on after 5–10 min. After 60±5 min the hardware glass walls are washed with less than 5 mL of ADF solution. The heater was turned off and solution filtered off. The sample was washed 3 times with 30 mL of hot (90–100 °C), distilled water allowing it to stay on sample for 3–5 min. After washing crucible with the sample was transferred to the cold extraction unit and washed with 25 mL of acetone and filtered. Then crucible with the sample was placed in the drying oven at 103±3 °C for more than 5 hours or 130±3 °C for more than 2 hours (LVS EN ISO 13906, 2008).

Second, after determining the ADF fraction, dried sample was transferred in high temperature resistant glass flask and proceeded the same as crude protein. For lignified protein determination sample weigh before ADF determination is used in calculations (Undersander et al., 1993).

Sodium and iron was determined according to LVS EN ISO 6869. After ash content was determined, ash was transferred in to volumetric flask and added 10 mL 6 M HCl solution. Solution was dried on hot plate. Then residue dissolved in 5 mL 6 M HCl and transferred with some 5 mL portions of hot water in 50 mL volumetric flask. Cooled and diluted to the mark with

water. After particles had settled quantity of test samples is diluted with water, 100 mL of lanthanum nitrate solution, 5 mL of caesium chloride solution and 5 mL of 6 M HCl acid solution. Then atomic absorbance is measured. Solution is diluted as much is needed to obtain absorbance in the linear part of calibration curve, which is made from known sodium or iron quantity solution. Blank sample is made using water instead of the test sample.

pH was determined according to ГОСТ 26180-84. 10 g of well crushed sample was placed in a volumetric flask. Water was added till 100 mL. After 5 hours pH was measured.

Colour of product was measured in CIE L*a*b* colour system using *Tristimulus* Colorimeter, measuring Hunter colour parameters by Colour Tec PCM/PSM. Colour values were recorded as L* (brightness) – the vertical co-ordinate runs from L* = 0 (black) through grey to L* = 100 (white); a* (-a, greenness, +a, redness) – the horizontal co-ordinate, that runs from -a* (green) through grey to +a* (red) and b* (-b, blueness, +b, yellowness) – another horizontal co-ordinate, that runs from -b* (blue) through grey to +b* (yellow) (Papadakis et al., 2000). Samples were crushed and bolted trough 1 mm sieve. Samples were densely pressed in a Petri plate with diameter 5 cm and colour was measured at least ten times at randomly selected locations of each sample.

Colour difference (ΔE*) of bean products from control sample was calculated using the following equation 1:

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

where ΔE^* − colour difference of the product, L^* − products colour intensity value, L_0^*− control samples colour intensity value, a^* − products value of colour component green − red, a_0^* – control samples value of colour component green − red, b^* − products value of colour component blue − yellow, b_0^* – control samples value of colour component blue − yellow.

During international exhibition “Riga Food 2013” 150 exhibition visitor as potential consumer opinion about such products were asked using 5 point scale for likeness. Where score 1 means “does not like at all” and score 5 – “likes very much”.

Data were analysed with correlation, ANOVA single factor analyses using Microsoft Excel 2007, Data Analysis, confidence level was taken 95% (α=0.05).

Results and Discussion

The dry matter content of prepared samples before extrusion was 38.0 g 100 g^{-1} in average (Figure 1). Sample with bell peppers contained only 34.6±0.5 % of dry matter, while samples with chia seeds and nettles– 38.8±0.5 % of dry matter each. The largest dry matter 39.30±0.5 % was detected in a sample with spinach additive.

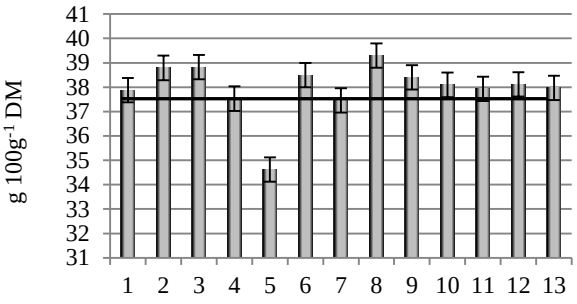

Figure 1. Dry matter of samples before extrusion
1 – control 2 –chia seeds; 3 –nettle; 4 –caraway seeds; 5 – bell peppers; 6 – salt; 7 –beetroot; 8 – spinach; 9 – almond essence; 10 – lemon essence; 11 – thyme; 12 – tomato; 13 – garlic; ── line for control sample

Dry matter in extruded bean products is shown in Figure 2. The highest dry matter content was in samples with bell peppers, 93.13±0.01 g 100 g^{-1}, and samples with salt 93.07±0.03 g 100g^{-1} but the lowest in a sample with almond essence – 88.45±0.02 g 100 g^{-1} and in control sample it was 95.13±0.05 g 100 g^{-1} of product, that could be explained with vaporization of essence.

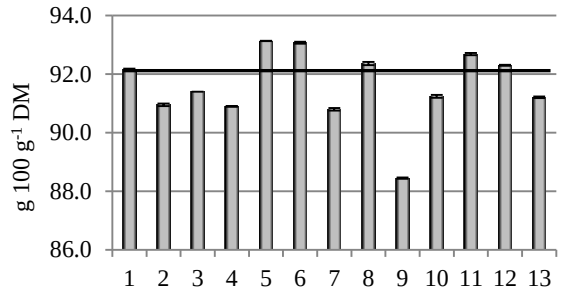

Figure 2. Dry matter in extruded bean products
1 – control 2 –chia seeds; 3 –nettle; 4 –caraway seeds; 5 – bell peppers; 6 – salt; 7 –beetroot; 8 – spinach; 9 – almond essence; 10 – lemon essence; 11 – thyme; 12 – tomato; 13 – garlic; ── line for control sample

Significant differences were found in protein content (Figure 3) among extruded products ($p=3.2\times10^{-6}$). The highest protein content was found in garlic containing products – 26.7±0.3 g 100 g^{-1} of product, but the less in those ones with tomato – 23.2±0.2 g 100 g^{-1} of product. They were the ones that made the changes significant. Mathematical analysis showed that there was no significant difference among other investigated sample protein content on dry matter. Protein content in a control sample was 26.2±0.2 g 100 g^{-1}. Relatively high protein content of samples with spinach can be explained b fact that fresh, raw spinach contains till 30% protein of dry matter. So the crude protein loss was not significant despite fresh material.

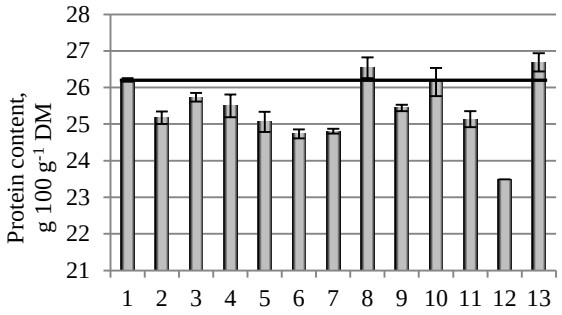

Figure 3. Protein content in extruded bean products
1 – control 2 –chia seeds; 3 –nettle; 4 –caraway seeds; 5 – bell peppers; 6 – salt; 7 –beetroot; 8 – spinach; 9 – almond essence; 10 – lemon essence; 11 – thyme; 12 – tomato; 13 – garlic — line for control sample

The content of ash in extruded been products is shown Figure 4. The lowest ash content is determined in control sample – 3.46±0.07 g 100 g^{-1} of products dry matter, sample with lemon essence – 3.49±0.04 g 100 g^{-1} of products dry matter and almond essence – 3.52±0.01 g 100 g^{-1} of products dry matter.

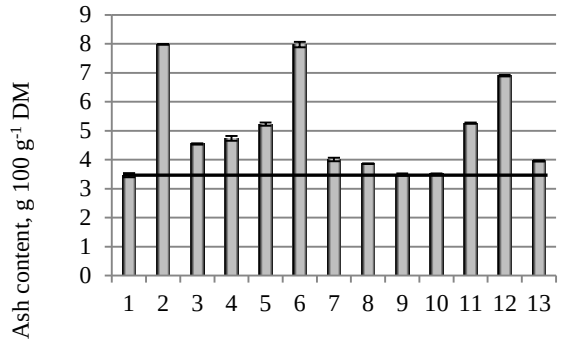

Figure 4.The content of ash in extruded bean products
1 – control 2 –chia seeds; 3 –nettle; 4 –caraway seeds; 5 – bell peppers; 6 – salt; 7 –beetroot; 8 – spinach; 9 – almond essence; 10 – lemon essence; 11 – thyme; 12 – tomato; 13 – garlic — line for control sample

However the highest ash content was detected in samples with salt and chia seed addition 7.98±0.01 g 100 g^{-1} and 7.97±0.09 g 100 g^{-1} of product, respectively. Significant differences were found between all samples ($p=3.54\times10^{-17}$) ash content, except for samples with almond and lemon essence who had no significant differences form control.

Lignified protein content of crude protein (CP) in samples was 3.40±0.04 g 100 g^{-1}, however in beans lignified protein of CP can be even 8.2 g 100 g^{-1}, as it is indicated in feed database (Interactive, 2014), so we can conclude, that no significant amount of lignified protein has been formed in extrusion process.

Sodium content in control sample was 0.02530±0.0002 g 100 g^{-1}, but with chia seeds – 1.93±0.03 g 100 g^{-1} and in the sample with salt 2.09±0.07 g 100 g^{-1} of product. Thus we can see control sample contains only little traces of sodium.

Figure 5 shows iron content of samples that had additives with elevated iron content.

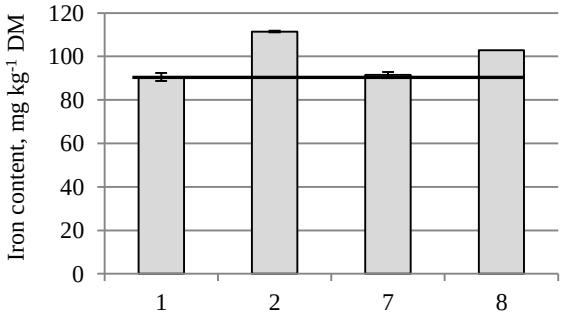

Figure 5.The content of iron in selected extruded bean products
1 – control 2 – chia seeds; 7–beetroot; 8 – spinach — line for control sample

Control sample and sample with beetroot had no significant differences. Control sample contained 90±2 mg kg^{-1} iron in dry matter of and sample with beetroot contained 91.4±0.3 mg kg^{-1} iron in dry matter. Samples with chia seeds and spinach had higher iron content 111±2 mg kg^{-1} and 103±1 mg kg^{-1}, respectively.

The values of extruded bean sample colour components L* a* b* are summarised in Table 2.

Table 2

Colour components of extruded bean products

Added additive	Value		
	L*	a*	b*
Control (without additive)	*82.9±0.3*	*-1.5±0.1*	*15.9±0.3*
Chia seeds	78.6±0.2	-1.1±0.3	14.3±0.4
Nettle	63.2±0.4	-1.7±0.1	16.6±0.4
Caraway-seeds	75.4±0.7	-1.5±0.3	17.5±0.4
Bell peppers	71.8±0.4	2.6±0.3	24.7±0.6
Salt	76.4±0.6	-1.1±0.3	15.5±0.8
Beetroot	55.9±0.5	16.8±0.4	1.1±0.2
Spinach	69.7±0.3	-2.0± 0.2	19.9±0.4
Almond essence	77.8±0.3	-1.4±0.1	17.2±0.3
Lemon essence	78.0±0.2	-1.7±0.2	16.4±0.6
Thyme	74.7±0.2	-1.5±0.3	15.5±0.4
Tomato	74.7±0.3	-0.8±0.2	22.2±0.3
Garlic	76.0±0.4	-1.3±0.2	16.3±0.5

Sample with added beetroot was darker, described by the lowest L* value 55.9±0.5 as well as the highest red one – 16.8±0.4 and the smallest b* value 1.1±0.2. While the lightest one was found control sample without additives described by colour components as follows: L*=82.9±0.3; a*=-1.5±0.1 and b*=15.9±0.3. The changes of colour components compared to a control sample are summarized in Table 3.

The colour of sample with beetroot differs from other extruded products.

Table 3

The changes of colour components compared to control sample

Added additive	Value			ΔE
	L*	a*	b*	
Chia seeds	-4.3	0.4	-1.6	4.6
Nettle	-19.7	-0.2	0.7	19.7
Caraway-seeds	-7.5	0.0	1.6	7.7
Bell peppers	-11.1	4.1	8.8	14.7
Salt	-6.4	0.4	-0.4	6.5
Beetroot	-27.0	18.3	-14.8	35.8
Spinach	-13.2	-1.2	3.9	13.8
Almond essence	-5.1	0.1	1.3	5.3
Lemon essence	-4.9	-0.2	0.5	4.9
Thyme	-8.2	0.0	-0.4	8.2
Tomato	-8.2	0.7	6.2	10.3
Garlic	-6.9	0.2	0.4	6.9

So we can see that beetroot gives samples the darkest colour, followed by nettle and spinach. Red colour is more in samples with bell peppers too, but not as much as in one with beetroot. Green colour can be obtained with nettle, spinach and lemon essence additives but yellow with bell peppers, tomato and spinach additives. pH was measured and significant differences were found ($p=1.15\times10^{-20}$). All samples can be divided in four groups, according to pH (Fig. 6).

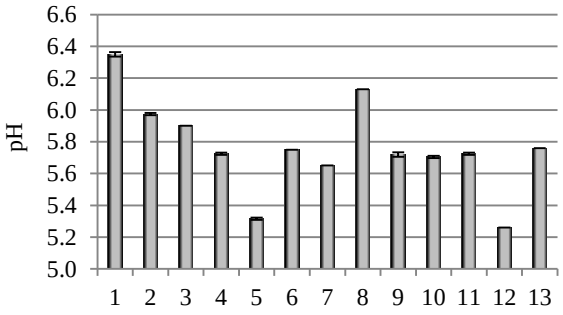

Figure 6. pH of extruded bean products

1 – control 2 – chia seeds; 3 –nettle; 4 –caraway seeds; 5 – bell peppers; 6 – salt; 7 – beetroot; 8 – spinach; 9 – almond essence; 10 – lemon essence; 11 – thyme; 12 – tomato; 13 – garlic

Products with pH>6.0 relates to the first group including control sample as well as sample with spinach additive, the second one with pH 5.8–6.0 would contain samples with nettle and chia seeds, the third one with pH 5.6–5.8 would be the largest one including samples with garlic, salt, thyme, caraway seed, almond an lemon essences and beetroot additives. In last group with pH<5.6 would be samples with bell peppers and tomato additives. The average pH value of all samples was 5.77±0.01, but for control sample it was 6.35±0.01.

Correlation was observed between Na and Fe content r=0.98; negative correlation between Na and pH r=0.96; dry matter and Fe content r=0.88; Na and dry matter content r=0.86; negative correlation between protein and Na – r=-0.68 and protein and pH content r=0.64.

The visitors in International exhibition "Riga Food 2014" were surveyed for their opinion of extruded bean products. Most likable to respondents seemed product with bell peppers and salt (3.6 and 3.3 points on average in 5 point scale), but the least they liked products with caraway seeds and lemon essence (2.6 and 2.8 in 5 point scale). Other tasted products were valued approximately by 3 points in 5 point scale.

Conclusions

Dry matter content of extruded bean products was in range from 88.45 g 100 g^{-1} to 93.13 g 100 g^{-1}, in a control sample it was 92.15 g 100 g^{-1}. Protein content was in range 23.49 g 100 g^{-1} till 26.69 g 100 g^{-1}, in control sample – 26.21 g 100 g^{-1}, and ash content 3.46 till 7.98 g 100 g^{-1}. Highest iron content was in samples with chia seeds 111±2 mg kg^{-1} on dry matter and spinach and 103±1 mg kg^{-1} on dry matter.

pH value was in range from 5.26 to 6.35. The highest pH has a control sample, all samples with additives had lower pH, except form lemon and almond essences. As expected high correlation have been observed between ash and sodium and iron content (r=0.98) and sodium and pH (r=-0.96). Not so close correlation was observed between dry matter and iron content (r=0.88), protein and sodium content (r=-0.68), protein and pH content (r=0.64) and sodium and dry matter content before extrusion (r=0.86).

References

1. Adsule R.N., Lawande K.M., Kadam S.S. (1989) Pea. **In**: *Handbook of world food legumes*: *Nutritional chemistry, processing technology and utilization* Vol. III. D. K. Salunkhe, S. S. Kadam (eds). Florida: CRC Press, p. 215–251.
2. Anderson J.W., Smith B.M., Washnock C.S. (1999) Cardiovascular and renal benefits of dry bean and soybean intake. *American Journal of Clinical Nutrition*, Vol. 70, p. 464–474.
3. Aguilera J.M., Crisafulli E.B., Lusas E.W., Uebersax M.A., Zabik M.E. (1984). Air classification and extrusion of navy bean fraction. *Journal of Food Science*, Vol. 49, No. 4, p. 543–548.
4. Deshpande S.S., Sathe S.K., Salunkhe D.K. (1984) Interrelationships between certain physical and chemical properties of dry bean (*Phaseolus vulgaris* L.). *Plant Foods for Human Nutrition*, Vol. 34, p. 53–65.
5. FAO (1993) Production Yearbook 1992, Vol. 26. *Food and Agriculture Organization of the United Nations*, Rome.
6. Gujska E., Czarnecki Z., Khan, K. (1996) High temperature extrusion of pinto bean (*Phaseolus vulgaris*) and field pea (*Pisum sativum*) flours. *Polish Journal of Food and Nutrition Science*, Vol. 46 No. 1, p. 51–60.
7. Gujska E., Khan, K. (1991) Functional properties of extrudates from high starch fractions of Navy and Pinto

beans and corn meal blended with legume high protein fractions. *Journal of Food Science*, Vol. 56, No. 3, p. 431–435.
8. Gupta Y. P. (1987) Antinutritional and toxic factors in food legumes: A review. *Plant Foods for Human Nutrition*, Vol. 37, p. 201–228.
9. Hangen L., Bennink M.R. (2002). Consumption of black beans and navy beans (*Phaseolus vulgaris*) reduced azoxymethane-induced colon cancer in rats. *Journal of Nutrition and Cancer*, Vol. 44, p. 60–65.
10. Interactive Feed Composition Libraries [accessed on 5.03.2014] Available at: http://dairyone.com/analytical-services/feed-and-forage/feed-composition-library/ interactive -feed-composition-library/
11. Kahlon T. S., Smith G.E., Shao Q. (2005) In vitro binding of bile acids by kidney bean (*Phaseolus vulgaris*), black matpe bean (*Vigna mungo*), Bengalgram (Cicera rietinum) and moth bean (Phaseolus aconitifolia). *Food Chemistry*, Vol. 90, p. 241–246.
12. Leathwood P., Pollet, R. (1988) Effects of slow release carbohydrates in the form of bean flakes on the evaluation of hunger and satiety in man. *Appetite*, No. 10, p. 1–11.
13. Melcion J. P., van der Poel A. F. B. (1993). Process technology and antinutritional factors: Principles, adequacy and process optimization. **In:** *Recent advances of research in antinutritional factors in legume seeds*. A. F. B. van der Poel, J. Hulsman, H. S. Saini (eds). Wageningen: Wageningen Press, p. 419–434.
14. Nasar-Abbas S.M., Plummer J.A., Siddique K.H.M., White P., Harris D., Dods K. (2008) Cooking quality of faba bean after storage at high temperature and the role of lignins and other phenolics in bean hardening. *LWT-Food Science and Technology*, Vol. 41 p. 1260–1267.
15. Papadakis S.E., Abudal-Malek S., Kamden R.E., Yam K.L. (2000) A Versatile and Inexpensive Techniques for Measuring Colour of Foods, *Food Technology*, Vol. 54, No. 12, p. 48–51.
16. Ravindran G., Carr A., Hardacre A., (2011) A comparative study of the effects of three galactomannans on the functionality of extruded pea–rice blends. *Food Chemistry*, Vol. 124, p. 1620–1626.
17. Rocha-Guzman N.E., Gallegos-Infante J.A., Gonzalez-Laredo R.F., Castillo-Antonio P.A., Delgado-Licon E., Ibarra-Perez F. (2006) Functional properties of three common bean (*Phaseolus vulgaris*) cultivars stored under accelerated conditions followed by extrusion, *LWT- Food science and technology* , Vol. 39 p. 6–10.
18. Salunkhe D.K. (1982) Legumes in human nutrition. Current status and future research needs. *Current Science*, Vol. 51, p. 387–394.
19. Shimelis E.A., Rakshit S.K. (2007) Effect of processing on antinutrients and in vitro protein digestibility of kidney bean (*Phaseolus vulgaris* L.) varieties grown in East Africa. *Food Chemistry*, Vol. 103, p. 161–172.
20. Sosulski F. (1988). Preparation and uses of vegetable food protein made by dry processes. **In:** *World congress "Vegetable protein utilization in human foods animal feed stuffs"*; Conference Proceedings, USA: Applewhite, p. 553.
21. Undersander D., Mertens D.R., Thiex N. (1993) *Forage analyses procedures, method 6 Acid Detergent Insoluble Nitrogen and Acid Detergent Fiber Crude Protein*, National Forage Testing Association USA, p. 99-105 [accessed on 11.03.2014] Available at: http://www. foragetesting.org/files/LaboratoryProcedures.pdf.
22. Viswanathan M., Ramachandran A., Indira P., Snehalatha C., Mohan V., Kymal P.K. (1989) Responses to legumes in NIDDM subjects lower plasma-glucose and higher insulin levels. *Nutrition Reports International*, Vol. 40, p. 803–812.

SHREDDED CARROTS QUALITY PROVIDING BY TREATMENT WITH HYDROGEN PEROXIDE

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Abstract

Carrots are a vegetable crop providing a source of important nutritional compounds, but their shelf-life, especially for shredded carrots, is not so long. Minimally processed carrots are very common in developed countries and are gaining popularity due to their convenience and freshness. Hydrogen peroxide (H_2O_2) is also a well-studied oxidant agent, directly toxic to pathogens. It has both bacteriostatic and bactericidal activity.

The purpose of the present research was to investigate H_2O_2 influence on shredded carrots chemical composition. Shredded carrots were treated with 0.5, 1.0 and 1.5% H_2O_2 water solution for 30±1 s, 60±1 s and 90±1 s. The major sugars (fructose, glucose and sucrose) were determined by applying the method of high performance liquid chromatography (HPLC). The total phenol content was determined spectrophotometrically according to the Folin-Ciocalteou method and antioxidant activity was determined using the $DPPH^{\bullet}$ (2.2-diphenyl-1-1-picrylhydrazyl radical) assay.

Significant influence of H_2O_2 water solution concentration and treatment time on analyzed shredded carrots quality parameters was found. It was detected that antioxidant activity decrease by approximately 28% in average in shredded carrots after treatment with disinfectant, total phenolic content by approximately 43% in average and total sugars by approximately 30% in average. For the maximal quality parameters preservation recommendable H_2O_2 water solution concentration is 1.5% and treatment time 30±1 s. As a result the content of total phenolic decreases by 35%, antioxidant activity by 20%, total sugars by 19%. As well, the content of fructose decreases by 8%, glucose by 18% and sucrose by 33%.

Keywords: total phenols, antioxidant activity, sugars, H_2O_2.

Introduction

Vegetables are an important part of our diet. They provide, not only the major dietary fiber component of our food, but also a range of micronutrients, including minerals, vitamins and antioxidant compounds, such as carotenoids and polyphenols. The nutritional value of fruit and vegetables is often associated with their antioxidant capacities (Singha et al., 2012).

Fresh-cut carrots (*Daucus carota* L.) could be finding on the market place as: whole peeled (baby), sticks, and sliced, shredded, grated and diced. Carrot is considered one of the vegetables whose consumption, both fresh and processed, has increased over the past years due not only to the nutritional and health benefits this vegetable provides, but also to the introduction of new carrot-derived products (Augspole, Rakcejeva, 2013).

One of the procedures involved in fresh-cut fruit and vegetable processing is cutting the product into smaller pieces. This cutting action essentially is causing the tissue to endure wounding stress. Wounding will cause some physiological effects to the tissue and it is understood that a more severe cutting process will elicit a greater wounding response decreasing the quality of the produce (Surjadinata, Cisneros-Zevallos, 2012).

Along with carotenoids, phenolic compounds also reveal antioxidative properties in vegetables. Phenolic compounds, especially flavonoids, show various types of biological activity, but the most important is the antioxidative activity. Still, it should be emphasized that phenolic compounds, beside coumarin compounds and lignins, are also synthesized in external carrot tissue as a result of mechanical injuries during harvest. This causes negative changes in the quality of storage roots, such as enzymatic browning or deterioration of taste and aroma as a result of enzymatic oxidation of pigments and unsaturated fatty acids (Smoleń, Sady; 2009). Phenolic compounds can act as antioxidants by interfering with oxidation processes through chain-breaking reaction activities (primary oxidation) or through scavenging of free radicals (secondary oxidation). The antioxidant properties of phenolic compounds stimulate the need to design strategies to enhance its content in plant tissues (Heredia, Cisneros-Zevallos, 2009).

The content of total phenolics in carrots ranges from 539.76 to 395.35 mg 100 g^{-1} (GAE) gallic acid equivalents of dry weight (Augspole et al., 2012). Phenolic acids and isocoumarins, predominant phenolics in carrots, contribute to plant tissue's defense mechanism against infections or injuries. Phenolic acid content in carrot peels may increase up to seven-fold when carrots are subjected to abiotic stress during postharvest handling and storage. Phenolic have also been associated with imparting bitter and sour tastes to stored and processed carrots and may result in color loss in processed strained carrots. The total content of phenolic acids in fresh carrots ranges from 77.2 mg kg^{-1} of fresh weight for yellow varieties to 746.4 mg kg^{-1} of fresh weight for purple varieties; in processed strained carrots the amount is between 24.9 and 156.2 mg kg^{-1} of dry weight (Socaciu, 2008).

Carrots, in particular, are noted for their rich antioxidants, especially β-carotene. In recent years, worldwide consumption of carrots has been steadily increasing because of their nutritional benefits. Carrots carry other potentially beneficial health effects, boasting anti-carcinogenic, anti-aging, antioxidant, and

immune-boosting properties, as well as the pro-vitamin activity of some carotenoids, all of which add to their importance in the diet (Yena et al., 2008). In the specific case of carrots, the most important micronutrient is β-carotene, which is a lipid-soluble carotenoid. It's typical chemical structure, consisting of a polyene chain with 11 conjugated double bonds and a β-ring at each end of the chain, gives β-carotene some health related properties (Knockaert et al., 2013).

Among the frequently used processes applied with the purpose of reducing/eliminating pathogens from food, heat-based treatments are the most efficient ones. Hydrogen peroxide (H_2O_2) is also a well-studied oxidant agent, directly toxic to pathogens. It has both bacteriostatic and bactericidal activity, also due to its capacity to generate other cytotoxic oxidizing species, such as hydroxyl radicals. At a concentration between 1 and 5%, hydrogen peroxide is generally used as sanitizer of some food contact surfaces and packaging materials in aseptic filling operations. The antimicrobial efficiency of hydrogen peroxide at higher concentrations (4–5%) is comparable to 100–200 ppm of chlorine treatment (Alexandre et al., 2012).

Fresh cut fruits and vegetables have been very popular for the bioavailability of numerous vitamins, minerals and other phytochemicals. However, they may naturally contain a wide variety of bacteria, fungi and yeast species. Commercial or homemade fresh cut fruits and vegetables are prepared by some simple treatments such as washing, cutting, grating, shredding and packaging. Among these steps, washing may be considered as the most critical step since it removes the soil particles and reduces the microbial load from the surface. Antimicrobial effects of chlorine based sanitizers on fresh cut produce have been previously reported, because they have been widely used as washing solution for fresh cut products to eliminate the microorganisms (Tornuka et al., 2012).

After summarizing data form scientific literature it was concluded, that hydrogen peroxide has mainly microbiological effect on treated food products. But not complete data was found about hydrogen peroxide influence on carrots chemical composition especially on antioxidant activity, phenols and sugars content. Therefore future experiments are needed. The purpose of the present research was as follow – to investigate hydrogen peroxide influence on shredded carrots chemical composition.

Materials and Methods

Materials

The research was accomplished on serotinous 'Nante' carrot (*Daucus carota* L.) hybrids 'Nante/Forto' harvested in Zemgale region (Latvia) in the first part of October 2012 and was immediately used for experiments.

Carrots were rinsed under the tap water, than peeled and shredded using Philips Comfort HR 7605 device.

Shredded carrots were treated with 0.5, 1.0 and 1.5% H_2O_2 water solution for 30±1 s, 60±1 s and 90±1 s.

As a control sample non-treated carrots was analysed for comparison.

H_2O_2 water solution preparation

To prevent degrading of the hydrogen peroxide solutions were prepared by mixing food grade concentrated hydrogen peroxide 30 g 100 g^{-1} (Peróxidos do Brasil Ltda, Curitiba, Brazil) with sterile deionized water; solution was prepared one minute before the treatment process (Delgado et al., 2012; Watson et al., 2007).

Total phenolic content

The total phenolic content of carrots was determined by using Folin-Ciocalteu assay. An aliquot (1 mL) of extracts or standard solution of gallic acid 20, 40, 60, 80 and 100 mg L^{-1}) was added to 25 ml volumetric flask, containing 9 ml of distilled deionized water. Reagent blank using distilled deionized water was prepared. One milliliter of Folin-Ciocalteu's phenol reagent was added to the mixture and shaken. After 5 min, 10 mL of 7% Na_2CO_3 solution was added to the mixture. The solution was diluted to volume 25 mL with distilled deionized water and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank was determined at 750 nm with spectrophotometer JENWAY 6300 (Baroworld Scientifid Ltd., UK). Total phenolic content of carrots was expressed as mg gallic acid equivalents (GAE) 100 g^{-1} in fresh weight (Baydar et al., 2006; Marinova et al., 2005). Then for result veracity a phenolic content of carrots was expressed as mg gallic acid equivalents (GAE) 100 g^{-1} in dry matter.

Antioxidant activity

The antioxidant activity was measured by the $DPPH^{\cdot}$ radical method according to A.L.K. Faller and E. Fialho, 2010 (Faller, Fialho; 2010). The antioxidant reaction was initiated by transferring 0.5 mL of carrot extract into a sample cavity containing 3.5 mL of freshly prepared $DPPH^{\cdot}$ methanol solution (0.004 g $DPPH^{\cdot}$ to 100 mL methanol). After 30 min of incubation in the dark at room temperature, the absorbance was measured at 517 nm with spectrophotometer JENWAY 6300 (Baroworld Scientifid Ltd., UK). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Each determination was performed in triplicate, and the results were expressed as mean ± SD. Inhibition of $DPPH^{\cdot}$ in percentage (I%) of each carrot sample was calculated from the decrease of the absorbance according to the relationship:

$$I\% = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100 \qquad (1)$$

where A_{blank} is the absorbance of the control reaction (methanol–water with $DPPH^{\cdot}$), and A_{sample} is the absorbance of a carrot sample (Faller, Fialho, 2010).

Sugars

The content of glucose, fructose and sucrose of carrots grown in Latvia is determined by applying the method of high performance liquid chromatography (HPLC).

The method is based on the fact that the chromatographic separation of glucose, fructose and sucrose is based on their delayed time differences. To 5±0.01 g of sample 20 mL of water was added into a 50 mL volumetric flask, heated for 20 min at 60 °C in a water bath and cooled to ambient temperature (20±2 °C). Then, 1ml of Carrez I and 1 mL of Carrez II solutions were added and shaken. A volumetric flask was filled up with water till the mark and shaken well. First, solution was filtered through the paper filter. The obtained extract was filtered through a membrane filter with pore size of 0.2 μm. Second, extract was placed in a vial and tested by HPLC Prominence (Shimadzu, Japan) equipped with SypelcosilTM LC-NH2 column (250×4.6 mm, particle size – 5 μm) and autosampler SIL-20A. Sugars were detected with a refractive index detector RID-10A (Shimadzu); acquired data were processed using Shimadzu LabSolutions software (LCsolution Version 1.21 SP1). Acetonitrile: water (80:20 v/v) was used as eluent while column temperature was held at 30 °C. The flow rate was 1.0 mL min^{-1}. Injection volume of samples was 10 μL. Calibration curve was acquired after two repeated HPLC runs of seven standard solutions of reference compounds.

The chromatography data processing system fixes the composition of glucose, fructose, and sucrose in carrots by comparing the carrot chromatography with the chromatography of sugar standard-solution.

Mathematical data processing

Data are expressed as mean ± standard deviation; for the mathematical data processing p-value at 0.05 (Two Way analysis of variance, ANOVA), was used to determine the significant differences. In case of establishing statistically significant differences, homogeneous groups were determined by Tukey's multiple comparison test the level of confidence α=0.05. The statistical analyses were performed using Microsoft Excel 2007.

Results and Discussion

Total phenolic content

The presence of phenolic compounds in carrots contributes to their sensory qualities, like colour, bitterness, or aroma. Therefore, the response of phenolic compounds could be used as a good indicator to evaluate the vegetables quality during processing and storage. Major phenols in carrots include chlorogenic, caffeic, and p-hydroxybenzoic acids along with numerous cinnamic acid derivatives. The different carrot tissues have similar composition, but the individual phenolic content differs and it decreases from the exterior (peel) to the interior (xylem). Moreover, the reported data may vary with the extraction method, the way to express the results, and other factors such as cultivars, post-harvest and processing conditions (Gonçalves et al., 2010).

In the present research significant influence ($p<0.05$) of H_2O_2 water solution concentration and treatment time on total phenolic content amount was found. The content of total phenolic content decreases equally by 43%, comparing with initial content of total phenolic, amount in non-treated shredded carrots (Fig. 1).

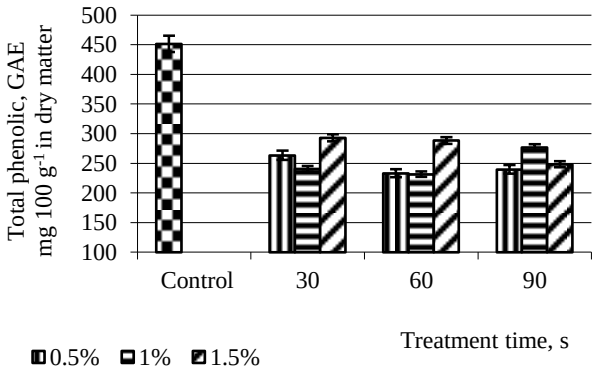

Figure 1. Total phenolic content in carrots

It was proved, that comparing the influence of different H_2O_2 concentrations on the content of total phenolic there are not significant differences ($p=0.962$). The similar tendency was observed analyzing the influence of treated time 30–90 s ($p=0.793$).

Acquired results mainly could be explained with possible intensive oxidation processes occurring in carrots. Very similar data was detected of shredded carrots samples treated with 1.5% H_2O_2 water solutions for 30 and 60 s (Fig. 1), however, the content of total phenolic was higher as 292.85±0.02 and 288.54±0.08 (GAE) mg 100 g^{-1} in dry matter respectively.

Antioxidant activity

Assessments of antioxidant properties of natural compounds are very important because of their uses in medicine, food and cosmetics (Mishra et al., 2012). During the last few years, the study of antioxidant capacity has received much attention, mainly due to the growing interest in the efficiency and function of natural antioxidants in food and biological systems. The antioxidative reactions of free radicals, which are molecules with unpaired electrons, are thought to contribute too many health problems, including cancer, cardiovascular diseases, and inflammatory problems and aging. Antioxidants are agents that, in one way or another, restrict the deleterious effects of these oxidant reactions, either scavenging free radical (eliminating them without generating more radical-induced damage) or other effects (i.e. preventing radical formation) (Fernandez-Orozco et al., 2011).

In the present experiments significant influence ($p<0.05$) of hydrogen peroxide concentration and shredded carrots processing time on antioxidant activity with disinfectant was established. It is indicate, that antioxidant activity decrease by 28% in average in shredded carrots after treatment with H_2O_2 disinfectant (Fig. 2).

After mathematical data processing was proved, that comparing the influence of different H_2O_2 concentrations and treated time 30–90 s on the antioxidant activity there are not significant differences significant difference ($p=0.617$) was not established in

antioxidant activity of with hydrogen peroxide treated shredded carrots. Obtained results are very similar with results on total phenolic contents (Fig 1).

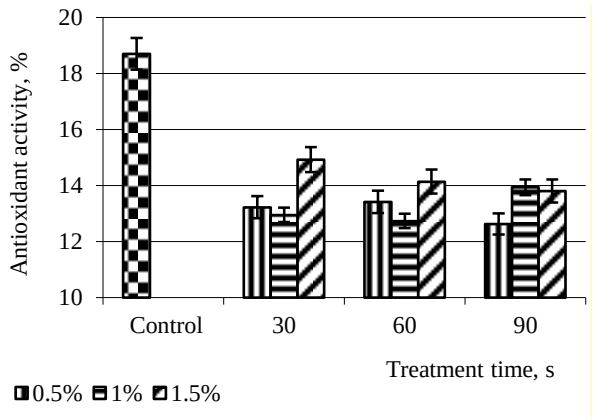

Figure 2. Carrots antioxidant activity

Maximally total antioxidant activity of shredded carrots is possible to maintain by processing with 1.5% hydrogen peroxide water solution for 30s, as a result antioxidant activity value was 14.92±0.03%, while, the total antioxidant activity of non-processed shredded carrots was 18.70±0.04%. Therefore, close interconnection (r=0.953) between total phenolic compounds and total antioxidant activity was detected in the present research.

Sugars

One of the most important qualities of vegetables is their sweetness, closely related to the soluble sugar content (Ozaki et al., 2009). Carrot is mainly constituted by water (approximately 90% of fresh weight) and carbohydrates, which account for 5% of carrot edible portion. In addition to terpenoids, carbohydrates have been reported to be one of the most important sensory indicators for consumer appreciation of this vegetable. As it is known, fructose, glucose and sucrose are the major sugars in carrot and extensive research has been published on their content in carrots of different variety and / or submitted to different processing and storage conditions (Soria et al., 2009).

In the present research negative influence of hydrogen peroxide on total sugar content and fructose, glucose and sucrose content in shredded carrots was detected (Table 1).

In the research obtained results demonstrate, there are not found significant differences ($p>0.05$) in total sugar content if shredded carrots was treated by 0.5% and 1.0% H_2O_2 water solution for 30–90 s. As a result the content of total sugars decreased by 33% in average compared to the control sample. Significant differences ($p<0.05$) in sugars content was found between shredded carrots samples treated with 1.5% H_2O_2 water solution for 30 and 90 s and for 60 and 90 s (Table 1). The content of total sugars decreased by 30% in average, if carrots were treated with different H_2O_2 concentrations for treated time 30–90 s compared to the non-processed shredded carrots. Mainly acquired results could be explained with possible oxidation processes influenced by hydrogen peroxide.

Table 1

Sugars content

H_2O_2 water solution, *%	Time, s**	Sugars, g 100 g^{-1}			Total sugars, g 100 g^{-1}
		Fructose	Glucose	Sucrose	
0	0	0.72±0.04	0.55±0.02	4.51±0.01	5.06±0.02
0.5	30±1	0.46±0.01	0.47±0.01	2.75±0.02	3.68±0.01
	60±1	0.26±0.01	0.45±0.02	2.69±0.01	3.40±0.02
	90±1	0.41±0.02	0.31±0.02	2.27±0.01	299±0.02
1.0	30±1	0.50±0.02	0.66±0.01	2.54±0.02	3.70±0.02
	60±1	0.44±0.04	0.13±0.01	2.45±0.01	3.02±0.01
	90±1	0.65±0.01	0.26±0.01	2.48±0.04	3.39±0.01
1.5	30±1	0.66±0.01	0.45±0.01	3.01±0.02	4,12±0.01
	60±1	0.26±0.01	0.19±0.03	2.76±0.06	3.21±0.01
	90±1	0.37±0.01	0.51±0.01	2.09±0.01	2.97±0.01

*concentration
**treatment time

Conclusions

In the present research significant influence ($p<0.05$) of H_2O_2 water solution concentration and treatment time on analyzed shredded carrots quality parameters was found.

It was detected that antioxidant activity decrease by approximately 28% in average in shredded carrots after treatment with disinfectant, total phenolic content by approximately 43% in average and sugars by approximately 30% in average compared to the non-processed shredded carrots.

For the maximal quality parameters preservation recommendable H_2O_2 water solution concentration is 1.5% and treatment time 30±1 s. In this treatment the

content of total phenolic compounds decrease by 35%, antioxidant activity by 20%, total sugars by 19%. As well, the content of fructose decreases by 8%, of glucose by 18% and of sucrose by 33%.

Acknowledgment

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References

1. Alexandre E.M.C., Brandão T.R.S., Silva C.L.M. (2012) Assessment of the impact of hydrogen peroxide solutions on microbial loads and quality factors of red bell peppers, strawberries and watercress. *Food Control*, Vol. 27, Iss. 2, p. 362–368.
2. Augspole I., Rakcejeva T. (2013) Effect of hydrogen peroxide on the quality parameters of shredded carrots. **In:** *Proceedings of Annual 19th International Scientific Conference Research for Rural Development*, Jelgava, Latvia, p. 91–97.
3. Augšpole I., Rakčejeva T., Dukaļska L. (2012) Changes of phenolic content and antiradical activity in hybrids of 'Nante' carrots during storage. *Chemine Technologija*, Vol. 4, p. 36–39.
4. Baydar N. G., Sagdic O., Ozkan G., Cetin S. (2006) Determination of antibacterial effects and total phenolic contents of grape (*Vitis Vinifera* L.) seed extracts. *Food science and technology*, Vol. 41, Iss. 7, p. 799–804.
5. Delgado D.A., Sant'Ana A.S., Granato D., Massaguer P.R. (2012) Inactivation of Neosartorya fischeri and Paecilomyces variotii on paperboard packaging material by hydrogen peroxide and heat. *Food Control*, Vol. 23, p. 165–170.
6. Faller A.L.K., Fialho E. (2010) Polyphenol content and antioxidant capacity in organic and conventional plant foods. *Food Composition and Analysis*, Vol. 23, Iss. 6, p. 561–568.
7. Fernandez-Orozco R., Roca M., Gandul-Rojas B., Gallardo-Guerrero L. (2011) DPPH-scavenging capacity of chloroplastic pigments and phenolic compounds of olive fruits (cv. Arbequina) during ripening. *Food Composition and Analysis*, Vol. 24, iss. 6, p. 858–864.
8. Gonçalves E.M., Pinheiro J., Abreu M., Brandão T.R.S., Silva C.L.M. (2010) Carrot (*Daucus carota* L.) peroxidase inactivation, phenolic content and physical changes kinetics due to blanching. *Food Engineering*, Vol. 97, Iss. 4, p. 574–581.
9. Heredia J.B., Cisneros-Zevallos L. (2009) The effect of exogenous ethylene and methyl jasmonate on pal activity, phenolic profiles and antioxidant capacity of carrots (Daucus carota) under different wounding intensities. *Postharvest Biology and Technology*, Vol. 51, Iss. 2, p. 242–249.
10. Knockaert G., Pulissery S.K., Lemmens L., Buggenhout S.V., Hendrickx M., Loey A.V. (2013) Isomerisation of carrot β-carotene in presence of oil during thermal and combined thermal/high pressure processing. *Food Chemistry*, Vol. 138, Iss. 2-3, p. 1515–1520.
11. Marinova D., Ribarova F., Atanassova M. (2005) Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *Chemical Technology and Metallurgy*, Vol. 40, Iss. 3, p. 255–260.
12. Mishra K., Ojha H., Chaudhury N.K. (2012) Estimation of antiradical properties of antioxidants using DPPH radical dot assay: A critical review and results. *Food Chemistry*, Vol. 130, iss. 4, p. 1036–1043.
13. Ozaki K., Uchida A., Takabe T., Shinagawa F., Tanaka Y., Takabe T., Hayashi T., Hattori T., Rai A.K., Takabe T. (2009) Enrichment of sugar content in melon fruits by hydrogen peroxide treatment. *Plant Physiology*, Vol. 166, Iss. 6, p. 569–578.
14. Singha D.P., McInerneya J.K., Daya L. (2012) Impact of boron, calcium and genetic factors on vitamin C, carotenoids, phenolic acids, anthocyanins and antioxidant capacity of carrots (Daucus carota). *Food Chemistry*, Vol. 132, Iss. 3, p. 1161–1170.
15. Smoleń S., Sady W. (2009) The effect of various nitrogen fertilization and foliar nutrition regimes on the concentrations of sugars, carotenoids and phenolic compounds in carrot (Daucus carota L.). *Scientia Horticulturae*, Vol. 120, Iss. 3, p. 315–324.
16. Socaciu K. (2008) *Food Colorants. Chemical and Functional Properties*. CRC Press, p. 429–547.
17. Soria A.C., Sanz M.L., Villamiel M. (2009) Determination of minor carbohydrates in carrot (Daucus carota L.) by GC-MS. *Food Chemistry*, Vol. 114, Iss. 2, p. 758–762.
18. Surjadinata B.B., Cisneros-Zevallos L. (2012) Biosynthesis of phenolic antioxidants in carrot tissue increases with wounding intensity. *Food Chemistry*, Vol. 134, Iss. 2, p. 615–624.
19. Tornuka F., Cankurta H., Ozturk I., Sagdic O., Bayram O., Yetim H. (2012) Efficacy of various plant hydrosols as natural food sanitizers in reducing Escherichia coli O157:H7 and Salmonella Typhimurium on fresh cut carrots and apples. *Food Microbiology*, Vol. 148, Iss. 1, p. 30–35.
20. Watson I., Tan B.K., Armstrong G., Stewart-Tull D., Marshall R. (2007) Shelf life extension of carrots and potatoes: A comparison of H_2O_2, laser, UV, and microwave treatments. *IOA Conference and Exhibition Valencia*, October, p. 29–31.
21. Yena Y.-H., Shiha C.-H., Chang C.-H. (2008) Effect of adding ascorbic acid and glucose on the antioxidative properties during storage of dried carrot. *Food Chemistry*, Vol. 107, Iss. 1, p. 265–272.

EFFICIENCY OF THE ADDITIONAL FERTILIZATION WITH NITROGEN FERTILIZERS GROWING CARROT OF EXCEPTIONAL QUALITY AND ITS INFLUENCE ON PRODUCTION STORAGE

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Abstract

Plant nutrition, among other agro-technical measures most influences yield, quality and production storability. The aim of investigations – instilling new technological elements of the growing of root-crop vegetables of exceptional quality to choose for the additional fertilization the most suitable nitrogen fertilizer and its rate in order the soil, environment was polluted as little as possible and the qualitative production was obtained. There were grown carrot 'Nerac' F_1 and 'Tito'. There was investigated the influence of nitrogen fertilizer used for additional fertilization (ammonium nitrate and calcium nitrate) and their rates (N_{30} and N_{15}) on carrot yield, and its impact on production storage. Additional fertilizations were carried out at
2–4 carrot leaves stage. Carrot were grown on profiled surface, sowing scheme 62+8, there were sown 1 mln. unt. ha^{-1} germinable seeds. Before sowing the field was fertilized with complex fertilizers. Carrots during vegetation period were additionally fertilized through leaves for three times applying soluble complex fertilizers. Experiments were carried out on sandy loam on light loam *Calc(ar)i-Epihypogleyic Luvisol* (*LVg-p-w-cc*). Data of investigations showed that carrot hybrid was more productive than cultivar. Calcium nitre was more effective both to hybrid and cultivar. Vegetables fertilized with it was preserved better than these fertilized with ammonium nitrate.

Keywords: nitrogen fertilizer, exceptional quality.

Introduction

The intensity of agricultural activities determines the level of maintenance of cultural landscapes, biodiversity, and the cultural identity of an area (Li et al., 2013). Success in vegetable culture in a temperate climate has been due to proper mineral nutrition of vegetables, because plant nutrition, among other agro-technical measures most influences yield, quality and production storability (Biesiada et al., 2011; Kant et al., 2011). However, the surplus of supply nutrients, especially nitrogen, may have an adverse effect on the environment as well as quality of the crops. More than 50% of N applied is effectively used by plants, but a large part is lost by leaching and it can lead the contamination of ground and surface water (Fageria et al. 2008; Wang et al., 2008). Optimization of technologies for growing plants is a method to increase plant productivity and to obtain quality yields (Cai, Ge, 2004; McAllister et al., 2012). The data of Cameron (2013) has shown that careful management of temperate systems using best management practices and newly developed technologies can increase the sustainability of agriculture and reduce its impact on the environment. In order to avoid negative consequences on the environment and to grow the production of exceptional quality, the amount of nitrogen scattered during vegetation was limited.

Carrot (*Daucus carota* L.) is the important vegetable crop, grown in temperate climate zone, which includes Lithuania. The objective of the study – instilling new technological elements of the growing of root-crop vegetables of exceptional quality to choose for the additional fertilization the most suitable nitrogen fertilizer and its rate in order the soil, environment was polluted as little as possible and the qualitative production was obtained.

Materials and methods

Experiments were carried out in the Institute of Horticulture in 2010 – 2012, on sandy loam on light loam *Calcari-Epihypogleyic Luvisols (LVg-p-w-cc).* Soil analysis in spring, before N fertilization, was carried out. The soil is characterized by the average content of humus (2.38–2.67), pH neutral to slightly alkaline (7.1-7.7) and by the presence of large amounts of Ca and Mg, which gives more favourable physical-chemical water and soil properties. Mineral nitrogen content in the layer 0–60 cm was low to average (44.2-87.7 mg kg^{-1}), while there was a good stock of soluble phosphorus (P_2O_5 – 166-262 mg kg^{-1}) and potassium (K_2O – 141-184 mg kg^{-1}).

The carrot 'Nerac' F_1 and 'Tito' (factor B) were grown on the profiled surface, 1.0 mln. ha^{-1} viable seed. Both the other cases the sowing scheme were 62 +8 cm.

Scheme of the fertilization (factor A): 1. Basic fertilization (handed before presowing tillage with additionally foliar application (Basic – B); 2. B+calcium nitrate N_{30} (B+CN N_{30}); 3. B+calcium nitrate N_{15} (B+CN N_{15}); 4. B+ammonium nitrate N_{30} (B+AN N_{30}); 5. B+ammonium nitrate N_{15} (B+AN N_{15}). Soil fertilization was carried out with complex mineral fertilizers using a ratio $N_{84}P_{84}K_{160,4}$. Fertilizers were applied before sowing of carrot. Nitrogen fertilizer for additional fertilization, introduced as ammonium nitrate (34% N, NO_3-N 17%, N-NH_4 17%.) and calcium nitrate (15.5% N, NO_3-N 14.4%, N-NH_4 1.1 % and 26.4 % CaO), was applied once during the growing season when carrot was at 2-4 leaf stage. Carrot during vegetation period were additionally fertilized through leaves for three times applying soluble complex fertilizers: twice with Ferticare 14 11 25 with microelements, 5 kg ha^{-1} by adding Delfan, 2 L ha^{-1}, once Ferticare 6 14 30 with microelements, kg ha^{-1} by

adding Final K, 2 L ha^{-1}. Experiments were carried out every year in 4 replications in systematic order. Area of record plot – 6.2 m^2.

Work of plant supervision was carried out according to vegetable growing technologies accepted in LIH, observing requirements of growing of exceptional quality production, where the use of nitrogen and pesticides is limited. Carrot yield was gathered, when vegetables reached technical maturity, classifying into marketable and nonmarketable. When harvesting according to the variants, with three replications, there were taken samples for biochemical investigations and 12–15 kg samples for root-crop storability investigations. Root-crops were stored in freezer, under stabile temperature (-1–+2° C) and relational humidity (85–90%), in the storage houses of Institute of Horticulture, LRCAF. Root-crop storability was inspected after 3 and 7 months, classifying well-preserved and diseased (rotten, partly rotten and wilted, i. e. not suitable for usage) red beet root-crops and establishing the natural loss, i. e., drying.

Table 1

Average monthly air temperature at the experiment site

Month	Air temperature °C			Multi annual
	2010	2011	2012	
May	13.5	15.6	13.2	12.3
Jule	14.6	18.9	14.6	15.9
June	19.9	20.2	18.7	17.3
August	17.8	17.7	16.5	16.7
September	9.9	13.7	13.1	12.1
October	3.8	11.8	6.7	7.1
Average	13.2	16.3	13.8	13.6

Investigations of carrot biochemical composition were carried out at the Laboratory of Biochemistry and Technology, Institute of Horticulture, LRCAF. There was established: dry matter (DM) – gravimetrically, after drying out at the temperature of 105±2 °C up to the unchangeable mass (Food analysis, 1986), dry soluble solids (DSS) – with refractometer (digital refractometer ATAGO) (AOAC, Official..., 1990b), sugars – by AOAC method (AOAC, Official..., 1990a), nitrates – by potentiometrical method with ion selective electrode (Metod. nurod., 1990), carotenes – spectrophotometrically (Davies, 1976).

Analysis of variance was performed using the ANOVA statistical package with two-way factors. Treatment means were compared using Fisher's protected least significant difference (LSD) test at $p \leq 0.05$. Contrasts were used to determine the effect of fertilization rates on yield, yield biochemical composition and quality of storage.

Meteorological conditions during the years of the experiment varied significantly (Tables 1, 2). The year 2010 was the colder, but with the higher temperature and higher amount of precipitation in July and August, as compared to multiannual.

Table 2

Average monthly precipitation at the experiment site

Month	Precipitation, mm			Multi annual
	2010	2011	2012	
May	71.4	15.6	57.8	50.7
Jule	72.6	48.0	99.6	71.2
June	119.2	203.6	135.4	75.3
August	105.4	135.2	81.0	78.4
September	51.0	75.8	59.8	58.7
October	0	4.2	69.8	50.5
Average	70.1	86.8	83.9	64.1

The highest and relatively uniform mean temperature during the whole season was in 2011. The year 2011 had the highest total precipitation, with especially humid July and August, but dry June. In 2012 air temperature was slightly higher than the multiannual average, but June and August were cool and June and August were wet, while July was hot and wet.

Results and Discussion

Using nitrogen in both variants, irrespective cultivars, forms and rates, carrot marketable yield increased averagely by 6.3 t ha % or 13.9%. The output of marketable yield increased correspondingly 9.2% (Fig. 1). Data of investigations carried out in various countries with various plants (Chohura, Kołota, 2009; Biesiada et al., 2011; Toivonen, Hodges, 2011) show that nitrogen determines the growth and productivity of plant most of all. According to the data of Zdravkovic et al. (1997), carrot fertilized with manure produced 48.4 t ha^{-1}, with calcium ammonium nitrate – 41.5 t ha^{-1} and their mixture – 41.5 t ha^{-1} of yield. The output of marketable yield is important parameter, which determines fertilizer suitability and corresponds to one of the main requirements of the optimal yield (Suojala, 2000; Zalatorius, Viškelis, 2005). Additionally fertilizing carrots with nitrogen at 2–4 leaf stage, marketable yield of cultivar 'Nerac' F_1 averagely increased 11.7%, the output of marketable yield – 3.4%. At the same time the yield of carrot cultivar 'Tito' increased 17.4%, output – 9.4%. The vegetable yields were not increased continuously with N rate increase, and oversupply of N reduced the plant growth, leading to a yield decline (Wang, Li, 2003). The data of our investigations showed that applying bigger nitrogen rate for additional fertilization the significant additional yield wasn't obtained – comparing with the case when smaller nitrogen rate for carrot fertilization was used. Both calcium nitrate and ammonium nitrate applied for additional fertilization, irrespective of the rates of nitrogen and cultivars, were equally effective for carrots. The best yields were obtained additionally using 30 kg ha^{-1} of nitrogen.

When the rate of nitrogen was decreased, marketable yield 'Nerac' F_1 decreased by 6.0 t ha^{-1} or 11.7%, but marketable yield of cultivar 'Tito' slightly increased. The output of marketable yield was slightly smaller fertilizing with bigger nitrogen rate, but significant differences between the variants weren't noted. Data of study Kolota et al. (2007) showed that application of ammonium nitrate, calcium nitrate and Entec 26 gave similar to each other and considerable better results than ammonium sulphate.

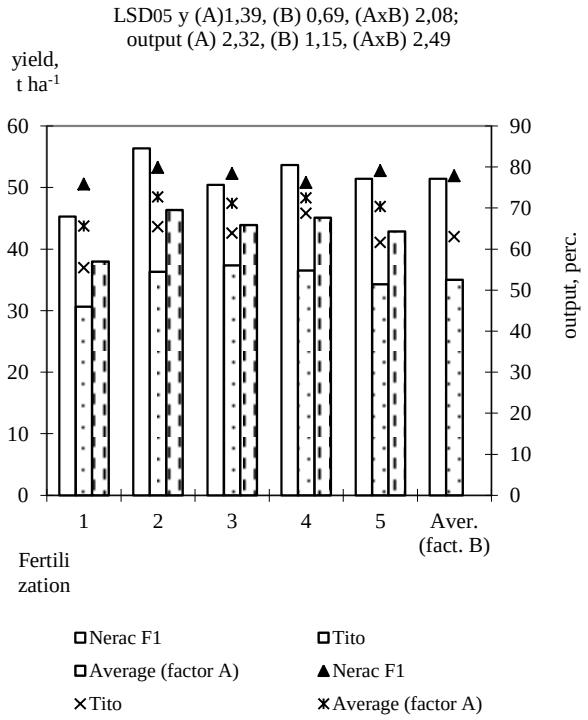

Figure 1. Influence of different nitrogen fertilizers and rates on marketable yield and output of marketable yield of exceptional quality carrot

Fertilization: 1 – Basic fertilization (B), 2 – B + CN N_{30}, 3 – B + CN N_{15}, 4 – B + AN N_{30}, 5 – B + AN N_{15}

Aytko (2004) indicate that in carrot there can be 8–12% of dry matter, 6–8% of sugars, 9–12 mg% of carotene, also potassium and microelements – boron and iodine. Ayaz et al. (2007), affirm that amounts of dry matter fluctuated in wide limits – from 6.40% to 11.43%; nitrates – from 8.1 mg kg^{-1} to 509 mg kg^{-1}. Their concentration in vegetables changes dependently on many factors: vegetable type and cultivar (Seljåsen et al., 2013, Chohura, Kołota, 2009; Rożek et al., 2000), soil, meteorological conditions (Rubatzky et al. 1999). Data of our investigations show that the amounts of dry matter and dry soluble solids fluctuated in very narrow limits and fertilization with nitrogen little influenced their changes (Table 3). Our present studies confirmed Polish (Pokluda, 2006) and Lithuanian (Pekarskas, Bartaševičienė, 2009; Karklelienė et al. 2007) investigators obtain data. They point out that differentiated soil fertilization with nitrogen doesn't significantly influence the amount of dry matter and dry soluble solids, carotenes, nitrates and phenols in carrot root-crops. Paoletti et al. (2012) also indicate that production systems do not affect the nutritional quality of carrot. The amounts of dry soluble so lids, as noted Suojala (2000), can increase when yield gathering is delayed. Slightly bigger amount of dry matter and dry soluble solids as shown in our studies was found in root-crops of cultivar 'Tito'. Big amount of sugars and carotenes, especially β-carotene, and small amount of nitrates are important carrot quality indices (Gajewski et al., 2009, 2010). Arscott and Tanumihardjo (2010) observed that their amounts depend on growth conditions, genotype and fertilization.

Table 3

Influence of different nitrogen fertilizers and rates on biochemical composition of exceptional quality carrot

Fertilization (fact A)	Biochemical composition				
	1	2	3	4	5
Hybrid 'Nerac' (factor B)					
Basic (B)	11.5	9.5	6.1	368.3	13.4
B+CN N_{30}	11.1	9.5	6.4	346.5	13.1
B+CN N_{15}	11.2	9.8	6.3	350.0	13.3
B+AN N_{30}	11.3	9.2	6.4	356.3	13.2
B+AN N_{15}	11.3	9.3	6.4	357.2	13.2
Cultivar 'Tito'(factor B)					
Basic (B)	11.8	9.4	6.3	361.8	13.4
B+CN N_{30}	11.5	9.7	6.3	353.5	13.8
B+CN N_{15}	11.7	9.8	6.2	344.7	14.2
B+AN N_{30}	11.9	9.6	6.4	354.2	12.4
B+AN N_{15}	11.7	9.5	6.2	349.0	12.6
Average fertilization (Factor A)					
Basic (B)	11.6	9.4	6.2	365.1	13.4
B+CN N_{30}	11.3	9.6	6.4	350.0	13.4
B+CN N_{15}	11.4	9.8	6.2	347.3	13.8
B+AN N_{30}	11.6	9.4	6.4	355.3	12.8
B+AN N_{15}	11.5	9.4	6.3	353.1	12.9
Average cultivars (factor B)					
Hybrid	11.3	9.5	13.2	355.7	13.2
Cultivar	11.7	9.6	13.3	352.6	13.3
LSD_{05}(A)	0.3	0.3	0.2	92.9	0.50
LSD_{05}(B)	0.2	0.2	0.1	47.0	0.25
LSD_{05}(AxB)	0.42	0.5	0.3	140.6	0.75

1 – dry matter, %, 2 – dry soluble solids, %, 3- sugars, %, 4 – nitrates, mg kg^{-1}, 5 – carotenes, mg 100 g^{-1}

Our studies have shown that the cultivar did not affect the sugar and carotene content in the roots, and additional nitrogen fertilization slightly increased the sugar content. Carotenes from additional nitrogen fertilizer use declined. Nitrogen fertilizer forms and rates had no significant effect on sugar and carotene

content. Too intensive fertilization, especially with nitrogen, according to many researchers (Ayaz et al., 2007; Kona 2006; Santamaria, 2005; Smoleń et al., 2011; Wang et al., 2008; Zeka et al., 2014) can cause unsuitable increases in some plants of nitrates, sugars and decreases of dry soluble solids, ascorbic acid (vitamin C), calcium and magnesium. There were found big differences between nitrates accumulation in different vegetable species (Gajewski et al., 2007). Our research has shown that tested cultivars did not affect the amount of nitrate in the roots. In the grown experiments in carrot root-crops irrespective cultivars there were 365.1 mg kg^{-1} (Table 3). Fertilizing with nitrogen the amount of nitrates increased 13.7 mg kg^{-1}.The least amount of nitrates in root-crops accumulated in carrot fertilized with calcium nitrate.

Table 4

Influence of different nitrogen fertilizers and rates on storability of exceptional quality carrot

Fertilization (fact A)	A			B		
	A1	A2	A3	B1	B2	B3
Hybrid 'Nerac' (factor B)						
Basic (B)	43.5	0.0	4.5	38.8	3.4	7.1
B + CN N_{30}	53.4	0.0	5.5	51.8	2.1	5.4
B + CN N_{15}	48.4	0.0	4.2	45.0	3.5	6.2
B + AN N_{30}	51.2	0.0	4.8	48.3	4.0	5.4
B + AN N_{15}	48.6	0.0	4.9	45.9	3.9	5.2
Cultivar 'Tito' (factor B)						
Basic (B)	28.9	0.0	5.8	26.3	5.3	7.4
B + CN N_{30}	34.3	0.0	5.8	32.0	3.5	8.4
B + CN N_{15}	35.1	0.0	5.9	31.4	8.8	5.9
B + AN N_{30}	34.4	0.0	6.4	31.7	5.9	6.1
B + AN N_{15}	32.5	0.0	5.6	30.8	4.3	5.9
Average fertilization (factor A)						
Basic (B)	36.2	0.0	5.1	32.5	4.4	7.2
B + CN N_{30}	43.8	0.0	5.7	41.9	2.8	6.9
B + CN N_{15}	41.7	0.0	5.1	38.2	6.2	6.0
B + AN N_{30}	42.8	0.0	5.6	40.0	4.9	5.8
B + AN N_{15}	40.6	0.0	5.3	38.3	4.1	5.5
Cultivars (factor B)						
Hybrid	49.0	0.0	4.	49.9	2.2	5.8
Cultivar	33.0	0.0	5.9	30.4	5.6	6.8
LSD_{05}(A)	4.7		0.9	4.7	1.9	1.2
LSD_{05}(B)	2.3		0.4	2.3	1.0	0.8
LSD_{05}(AxB)	7.0		1.3	7.0	2.9	2.4

A – after short-term storage, B – after long-term storage; A1, B1 – marketable production, t ha^{-1}, A2, B2 – sick, %, A3, B3 – natural losses

Vegetable storability is influenced by climatic conditions, soil properties, cultivars, fertilization and forms of fertilizers, as well as the time of harvesting (Rożek et al., 2000, Fikseliová et al., 2010; Wrzodak et al., 2012). To store carrot is more difficult than other root-crop vegetables, because their root-crop, as observed Autko (2004) have a thin epithelium tissue (4–8 layers of periderma, while potato – 9-11 layers), which during yield gathering with mechanical means very often is injured. Therefore water is evaporated more intensively and root-crops of carrot quickly wilt, and wilted are less resistant to diseases, but small mechanical injures root-crop is able to "heal up". According to Suslov et al. (2009), storage of carrots for a pro-longed period of time at temperatures above 2 °C results in heavy sprouting of the tops and lateral roots. When carrot cultivar 'Nerac' F_1 was stored for short time (up till New Year) marketable production comprised averagely 49.0 t ha^{-1}, and after long-time storage (up till May) – 45.9 t ha^{-1}, and cultivar 'Tito' correspondingly 33.0 and 30.4 t ha^{-1} (Table 4). Storage losses correspondingly were 4.8% and 5.9%, and after long-term storage – 9.2 and 12.3%. Losses of cultivar short-term and long-term storage were slightly bigger than these ones of hybrid. Marketable production of carrot fertilized with nitrogen fertilizers after short-time storage, irrespective of cultivars, increased on the average 6.0 t ha^{-1} or 16.7%, after long-time storage – 7.1 t ha^{-1} or 21.8%. This is due to the fact that nitrogen fertilizer increased yields. Nitrogen fertilizer forms and rates used for additional fertilization did not affect the storability quality of carrot. Wrzodiak et al. (2012) observes that there were marked differences in storage life depending on the cultivar, cultivation method and storage period.

Conclusions

The best marketable yield was obtained additionally using 30 kg ha^{-1} of nitrogen in form of calcium nitrate. The marketable yield of carrot 'Nerac' F_1 was 56.4 t ha^{-1}, cultivar 'Tito' – 36.3 t ha^{-1}.

The amounts of dry matter and dry soluble solids fluctuated in very narrow limits and fertilization with nitrogen little influenced their changes. Slightly bigger amount of dry matter and dry soluble solids as shown in our studies was found in root-crops of cultivar 'Tito'. Additional nitrogen fertilization slightly increased sugar content. Carotenes from additional nitrogen fertilizer use declined. Fertilizing with nitrogen the amount of nitrates increased 13.7 mg kg^{-1}.The least amount of nitrates in root-crops accumulated in carrot fertilized with calcium nitrate.

Used for additional fertilization nitrogen fertilizer forms and rates did not affect the storability quality of carrot. Losses of cultivar short-term and long-term storage were slightly bigger than there ones of hybrid. The marketable production after short-time storage irrespective of cultivars increased on the average 6.0 t ha^{-1} or 16.7%, after long-time storage – 7.1 t ha^{-1} or 21.8%.

References

1. AOAC (1990a) Sucrose in fruits and fruit products. In: *Official Methods of Analysis*. 15th edition. Helrich K (ed.), AOAC Inc., Arlington, VA: 922 p.
2. AOAC (1990b) Solids (soluble) in fruits and fruit products. In: *Official Methods of Analysis*. 15th edition, Helrich K. (ed.). AOAC Inc., Arlington, VA: 915 p.
3. Arscott S. A., Tanumihardjo S. A. (2010) Carrots of many colours provide basic nutrition and bioavailable phytochemicals acting as a functional food. *Comprehesive Reviews in Food Science and Food Safety*, Vol. 9, p. 223-239.
4. Ayaz A, Topçy A., Yurttagul M. (2007) Survey of nitrate and nitrite levels of fresh vegetables in Turkey. *Journal of Food Technology*, Vol. 5, Vol. 2, p. 177-179.
5. Biesiada A., Nawirska A., Kucharska A., Anna Sokół-Łętowska A. (2011) The effect of nitrogen fertilization methodson yield and chemical composition of pumpkin (Cucurbita maxima) fruits before and after storage. *Vegetable Crops Research Bulletin*, Vol. 70, p. 203–211.
6. Cai B., Ge J. (2004) He effect of nitrogen level on main nutrient of sugar beet. *Nature and Science*, Vol. 2, No 4, p.79-83.
7. Cameron K.C., Di H. J., Moir J.L. (2013) Nitrogen losses from the soil/plant system: a review. *Annals of Applied Biology*, Vol. 162, p. 145–173.
8. Chohura P., Kołota E. (2009) Effect of nitrogen fertilization on the yield and quality of field-grown leaf lettuce for spring harvest. *Vegetable Crops Research Bulletin*, Vol. 71, p.41-49.
9. Davies B. H. (1976) Carotenoids. In: Chemistry and Biochemistry of Pla, ISBN 10 0122899016, Lon nt Pigments, Vol. 1, ed. Goodwin T. W., Academic Press. p. 35-165. London, New York
10. Fageria N. K., Baligar V. C., Li Y. C. (2008) The Role of Nutrient Efficient Plants in Improving Crop Yields in the Twenty First Century. *Journal of Plant Nutrition*, Vol. 31, p. 1121-1157.
11. Fikselová M., Mareček J., Mellen M. (2010). Carotenes content in carrot roots (Daucus carota L.) as affected by cultivation and storage. *Vegetable Crops Research Bulletin*, Vol. 73, p. 47-54.
12. Food analysis: general techniques, additives, contaminants and composition (1986) Rome: FAO, 205 p.
13. Gajewski M., Szymczak P., Elkner K., Dąbrowska A., Kret A., Danilcenko H. (2007) Some aspects of nutritive and biological valueof carrot cultivars with orange, yellowand purple-coloured roots. *Vegetable Crops Research Bulletin*, Vol. 67, p. 149-161.
14. Gajewski M., Węglarz Z., Sereda A., Bajer M., Kuczkowska A., Majewski M. (2010) Carotenoid Accumulation by Carrot Storage Roots in Relation to Nitrogen Fertilization Level. *Notulae Botanicae Horti Agrobotanici Cluj Napoca*, Vol. 38, Vol. 1, p. 71–75.
15. Gajewski M., Węglarz Z., Sereda A., Bajer M., Kuczkowska A., Majewski M. (2009). Quality of carrots grown for processingas affected by nitrogen fertilizationand harvest term. *Vegetable Crops Research Bulletin*, Vol. 70, p. 135-144.
16. Kant S., Bi Y.-M., Rothstein S. J. (2011) Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. *Journal of Experimental Botany*, Vol. 62, p. 1499–1509.
17. Karklelienė R., Juškevičienė D., Viškelis P. (2007) Productivity and quality of carrotr (Daucus sativus Röhl.) and onion (Allium cepa L.) cultivars and hybrids. *Sodininkystė ir daržininkystė*, Vol 26, p. 208-216.
18. Kołota E., Adamczewska-Sowińska K., Krężel J. (2007) Suitability of Entec 26 as a source of nitrogen for red beet and celeriac. *Vegetable Crops Research Bulletin*, Vol. 67, p.47-54.
19. Kóňa, J. (2006). Nitrate accumulation in different parts of carrot root during vegetation period. *Acta Horticulturae et Regiotecturae*, Vol. 9, p. 22–24.
20. Li S-H., Wang Z-H., B.A. Stewart B. A. (2013) Responses of Crop Plants to Ammonium and Nitrate N. *Advances in Agronomy*, Vol. 118, p. 205–397.
21. McAllister C. H., Beatty P. H., Good A. G. (2012) Engineering nitrogen use efficient crop plants: the current. *Plant Biotechnology Journal*, Vol. 10, p. 1011–1025.
22. Paoletti F., Raffo A., Kristensen H. L., Thorup-Kristensen K., Seljåsen R., Torp T., Busscher N., Ploeger A., Kahl J. (2012) Multi-method comparison of carrot quality from a conventional and three organic cropping systems with increasing levels of nutrient recycling. *Journal of the Science of Food and Agriculture*, Vol. 92, p. 2855–2869.
23. Pekarskas J., Bartaševičienė D. (2009) Ekologiškai augintų morkų veislių derlingumas ir biocheminė sudėtis. *Sodininkystė ir daržininkystė*, Vol. 28, p. 99-105.
24. Pokluda R. (2006) An assessment of the nutritional value of vegetables using an ascorbate-nitrate index. *Vegetable Crops Research Bulletin*, Vol. 64, p. 28-37.
25. Rożek S., Leja M., Wojciechovska R. (2000) Effect of differentiated nitrogen fertilization on chantes of certain compounds in stored carrot roots. *Folia Horticulturae*, Vol. 12, p. 21-34.
26. Rubatzky V. E., Quiros C. F., Simon P. W. (1999) *Carrots and related vegetable umbelliferae*. USA, University of California, Davis, University of Viskonsin, Madison: C A B Intl, 294 p.
27. Santamaria P. (2006) Nitrate in vegetables: toxicity, content, intake and EC regulation. *Journal of the Science of Food and Agriculture*, Vol 86, p. 10-17.
28. Seljåsen R., Kristensen H. L., Lauridsen Ch., Wyss G. S., Kretzschmar U., Birlouez-Aragone I., Kahl J. (2013) Quality of carrots as affected by pre- and postharvest factors and processing. *Journal of the Science of Food and Agriculture*, Vol. 93, p. 2611-2626
29. Smoleń S., Sady W., Wierzbińska J. (2011) The effect of various nitrogen fertilization regimes on the concentration of thirty three elements in carrot (*Daucus carota* L.) storage roots. *Vegetable Crops Research Bulletin*, Vol. 74, p. 61–76.
30. Suojala T. (2000) Variation in sugar content and composition of carrot storage roots at harvest and during storage. *Scientia Horticulturae*, Vol. 85, p. 1–19.
31. Suslow T.V., Mitchell J., Cantwell M. 2009. Carrot. Recommendation for maintaining postharvest quality.Disponívelem:<http://postharvest.ucdavis.edu/Produce/ProduceFacts/Veg/carrot.
32. Toivonen P. M. A., Hodges D. M (2011) Abiotic Stress in Harvested Fruits and Vegetables, AbioticStress in Plants - Mechanisms and Adaptations, ed. A. Shanker, Available from: http://www. intechopen.com/books/abiotic-stress-in-plants-mechanisms-andadaptations/ abiotic-stress-in-harvested-fruits-and-vegetables

33. Zdravkovic M., Damjanovic M., Corokalo D. (1997) The influence of fertilization on yield of different carrot varietes. *Acta Horticulturae*, vol. 462, p. 93–96.
34. Zalatorius V., Viškelis P. (2005) Papildomo juostinio tręšimo per lapus įtaka morkų produktyvumui ir laikymuisi. *Sodininkystė ir daržininkystė*, Vol. 24, p. 72-79.
35. Zeka N., Mero G., Skenderasi B., Gjançi S. (2014) Effects of nitrogen sources and levels on yield and nutritive values of spinach (*Spinacia Oleracea* L.). *Journal of International Academic Research for Multidisciplinary*, Vol. 1, p. 327-337.
36. Wang Z. H., Li S. X. (2003). Effects of N forms and rates on vegetable growth and nitrate accumulation. *Pedosphere*, Vol. 13, No 4, p. 309-316.
37. Wang Z. H., Li S. H, Malhi S. (2008) Effects of fertilization and other agronomic measures on nutritional quality of crops. *Review. Journal of the Science of Food and Agriculture*, Vol. 88, p. 7-23.
38. Wrzodak A., Szwejda-Grzybowska J., Elkner K., Babik I. (2012) Comparison of the nutritional value and storage life of carrot roots from organic and conventional cultivation. *Vegetable Crops Research Bulletin*, Vol. 76, p. 137–150.
39. Аутко А.А. (2004). *В мире овощей*. Минск, УП «Технопринт», 564 p.

IMPACT OF THE DEGREE OF MATURITY ON APPLE QUALITY DURING THE SHELF LIFE

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Abstract

The main objective of this work was to determine optimum harvest date in apples, which were kept in long term as well as shelf-life storage (supermarket, T=18 °C). The effect of storage conditions and inhibitor of ethylene 1-methylcyclopropene (1-MCP) treatment on ethylene production and quality of apples stored in normal and controlled atmosphere conditions was evaluated during one successive season 2012/2013. Experiments were carried out at the Latvia State Institute of Fruit-Growing. Treated and untreated (control) apples were stored for six months in the cold storage rooms at 2±0.5 °C under normal atmosphere (NA) conditions or controlled atmosphere (CA) with 1.0% O_2, 2.0% CO_2, 97% N_2 and 1.5% O_2, 2.5% CO_2., 96% N_2. Directly after storage and after shelf-life (additionally 14 days at 18 °C) internal ethylene concentration was determined. For estimation of apple quality sensory evaluation and density were chosen. The obtained results give the possibility to conclude that almost all cultivars which are early harvested were suitable for storage in modified atmosphere. The apple storage in modified atmosphere gives the opportunity to extend the apple shelf-life period more than two weeks simultaneously without any qualitative changes.

Keywords: apples, quality, shelf-life, storage.

Introduction

Consumers mainly associate quality of apple with their firmness, juiciness, and sweetness. The softness of apples (mealiness) characterise a fruit on with low quality and suggest that fruits were stored too long (Galmarini et al., 2013). The shelf-life period of apples is determined by number of indicators. It has been reported by Gregersons from University of Illinois USA that apple expiration date mainly depends from chemical composition, especially from content of water and sugar (Gregersons, 2009), another authors suggested that it's depended from thickness of the wax layer and from the cultivars (Soliva-Fortuny et al., 2002). Winter cultivars which contain lower amount of water comparing with summer cultivars, always kept longer. There are a lot of factors which can affect the apples expiration date. Basically significant affect renders the ripeness of apples, for example 'Granny Smith' cultivar which was harvested slightly green and stored in controlled modified atmosphere for about a year, will be kept in shelf-life approximately 6–8 weeks, however the apple will not have a strong taste and aroma. According to Raffo and colleagues the apple storage in controlled modified atmosphere extend the expiration date up to 60 days, on the other hand it affect the sensory qualities of the products (Rafo et al., 2009). This type of storage not only delay the aging process, but also suppress the volatile organic compounds (esters, aldehydes, alcohols and terpenes), which is responsible for the fruit aroma development. Fruit quality on shelves is also dependent from the storage temperature and air relative humidity in the storage chamber. Traditionally the storage camera (refrigerator) can impair the texture of apples, as a result, during the expiration time these fruits can promptly become soft and mealy (Hui 2010). One of the most important indicators during the storage of apples is the placement in the shop. During the apple respiration processes the ethylene production increase, which accelerate the aging of the apple, and if they are stored near by the fruits which are also produce ethylene (pears, peaches, nectarines, plums, tomatoes, avocados, bananas, kiwi, mango and apricot), the ripening process become up to 10 times intensively, as a result maximal expiration date for this product will be 1–2 weeks. One of the possibility how can prolong the expiration date of fruit is 1-MCP treatment with the aim to delay the ethylene production. It gives the opportunity to extend the shelf-life period up to 4–6 weeks. However, there is still a risk that during the storage a receptor of ethylene will recovered and the cells will become susceptible to ethylene molecules (Abadi et al., 2009). Scientist from Spain Valera (2008) has determined the maximal shelf-life period for apples after storage in modified atmosphere. It has been reported that after 61 day storage of apples in a shop at 20 °C temperature, consumer evaluation showed that the quality did not changed significantly, while after 70 days of storage apples became softer. The aim of the work was determine optimal harvest date in apples, which are kept in long term as well as shelf-life storage (supermarket, T=18 °C).

Materials and Methods

The research has been conducted at the Latvia State Institute of Fruit-Growing (LSIFG) with the seven different commercial apple cultivars: 'Auksis', 'Orlik', 'Gita' (autumn), 'Antej', 'Belorusskoje Malinovoje', 'Sinap Orlovskij', 'Zarja Alatau' (winter). Apples were preserved in controlled modified atmosphere T=2±1 °C for six months, and two weeks stored in natural atmosphere (shelf-life) T=18±1 °C. The storage time was choosen accordingly with the report from Гудковский (2012). ULO1 chamber with gas content O_2=1.00%, CO_2=2.00%, and ULO2 chamber with gas content O_2=1.50%, CO_2=2.50% was used for preservation in controlled modified environment, and control samples

were stored in cooling chamber at temperature 2±1 °C with relative humidity 85%. Treatment with 1-MCP was performed when fruit were ready for consumption. 1-MCP was obtained from Hangzhou Ruijiang Chemical Co., Ltd. 1-MCP was dissolved in warm water at ratio 1 : 30 in a sealed container. The container with 1-MCP was placed into a hermetically closed fruit processing cabinet; then the plug was opened and the treatment was performed for 24 h at 18±1 °C. The final concentration of 1-MCP in processing cabinet was 0.625 $\mu L \cdot L^{-1}$.

For optimal long term storage the apples with following parameters were chosen (Table 1 and 2).

Table 1

Suitable parameters of different type of storage for determination of optimal harvesting time

Cultivar	Concentration of ethylene, $\mu L\ h^{-1}\ L^{-1}\ kg^{-1}$	Firmness, kg cm^{-2}	The ration of soluble solids / titratable acidity, °Brix / %
'Auksis'	0.174	9.400	18.177
'Orlik'	0.181	8.490	13.301
'Gita'	0.170	6.070	10.989
'Antej'	0.221–0.252	6.320–6.440	16.879–17.530
'Belorusskoje Malinovoje'	0.187–0.204	8.640–6.770	11.432–13.573
'Sinap Orlovskij'	0.177–0.188	8.640–8.810	13.561–15.377
'Zarja Alatau'	0.187–0.215	6.050–6.920	17.765–19.077

Data were obtained in our previous investigations.

Table 2

Suitable parameters of different type of storage for determination of optimal harvesting time

Cultivar	Iodine starch test (1–10)	Streif's index	De Jager's index	FARS index
'Auksis'	5.0	0.167	5.004	0.103
'Orlik'	4.0	0.192	5.383	0.160
'Gita'	5.0	0.116	3.489	0.110
'Antej'	4.5–5.0	0.109–0.128	3.27–3.758	0.072–0.085
'Belorusskoje Malinovoje'	5.0	0.133–0.187	3.990–5.598	0.100–0.151
'Sinap Orlovskij'	4.0–4.8	0.152–0.198	4.524–5.546	0.117–0.162
'Zarja Alatau'	4.5–6.7	0.073–0.127	2.098–3.724	0.047–0.087

Data were obtained in our previous investigations

Determination of physicochemical parameters

Soluble solids content

The soluble solids content was determined according to LVS EN 12143:2001 standard.

Titratable acidity

The changes in acidity characterize the degree of apples ripeness. Titratable acid content is determined according to AOAC 942.15 standard.

Apple density

The changes in the fruit pulp density describe the degree of ripeness of apple. The density was measured with a digital penetrometer. Procedure and data collection was done according to LVS EN 1131:2001 standard.

Iodine-starch test

Iodine-starch test is based on the reaction of iodine with starch and expressed by specific blue colouring. During comparison, only the coloured field sizes and proportions were evaluated, but not the colouration intensity or colour tone. The standard scale range is 10 points where 1 point – clear blue, apple ripening has not started, 10 points – the starch is broken up and the apple remains untangled (Lopez Camelo, 2004).

The following indices were calculated:

TSS/TA ratio;

Streif's index (SI) F/(R*S) (Streif, 1996);

De Jager's index (PFW-1)F * (11 – S) / R (De Jager et al., 1996);

FARS index (F*A)/(R*S).

where: F – firmness, kg cm^{-2},

A – titratable acidity (TA), %,

R – soluble solids content (TSS), ºBrix,

S – starch index (on a scale from 1 to 10).

Determination of ethylene production

Ethylene production rate was determined with the Ethylene analyzer ICA 56 based on quantity of ethylene formed by the fruits during the postharvest storage (Barker, 2002).

For ethylene determination one apple from each treatment was enclosed in about 4 L airtight jar for 24 h at 20 °C, and then ethylene production rates were measured. Ethylene production was given as $\mu L\ h^{-1}\ L^{-1}\ kg^{-1}$ (Oz, Ergun, 2009).

Sensory evaluation

The line scale method, based on the ISO 4121:2003 (Sensory analysis – Guidelines for the use of quantitative response scales) was used for this study. The samples were evaluated for taste, aroma, acidity, sweetness, juiciness, and colour change.

Statistical analysis

Data was processed by SPSS software version 17.0. Data was analysed using descriptive statistics and processed by one-way analysis of variance ANOVA (one way ANOVA), as well as for comparing all apple samples depending from the storage of type two-way analysis of variance ANOVA were used (two-way ANOVA). Microsoft Excel software version 2007 was used to determine significant differences between the samples.

Results and Discussion

The most important aspect in consumer's choice of fruits and further inclusion in diet is sensory evaluation. The high quality apples were chosen to estimate fruit quality both after six months in different types of storage as well as after the shelves storage (shelf-life).

Sensory evaluation

Dramatic changes in apples quality have occurred during the storage in cooling chamber. From the seven of apple cultivars were retained only four of them. Analyzing data from sensory evaluation of apple colour (Figure 1a), which were stored in cooling chamber it was detected that highest assessment was for cultivar 'Sinap Orlovskij', while the lowest for 'Auksis' and 'Zarja Alatau'. Statistical evaluation of colour data showed that between the samples there were no significant differences ($p>0.05$). Similar results were obtained during the evaluation of aroma changes in apple samples. The highest intensity of aroma was recorded for cultivar 'Auksis', which corresponds to 6.87 points. On the other hand the lowest intensity of aroma was observed for cultivar 'Sinap Orlovskij', which corresponds to 4.21 points. Comparing the data statistically it is seen that there were significant differences ($p<0.05$).

Comparing the data obtained after sensory evaluation of taste the intensity had the greatest reduction similarly like previously in 'Sinap Orlovskij', which conform 4.32 points. On the other side the highest intensity of taste was found in 'Zarja Alatau' and this amount corresponds to 7.89 points. Analyzing data obtained after sensory evaluation of apple acidity, experts were concluded that the intensity of acidity of cultivar 'Sinap Orlovskij' was not expressed so well (3.09 points). Comparing the data of the sweetness of apples there were no significant differences between the cultivars. Evaluation of apple juiciness showed that maximal value was recorded for cultivar 'Belorusskoje Malinovoje' (9.46 points), while the minimal value was for cultivar 'Auksis' (4.82 points). Comparing the data of apples after six months of storage, which had been stored in cooling chamber as well as after storage on shelves (shelf-life) it was found that the colour of apples after shelf-life period became more intensive for all cultivars. On the other side analyzing the data of apple sweetness was recorded that it's became more intensive for cultivar 'Belarusskoje Malinovoje', while other attributes became less intensive (Figure 1b).

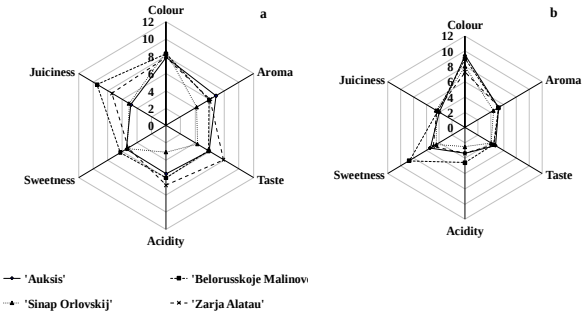

Figure 1. Diagram of apple sensory evaluation of control in cooling chamber: *a* – after six months of storage, *b* – shelf-life period

Obtained results suggest that apple storage in cooling chamber combination with 1-MCP treatment gives the possibility maintain and prolong the fruit expiration date till six months. Analyzing the obtained data after sensory evaluation (Figure 2a), it was found that the attributes like colour and aroma got significantly ($p<0.05$) lower assessments, while taste and juiciness has been more intensive comparing with control samples. The effectiveness of 1-MCP in decreasing the impact of aging on fruit physiology has been shown in a number of studies (Abdi et al., 1998; Golding et al., 1998; Fan, Mattheis 1999; Lurie et al., 2002; Defilippi et al., 2004; Kondo et al., 2005; Mattheis et al., 2005). On the other hand studies from Latvia (Juhnevica et al., 2013; Juhnevica-Radenkova et al., 2013) imply that 1-MCP treatment had a positive effect on apple sensory attributes.

Scientific studies from Michman and colleagues suggest that one of the most important negative aspects of 1-MCP is that, by eliminating sensitivity to ethylene, the production of volatile compounds is also reduced. These compounds contribute significantly to apple flavour and aroma (Michman et al., 2001). Our results coincide with the conclusions before, and unfortunately they are not recovered or partially recovering after two weeks of shelf-life (Figure 2b).

During the storage in ULO type cameras the colour and aroma of the samples (Figure 3a and Figure 4a) were partially developed. Statistically comparing the data it's seen that there were no significant differences between the ULO cameras ($p<0.05$). During the storage in ULO 1 camera it was observed one exception for cultivar 'Gita'. During the storage of this cultivar intensive weight loss was occurred, hence this cultivar had not appropriate quality.

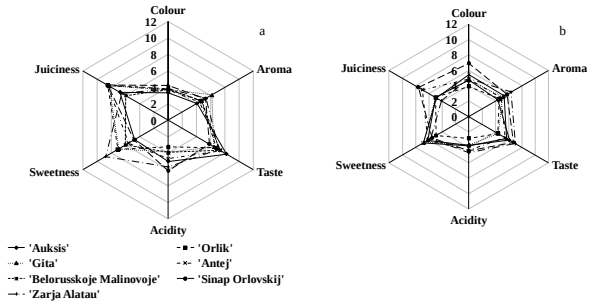

Figure 2. Diagram of apple sensory evaluation of control +1-MCP: *a* – after six months of storage, *b* – shelf-life period

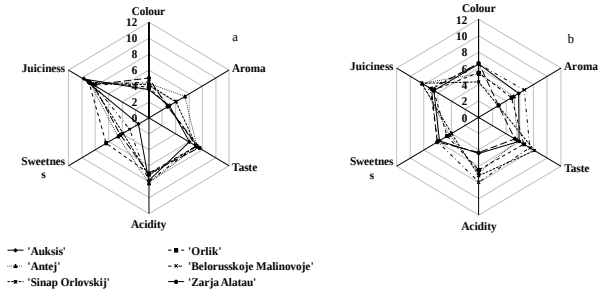

Figure 3. Diagram of apple sensory evaluation of ULO 1 chamber: *a* – after six months of storage, *b* – shelf-life period

The intensive weight loss can be explainable by the fact that this cultivar was harvested too early. Comparing the data of sensory attributes (taste, acidity and juiciness) it is seen that there were no significant differences between the cultivars in ULO cameras (ULO 1 and ULO 2).

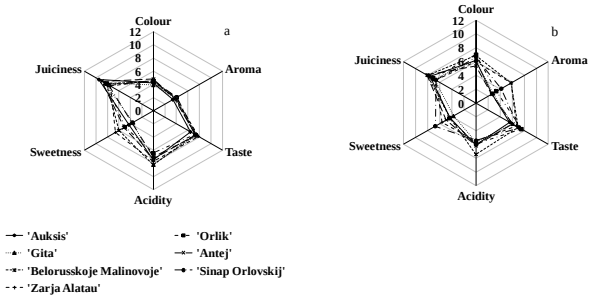

Figure 4. Diagram of apple sensory evaluation of ULO 2 chamber: *a* – after six months of storage, *b* – shelf-life period

Taken into account the evaluation of apple quality, it was recorded that apples, which were stored in ULO type cameras have a distinctive juiciness and fresh taste. Scientists from Poland were gained the similar results (Kruczyńska, Rutkowski, 2006). Mainly all apples which were stored in ULO type cameras can be characterized like green apples. One part of panellists noted, that the tastes of these apples are not so suitable for consumption like other apples, which were stored in cooling chamber in combination with 1-MCP treatment.

The results of this study showed that colour became more intense in all cultivars, while aroma was recovered in one part of cultivars (ULO 1: 'Orlik', 'Belorusskoje Malinovoje') and (ULO 2: 'Belorusskoje Malinovoje' and 'Zarja Alatau') (Figure 3b and Figure 4b). The evaluations of attributes like taste, acidity, sweetness and juiciness almost for all cultivars were much higher than for control samples and control, which was treated with 1-MCP. Obtained results can be explainable by the fact that during the storage in modified atmosphere decreased level of O_2 and increased level of CO_2 has a retarding effect on enzymatic activity and on morphological changes at a cellular level i.e. respiratory intensity, breakdown of membrane phospholipids and decrease of the volume of cytoplasma and mitochondria are all inhibited (Herregods, 1999).

Apple firmness

In connection with reports from Poland scientists (Rutkowski et al., 2008) and scientists from Latvia (Juhnevica et al., 2009) another important indicator of fruit quality is tissue firmness, which play an important role in fruits choosing by consumers.

The softening and cell separation in many fruits is associated with the increased proportion of readily soluble pectins. There are stages of cell wall breakdown during the ripening of apples. In the first stage fruit firmness declines slowly and wall galactans decrease. In the second stage, firmness decreases more rapidly and soluble polyuronides increase (Herregods, 1999). Reduction of the oxygen concentration to O_2 2%, give the possibility significantly delay the second stage of fruit ripening (Knee, 1974). At the moment the standards for estimating the firmness of apple tissue, which characterize the quality of these fruits does not exist in Latvia. Based on the scientist from Poland (Rutkowski) and his unpublished works, apples can be considered like qualitative, when the firmness of the tissue not lower than 4.0 kg cm^{-2}, while the another scientist from America submits that firmness of qualitative apple should be not lower than 4.4 kg cm^{-2} (Kupferman, 1992).

Taken into account the works from Poland scientists as well as after estimating the obtained data it is seen that our obtained results partially similar with Polish standards. Obtained results from ULO 1 type camera (Figure 5) showed that firmness of cultivar 'Auksis' is (4.7 kg cm^{-2}) and 'Sinap Orlovskij' (4.6 kg cm^{-2}).

Comparing the data obtained in ULO 2 type camera practically all cultivars are comply with the Polish standards, except of cultivar 'Orlik' (2.9 kg cm^{-2}). Although after two weeks of apple storage on shelves (shelf-life) comparing with after six months of storage there were no found significant differences ($p>0.05$) between ULO cameras, and these results were no complying with Polish standards.

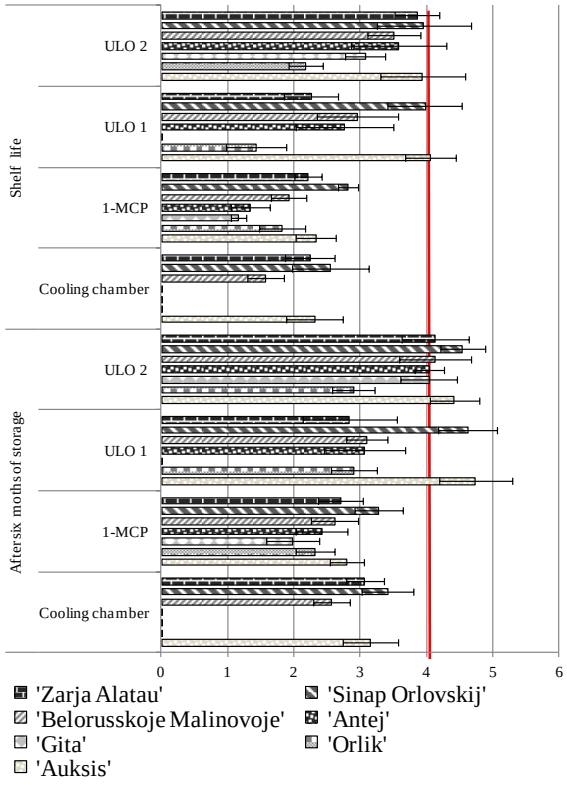

Figure 5. Firmness, kg cm^{-2}, depending on storage conditions

Detected only one exception for cultivar 'Auksis', which was stored in ULO 1 and the firmness corresponds with the standards (4.0 kg cm^{-2}).

Conclusions

Obtained results indicate that fruits, which were stored in modified atmosphere, as well as were treated with 1-MCP have less intensive aroma, flavour and colour. One of the possibilities how recover partially these attributes by storing the fruits for a short time (two weeks) on shelves (shelf-life).

Taken into account the evaluation of apple quality was recorded that apples which were stored in ULO type cameras have a distinctive juiciness and fresh taste.

After six months of apple storage was detected that more appropriate storage for maintaining of apple firmness was ULO 2 type camera. All of these results comply with quality standards.

On the other hand after two weeks of apple storage only one cultivar 'Auksis' (ULO 1 type) showed a positive value of firmness, while the other cultivars are not complying with the quality standards.

Acknowledgment

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References

1. Abadi D. H., Kaviani B., Hoor S.S., Torkashvand A.M., Zarei R. (2009) Quality management of cut carnation Tempo with 1-MCP. *African Journal of Biotechnology*, No. 8, p. 5351–5357.
2. Abdi N., McGlasson W. B., Holford P., Williams M., Mizrahi Y. (1998) Response of climacteric and suppressed climacteric plums to treatment with propylene and 1-methylcyclopropene. *Postharvest Biology and Technology*, No. 14, p. 29–39.
3. Barker L. R. (2002) *Postharvest technical training handbook*. Queensland Department of Primary Industries (QDPI, Australia).
4. De Jager A., Roelofs F. P. (1996) Prediction of optimum harvest date of Jonagold. **In:** *COST 94. The postharvest treatment of fruit and vegetables: Determination and prediction of optimum harvest date of apple and pears*. Lofthus, Norway, p. 21–32.
5. Defilippi B.G., Dandekar A.M., Kader A.A. (2004) Impact of suppression of ethylene action or biosynthesis on flavor metabolites in apple (*Malus domestica Borkh*) fruits. *Journal of Agricultural and Food Chemistry*, No. 52, p. 5694–5701.
6. Fan X., Mattheis J.P. (1999) Methyl jasmonate promotes degreening of apple fruit independent of ethylene action. *HortScience*, No. 34, p. 310–312.
7. Galmarini M. V., Symoneaux R., Chollet S., Zamora M.C. (2013) Understanding apple consumers expectations in terms of likes and dislikes: use of comment analysis in a cross-cultural study. *Appetite*, No. 62, p. 27–36.
8. Golding J.B., Shearer D., Wyllie S.G., McGlasson W.B. (1998) Application of 1-MCP and propylene to identify ethylene dependent ripening processes in mature banana fruit. *Postharvest Biology and Technology*, No. 14, p. 87–98.
9. Gregersons J. (2009) *Apples*. Shelf Life Advice. [accessed on 02.06.2009.]. Available at: http://shelflifeadvice.com/fruit/fresh-fruit/apples
10. Herregods M. (1999) Ca - storage of fruits and vegetables. **In:** *Post-harvest losses of perishable horticultural products in the Mediterranean region*. Chania: CIHEAM, p. 3-9.
11. Hui Y. H. (2010) **In:** *Handbook of Fruit and Vegetable Flavors*. *John Wiley and Sons*. Canada, p. 1083.
12. Juhnevica K., Segliņa D., Krasnova I., Skudra G., Kļava D., Skudra L. (2009) The Evaluation Of Apple Quality during Storage at Modified Atmosphere. *Journal Chemine Technologija*, No. 3, p. 30– 37.
13. Juhnevica K., Skudra L., Skrivele M., Radenkovs V., Seglina D., Stepanovs A. (2013) Effect of 1-methylcyclopropene treatment on sensory characteristics of apple fruit. *Environmental and Experimental Biology*, No. 11, p. 99–105.
14. Juhņeviča-Radenkova K., Drudze I., Segliņa D., Krasnova I., Olšteine A., Kaufmane E. (2013) 1-Metilciklopropēna (1-MCP) ietekme uz ābolu kvalitāti glabāšanas laikā (The influence of 1-Methylcyclopropene on apple quality during storage). **In:** *Vietējo Resursu (zemes dzīļu, meža, pārtikas un transporta) ilgtspējīga izmantošana - jauni produkti un tehnoloģijas (Nat Res) (State Research Programme "Sustainable use of local resources (earth, food, and*

transport) – new products and technologies (NatRes). B. Andersons, V. Segliņš, D. Dubrovskis, R. Galoburda, A. Paeglītis (eds.). Riga, Latvia, p. 207–211. (In Latvian)
15. Knee M. (1974) Changes in structural polysaccharides of apples ripening during storage. *Colloques nationaux. Centre national de la recherche scientifique*, No. 238, p. 341–345
16. Kondo S., Setha S., Rudell D.R., Buchanan D.A., Mattheis J.P. (2005) Aroma volatile biosynthesis in apples affected by 1-MCP and methyl jasmonate. *Postharvest Biology and Technology*, No. 36, p. 61–68.
17. Kruczyńska D. E., Rutkowski K. P. (2006) Quality and storage of Czech scab resistant apple cultivars. *Polish Phytopathological Society*, No. 39, p. 53–61.
18. Kupferman E. (1992) Retaining the Firmness of Apples in Storage. Tree Fruit Postharvest Journal, No. 3, p. 4–6.
19. López Camelo A. F. (2004) Manual for the preparation and sale of fruits and vegetables. From field to market. *Instituto Nacional de Tecnología Agropecuaria Estación Experimental Agropecuaria*, No. 1, p. 11–26.
20. Lurie S., Pre-Aymard C., Ravid U., Larkov O. Fallik E. (2002) Effect of 1-methylcyclopropene on volatile emission and aroma in cv Anna apples. *Journal of Agricultural and Food Chemistry*, No. 50, p. 4251–4256.
21. Mattheis J. P., Fan X., Argenta L. C. (2005) Interactive responses of 'Gala' apple fruit volatile production to controlled atmosphere storage and chemcial inhibition of ethylene action. *Journal of Agricultural and Food Chemistry*, No. 53, p. 4510–4516.
22. Mitcham B., Mattheis J., Bower J., Biasi B., Clayton M. (2001) Responses of European Pears to 1-MCP. *Perishables Handling Quarterly*, No. 108, p. 16–19.
23. Oz A. T., Ergun N. (2009) Effect of harvest maturity on shelf-life of 'Harbiye' persimmon fruit. **In:** *The 10th International Controlled and Modified Atmosphere Research Conference*, Antalya, Turkey, No. 876, p. 395–398.
24. Raffo A., Kelderer M., Paoletti F., Zanella A. (2009) Impact of innovative controlled atmosphere storage technologies and postharvest treatments on volatile compounds production in Cv. Pinova Apples. *Journal of Agriculture Food Chemistry*, No. 57, p. 915–923.
25. Rutkowski K., Michalczuk B., Konopacki P. (2008) Nondestructive determination of 'Golden Delicious' apple quality and harvest maturity. *Journal of Fruit and Ornamental Plant Research*, No. 16, p. 39–52.
26. Soliva-Fortuny R., Oms-Oliu G., Martin-Belloso O. (2002) Effects of ripeness stages on the storage atmosphere, color and textural properties of minimally processed apple slices. *Journal of the Science of Food and Agriculture*, No. 67, p. 1958–1962.
27. Streif J. (1996) Optimum harvest date for different apple cultivars in the 'Bodensee' area. **In:** *COST 94. The Postharvest Treatment of Fruit and Vegetables: Determination and Prediction of Optimum Harvest Date of Apple and Pears*. A. De Jager, D. Johnson, E. Hohn (eds). ECSC-EC-EAEC, Brussels, p. 15–20.
28. Varela P., Salvador A., Fiszman S. (2008) Shelf-life estimation of 'Fuji' apples: The behaviour of recently harvested fruit during storage at ambient conditions. *Postharvest Biology and Technology*, No. 50, p. 64–69.
29. Гудковский А.В., Кожина В.Л., Балакирев А.Е., Назаров Ю.Б. (2012) Современная система хранения плодов и ягод. *Перспективы развития технологий хранения и переработки плодов и ягод в современных экономических условиях*, стр. 75–83.

PHYSICAL AND CHEMICAL PARAMETERS OF LATVIAN FRESH CRANBERRIES

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Abstract

Cranberries belong to a group of evergreen dwarf shrubs or trailing vines in the genus *Vaccinium* subgenus *Oxycoccos*. Berries contain a diverse array of nutrients with recognized biological activities that promote or contribute to health. The aim of the research was to evaluate physically-chemical parameters of Latvian fresh cranberries. The research was accomplished on fresh Latvian wild and large-berry cranberries harvested in Kurzeme region: wild cranberries and large-berry cranberries variety 'Steven', 'Ben Lear', 'Bergman', 'Pilgrim' and 'Early Black'. The following quality parameters of cranberries were analysed using standard methods: anthocyanins (spectrophotometric), colour (using the colour system CIE L* a* b*), organic acids (high-performance liquid chromatography (HPLC)), polyphenols (HPLC), and pH value (LVS ISO 1132:2001). The research results confirm a close interaction (strong positive correlation (r=0.919) between the colour a* component intensity and anthocyanin content. The highest content of benzoic acid was determined in large-berry cranberries of the cultivar ′Early Black′ – 13.66 mg 100 g^{-1}, which was by 56% higher than in wild cranberries – 5.98 mg 100 g^{-1}, and by 65% higher than in the cultivar ′Steven′ berries – 4.82 mg 100 g^{-1}. The highest content of polyphenol compounds was found in cranberries of the cultivars ′Pilgrim′, ′Early Black′, and ′Steven′; while the lowest – in cranberries of the cultivar ′Bergman′. The average pH value for all samples was 2.465; it is slightly lower than found in the scientific literature.

Keywords: anthocyanins, organic acids, polyphenols, cranberries.

Introduction

Cranberries are extremely versatile berries. When incorporated into other food products, they provide refreshing flavour as well as a characteristic red colour. Used in combination with other fruits and berries, cranberries can accentuate and enhance the flavours of these fruits and berries. Because of their health gains, cranberries are experiencing an expansion into the food and beverage industry. Available year-round and in a variety of forms, cranberries can be used to improve numerous products and applications in the food and beverage industry (Hui et al., 2006).

Anthocyanins (of the Greek *anthos* = flower and *kianos* = blue) are the most important pigments of the vascular plants; they are harmless and of easy incorporation in aqueous media, which makes them interesting for its use as natural water-soluble colorants (Pazmiño-Durán et al., 2001). Anthocyanins are glycosylated polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium (flavylium) salt which are natural colorants widely distributed in nature (Bordignon-Luiz et al., 2007; Hendry, Houghton 1996). Many researches indicate that anthocyanins are not only nontoxic and nonmutagenic, but they have positive therapeutic properties such as antioxidant, anti-inflammatory, anticarcinogenic, antiviral, and antibacterial properties (Tall et al., 2004).

A limiting factor for the incorporation of anthocyanins in foods is the low stability of these pigments which are influenced by several factors: most important the pH of a system. They are susceptible to light and temperature, different agents can also cause their degradation (oxygen, enzymes etc.) (Jackman, Smith, 1996). The content of anthocyanins in cranberries equals to 20–360 mg·100 g^{-1} (Wang, Stretch, 2001; Prior et al., 2001).

Colour is an important factor in the consumer's choice of food products. It is one of the most important characteristics used to define the quality of food and has a decisive influence on the acceptance by the consumer (Hendry et al., 1996). CIELab is a nonlinear transformation of XYZ into coordinates L*, a*, b* and it is used for the colour measurement interpretation (Hoffmann, 2008).

Organic acids are primary metabolites, which can be found in great amounts in all plants, especially in berries. Organic acids are naturally found in fruits and berries. Many berries contain a variety of free acids which are colourless compounds soluble in water, ethanol; but insoluble in the nonpolar solvents benzene or petroleum ether. These acids are weakly acidic and can accumulate in the cellular vacuoles of plants. Some of the acids are components of the citric acid cycle, while others are intermediates in the pathway leading from carbohydrates to either aromatic compounds or isoprenoid derivatives (Arslan, Özcan, 2011; Dashek, Micales, 1997). Organic acids are biologically important because they form part of different biochemical metabolic routes such as the Krebs cycle (Arslan, Özcan, 2011; Silva et al., 1991). The concentration of organic acids is an important factor influencing the organoleptic properties of berries (Koyuncu, Dilmaçünal, 2010; Lee, 1993).

Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate and phenylpropanoid pathway in plants. These compounds one of the most widely occurring groups of phytochemicals are of considerable physiological and morphological importance in plants (Pupponen-Pimia, 2001). Many small fruit phenolic compounds are good sources of natural antioxidants and have inhibitory effects on mutagenesis and carcinogenesis. During the past decades, extensive analytical research has been carried out on the separation and determination of

phenolic constituents in various fresh fruit products and environmental samples. The unique antibacterial activities of cranberry implicate that cranberry may possess a very different flavonoid and phenolic composition from other kinds of fruits. An efficient separation and quantitation method is essential for understanding the components of flavonoid and phenolic antioxidants in cranberry and their health benefits. Phenolic compounds are not temperature resistant; therefore treatment at elevated temperatures will negatively influence polyphenol compound activity (Chen et al., 2001).

Therefore the aim of the current research was to evaluate physically-chemical parameters of Latvian fresh cranberries.

Materials and Methods

The research was accomplished on fresh Latvian wild and large-berry cranberries harvested in Kurzeme region in 2010: wild cranberries and large-berry cranberries variety 'Steven', 'Ben Lear', 'Bergman', 'Pilgrim' and 'Early Black'.

During the experiments, the following quality parameters of cranberries were controlled using standard methods:

- anthocyanins were determined by means of "Spectrophotometer Anthocyanins Determination Method" using a 6705 UV/VIS Spectrophotometer JENWAY (Bordignon-Luiz et al., 2007);
- a colour parameters of fresh cranberries were determined by direct reading with a COLOR TEC PMC. The colour parameters such as luminance (L*) ranging from 0 (black) to 100 (white) on a vertical axis, red saturation index (a*), and yellow saturation index (b*), were evaluated (McGuire, 1992);
- pH of berries was measured using potentiometric method LVS ISO 1132:2001 using Jenway 3510 pH metre. Prior to the determination of pH, cranberries were chopped by the blender; organic acids were extracted with water using the method of Hernandez et al. (2009);
- phenolic compounds were determined using a high-performance liquid chromatography (HPLC) with UV detection (at 280 nm) (Berregi et al., 2003).

Microsoft Excel software was used for the research purpose to calculate mean arithmetical values and standard deviations of the mathematical data used in the research. SPSS 20.0 software was used to determine the significance of research results, which were analysed using the following test methods: Sheffe test, two-factor ANOVA, and three-factor ANOVA analyses to explore the impact of factors and their interaction, and the significance effect (p-value).

Results and Discussion

Anthocyanins

The anthocyanins give to cranberries bright red colour and work like antioxidant. The changes of temperature during berries growing are closely related with low content of anthocyanins in the cranberries. For example, the high temperature inhibits the formation of anthocyanins in the berries (Prior et al., 2001). During present research it was detected that that the highest anthocyanin content was in large-berry cranberries variety 'Pilgrim' and 'Early Black', respectively 898±12 mg 100 g^{-1} and 839±8 mg 100 g^{-1} in dry matter what was by 66% and 63% higher comparing to anthocyanin content in wild cranberries. The lowest anthocyanin content was detected in wild cranberries, as 306±4 mg 100 g^{-1} (Figure 1), and in large-berry cranberries variety 'Ben Lear' – 492±4 mg 100 g^{-1}. Obtained results suggest that in the large-berry cranberries varieties 'Pilgrim' and 'Early Black' is the lowest red pigment intensity – they are not so red.

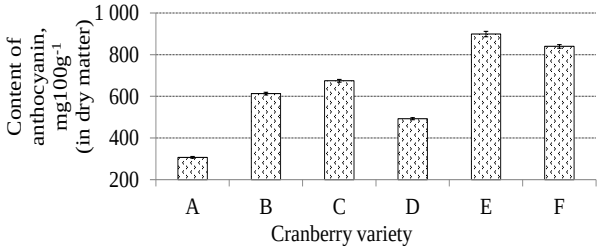

Figure 1. Content of anthocyanins

A – wild, B –'Steven', C – 'Bergman', D –'Ben Lear', E –'Pilgrim', F –'Early Black'

In scientific literature is mentioned, that the anthocyanin content in Latvian large cranberries is in the range from 186 mg% to 604 mg% (Ripa, 1992). Different results were obtained by Wang, Stretch (2001) and Prior et al., (2001), where anthocyanin content in cranberries is in the range from 360 mg 100 g^{-1}. Such differences could be explained with the berry cultivar and the characteristics of growing conditions.

Intensity of colour compounds

The L* colour compound value indicates colour saturation level in the product. The research data show, that the colour of wild cranberries (23.39±1.22) is darker then colour of large-berry cranberries (Figure 2). If compare the large-berry cranberries ′Steven′ and wild cranberries, the ′Steven′ berries are brighter – L* colour compound value is 17.00±0.69.

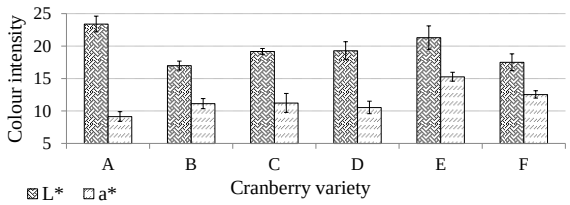

Figure 2. Intensity of L* and a* colour

A – wild, B –'Steven', C – 'Bergman', D –'Ben Lear', E –'Pilgrim', F –'Early Black'

The a* colour compound value mainly indicate the red colour of product. As results of the current research demonstrate, cranberry of variety ′Pilgrim′ are redder

(a* colour compound value is 15.28±0.74) (Figure 3). The ′Pilgrim′ berries red colour is on average by 40% higher comparing to wild cranberries – the red colour value was 9.15±0.69. Red colour intensity changes are closely related with the anthocyanins content in the berries. It is the reason, why large cranberries have more pronounced colours (Figure 1), what mainly could be explained with higher content of anthocyanins in berries.

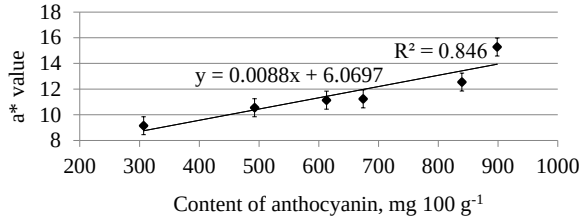

Figure 3. The a* colour intensity and anthocyanin content correlation analysis of fresh cranberries

The research results confirm a good correlation (r=0.919) (Figure 3) between the colour a* component intensity and anthocyanin content in analysed cranberries.

pH

Significant difference in pH value vas detected between cranberries varieties ′Steven′ and ′Pilgrim′ (p=0.020; α=0.050), and between ′Steven′ and ′Early Black′ (p=0.020; α=0.050) (Figure 4). The average pH value for all berries samples was 2.465; it is slightly lower than found in the scientific literature – the pH value of cranberry juice is 3.000 (Ripa, 1992).

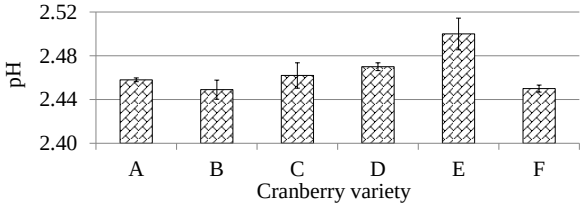

Figure 4. pH value

A – wild, B –'Steven', C – 'Bergman', D –'Ben Lear', E –'Pilgrim', F –'Early Black'

Possible presence and abundance of organic acids, mainly citric, malic, quinic, and benzoic acids, are responsible for cranberries characteristically low pH (Figure 4). These acids also aid in the stabilization and protection of anthocyanins, and help protect the much desired red color of cranberries. In theory, the pH should also affect the cranberry phenolic ability to inhibit lipid peroxidation and their free radical-scavenging capacity (Cailleta et al., 2011).

Organic acids

Cranberries are rich in bioactive components, such as, phenols and organic acids, which can provide berry antimicrobial activity (Puupponen-Pimia et al., 2001; Rauha et al., 2000).

Organic acids were determined in cranberries as follow: tartaric acid, qinic acid, malic acid, succinic acid, benzoic acid (Fig. 5).

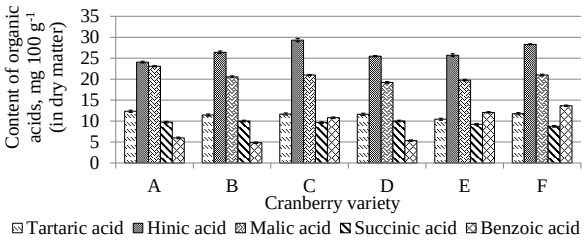

Figure 5. Content of organic acids

A – wild, B –'Steven', C – 'Bergman', D –'Ben Lear', E –'Pilgrim', F –'Early Black'

It should be noted that the benzoic acid content is one of the most important parameters for berry bactericidal properties. The highest content of benzoic acid was determined in large-berry cranberries of the variety ′Early Black′ – 13.66 mg 100 g^{-1}, which was by 56% higher than in wild cranberries – 5.98 mg 100 g^{-1}, and by 56% higher than in the variety ′Steven′ berries – 4.82 mg 100 g^{-1} (Figure 5). The mathematical data processing outlines that the benzoic acid content is significantly different in all cranberry varieties (p=0.0001; α=0.0500), except wild cranberries and cranberries of the variety ′Ben Lear′ (p=0.0430; α=0.0500). There are no significant differences between the large-berry variety ′Steven′ and ′Ben Lear′ (p=0. 0500; α=0.0500) cranberries.

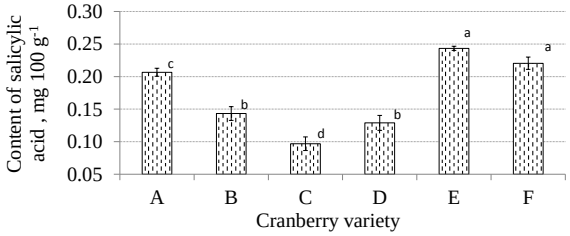

Figure 6. Content of salicylic acid

A – wild, B –'Steven', C – 'Bergman', D –'Ben Lear', E –'Pilgrim', F –'Early Black'

The highest salicylic acid content was found in cranberry variety 'Pilgrim' – 0.2434±0.0032 mg 100 g^{-1} it was by 15% higher comparing to salicylic acid content in wild cranberries – 0.2066±0.0062 mg 100 g^{-1} and by 60% higher comparing to salicylic acid content in 'Bergman' cranberries – 0.0968±0.0105 mg 100 g^{-1} in dry matter (Figure 6). In the present research it was proved, that in cranberries is highest hinic acid content (24.0000–29.0000 mg 100 g^{-1}) (Figure 5), however lowest (Figure 6) – salicylic acid content (0.0968–0.2434 mg 100 g^{-1}). Such difference mainly could be explained with individuality of the variety (Celik et al., 2008).

Polyphenols

The most important polyphenol function is protection of plant decay modes (Dixon, Paiva, 1995; Bennet, Wallsgrove, 1994). There are caused by phenol

changes, like excessive exposure to UV light, mechanical damage or microbial infection. The environmental factors have the significant effect on the content of phenolic compounds in berries. Within present research it was established that in cranberry more popular polyphenols are rutin, catechin, caffeic acid and chlorogene acid (Figure 7).

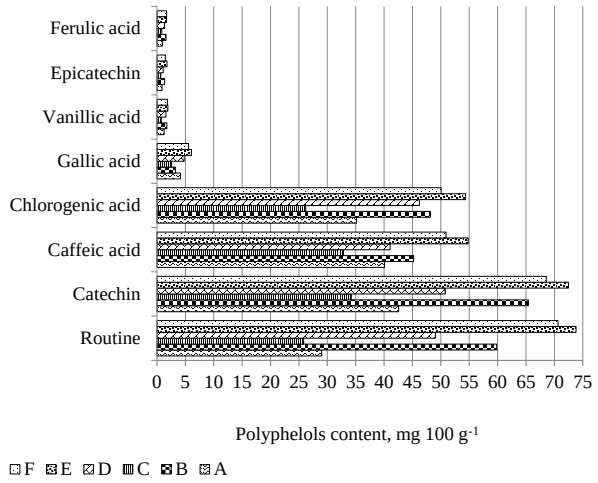

Figure 7. Content of polyphenols in fresh cranberries (in dry matter)

A – wild, B –'Steven', C – 'Bergman', D –'Ben Lear', E –'Pilgrim', F –'Early Black'

The highest content of analysed compounds was found in cranberries of the variety ′Pilgrim′, ′Early Black′, and ′Steven′; while the lowest – in cranberries of the variety ′Bergman′ (Figure 7). There is a significant difference ($p=0.0001$, $\alpha=0.0500$) in the content of polyphenol in cranberries.

Conclusions

The highest anthocyanin content was found in cranberry varieties 'Pilgrim' and 'Early Black', the lowest in wild cranberries.

The colour of wild cranberries is darker then the colour of large-berry cranberries.

The highest content of benzoic acid was determined in large-berry cranberries of the variety ′Early Black′, which was by 56% higher than in wild cranberries – 5.98 mg 100 g^{-1}, and by 56% higher than in the variety ′Steven′ berries – 4.82 mg 100 g^{-1} in dry matter.

The highest salicylic acid content was found in cranberry variety 'Pilgrim' berries; it was by 15% higher comparing to salicylic acid content in wild cranberries and by 60% higher comparing to salicylic acid content in 'Bergman' cranberries.

The highest content of polyphenol compounds was found in cranberries of the variety ′Pilgrim′, ′Early Black′, and ′Steven′; while the lowest – in cranberries of the variety ′Bergman′.

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References

1. Arslan D., Özcan M.M. (2011) Influence of growing area and harvest date on the organic acid composition of olive fruits from Gemlik variety. *Journal of Scientia Horticulturae*, Vol. 130, p. 633–641.
2. Bennet R.C., Wallsgrove R.M. (1994) Secondary metabolites in plant defence mechanisms. *Tansley Review*, No. 72. New Phytol, Vol. 127, p. 617–633.
3. Berregi I., Santos J. I., Gloria del Campo, Miranda J.I. (2003) Quantitative Determination of (-)- Epicatechin in Cider Apple Juice by HNMR. *Talanta*, Vol. 61, Iss. 2, p. 139–145.
4. Bordignon-Luiz M.T., Gauche C., Gris E.F., Falcao L.D. (2007) Colour stability of anthocyanins from Isabel Grapes *(Vitis labrusca L.)* in model systems. *LWT-Food Science and Technology*, Vol. 40, Iss. 4, p. 594–599.
5. Cailleta S., Côtéa J., Doyonb G., Sylvainc J.-F., Lacroixa M. (2011) Antioxidant and antiradical properties of cranberry juice and extracts. *Food Research International*, Vol. 44, Iss. 5, p. 1408–1413.
6. Celik H., Ozgen M., Serce S., Kaya C. (2008) Phytochemical accumulation and antioxidant capacity at four maturity stages of cranberry fruit. *Journal of Scientia Horticulturae,* Vol. 117, p. 345–348.
7. Chen H., Zuo Y., Deng Y. (2001) Seperation and determination of flavonoids and other phenolic compounds in cranberry Juice by high-performance liquid chromatography. *Journal of Chromatography A,* Vol. 913, Iss. 1–2, p. 387–395.
8. Dashek W.V., Micales A.M. (1997) Methods in plant biochemistry and molecular biology. **In:** *Isolation, Separation, and Characterization of organic Acids.* Dashek W.V. (Ed.), CRC Press, Boca Ration, FL, USA, p. 107–113.
9. Dixon R.A., Paiva N.L. (1995) Stress-induced phenylpropanoid metabolism. *Journal of Plant Cell,* Vol. 7, p. 1085–1097.
10. Hendry G.A., Houghton J.D. (1996) *Natural Food Colorants*. Glasgow: Backie Academic and Professional. p. 348.
11. Hernandez Y., Lobo M.G., Gonzalez M. (2009) Factors affecting sample extraction in the liquid chromatographic determination of organic acids in papaya and pineapple. *Food Chemistry*, Vol. 114, Iss. 2, p. 734–741.
12. Hoffmann G. (2008) *CIELab Color Space.* p. 2–3.
13. Hui H.Y., Barta J., Pilar Cano M., Gusek T., Sidhu J.S., Sinha N. (2006) *Handbook of Fruits and Fruit Processing*. USA: Blackwell Publishing. 697 p.
14. Jackman R.L., Smith J.L. (1996) *Natural Food Colourants*. Glasgow: London, p. 244–309.

15. Koyuncu M.A., Dilmaçünal T. (2010) Determination of vitamin C and organic acid changes in strawberry by HPLC during cold storage. *Journal Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, Vol. 38 Iss. 3, p. 95–98.
16. Lee H.S. (1993) HPLC method for separation and determination of non-volatile organic acids in orange juice. *Journal of Agriculture and Food Chemistry*, Vol. 41, p. 1991–1993.
17. McGuire R.G. (1992) Reporting of objective color measurements. *Journal of Horticultural Science*, Vol. 27, p. 1254–1255.
18. Pazmiño-Durán A.E., Giusti M.M., Wrolstad R.E., Glória B.A. (2001) Anthocyanins from oxalis triangularis as potentital food colorants. *Journal of Food Chemistry*, Vol. 75, Iss. 2, p.211–216.
19. Prior R.L., Lazarus S.A., Cao G., Muccitelli H., Hammerstone J.F. (2001) Identification of procyanidins and anthocyanins in blueberries and cranberries *(Vaccinium Spp*.) using high-performance liquid chromatography/mass spectrometry. *Journal of Agricultural and Food Chemistry*, Vol. 49, p. 1270–1276.
20. Puupponen-Pimia R., Nohynek L., Meier C., Kahkonen M., Hoinonen M., Hopia A., Oksman-Caldentey K.M. (2001) Antimicrobial properties of phenolic compounds from Finnish berries. *Journal of Applied Microbiology*, Vol. 90, p. 494–507.
21. Rauha J.P., Remes S., Heinonen M., Hopia A., Kahkonen M., Kujala T., Pihlaja K., Vuorela H., Vuorela P. (2000) Antimicrobial effects of Finish plant extracts containing flavonoids and other phenolic compounds. *International Journal of Food Microbiology*, Vol. 56, p. 3–12.
22. Ripa A. (1992) *Cranberries, bog bilberry, cowberry in the garden.* Riga: Avots. 104 p. (in Latvian)
23. Silva G.H., Chase R.W., Hammercshmidt R., Cash J.N. (1991) After-cooking darkening of Spartan Pearl potatoes as influenced by location, phenolic acids, and citric acid. *Journal of Agricultural and Food Chemistry,* Vol. 39, p. 871–873.
24. Tall J.M., Seeram N.P., Zhao C., Nair M.G., Meyer R.A., Raja S.N. (2004) Tart cherry anthocyanins suppress inflammation-induced pain behaviour in rat. *Journal of Behaviour Brain Research*, Vol. 153, Iss. 1, p. 181–188.
25. Wang S.J., Stretch A.W. (2001) Antioxidant capacity in cranberry is influenced by cultivar and storage temperature. *Journal of Agricultural and Food Chemistry*, Vol. 49, p. 969–974.

PHYSICAL AND CHEMICAL PARAMETERS OF STRAWBERRY PUREE

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Abstract

Practice of the world shows, that more and more often a consumer chooses products, which can be used in nutrition without spending much time for their preparation. Use of fruit desserts in nutrition is one of the ways how to consume vitamins and minerals. The aim was to investigate physical and chemical parameters of strawberry puree from different cultivars and the effect of freezing on its quality. The strawberry cultivars - 'Polka', 'Honeoye', and 'Senga Sengana' harvested in Latvia were used in the study. The strawberries were processed in a blender until obtaining a homogenous mass. The strawberry mass was analyzed fresh and after storage at –18 °C. Content of soluble solids was determined according to ISO 2173:2003 using digital refractometer; content of total phenols by spectrophotometer method. Content of total acids was explicit as citric acid. Content of sugars (sucrose, glucose, and fructose) was evaluated by high performance liquid chromatography (HPLC). Content of anthocyanins was evaluated by spectrophotometer method. pH was measured according to LVS 1132:2001, content of vitamin C - AOAC Official Method 967.21. Colour was measured in CIE L* a* b* system. Content of vitamin C in strawberry puree essentially differs ($p<0.01$) among the researched cultivars and among the types of thermal treatment. Content of vitamin C in strawberry puree has a close correlation with content of total sugars. Close correlation of a* (r=0.810) and b* (r=0.851) with content of total sugars was established. This could be explained with the changes of the content of anthocyanins in strawberry puree. Content of total acids in fresh strawberry puree from the researched cultivars was from 0.85±0.03 to 0.87±0.03 mg 100 g^{-1}, which increases in average for 4.4% as a result of freezing. The highest content of anthocyanins was in fresh strawberry puree from the cultivar 'Polka', whereas the lowest – from the cultivar 'Honeoye'.

Keywords: strawberry puree, freezing, anthocyanins, sugars, vitamin C, colour.

Introduction

Practice of the world shows, that more and more often a consumer chooses products, which can be used in nutrition without spending much time for their preparation. Use of fruit desserts in nutrition is one of the ways how to consume vitamins and minerals.

Strawberries contain 80–90% water, 0.9–1.2% fibre, 4.5–10% sugar, 0.17–0.25% tannins, vitamins B_1, B_6, K, carotene, folic acid, iron, potassium, calcium (Blanda et al., 2009). Strawberries are known as a good source of vitamin C, folates (folic acid) and recently also as a product with high content of various phenols (Proteggente et al., 2002), majority of which exhibits antioxidative capacity (Tulipani et al., 2011). Strawberry quality is also described by content of minerals, carbohydrates, organic acids – malic acid, tartaric acid, citric acid. Citric acid forms around 90% of organic acids present in strawberries (Sturm et al., 2003). It is reduced along ripening and depends on the cultivar (Brasileiro et al., 2011).

According to Sturm et al. (2003) and Pelayo et al. (2003) the most important strawberry quality indicators are content of sugars and total acids, which can be described as a sugar / acid ratio (Pineli et al., 2011). Sugar / acid ratio characterizes degree of sweetness, which depends on cultivar, ripeness, and weather conditions. Sturm et al. (2003) in their research established different results, where total content of acids in some cultivars increased during fruit ripening (content of citric acid in strawberries of cultivar 'Miss' increased from 0.40 g 100 g^{-1} till 1.00 g 100 g^{-1}). This study indicated presence of tartaric acid in amount of 0.10±0.04 g 100 g^{-1}. The content of sucrose and glucose was the smallest among all sugars detected in studied strawberries, whereas fructose content was the highest (Sturm et al., 2003). According to the study of Pelayo et al. (2003) dry matter in strawberries was 7.8–16.6%; total sugars (fructose, glucose, sucrose) were 4.4–10.8%, organic acids – 0.49–1.61%. Pineli et al. (2011) observed that values of total anthocyanins increased approximately 9–13-fold from green to pink stage. Cyanindin-3-glucoside contributes to a red colour, whereas pelargonidin is a red-orange pigment.

Strawberries are one of the most widely consumed fruits both in fresh and processed form – such as juice, beverage, jam, puree or powder. Storage of fresh strawberries is complicated because they are perishable soft fruits exhibiting an extremely short postharvest shelf-life due to high content of water. Freezing is the best and most effective method of fruit preservation, which maintains taste, appearance, flavour and nutritional value of fresh fruits (Sturm et al., 2003; Ancos et al., 2000; Skrede, 1996). More and more often fruit is frozen for further processing and it is important to study effect of freezing on quality of final products (Skrupskis et al., 2011; Kampuse et al., 2005). Only few researches are available on freezing of fruit puree (Huang et al., 2013). Researchers concluded that fruit with high content of soluble solids are less suitable for freezing (Modise, 2008; Kampuse et al., 2003). Sour fruit juice (Boca et al., 2011), sugar, sugar syrup or honey can be used for puree colour stabilisation during its storage and thawing. Quality of fruit puree is influenced by many factors – differences in cultivars, changes of physical and chemical and

textural parameters during thermal treatment and storage, sensory parameters, as well as microbiological quality.

The aim of the current research was to investigate physical and chemical parameters of strawberry puree from different cultivars and the effect of freezing on its quality.

Materials and Methods

Materials

Strawberries of the cultivars 'Polka', 'Honeoye', and 'Senga Sengana' at full ripeness stage, harvested at the end of June or at the beginning of July depending on the type of cultivar, were used for the study. After harvesting, the strawberries were sorted, cleaned, washed, processed in a HR 2000/70 blender (Philips, China) until obtaining a homogenous mass, and placed into 200 mL plastic vessels. The prepared mass was analysed – fresh (just after preparation) and frozen (frozen at -25±2 °C for 20 h, stored in a frozen condition at -18±2 °C for three months, thawed for 16 h till the product temperature reached +4±2 °C; further in the text – frozen puree).

Methods

For measurement of *soluble solids content*, an ATAGO N20 digital refractometer (Atago Co., Ltd, Japan) was used according to ISO 2173:2003.

pH was determined using a Jenway pH meter 3510 (Jenway, UK) according to the standard LVS EN 1132 "pH Determination of Fruit and Vegetable Juice".

Content of vitamin C was determined by 2,6-dichloroindophenol titrimetric method (AOAC Official Method 967.21).

Content of sugars (sucrose, glucose, and fructose) was determined using a LC-10AD Prominence (Shimadzu Japan) high performance liquid chromatograph (HPLC) equipped with refractive index detector RID-10A and autosampler SIL-10AF. Test conditions: mobile phase mixture of acetonitrile with deionized water in ratio 80 : 20 (v/v); flow rate – 0.6 mL min^{-1}; temperature of column and detector – +50 °C; volume of the injected sample – 10 μL.

Strawberry puree samples for sugar testing were prepared adding 40 mL of deionized water (+80 °C) to 10 grams of puree and mixing it for 1 min. Then sample was centrifuged (10 000 g / 5 min at +20 °C). The procedure was repeated twice, then supernatant was cleared, and finally filtered through membrane filter (0.45 μm). Each sample was analysed in triplicate. Chromatograph software calculated content of glucose, fructose, and sucrose comparing chromatograms of puree with standard curves of respective sugar.

Content of total acids was determined by potentiometric titration method and was converted to citric acid.

The *total phenolic content* (TPC) of strawberry puree was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999) with some modifications. For analysis 4–6 grams of puree was taken. The absorbance was measured at 765 nm using a UV–1650 PC spectrophotometer (Shimadzu, Japan) twice for each of the duplicate samples. Total phenols were expressed as gallic acid equivalents (GAE) per 100 grams of puree.

The *content of anthocyanins* was determined by the spectrophotometric method (Moor et al., 2005). For determination, 20 grams of strawberry puree was homogenized together with 40 g of ethanol and hydrochloric acid mixture (85 : 15 v/v) for 1 min. After filtration sediments were rinsed three times with 10 mL ethanol and 1.5 M HCl reagent (3 x 10 mL). The absorbance of filtrate was measured at 535 nm using a UV-1650 PC spectrophotometer (Shimadzu, Japan) in triplicate.

Colour was measured in CIE L*a*b* colour system by a colorimeter (Tec PCM/PSM, USA). Strawberry puree before colour measurement was placed in a 100 mL PET containers, to have the same thickness of puree layer. Colour was measured in eight randomly selected spots. Data was processed in ColorSoft software.

A colour spectrum was determined in a three coordinate system: L* represents lightness, where L*=100 – white, L*=0 – black. Value of colour component a* is from -a* (green) to +a* (red), component b* – from -b* (blue) to +b* (yellow). Colour changes in puree after freezing compared to fresh puree were described by total colour difference ΔE* (MacDougall et al., 2002).

Total colour difference (ΔE*) was calculated according to the equation:

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

where L_0^*; a_0^*; b_0^* – values for fresh sample;

L^*; a^*; b^* – values for frozen sample.

In the research calculations were carried using *MS Excel* program and *SPSS 16* statistics software. The hypothesis set was checked with the p-value method. Factors were estimated as significant when the p-value was $<\alpha_{0.05}$. For interpretation of the results it is assumed, that α=0.05 with 95% reliability, if not indicated otherwise. The following tests and analyses were used: one-factor and two-factor variance analysis (ANOVA), correlation and regression analysis. If the correlation between variables is linear, the determination coefficient coincides with the correlation coefficient: R^2 is equal to r^2. If the value of correlation coefficient is $0.5 \leq |r| \leq 0.8$, then there is medium close linear correlation between the examined variables. If $|r|>0.8$, then there is a close linear correlation between the examined variables (Arhipova, Bāliņa, 2006).

Results and Discussion

Soluble solids

Soluble solids content in fresh strawberry puree samples ranged from 8.54 °Brix to 10.50 °Brix

(Table 1). After freezing and thawing cycle content of soluble solids decreased possibly due to drip loss.

Table 1

Content of soluble solids in fresh and frozen strawberry puree

Cultivar	Soluble solids, °Brix	
	fresh	frozen
'Senga Sengana'	9.32±0.06	8.24±0.08
'Polka'	10.50±0.08	9.98±0.05
'Honeoye'	8.54±0.19	8.39±0.05

Pineli et al. (2011) observed lower content of soluble solids (5.0–7.9 °Brix).

pH

Values found for pH tended to be lower for strawberries of cultivar 'Honeoye' (Table 2).

Table 2

pH in fresh and frozen strawberry puree

Cultivar	pH	
	fresh	frozen
'Senga Sengana'	3.43±0.02	3.32±0.01
'Polka'	3.44±0.01	3.32±0.01
'Honeoye'	3.24±0.02	3.18±0.03

pH determined for strawberry purees in the current research are within the same range as observed by Pineli et al. (2011).

Content of vitamin C

Content of vitamin C in strawberry puree essentially differed ($p<0.01$) among the researched cultivars (Table 3).

Table 3

Content of vitamin C in fresh and frozen strawberry puree

Cultivar	Content, mg 100 g^{-1}	
	fresh	frozen
'Senga Sengana'	23.51±0.38	21.63±0.26
'Polka'	21.13±0.23	15.81±0.32
'Honeoye'	19.40±0.27	14.50±0.30

The most significant differences were found between puree made from 'Senga Sengana' and 'Polka', as well as puree of 'Senga Sengana' and 'Honeoye'. Content of vitamin C in fresh puree was between 19.40±0.27 mg 100 g^{-1} ('Honeoye') and 23.51±0.38 mg 100 g^{-1} ('Senga Sengana'). During fruit homogenization air is incorporated in puree, consequently content of vitamin C is reduced due to presence of oxygen. As a result fruit puree contains smaller amount of vitamin C compared to whole fruits. Literature data show, that vitamin C content in strawberries (not puree) of cultivar 'Polka' was 58–63 mg 100 g^{-1} (Laugale et al., 2000); Finnish researchers depending on harvest year detected its content from 48.0±1.6 to 62.0±1.6 mg 100 g^{-1} (Hakala et al., 2003). Whereas strawberries of cultivar 'Honeoye' contained more vitamin C – 80–87 mg 100 g^{-1} (Laugale et al., 2012) and 54.1±3.7–71.3±3.1 mg 100 g^{-1} (Hakala et al., 2003). In cultivar 'Senga Sengana' it was from 43.6±2.5 to 54.2±2.5 mg 100 g^{-1} (Hakala et al., 2003). Another Finnish study (Hägg et al., 1995) in various fresh strawberries determined 56–99 mg 100 g^{-1} of vitamin C, which decreased by 34% as aresult of freezing. Our research reveals that content of vitamin C is significantly higher ($p<0.01$) in fresh puree compared to puree after freezing. The decrease of vitamin C content in all studied purees was 18.8% on average. Velde et al. (2013) demonstrated that content of vitamin C in frozen strawberries was 44.5±3.2 mg 100 g^{-1}.

Vitamin C content has a close correlation with total sugar content in strawberry puree ($r=0.830$). Data shows: the higher total sugar content in strawberry puree, the higher vitamin C content (Fig. 1).

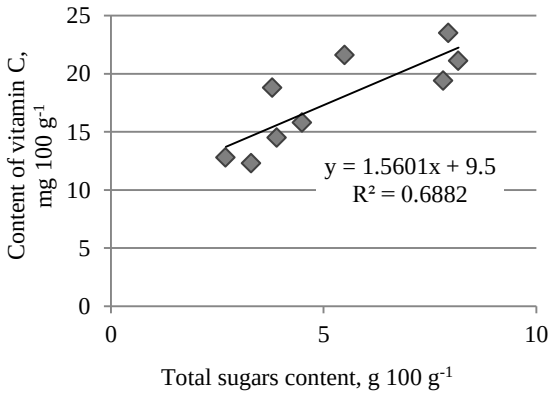

Figure 1. Correlation between content of vitamin C and total sugars in strawberry puree

Regression equation shows – if total sugar content is increased by 1 g 100 g^{-1}, content of vitamin C will increase by 1.56 mg 100 g^{-1}. It can be explained by the processes taking place during fruit maturation – both synthesis of vitamin C and sugars occurs simultaneously.

Content of total sugars

There is no essential difference ($p=0.117$) in content of total sugars of strawberry puree between cultivars, whereas it is essentially ($p<0.001$) influenced by the freezing (Fig. 2). Total sugar content in fresh strawberry puree is from 7.82 to 8.17 g 100 g^{-1}, which is similar to the result established for strawberry variety 'Mohawk' – 7.41 g 100 g^{-1} (Sturm et al., 2003); whereas German study (Keutgen et al., 2007) show total sugar content of 6.33±0.39 g 100 g^{-1}, which is lower compared to our study. Total sugar content in strawberry puree decreased during freezing in average by 41.9%.

Content of individual sugars – sucrose, glucose and fructose in both fresh and frozen strawberry puree is presented in Figure 3.

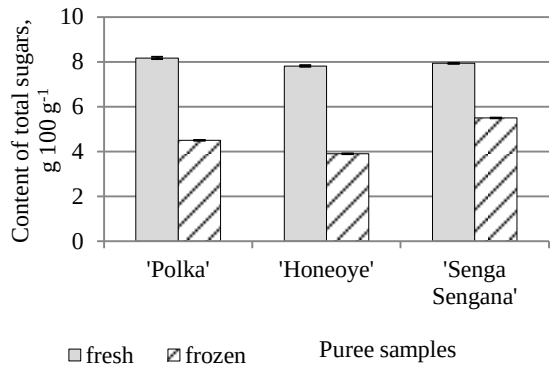

Figure 2. Content of total sugars in strawberry puree

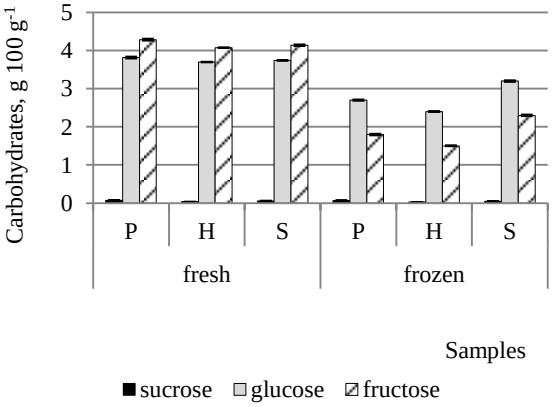

Figure 3. Content of sucrose, fructose and glucose in strawberry puree

P – 'Polka'; H – 'Honeoye'; S – 'Senga Sengana'

Evaluation of individual sugars (sucrose, glucose, and fructose) in strawberry puree show that the highest content exhibited *fructose* from 4.08±0.01 to 4.29±0.01 g 100 g^{-1}. Its content did no differ among studied cultivars (p=0.124). Sturm et al. (2003) in their study found that fresh strawberries of various cultivars contained 0.04–3.30 g 100 g^{-1}, which is lower compared to our results. Essential differences in content of fructose (p<0.001) are established between fresh and frozen strawberry puree. Its content decreases as a result of freezing (approx. by 55.2%).

Content of *glucose* in fresh strawberry puree is from 3.70±0.01 to 3.81±0.03 g 100 g^{-1}, what is less than content of fructose in it. Content of glucose does not differ among studied cultivar (p=0.109) purees. Slovenian research (Sturm et al., 2003) established content of glucose in strawberries of various cultivars in the range from 1.63 to 2.82 g 100 g^{-1}, which is lower compared to our study. Content of glucose in strawberry puree has a tendency to decrease as a result of freezing (in average for 26%), as it was observed with content of fructose. Comparing content of sugars in fresh strawberry purees from all the researched cultivars, content of *sucrose* in them is the lowest. It is from 0.04±0.001 to 0.07±0.003 g 100 g^{-1} and it does not differ substantially among cultivars (p=0.301), however it is essentially (p<0.001) influenced by the freezing.

Content of total acids

Content of total acids in fresh strawberry puree does not essentially (p=0.164) differ among cultivars and it is from 0.85±0.03 to 0.87±0.03 mg 100 g^{-1}, which increases in average for 4.4% as a result of freezing (Table 4).

Table 4

Content of total acids in strawberry puree

Cultivar	Content, mg 100 g^{-1}	
	fresh	frozen
'Senga Sengana'	0.86±0.03	0.89±0.02
'Polka'	0.87±0.03	0.90±0.03
'Honeoye'	0.85±0.03	0.90±0.02

Content of total acids has a negative close correlation with content of vitamin C in strawberry puree (r=0.820). Data show that content of vitamin C is lower in strawberry puree, which has higher content of total acids. This can be explained by the ripeness stage of strawberries. This is also confirmed by close negative correlation (r=0.916), which exists between content of total sugars and acids in strawberry puree (Figure 4).

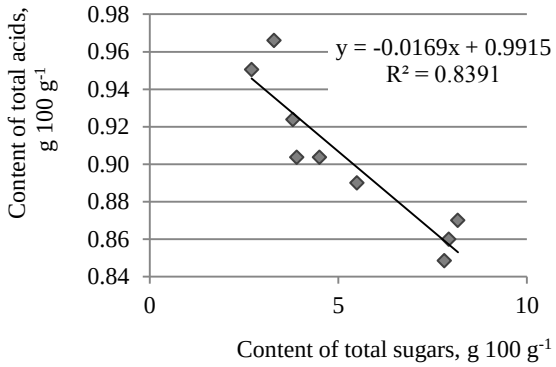

Figure 4. Correlation between content of total acids and sugars in strawberry puree

The correlation shows that the increase of total sugars by 1 g 100 g^{-1}, results in decrease of total acids in strawberry puree by 0.0169 g 100 g^{-1}. This might be due to the physiological properties of fruits when with increased stage of ripeness increase in sugar content and decrease in total acids is observed (Brasileiro et al., 2011). *Sugar/acid ratio* was not significantly different among cultivars (p=0.138) and it ranged from 9.2 to 9.4. Fresh strawberry purees from researched cultivars may be characterized as "sour", which sugar/acid ratio does not change essentially during freezing – they also can be characterised as "sour". Sturm et al. (2003) found sugar/acid ratio at full maturity stage around 6.5 in average, with higher values for some cultivars – 8.7 (cultivar 'Mohawka'), whereas Keutgen et al. (2007) reported sugar / acid ratio around 10.13, which is similar to our result.

Content of total phenolic compounds

Content of total phenolic compounds in strawberry puree (Table 5) does not significantly differ among cultivars (p=0.471).

Table 5

Content of total phenols in strawberry puree

Cultivar	Content, mg 100 g^{-1}	
	fresh	frozen
'Senga Sengana'	141±14	1591±28
'Polka'	181±16	1733±27
'Honeoye'	130±11	2180±29

Pineli et al. (2011) reported higher content of phenols in fresh strawberries compared to our results 223.46±6.96 mg 100 g^{-1}. Our research revealed that content of total phenols in fresh strawberry puree essentially differs (p=0.001) from their content in frozen puree – in average by 91%. The same conclusion was drawn in research of Blanda et al. (2009), who established higher total phenol content in frozen fruits compared to fresh ones. Higher content possibly was formed in hydrolysis of polymers or in metabolism processes, which is created by osmotic stress in fruits (Blanda et al., 2009).

Content of anthocyanins

Content of anthocyanins in strawberry puree was essentially (p<0.01) different among the researched cultivars (Figure 5). The highest content of anthocyanins was in fresh strawberry puree from the cultivar 'Polka' (30.04±0.63 mg 100 g^{-1}), whereas the lowest – from the cultivar 'Honeoye' (14.91±0.41 mg 100 g^{-1}).

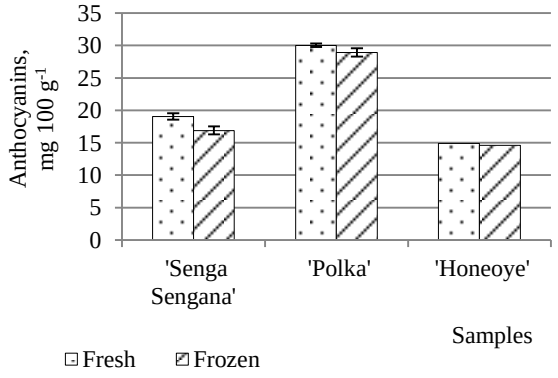

Figure 5. Content of anthocyanins in strawberry puree

During freezing their content decreased for 2.05% to 11.32%. During freezing content of anthocyanins was preserved better than under the influence of pasteurisation, alongside better preserving the red colour characteristic to strawberries (Boca et al., 2011). Content of anthocyanins has a close correlation with the content of soluble solids in strawberry purees (r=0.833). In strawberry purees, with higher content of soluble solids, the content of anthocyanins was higher.

Literature show anthocyanins content in strawberries 10–80 mg 100 g^{-1}. Pineli et al. (2011) indicated that anthocyanins content in unripe berries was 2.35±0.35 mg 100 g^{-1}, and it increased till 22.64±0.66 mg 100 g^{-1} upon ripening. Aaby et al. (2012) study demonstrated higher anthocyanins content, in fresh strawberries of cultivar 'Polka' it was 37.2 mg 100 g^{-1}, in 'Senga Sengana' – 27.4 mg 100 g^{-1}. Whereas Velde et al. (2013) in frozen strawberries established similar anthocyanins content to our study 22.1±4.2 mg 100 g^{-1}.

Strawberry puree colour

Colour component a*, b* and L* values of fresh and frozen strawberry puree does not differ essentially in cultivars (p>0.05), whereas there are essential (p<0.001) differences of them in the types of treatment. This is also shown by total colour changes (Fig. 6).

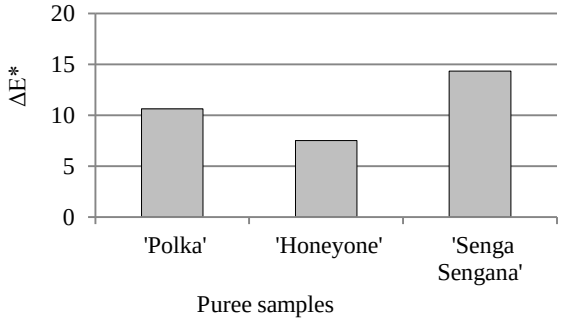

Figure 6. The influence of freezing on the total colour difference (ΔE*) of strawberry puree

Colour component a* and b* value of strawberry puree has a close correlation with content of total sugars in strawberry puree. Data, displaying the close correlation a* (r=0.810) and b* (r=0.851) for the value with content of total sugars in strawberry puree, show that in samples, where content of total sugars is higher, red colour (a*) and yellow (b*) colour intensity increases. This could be explained with the changes of the content of anthocyanins in strawberry puree, because they form red colour characteristic to strawberries and they are glucosidically bound with sugar.

Conclusions

Content of vitamin C in strawberry puree essentially differs (p<0.01) among the researched cultivars and between fresh and frozen-thawed puree. Close correlation of colour components a* (r=0.810) and b* (r=0.851) with content of total sugars was established. Content of total acids in fresh strawberry puree from the researched cultivars was from 0.85±0.03 to 0.87±0.03 mg 100 g^{-1}, which increases in average by 4.4% as a result of freezing. The highest content of anthocyanins was in fresh strawberry puree from the cultivar 'Polka', whereas the lowest – from the cultivar 'Honeoye'.

References

1. Aaby K., Mazur S., Nes A., Skrede G. (2012) Phenolic compounds in strawberry (Fragaria x ananassa Duch.)

fruits: Composition in 27 cultivars and changes during ripening. *Food Chemistry*, Vol. 132, p. 86–97.
2. Ancos B., Gonzlez E., Cano M. (2000) Ellagic acid, vitamin C, and total phenolic contents and radical scavenging capacity affected by freezing and frozen storage in raspberry fruit. *Journal of Agricultural and Food Chemistry*, Vol. 48, No. 10, p. 4565–4570.
3. Arhipova I., Bāliņa S. (2003) *Statistika ekonomikā. Risinājumi ar SSPS un Microsoft Excel: mācību līdzeklis*. Rīga: Datorzinību centrs. 352 lpp.
4. Blanda G., Cerretani L., Cardinali A., Barbieri S., Bendini A., Lercker G. (2009) Osmotic dehydrofreezing of strawberries: Polyphenolic content, volatile profile and consumer acceptance. *Food Science and Technology*, Vol. 42, p. 30–36.
5. Boca S., Krasnova I., Seglina D., Aboltins A., Skrupskis I. (2011) Influence of natural berry juices on stability of anthocyanins in strawberry mass. **In:** *Proceeding 6th International CIGR Technical Symposium „Towards a Sustainable Food Chain: Food Process, Bioprocessing and Food Quality Management".* Nantes, France, 18th – 20th April, 2011. Nantes, p. 91–94.
6. Brasileiro A.V., Cordova A.C., Chiarello M.D. (2011) Antioxidants and other chemical and physical characteristics of two strawberry cultivars at different ripeness stages. *Journal of Food Composition and Analysis*, Vol. 24, p. 11–16.
7. Hakala M., Lapvetel A., Huopalahti R., Kallio H., Tahvonen R. (2003) Modifications of taste-relevant compounds in strawberry fruit under NaCl salinity, *Journal of Food Composition and Analysis*, Vol. 16, p. 67–80.
8. Hägg M., Ylikoski S., Kumpulainen J. (1995) Vitamin C Content in Fruits and Berries Consumed in Finland, *Journal of Food Composition and Analysis*, Vol. 8, Iss. 1, p. 12–20.
9. Huang Y., Ye M., Chen H. (2013) Inactivation of Escherichia coli O157:H7 and *Salmonella* spp. in strawberry puree by high hydrostatic pressure with/without subsequent frozen storage. *International Journal of Food Microbiology*, Vol. 160, p. 337–343.
10. Kampuse S., Skrupskis I., Kampuss K. (2003) Quality evaluation of different frozen berry cultivars. *Horticulture and Vegetable Growing*, Vol. 22, No. 4, p. 167–177.
11. Kampuse S., Segliņa D., Skrupskis I. (2005) Changes of blackcurrant puree chemical components after frozen storage. *Плодоводство*, Том 17, Часть 2, Proceedings of the international conference of Belarus, p. 282–286. Материалы международной научной конференции "Современное плодоводство: состояние и перспективы развития" (2005: Самохваловичи).
12. Keutgen A., Pawelzik E. (2007) Modifications of taste-relevant compounds in strawberry fruit under NaCl salinity. *Food Chemistry*, Vol. 105, p. 1487–1494.
13. Laugale V. (2000) Remontanto zemeņu šķirņu izmantošana ražošanas sezonas pagarināšanai Latvijas apstākļos. **No:** *Zinātne, Latvija, Eiropa*. Starptautiskās zinātniskās konferences materiāli. Latvija, Jelgava, 71. – 76. lpp.
14. MacDougall D. B. (2002) Colour measurement of food: principles and practice. **In**: *Colour in Food. Improving Quality*. Ed.: D. B. MacDougall. Cambridge, England: Woodhead Publishing Ltd. CRC Press LLC, p. 33–63.
15. Modise D.M. (2008) Does freezing and thawing affect the volatile profile of strawberry fruit (Fragaria×ananassa Duch.)? *Postharvest Biology and Technology*, Vol. 50, p. 25–30.
16. Moor U., Karp K., Põldma P., Pae A. (2005) Cultural Systems Affect Content of Anthocyanins and Vitamin C in Strawberry Fruits. *European Journal of Horticultural Science*, Vol. 70, No. 4, p. 195–201.
17. Pelayo C., Ebeler S.E., Kader A.A. (2003) Postharvest life and flavor quality of three strawberry cultivars kept at 5 °C in air or air +20 kPa CO_2. *Postharvest Biology and Technology*, Vol. 27, p. 171–183.
18. Pineli L., Moretti C., Santos M., Campos A. (2011) Antioxidants and other chemical and physical characteristics of two strawberry cultivars at different ripeness stages. *Journal of Food Composition and Analysis*, Vol. 24, p. 11–16.
19. Proteggente A.R., Pannala A.S., Paganga G., Van Buren L., Wagner E., Wiseman S., Van De Put F., Dacombe C., Rice-Evans C.A. (2002) The antioxidant capacity of regular consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radical Research*, Vol. 36, p. 217–233.
20. Singleton V.L., Orthofe, R., and Lamuela-Raventos R.M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. *Methods in Enzymology*, Vol. 299, p. 152–178.
21. Skrede G. (1996) Fruits. **In:** *Freezing Effects on Food Quality*. E.J. Lester (ed.). New York, NY: Marcel Dekker, Inc., p. 183–245.
22. Skrupskis I., Boca S., Rucins M., Rozenbergs V., Iljins U. (2011) Research of Half-Finished Frozen Fruit and Berry Products. **In**: *Proceedings of the 23rd IIR International congress of refrigeration "Refrigeration for Sustainable Development"*, Prague, Czech Republic, August 21–26, 2011. Czech Republic, Prague, p. 7.
23. Sturm K., Koron D., Stampar F. (2003) The composition of fruit of different strawberry varieties depending on maturity stage. *Food Chemistry*, Vol. 83. p. 417–422.
24. Tulipani S., Marzban G., Herndl A., Laimer M., Mezzetti B. (2011) Influence of environmental and genetic factors on health-related compounds in strawberry. *Food Chemistry*, Vol. 124, p. 906–913.
25. Velde F., Tarola A.M., Güemes D., Pirovani M.E. (2013) Bioactive Compounds and Antioxidant Capacity of Camarosa and Selva Strawberries (*Fragaria* x *ananassa* Duch.). *Foods*, Vol. 2, No. 2, p. 120–131.

INFLUENCE OF *OENOCOCCUS OENI* AND OAK CHIPS ON THE CHEMICAL COMPOSITION AND SENSORY PROPERTIES OF CIDER

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Abstract

Cider quality is influenced by several factors, namely, apple variety, yeast strains, fermentation and maturation conditions. The aim of current research was to evaluate influence of lactic acid bacteria *Oenococcus oeni* and oak chips on the quality of apple cider. After main fermentation lactic acid bacteria *Oenococcus oeni* (LAB) and two types of oak chips: unroasted oak chips (U_OC) and medium roasted oak chips (MR_OC) were added to cider and samples were matured for four weeks. For matured ciders total phenolic compounds (TPC) were determined spectrophotometrically, individual phenolic compounds by HPLC, volatile compounds by SPME followed by GC/MS and sensory properties using line scale and ranking test. The TPCin ciders ranged from1028 mg L^{-1}in cider LA to 1526 mg L^{-1}in cider MR_OC. Among analysed phenolic compounds chlorogenic acid dominated in all samples. In cider MR_OC comparing with control sample higher content of caffeic acid, epicatechin, ferulic acid and vanillin was determined. The highest total peak area was determined in cider U_OC. Principial component analysis showed that profile of volatile compounds can be explained by three factors; the first two represent 81% of the total variances. The characteristic volatiles of cider LAB were acetic acid, ethyl-9-decanoate, etildecanoate, octanoic acid, and etylhexanote, in cider MR_OC 3-methyl-1-butanol acetate, butyl acetate and ethyl acetate, whereas in cider U_OC 3-methyl-1-butanol and phenylalcohol. Preference ranking test results showed that the assessors preferred cider LAB. Addition of *Oenococcus oeni*, unroasted and roasted chips during maturation significantly influence chemical composition and sensory properties intensity of cider.

Keywords: cider,maturation, *Oenococcus oeni*, oak chips

Introduction

The quality of fermented drinks like cider depends on presence of aroma compounds in product, that are influenced by several factors, namely apple variety, yeast strains, fermentation conditions, the production process and fining treatments (Hidalgo et al., 2004; Martınez-Rodrıguez, Polo, 2003). Quality of ciders could be improved during maturation process by malolactic fermentation and maturation on oak chips.

Malolactic fermentation can be considered as the part of maturation process (Buglass, 2011). *Oenococcus oeni* are the main bacteria responsible for malolactic fermentation (Fugelsang, 1997) converting L-malic acid to L-lactic acid and CO_2. Bacteria's can adapt to rough environment of wines with high alcohol content, low pH and presence of sulphur dioxide (Lonvaud-Funel, 1999). In cider production malolactic fermentation is significant, and usually it starts after alcoholic fermentation. Exception is Asturian ciders because there both fermentation processes occurs simultaneously (Blanco Gomis et al., 2003) and efficiency depends on temperature and nutrients. In ciders from unpasteurized and unsulphured juices, can develop wild lactic acid bacterias (Lea, Drilleau, 2003). Winemakers assess the benefits of controlled malolactic fermentation (Krieger-Weber, 2009). Inoculation of starter, that mainly contains *O. oeni* bacteria, winemakers can reduce risks related to potential bacterial or bacteriopfage spoiling, and accelerate beginning of malolactic fermentation and harmonize wine taste and fullness.

In traditional winemaking fermentation and/or maturation occurs in oak barrels and it influence wine quality positively. Wine is fortified with substances extracted from oak, forming more complex aroma and taste of wines (Návojská et al., 2012). Oak barrels are expensive, takes up much area, and also it is more difficult to clean them. Based on these statements alternative methods for improvement of wine quality (Rodríguez-Rodríguez et al., 2011; Návojská et al., 2012) are developed, for example, use of wood chips. In England small and medium cider producers still use oak barels and use of chips are not so popular comparing to wine industry (Buglass, 2011). Oak chips are obtained from wood processing byproducts, by using tradicional methods of treatment – boiling and roasting (Bozalongo et al., 2007). Fan et al. (2011) investigations showed influence of different type oak chips (different raw materials, rosating degree) to the volatiles in cider. Oak typical aroma is influenced by wine and wood contact duration, temperatures, wood properties, for example, species, geographic origin, roasting degree (Garde-Cerdán, Ancin-Azpilicueta, 2006; Garde-Cerdán, 2010, Návojská et al., 2012). Several compounds are typicsal for unroasted oak (*cis* oak lactones) and others as vanillin, 4-methylguaiacol and furfurol, mainly are formed from oak polymers that degradates and hydrolises during storage (Hale et al., 1999). High roasting temperature influence degradation of wood polymers as lignins and celulose, forming aldehydes, phenols, furfurol derivatives, lactones (Nonier et al., 2006). In experiments about oak influence to the aroma of wine, positive correlation between concentration of vanillin and smoke and cinnamon aroma were determined (Spillman et al., 1998). Content of total phenolic compounds increases by increasing roasting intensity(Cabrita et al., 2011).

Development and improvements of technology should be designed taking into account sensory evaluation. In order to match the instrumental analysis with the consumer requirements, a sensorial profile of the cider

is necessary. Sensory quality is related to consumer acceptance and confidence in the product, being defined by the interaction between the food and the consumer. Thus, sensory quality depends on both the sensory characteristics of the food and how consumers perceive them (Cardello, 1995; Costell, 2002; Ares et al., 2009). Sensory descriptive analysis is a primary tool of food scientists, which involves the evaluation of both the qualitative and quantitative sensory characteristics of products (Meilgaard et al., 1999).

The aim of current research was to evaluate influence of lactic acid bacteria *Oenococcus oeni* and oak chips on the quality of apple cider.

Materials and Methods

Experiments were carried out at the Faculty of Food Technology, Latvia University of Agriculture in 2012.

Materials

Apples grown at the Latvian State Institute of Fruit Growing were used in the experiments. Apples were harvested and stored for 1–2 weeks at +3±1 °C with relative ambient humidity of 90–95%. In the present study juices of three varieties 'Auksis', 'Lietuvas Pepiņš', 'Kerr' in proportion 2 : 1 : 2 (by volume) were used. Fermentation was performed using the commercial *Saccharomyces cerevisiae* yeast strain '71B-1122' (Lalvin, Lallemand Inc., Canada). Fermentation was carried out at 16±1 °C for 28 days.

In order to reduce the sharp acidity of cider (malic acid) lactic acid bacteria (Bacchus Malolactic Bacteria Culture *Oenococcus oeni*) (Lalvin, France) was added to the cider at a concentration of 0.05 g L^{-1} (7.3×10^5 cells L^{-1}).

In order to improve the taste and aroma of cider, two types of oak chips were added:

- unroasted oak chips 'French Oak Chips' (Young's Brew, UK);
- medium roasted oak chips 'American oak chips' (Browland, Belgium).

Oak chips were added in concentration 1.5 g L^{-1} of cider.

All cider samples were matured at +16±1 °C for four weeks.

In experiment the following samples were analysed:

- control – control sample stored without additives;
- LAB – sample matured with addition of *Oenococcus oeni*;
- U_OC – sample matured with unroasted oak chips;
- MR_OC – sample matured with medium roasted chips.

Determination of total acids, solids and alcohol content

Determination of titratable acidity (expressed as total acids) was performed according to method LVS EN 12147:2001 and expressed in g L^{-1}. Alcohol content was determined by volume % (ГОСТ 12787–81) and solids were determined after removal of alcohol gravimetrically.

Determination of total phenolic content

The total phenolic concentration was determined spectrophotometrically according to the Folin-Ciocalteu colometric method (Singleton, 1999). Cider was diluted with ethanol/acetic acid solution (1:20 v/v). The ethanol/acetic acid solution was prepared using an acetic acid water solution (2.5%) and ethanol (98% vol.) in ratio the of 10:90 (v/v). To 0.5 mL of aliquot 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times with water) was added and, after 3 minutes 2 mL of sodium carbonate (Na_2CO_3) (75 g L^{-1}) was added. After 30 minutes of incubation at room temperature, the absorbance was measured at 765 nm using a JENWAY 6300 spectrophotometer (Baroworld Scientifid Ltd., UK). Results were expressed as chlorogenic acid equivalents.

Determination of individual polyphenols

The concentration of all individual polyphenols was determined by High-performance liquid chromatography (HPLC) Shimadzu LC-20AD Prominence with diode array detector (SPD-M20A). Separation was performed in a PerkinElmer C18 4.6 mm×250 mm column (thermostated at 27 °C). Eluting solvents are methanol (A, 20%), water (B, 78,4%) and acetic acid (1.6%) used in a gradient mode and at 17.50 minutes solvents ratio are as follows – A concentration 40.3%, B concentration 58.5%, C concentration of 1.2%. 10 µL of the sample was injected in the chromatograph using automatic sample injection system SIL-20 AC. The total duration of the analysis was 35 minutes. For the detection and quantification of compounds, several wavelengths were used: 253 nm for 4-hydroxybenzoic acid and rutine, 263 nm for gallic acid, 278 nm for catechin, caffeic acid, syringic acid, 298 nm for chlorogenic acid, epicatechin, coumaric acid, sinapic acid and ferulic acid.

Determination of volatile compounds

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

quantitative measure, the share in the total GC peak area for each compound is given.

Sensory analysis

Sensory evaluation of the ciders was carried out with trained panellists (33 women and 3 men, aged 21–71). The panellists had studied the basics of sensory evaluation methods and were experienced in sensory panels. This group included students and staff of the Latvia University of Agriculture Faculty of Food Technology. Line scale (ISO 4121:2003) for determination of the intensity of sensory properties (clarity, apple, fruit and yeast aroma, apple, yeast, sour, astringent and bitter taste) was used. Ranking test (ISO 8587:2006) was used to rank samples according to their degree of liking.

Statistical analysis

The analysis of variance was performed by the ANOVA procedure and $p<0.05$ was considered as statistically significant. Principal component analysis was performed with the software Multibase 2014 for Windows.

Results and Discussion

Sensory properties of cider can be improved by the maturation process using different technologies. Quality parameters of matured ciders are presented in Table1.

Table 1

Quality parameters of matured ciders

Sample	Total acids, g L^{-1}	Solids, g L^{-1}	Alcohol, vol %
Control	7.85 ± 0.31^{c}*	0.87 ± 0.06^{b}	5.58 ± 0.23^{ab}
LAB	3.99 ± 0.21^{a}	0.65 ± 0.08^{a}	5.60 ± 0.21^{b}
U_OC	7.16 ± 0.25^{b}	0.85 ± 0.05^{b}	5.52 ± 0.25^{a}
MR_OC	6.97 ± 0.19^{b}	0.84 ± 0.06^{b}	5.54 ± 0.20^{ab}

* The different letters in the same column represents significant diferences between values ($p<0.05$).

In cider LAB significantly ($p<0.05$) lower total acid content and higher pH was detected. Also dry matter in this sample is significantly lower than in all other samples. The alcohol content and pH are important factors affecting the growth of lactic acid bacteria and their activity. Solier et al. (2010) study shows that the low pH of the wine significantly ($p<0.05$) negative effects malolactic fermentation. Gockowiak and Henschke (2003) studies have shown that the pH from 2.9 to 3.5 have a negative impact on the viability of bacteria, but at pH 3.5 malolactic fermentation takes place successfully, regardless of the alcohol content. But the range of pH from 3.0 to 3.2 together with an alcohol content of 78.9 and 102.6 g L^{-1} inhibited the lactic acid bacteria (Solier et al., 2010). Addition of oak chips to maturing process significantly ($p<0.05$) influenced only the content of total acids.

The total phenolic content in the analyzed ciders varied from 1028 mg L^{-1} in the sample LAB to 1526 mg L^{-1} in the sample MR_OC; the total phenolic content in the samples matured with oak chip was significantly ($p<0.05$) higher (Fig.1.).

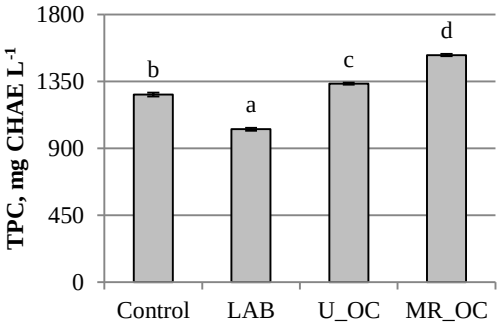

Figure 1. TPC of matured ciders

Chlorogenic acid was the most important identified phenolic compound in all samples of ciders (Tab. 2). The highest content of chlorogenic acid was identified in the control sample, but the lowest content was identified in the sample LAB. Cabrita et al. (2008) in the research on wines revealed that the content of gallic acid, ferulic acid and caffeic acid increased after malolactic fermentation, but vanillin and syringic acid remained stable. The research of Figueiredo-Gonzįlez et al. (2014) showed that the content of catechine and epicatechine decreased during wine maturing in oak barrels. The sample MR_OC had significantly higher content of caffeic acid, epicatechine, ferulic acid and vanillin. At high temperature during roasting process lignina nd cellulose polymers contained in oak chips degradates forming aldehydes, phenols, furfural derivatives, lactones and other compounds (Nonier et al., 2006). Similarly, Bozalongo et al. (2007) found that oak roasting increased the content of compounds created by lignin thermal degradation (vanillin, eugenol etc.).

The highest content of 4-hydroxybenzoic acid was identified in the sample U_OC that corresponded to the findings of Cadahía et al. (2009) where the content of 4-hydroxybensoic acid and its derivatives increased when wines were matured with French oak tree chips.

In analysed samples 22 volatiles were identified. The highest total peak area for sample U_OC and the lowest peak are for control sample were detected. Main volatile compounds are alcohols forming 55.5% (LAB) to 69.1% (U_OC) and esters forming 25.5% (control) to 39.1% (LAB) from total peak area of identified compounds.

The analysis of variance showed a significant difference ($p<0.05$) in all sensory properties intensity except for the intensity of fruit aroma. The addition of lactic acid bacteria caused less distinct clarity in matured ciders with the most intensive apple aroma and taste, but with the least astringent, yeast and sour taste intensity.

The cider samples matured with unroasted and medium-roasted oak chips showed higher bitter taste intensity and lower sour taste intensity (Figure 2).

Table 2

Individual phenolic compounds in ciders (mg L^{-1})

Compounds	Control	LAB	U_OC	MR_OC
Chlorogenic acid	191.25±9.56bd	167.08±7.96a	190.69±9.08bd	188.41±6.28b
Caffeic acid	6.10±0.29 b	4.55±0.23 a	6.96±0.33 b	23.83±0.88c
Syringic acid	7.03±0.25 b	6.38±0.24 a	8.20±0.30c	6.20±0.31 a
Gallic acid	0.10 ±0.01	n.i.	0.60±0.02	0.38±0.01
Vanillin	0.44±0.02 a	0.82±0.03 b	0.86±0.04 b	1.61±0.07 c
Hydroxybenzoic acid	0.30±0.02 b	0.17±0.01 a	0.86±0.03d	0.35±0.01 c
Catechin	2.82±0.12 d	3.26±0.13 c	0.78±0.03 a	1.18±0.06 b
Epicatechine	0.27±0.01d	0.08±0.01 b	0.05±0.01 a	3.16±0.14 c
Sinapic acid	0.11±0.01	0.31±0.01	n.i.	2.21±0.08
Ferulic acid	0.63±0.02 a	1.33±0.06 d	0.72±0.04 b	3.67±0.17 c

n.i. – not identified

* The different letters in the same row represents significant diferences between values ($p<0.05$).

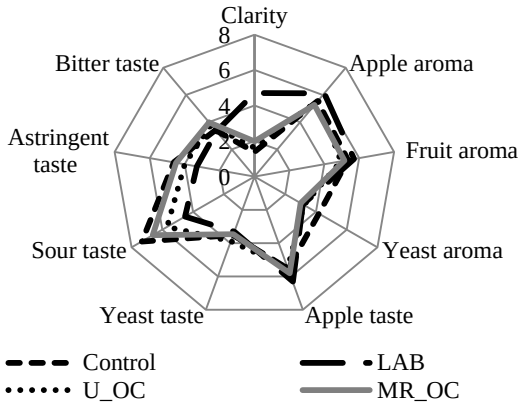

Figure 2. Spider plot for sensory properties of matured ciders

Principal component analysis of volatile compounds and sensory properties showed that differences can be explained by three factors: the first two of them accounted for 82% of the total variable set (Fig. 3).

The results showed that different cider maturing technologies influenced the content of volatile compounds in ciders. The control sample was characterized by the most intensive yeast aroma. The LAB sample was characterized by the most intensive fruit, apple aroma and the most intensive apple taste. The dominating volatile substances in the LAB sample were acetic acid, ethyl-9-decanoate, ethyl decanoate, octanoic acid, ethyl hexanoate; on the whole sweet, oily, fruit (grape), flower aromas, dominated in this sample; less dominant aromas were stale, bitter, soap and wax.

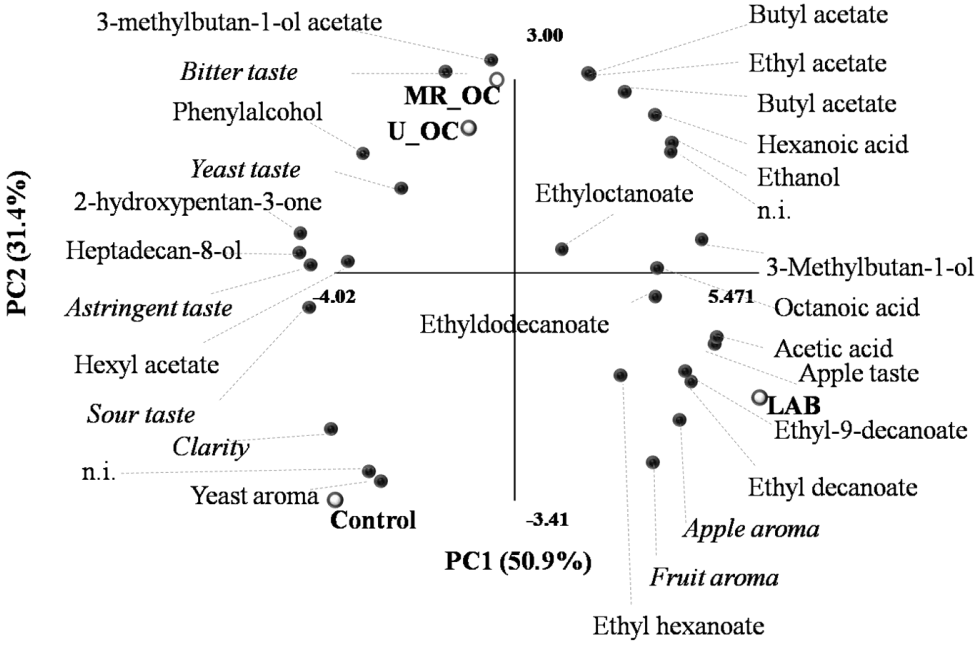

Figure 3. PCA of volatiles and sensory properties of matured ciders

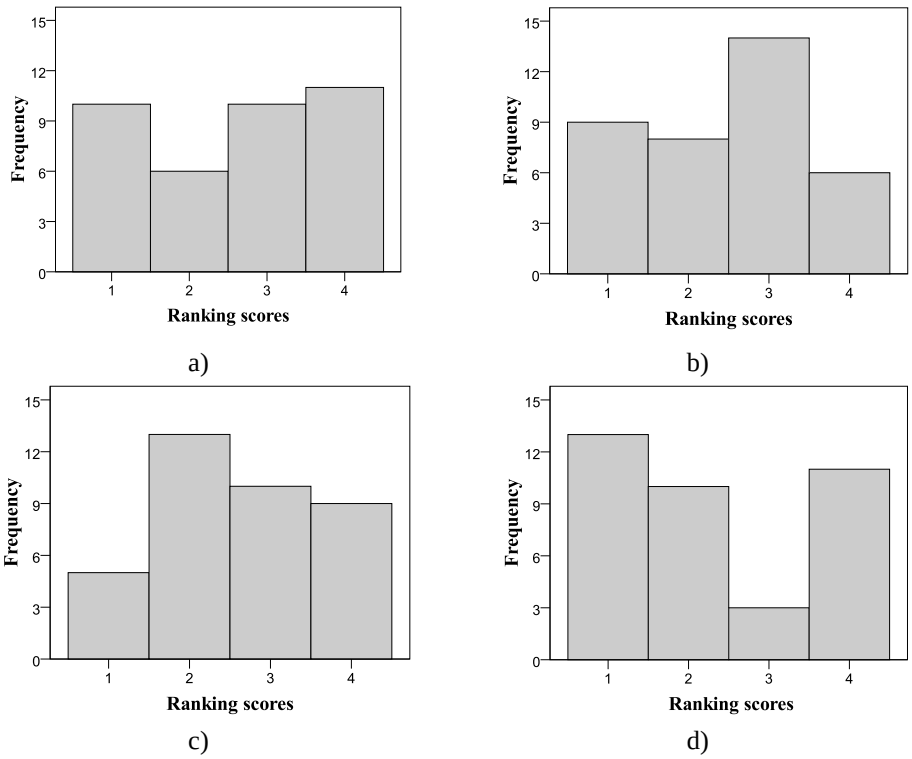

Figure 4. Histograms of matured cider ranking
a) control, b) U_OC, c) MR_OC, d) LAB

The ranking test results are presented as histograms showing frequency of each ranking score (1 – the highest rank; 4 – the lowest rank) evaluated by panellists (Figure 4).
Preference ranking test results showed that the assessors preferred cider LAB.

Conclusions

Addition of lactic acid bacteria *Oenococcus oeni* during cider maturing process changed ciders' chemical properties reducing the total content of acid, soluble solids, phenols, content of volatile compounds. Ciders matured with unroasted chips and medium-roasted chips have lower total content of acids and higher total phenolic content. Maturing with oak chips as well as addition of lactic acid bacteria influenced the ratio of volatile substances, i.e., content of esters and volatile acids increased, but content of alcohol decreased. Ciders matured by adding lactic acid bacteria *Oenococcus oeni* have the most intensive apple aroma and taste, the lowest astringent, yeast and sour taste intensity. The cider sample matured by unroasted and medium-roasted oak chips showed higher intensity of bitter taste and lower intensity of sour taste. The ranking test sensory results showed that the highest rank was awarded to the sample that had maturated by adding lactic acid bacteria *Oenococcus oeni*.

Acknowledgment

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References

1. Ares G., Barrios S., Lareo C., Lema P. (2009) Development of a sensory quality index for strawberries based on correlation between sensory data and consumer perception. *Postharvest Biology and Technology*, Vol. 52, p. 97–102.
2. Blanco Gomis D., Mangas-Alonso J.J., Junco-Corjuedo S. (2003) Ciders produced by two types of presses and fermented in stainless steel and wooden vats. *The Journal of Institute of Brewing*, Vol. 109, p. 342–348.
3. Bozalongo R., Carrillo J.D., Torroba M.A.F., Tena M.T. (2007) Analysis of French and American oak chips with different toasting degrees by headspace solid-phase microextraction-gas chromatography-mass spectrometry. *Journal of Chromatograph A*, Vol. 1173, No. 1-2, p. 10–17.
4. Buglass A. J. (2011) Cider and Perry. In: Handbook of Alcoholic Beverages: Technical, Analytical and Nutritional Aspects. Edited by: J.Alan. Chichester: John Wiley & Sons, p. 231–265.
5. Cabrita M.J., Barrocas Dias C., Costa Freitas A.M. (2011) Phenolic Acids, Phenolic Aldehydes and Furanic Derivatives in Oak Chips: American vs. French Oaks.

South African Journal for Enology and Viticulture, Vol. 32, No. 2 , p. 204–210.
6. Cadahia E., Fernindez de Simón B., Sanz M., Poveda P., Colio J. (2009) Chemical and chromatic characteristics of Tempranillo, Cabernet Sauvignon and Merlot wines from DO Navarra aged in Spanish and French oak barrels. *Food Chemistry*, Vol. 115, p. 639–649.
7. Cardello A.V. (1995) Food quality: conceptual and sensory aspects. *Food Qualilty Preference*, Vol. 6, p. 163–168.
8. Costell E. (2002) A comparison of sensory methods in quality control. Food Qualilty Preference, 13, p. 341–353.
9. Fan W., Xu Y., Yu A. (2006) Influence of Oak Chips Geographical Origin, Toast Level, Dosage and Aging Time on Volatile Compounds of Apple Cider. *Journal of the Institute of Brewing*, Vol. 112 (3), p. 255–263.
10. Figueiredo-González M., Cancho-Grande B., Simal-Gándara, J., Teixeira N., Mateus N., De Freitas V. (2014) The phenolic chemistry and spectrochemistry of red sweet wine-making and oak-aging, *Food Chemistry*, Vol. 152, p. 522–530.
11. Fugelsang K.C. (1997) *Wine Microbiology*. Chapman & Hall, New York, USA.
12. Garde-Cerdán T., Ancin-Azpilicueta C. (2006) Effect of oak barrel type on the volatile composition of wine: Storage time optimization. *Food Science and Technology*, Vol. 39, No. 3, p. 199–205.
13. Garde-Cerdán T., Lorenco C., Carot J.M., Esteve M.D., Climent M.D., Salinas M.R. (2010) Effects of composition, storage time, geographic origin and oak type on the accumulation of some volatile oak compounds and ethylphenols in wines. *Food Chemistry*, Vol. 122, p. 1076–1082.
14. Gockowiak H., Henschke P. A. (2003) Interaction of pH, ethanol concentration and wine matrix on induction of malolactic fermentation with commercial 'direct inoculation' starter cultures. *Australian Journal of Grape and Wine Research*, Vol. 9, No. 3, p. 200–209.
15. Hale M.D., McCafferty K., Larmie E.; Newton J., Swan J.S. (1999) The influence of oak seasoning and toasting parameters on the composition and quality of wine. *American Journal of Enology and Viticulture*, Vol. 50, p. 495–502.
16. Hidalgo P., Pueyo E., Pozo-Bayón M.A., Martınez-Rodrıguez A.J., Martın- Alvarez P., Polo M.C. (2004) Sensory and analytical study of rosé sparkling wines manufactured by second fermentation in the bottle. *Journal of Agricultural and Food Chemistry*, Vol. 52, p. 6640–6645.
17. Krieger-Weber S. (2009) Application of yeast and bacteria as starter cultures. **In:** *Biology of Microorganisms on Grapes, in Must and in Wine*. Edited by H.König, G.Unden, J.Fröhlich. Berlin: Springer, p. 498–511.
18. Lea A.G.H., Drileau J. F. (2003) Cider making. In: Fermented beverage production. Lea AGH. Pigott JR. editors. NY, Kluwer Acad emic/Plenum Publisheres. p. 59–87.
19. Lonvaud-Funel A. (1999) Lactic acid bacteria in the quality improvement and depreciation of wine. *A. van Leeuw*. 76, p. 317–331.
20. Martınez-Rodrıguez A. J., Polo M. C. (2003) Effect of the addition of bentonite to the tirage solution on the nitrogen composition and sensory quality of sparkling wines. *Food Chemistry*, Vol. 81, p. 383–388.
21. Meilgaard M., Civille G., Carr B. T. (1999) Sensory evaluation Techniques, CRC Press: Boca Raton, New York.
22. Návojská J., Brandes W., Nauer S., Eder R., Frančáková H. (2012) Influence of different oak chips on aroma compounds in wine. *Journal of Microbiology, Biotechnology and Food Sciences*, Vol. 1, No. 4, p. 957–971.
23. Nonier M.F., Vivas N., Vivas de Gaulejac N., Absalon C., Soulie P., Fouquet E. (2006) Pyrolysis-gas chromatography/mass spectrometry of Quercus sp. wood. Application to structural elucidation of macromolecules and aromatic profiles of different species. *Journal of Analytical and Applied Pyrolysis*, Vol. 75, p. 181–193.
24. Rodríguez-Rodríguez P., Gómez-Plaza E. (2011) Effect of Volume and Toast Level of French Oak Barrels (*Quercus petraea* L.) on Cabernet Sauvignon Wine Characteristics. *American Journal of Enology and Viticulture*, Vol.62, p. 359–365.
25. Singleton V.L., Orthofer R., Lamuela-Raventos R.M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, Vol. 29, p. 152–178.
26. Spillman, P. J., Iland, P. G., Sefton, M. A. (1998) Accumulation of volatile oak compounds in a model wine stored in American andLimousin oak barrels. *Australian Journal of Grape and Wine Research*, Vol. 4, p. 67–73
27. Solieri L., Genova F., Paola M. D., Giudici P. (2010) Characterization and technological properties of Oenococcus oeni strains from wine spontaneous malolactic fermentations: a framework for selection of new starter cultures. *Journal of Applied Microbiology*, Vol. 108, No. 1, p. 285–298.

BERRY AND FRUIT JUICES AS POTENTIAL UNTRADITIONAL ACIDITY REGULATORS IN MASHING

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Abstract

Acids traditionally used for acidification of mash (lactic acid, phosphorus acid) provide optimal medium pH, however, it is theoretically possible to choose such agents that would complete several tasks, ensuring the regulation of pH. Berry and fruit juices (cranberry, black currant, red currant, quince, apple and lemon) containing different organic acids, such as citric acid, malic acid, tartaric acid and fumaric acid, have similar properties, although they can not only acidify mash but also increase the content of extract substances in wort. In berries and fruits juices titratable acidity and pH was measured potentiometrically using pH meter. The highest titratable acidity of berry and fruit juices was in lemon (5.71 mmol L^{-1}) and quinic juice (5.80 mmol L^{-1}). Lemon juice has a lower pH 2.40 and apple juice has the highest pH 4.82. Results of the analysis of mash pH changes showed, that it is possible to reduce pH replacing traditional acidification regulators (lactic acid, phosphoric acid) with berry and fruit juices. The pH was practically in all the mashing stages in the limits of 5.14±0.02 up to 5.19±0.02. The content of wort extract was analyzed using beer analysing system – Anton Paar „Alcolaizer" analysis. Using HPLC the Carbohydrates like glucose and maltose in wort were detected and quantified. The optimal berry and fruit mashing acidification regulators were quince and cranberry juices.

Keywords: acidity regulators, pH, mashing, mash, wort.

Introduction

In brewing, the raw materials that give the beer its specific sensory properties are water, barley malt, hop, and yeast. The most important technological process in producing of wort is the mashing what is the first stage of beer preparing (Heyse, 1995., Kunze, 1998). The factors that affect the output of malt extract are the quality of water, medium pH and temperature. In the process of mashing, it is important to provide the optimal conditions for starch hydrolysis ferments α-amylase and β-amylase activity. The largest α-amylase activity was stated when medium pH was 5.1–5.2. In the mashing process mash pH is higher and optimal pH is obtained by adding traditional acidificators: phosphor acid, lactic acid or gypsum to the mixture (Kunze, 1998; Narziβ, 2004; Меледина et al., 2006). However, these traditional materials can be partially replaced with other products. Berry and fruit juices (cranberry, black currant, red currant, quince, apple and lemon) containing different organic acids, such as citric acid, malic acid, tartaric acid and fumaric acid, have similar properties, although they can not only acidify mash but also increase the content of extract substances in wort.

The aim of the research was to produce the wort using untraditional acidity regulators as fruit and berry juices and to analyse its chemical composition.

Materials and Methods

Acidity regulators

Samples of berry and fruit: (black currant (*Ribes nigrum*), red currant (*Ribes rubrum),* quince (*Cydonia oblonga*), cranberry (*Vaccinium microcarpum*), apple (Antonovka) (*Malus sylvestris*) were collected at Cesis region in Latvia. Lemon juice is obtained from Lemon samples (*Citrus x lemon*) buy in local retail market Maxima. After collection, the berries and fruits were extracted in juice extractor Moulinex A7534K, and pasteurized 80 °C, 1.5 L min^{-1} with Voran PA 90.

Chemical acidity regulator reagents: lactic, phosphoric, fumaric, quinic, malic and citric acids HPLC–grade>99.0% (Fluka, Germany)

An amount of acidity regulators (Fig. 1.) added to experimental mash with initial pH.

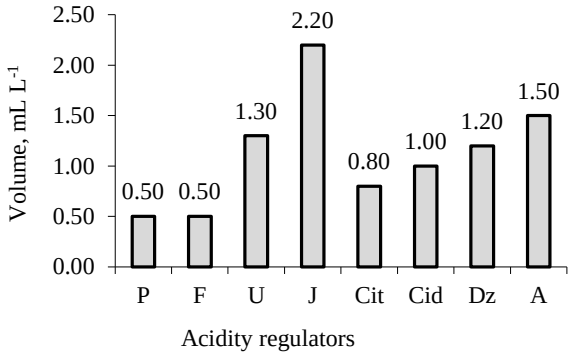

Figure. 1. An amount of added acidity regulators in mash

P – lactic acid, F – phosphoric acid, U – black currants juice, J – red currants juice, Cit – lemon juice, Cid – quinice juice, Dz – cranberries juice, A – apple juice

Raw materials of mashing

Light barley malt (Brupak, England), water (SIA „Griģis un Co") in accordance with the law of LR MK Nr.235/29.04.2003.

The titratable acidity and pH

The titratable acidity and pH was measured potentiometrically using pH meter – WTW inoLab® pH 720.

Wort extract

Content of wort extract was analysed using beer analysing system - Anton Paar „Alcolaizer analysis".

Carbohydrates

The Carbohydrates was determined by HPLC using Shimadzu LC20 Prominence chromatographer. Determination parameters: Refractive Index Detection RID-10A; Column: Alltech NH_2, 4.6x250mm, 5μm; Temperature of the column: +30 °C; Mobile phase: A – acetonitrile and water (70:30); Isocratical regime; Capacity of the injection sample; 10 μL; Rate of the flow: 1.3 mL min^{-1}; Total time of the analysis: up to 20 minutes.

Technological process of mashing and obtaining wort (Fig.2.)

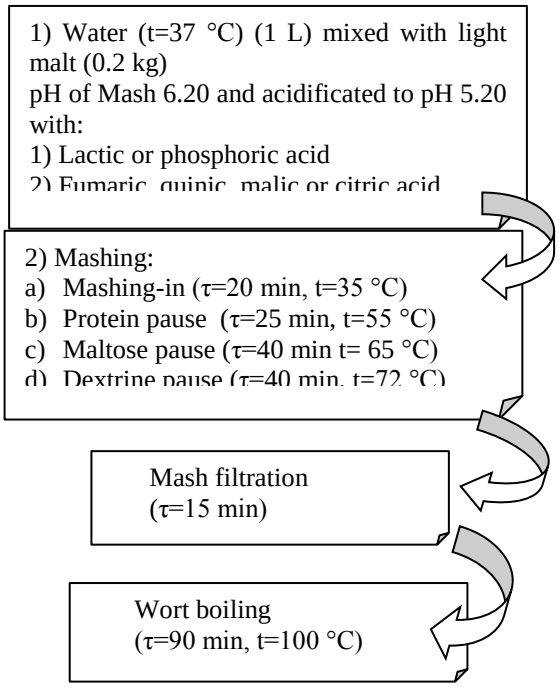

Figure. 2. Technological process of preparation mash and wort

Mathematical data processing

Data obtained were analysed using statistical software Minitab 15 for Windows.

Results and Discussion

The titratable acidity and pH of berry and fruit juices.

The pH in the samples is characterized in Figure 3.

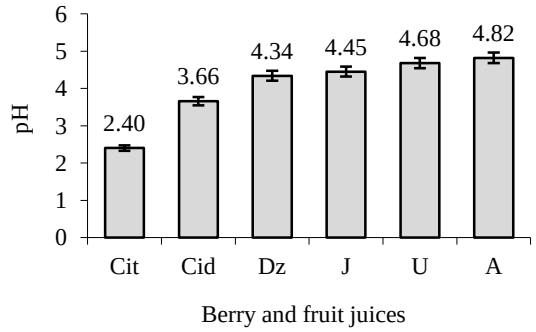

Figure. 3. The pH of berry and fruit juices

U–black currants juice, J–red currants juice, Cit–lemon juice, Cid–quinice juice, Dz–cranberries juice, A–apple juice

It should be concluded from the data presented in Figure 3, that from the berry and fruit juices the lemon juice has a lower pH 2.40 and apple juice has the highest pH 4.82.

The total titratable acidity of berry and fruit juices are showed in Figure 4.

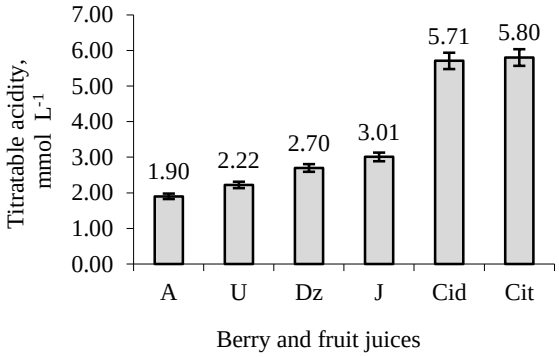

Figure 4. The titretable acidity of berry and fruit juices

U – black currants juice, J – red currants juice, Cit – lemon juice, Cid – quinice juice, Dz – cranberries juice, A – apple juice

The highest titratable acidity of berry and fruit juices was in lemon (5.71 mmol L^{-1}) and quinic juice (5.80 mmol L^{-1}).

Changes of pH in mashing process and evaluation of extract substance content

It is possible to reduce pH mash during mashing using cranberry, black currant, red currant, quince, apple, lemon fruit and berry juices up to pH 5.20±0.02. The changes of mash pH by fruit and berry juices are slight and vary between 5.19±0.02 and 5.14±0.02, thus providing amylase activity of α- and β-enzymes and optimal conditions for starch hydrolysis (Kunze, 1998, Montanari et al., 2005).

The content of measured extract substances in wort obtained during the experiments using traditional and untraditional acidity regulators is displayed in Fig. 5.

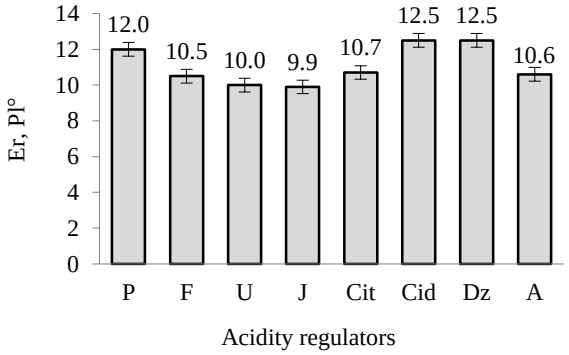

Figure. 5. The content of extract substances in wort (Er), using various acidity regulators

U–black currants juice, J–red currants juice, Cit–lemon juice, Ci–quinice juice, Dz–cranberries juice, A–apple juice

Optimal results of extract substances are gained using cranberry (12.5 Pl°) and quince (12.5 Pl°) juices, the lowest results – using red currant 9.9 (Pl°) and black

currant juices. The obtained results using traditional acidity regulators, lactic acid and phosphorus acid, are 12.0 Pl° and 10.5 Pl° (Fig. 5.). Comparing quinic and cranberries juices to the traditional acidity regulators lactic acid, the quinic and cranberries juice's acquisition of extract substances is more efficient by 0.5 Pl°. Using lemon, apple, black currant and red currant juices for acidification of mash there were fewer amounts (by 14.4–23.2%) of extract substances extracted comparing to cranberry and quince juices.

Content of carbohydrates in the wort

Starch in malt hydrolyses to dextrin in mashing process where enzymes α- and β-amylases are active, thus forming maltose and glucose. The amount of maltose and glucose in wort depends on mashing conditions and certain organic acid used for acidification. Measured content of carbohydrates is displayed in Table 1 and 2.

Table 1

Content of carbohydrates in the wort, %

Carbohydrate	Acidity regulators			
	P	F	U	J
	(n=5) $\bar{x}_i$ ±SD%	(n=5) $\bar{x}_i$ ±SD%	(n=5) $\bar{x}_i$ ±SD%	(n=5) $\bar{x}_i$ ±SD%
Fructose	–	–	0.13±0.05	0.11±0.05
Glucose	1.36±0.03	1.17±0.03	1.08±0.03	0.91±0.03
Sucrose	0.40±0.06	0.34±0.06	0.32±0.06	0.26±0.06
Maltose	7.38±0.05	6.30±0.05	5.83±0.05	4.91±0.05

P – lactic acid, F – phosphoric acid, U – black currants juice, J – currants juice

Table 2

Content of carbohydrates in the wort, %

Carbohydrate	Acidity regulators			
	Cit	Cid	Dz	A
	(n=5) $\bar{x}_i$ ±SD%	(n=5) $\bar{x}_i$ ±SD%	(n=5) $\bar{x}_i$ ±SD%	(n=5) $\bar{x}_i$ ±SD%
Fructose	0.08±0.05	0.07±0.05	0.07±0.05	0.11±0.05
Glucose	1.19±0.03	1.35±0.03	1.37±0.03	1.00±0.03
Sucrose	0.34±0.06	0.39±0.06	0.37±0.06	0.54±0.06
Maltose	6.50±0.05	7.42±0.05	7.44±0.05	7.46±0.05

Cit–lemon juice, Cid–quinic juice, Dz–cranberries juice, A–apple juice

As the amount of extract substances is different when using various acidity regulators in mashing process (Fig. 5), the concentration of maltose and glucose also differ significantly (Table 1 and 2). There was also a little amount of fructose (0.07–0.13%) and sucrose (0.26–0.54%) found in wort (Table 1 and 2). There was no fructose found in wort extracted using lactic and phosphorus acids, because the fructose come from juices.

Acidity regulators – black currant, red currant, lemon, quince and cranberry juices – themselves contain fructose and glucose (Table 3).

The obtained data (Table 3) show that the content of fructose in black current (4.29%), red current (3.95%) and apple (3.60%) juices is the largest comparing to lemon (1.62%), quince (1.51%) and cranberry (1.64%) juices. The amount of glucose is also bigger in black currant and red currant juices (3.80–3.66%), however the glucose amount in apple juice is the least (0.50%). Black currant and red currant juices contain 52–58% more glucose and 35–38% more fructose that lemon, quince and cranberry juices. The total content of glucose and fructose is arranged as follows: black currant (8.09%) > red currant (7.64%) > apple (4.10%) > cranberry (3.93%) > lemons (3.82%) > quince (3.51%).

Table 3

The content of glucose and fructose in berry and fruit juices, %

Berry and fruit juices	Fructose	Glucose	Total content of glucose and fructose
Black currants	4.29±0.21	3.80±0.19	8.09±0.20
Red currants	3.95±0.19	3.69±0.18	7.64±0.18
Apples	3.60±0.18	0.50±0.02	4.10±0.10
Cranberries	1.64±0.08	2.29±0.11	3.93±0.09
Lemons	1.62±0.08	2.20±0.11	3.84±0.09
Quince	1.51±0.07	2.00±0.10	3.51±0.08

Summarizing the results, the biggest amount of glucose and fructose is found in black currant and red currant juices, but the least – in quince juice.

Fumaric, quinic, malic and citric acids like acidity regulators

Due to the fact that berry and fruit juices differ because of various qualitative and quantitative content of acids, the following hypothesis has appeared: high outcomes of extract substances are connected with the concentration of malic acid and quinic acid in berry and fruit juices. Dominant acid in black currant, red currant and lemon juices is citric acid. Cranberry and quince juices contain not only citric acid, but also malic acid, quinic acid, but quince also contains fumaric acid. In order to verify the hypothesis that dominant organic acids that significantly affect the outcome of extract substances are malic acid and quinic acid, separate addition of these acids, citric acid and fumaric acid in mashing process was performed (Fig. 6). The obtained data displayed that added organic acids affect the content of glucose and maltose in wort to a great extent. The highest amount of glucose and maltose was extracted using quinic acid and malic acid as acidification agents, where the total amount of carbohydrates was 9.9±0.04% and 9.5±0.04%.

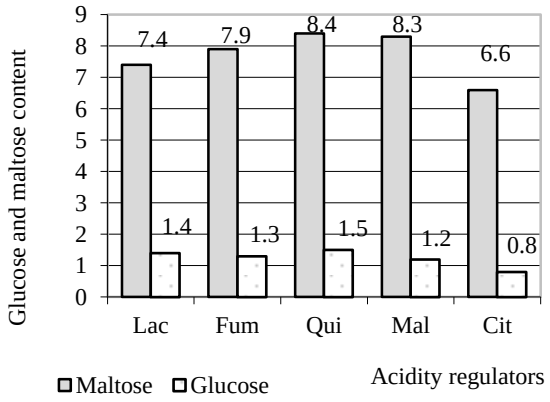

Fig. 6.The content of glucose and maltose in wort
Lac–Lactic acid, Fum-fumaric acid, Qui–quinice acid, Mal–malic acid, Cit–citric acid

Slightly smaller content of carbohydrates was measured when acidifying mash with fumaric acid (9.2±0.04%). Comparing quinic acid, malic acid and citric acid as acidity regulators together with regulation of pH these acids also affect the content of glucose and maltose, using citric acid 26.7% smaller amount of total carbohydrates content is extracted. Thus, experiments helped to find out that using lemon, apple, black currant and red currant juices for mash acidification it is possible to extract less amount of extract substances than by using cranberry and quince juices, as the dominant acid in lemon, black currant and red currant juices is citric acid.

Conclusions

The lemon juice has a lower pH 2.40 and apple juice has the highest pH 4.82. The highest titratable acidity of berry and fruit juices was in lemon (5.71 mmol L^{-1}) and quinic juice (5.80 mmol L^{-1}). The changes of mash pH by fruit and berry juices are slight and vary between 5.19±0.02 and 5.14±0.02. Optimal results of extract substances are gained using cranberry (12.5 Pl°) and quince (12.5 Pl°) juices, the lowest results – using red currant 9.9 (Pl°) and black currant juices. The highest amount of glucose and maltose was extracted using tartaric acid and malic acid as acidity regulators, where the total amount of carbohydrates was 9.5±0.04%. Slightly smaller content of carbohydrates was measured when acidifying mash with fumaric acid (9.2±0.04%). The optimal berry and fruit mashing acidification regulators were quince and cranberry juices.

References

1. Heyse K.U. (1995) *Handbuch der Brauerei – Praxis*, 3, verbesserte und erweiterte Auflage; verlag Hans Carl, Getranke Fachferlag, p. 547.
2. Kunze W. (1998) *Technology of malting and brewing*, VLB: Berlin p. 1074.
3. Narziβ L. (2004) *Abriβ der Bierbrauerei*. Wiley-VCH, p. 450.
4. Montanari L., Floridi S., Marconi O., Tironzelli M., Fantozzi P. (2005) Effect of mashing procedures on brewing. *European Food Research and Technology*. Vol. 221, Nos. 1–2, p. 175–179.
5. Меледина Т. В., Дедегкаев А. Т., Баланов П. Е. (2006) *Технология пивного сусла*. Феникс, 224 стр.

QUALITY CHANGES OF NATURALLY FERMENTED KVASS DURING PRODUCTION STAGES

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Abstract

Commercially available beverages sold as kvass are kvass drinks and malt extract drinks, made by diluting grain extract concentrates with water and adding colourings, different flavours and artificial sweeteners. Kvass quality parameters are defined by the Regulation No 926/2010 *Quality and classification requirements for kvass and kvass (malt) beverage* of the Cabinet of Ministers of the Republic of Latvia Naturally fermented kvass is made from rye bread rusks without additional additives. The aim of this research was to assess the quality changes of naturally fermented kvass during production stages. Experiments were carried out at the Latvia University of Agriculture Department of Food Technology. Dry matter (refractometer, ISO 6496), active acidity (LVS EN ISO 10523:2012) and sensory properties (25 panellists; line scale ISO 4121:2003) were analysed in kvass samples during production stages. During fermentation stage of naturally fermented kvass, pH drops from 4.08 to 3.77 and in later production stages pH is between 3.82 and 3.88, pH levels do not exceed the index values of the Regulation of the Cabinet of Ministers. Relative dry matter content reduced from 5.96% to 4.94%. Sensory evaluation showed that the intensity of flavour, aroma and acidity was most pronounced in kvass sample C (total production time 156 h), however, colour was most pronounced in kvass sample A (total production time 36 h). Longer maturation process aids in the formation of more robust flavour as well as yeast and protein residue.

Keywords: kvass, dry matter, active acidity, sensory evaluation.

Introduction

Kvass is a non-alcoholic beverage that can be used without restriction, its effects on the human body is similar to kefir. This is due to lactic acid which is formed by lactic acid bacteria (Costa et al., 2013). Kvass has beneficial effects on the digestive tract (Feik et al., 2010), furthermore energy value of naturally fermented kvass is only 25 kcal (105 kJ) per 100 mL (Costa et al., 2013). Kvass has thirst soothing and diuretic properties.

Fermented bread kvass has the positive qualities of beer, as well as minerals and vitamins. It contains more than 30 minerals and trace elements. Kvass contains such minerals as copper, phosphorus, potassium, zinc, iron, and fluorine and B vitamins – thiamine, riboflavin, niacin and folic acid (Zariņš, 1991); the content of thiamine (B_1) in naturally fermented kvass is up to 0.04 mg per 100 mL (Покровский, 1976). There are no fat, cholesterol and nitrates in kvass. Most of the beneficial substances come from the raw materials used in naturally fermented kvass production – rye bread and malt.

Kvass is very low in sodium, so it promotes the excretion of fluid and it can recommend instead of other soft drinks for people who want to lower their blood pressure with food restrictions (Рудольф, 1982).

Commercially available beverages sold as kvass are kvass drinks and malt extract drinks, made by diluting grain extract concentrates with water and adding colourings, preservatives, different flavours and artificial sweeteners. The most commonly used preservatives in soft drink production are benzoic acid, sorbic acid and sodium benzoate (Дотарецкий, 1990).

Regulation No 926/2010 of Cabinet of Ministers of the Republic of Latvia indicates that it is allowed to use these raw materials if they comply with the requirements set in food laws and regulations for kvass production: spring water, drinking water, fruit juice and puree, vegetable juice and puree, fruit juice concentrate, sugar, kvass mash concentrate, bread rusks, cereals, malt and grain products, raw plant extracts, kvass concentrate, carbon dioxide, compressed bakers' yeast or cultured yeast, honey, sweeteners and flavourings (except in the production of kvass), food additives.

Maximum cleanliness and hygiene conditions associated with good manufacturing practice must be provided during kvass production therefore stainless steel tanks are used (Bidzāne, 2000).

Water quality affects the formation of kvass sensory indicators. Kvass consistency is better if softer water is used. Elevated sulphate content in the water causes kvass to taste bitter, silicates interfere with the fermentation process and causes sludge, chlorides lead to unpleasantly sweet taste, iron and manganese affect kvass colour and foaming (Hugenholtz, 2013).

Malt for kvass production is usually obtained from spring barley, which has low levels of protein (8–11%) and higher levels of starch which contains the necessary sugars for fermentation. Germination of grains activates the break down starch and proteins during mash cooking (Sacher, 2013), thus obtaining necessary nutrients for yeasts and lactic acid bacteria.

Rye bread rusks which are the basic raw material in naturally fermented kvass production have a strong flavour and sour taste. Rye bread rusks are obtained by drying sliced or diced rye bread.

Bread yeast *Saccharomyces cerevisiae* is used for kvass production; yeast cells cause intense ethanol fermentation during anaerobic fermentation forming alcohol and carbon dioxide. To control fermentation and avoid formation of too much alcohol, oxygen is supplied during this process (Birch et al., 2013).

Active lactic acid bacteria growth takes place simultaneously with yeast cell growth during mash fermentation; lactic acid bacteria produce lactic acid.

Bread kvass made from pure cultures of lactic acid bacteria and yeasts is clearer and has increased resilience. Yeast and lactic acid bacteria that give refreshing taste and aroma are most commonly used. (Dlusskaya et al., 2008) Malt extract and bread fermentation process provides proteins, sugars, organic acids and vitamins.

Usually, preservatives which extend shelf life are added or kvass pasteurization is used. These processes shorten production time and costs, and also impact such sensory properties as taste and smell, as well as biologically active compounds. Naturally fermented kvass is made without preservatives and is not pasteurized, thus saving the maximum quantity of vitamins and minerals, as well as the flavour and aroma.

Before the production of a new type of kvass it is necessary to define sensory scores, which can significantly affect consumer beverage choices. Therefore the aim of this research was to assess the quality changes of naturally fermented kvass during production stages.

Materials and Methods

All analyses were completed at Microbiology Research laboratory, laboratory of Sensory analyses and laboratory of Bread technology at Latvia University of Agriculture.

Kvass production

For the bread kvass production the following materials were used: rye bread rusks (Ltd *Liepkalni*), baker's yeast *Saccharomyces cerevisiae* (Sp.z.o.o. *Lallemand*), lactic acid bacteria *Leuconostoc mesentericus* (Ltd Chr. Hansen), beet sugar (Ltd *Dansukker*) and dark malt (Ltd *Liepkalni*).

To prepare 1 litre of kvass mash, 200 g of rye bread rusks and 2 g dark malt were soaked in 2 litres of hot water (78±2 °C). Bread rusks were left to soak for 3 hours, then the water-bread rusk suspension was filtered (300 microns) and the liquid fraction was cooled down and used in further kvass production stages.

1 g baker's yeast, 2 units of lactic acid starter and 30 % of the estimated quantity of sugar were added to 1 litre of kvass mash. The total quantity of sugar for kvass production is 30 g; therefore 10 g of sugar were added prior to fermentation. The fermentation of kvass mash took 9 hours at 27±1 °C.

After fermentation kvass was placed in a refrigeration chamber to cool down to 3±1 °C. After cooling, the yeasts were filtered (5 microns) and the remaining sugar was added (blending). Kvass was maturated for 12 hours at 6±1 °C and then it was ready for drinking (total production time 25 hours).

Afterwards kvass was filled in 0.5Ll plastic bottles and stored for 156 h in total, in order to complete physicochemical and sensory analysis. Technological process of naturally fermented kvass production is given in Figure 1. Shelf life of naturally fermented kvass is 125±5 hours (about 5 days).

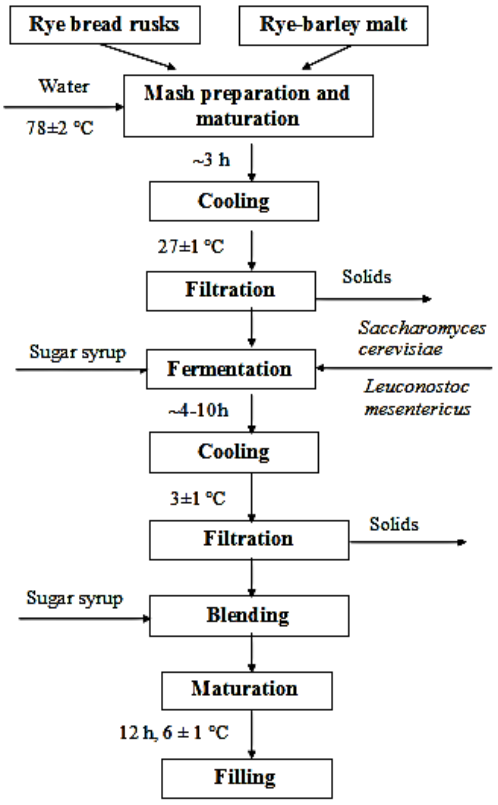

Figure 1. Technological process of naturally fermented kvass production

Physicochemical analyses

The Regulation No 926/2010 defines such kvass quality parameters:

1) dry matter content – 3.0 to 14.0 percent by weight,
2) acidity – 2.0 to 3.5, expressed as mL of 1N NaOH per 100 mL.

Active acidity (pH) and dry matter content was determined in kvass during 8 production stages (Table 1). Active acidity (pH) was determined according to the standard (AACC 02-31) and dry matter was determined with table refractometer according to the standard ISO 6496).

Microbiological analyses

Lactic acid bacteria were determined in kvass during 8 production stages (Table 1) according to the standard LVS ISO 15214:1998.

Sensory analyses

Kvass samples were evaluated sensory by 25 trained panellists (36% men and 64% women), average age 21 years.

Each panellist was served 3 samples of kvass in a randomized serving sequence: kvass stored for 11 h (sample A, total production time 36 h), kvass stored for 59 h (sample B, total production time 84 h), and kvass stored for 131 h (sample C, total production time 156 h). Line scale was used to evaluate the intensity of kvass sensory properties (aroma, flavour, acidity, and colour) (ISO 4121:2003). Kvass samples for sensory

evaluation were chosen according to organoleptic evaluation by research funder.

Table 1

Stages of kvass production process

Stage	Materials and technological process	Time, h
S_0	Rye bread rusks, before soaking	0
S_1	Kvass after fermentation	12
S_2	Kvass after blending	13
S_3	Kvass after maturation	36
S_4	Kvass during storage	60
S_5	Kvass during storage	84
S_6	Kvass during storage	132
S_7	Kvass during storage	156

Data analyses

The obtained data processing was performed with the Microsoft Excel 13 for Windows; arithmetic mean, standard deviation and standard error were calculated (Arhipova et al., 1999). For data cross-comparison ANOVA, Regression and other statistical calculation functions were used. Both t-test and F-test were used in order to assess the significance of changes and inter-comparison of the obtained data. For the interpretation of the results it is assumed that α=0.05 with 95% confidence (Næs et al., 2011).

Results and Discussion

The changes detected in pH during kvass production stages are given in Figure 2.

The numerical value of pH decreased slightly during kvass fermentation; the initial pH was 4.08 and pH after fermentation was 3.77. During the rest stages of kvass production pH stabilized and was around pH 3.85. Compared to the indexed values of the Regulation No 926/2010, pH value of laboratory produced kvass corresponded to the regulatory scale (experimentally determined conversion factor approximately1.8). pH value did not decrease to interval lower limit value (2.0 mL 1 N NaOH) in any of the controlled production stages; at the end a trend in pH increase was observed. The increase of pH in kvass at the end of production stages could be explained by the formation of new substances because yeast and lactic acid bacteria cells gradually die.

A strong, negative correlation (r=-0.92) was observed between pH and lactic acid bacteria count changes in kvass. Correlation is significant (p<0.05) primarily during the first three stages of kvass production (Lidums, 2011).

During later stages, at the onset of lactic culture gradual degradation and stabilization of pH, correlation is weak and negative (r= -0.11). As pH is the logarithm of H^+ ion concentration, correlation is compared to the logarithmic values of lactic acid bacteria count. A moderate, negative correlation (r= -0.50) was observed using pairwise correlation calculation between pH and the absolute number of lactic acid bacteria.

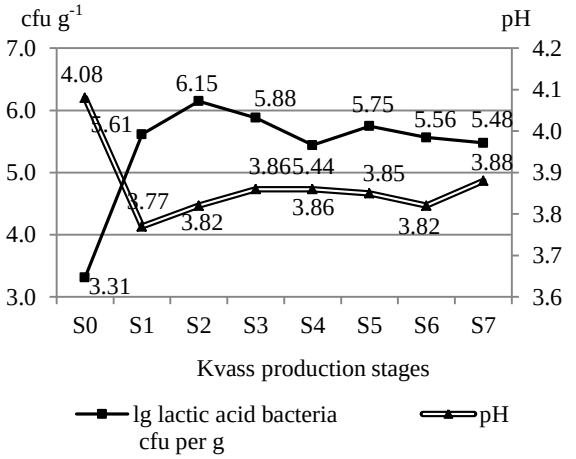

Figure 2. Changes in pH and lactic acid bacteria count during kvass production stages

S0 – rye bread rusks, before soaking, S1 – kvass after fermentation, S2 – kvass after blending, S3 – kvass after maturation, S4, S5, S6, S7 – kvass during storage

During kvass production stages dry matter content (%) experienced a slight decrease at the expense of volatile fermentation product formation (mainly alcohol). At times a rise in dry matter content can be observed at the expense of increased fermentative microorganism total cell count.

Decrease in dry matter content was found after intensive fermentation stage (S_1). Relative dry matter content changed from 5.96% to 4.94%. Decrease in the intensity of fermentation caused sedimentation of some substances that were not fully included in the test sample. In later stages of kvass production significant changes on dry matter content were not observed (p>0.05).

The changes in indicator value were characterized by this polynomial division:

$$y = 0.00005x^2 - 0.0148x + 6.0285 \quad (1),$$

where y – the dry matter content (%) after a certain time, x – fermentation, h.

The process is characterized by regression $R^2 = 0.99$. The first right-hand number in the division (1) is a very low figure, so it can be dropped and the calculations performed with a linear response without significant mistakes:

$$y = -0.015x + S0 \quad (2),$$

where y – the dry matter content, %; x – fermentation, h; S0 – initial dry matter content, %.

It should be noted that the decrease in dry matter content affects physical as well as chemical and biogenic elements, so for each particular set of circumstances of kvass production stages a calculation formula must be found. In general form is looks like this:

$$\text{Dry matter (\%)} = -k \times (\text{hours}) + \text{initial dry matter (\%)} \quad (3),$$

where k – coefficient of proportionality, determined with control measurements.

Changes in dry matter content during kvass production stages are given in Figure 3.

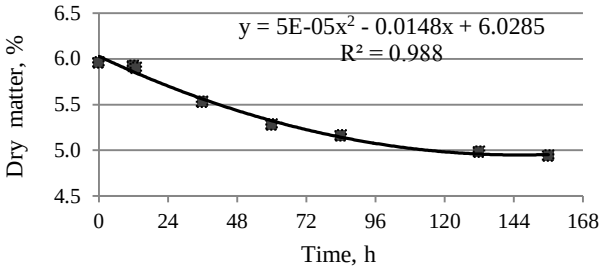

Figure 3. Changes in dry matter content (%) during kvass production stages

Dry matter content decreased during kvass fermentation, as most of the dry ingredients and sugar were used for yeast and lactic acid bacteria development.

Since in kvass production normative documents no strict parameters have been set for acidity (affects flavour) and dry matter (affects clarity), solely the recommended value intervals, many versions and combinations (market brands) of kvass quality and sensory properties are possible.

The intensity of sensory properties of three kvass samples with different storage (total production) times was evaluated (Fig. 4).

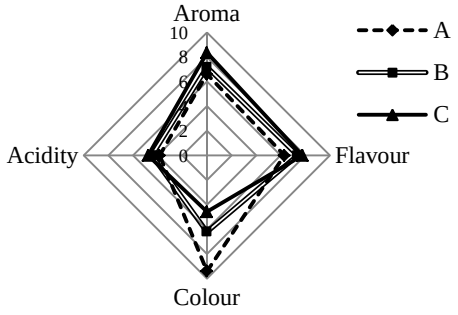

Figure 4. The intensity of kvass sensory properties

The results show that the intensity of aroma was most pronounced in kvass sample C (total production time 156 h) and the least pronounced in kvass sample A (total production time 36 h) (p=0.0230). This is due to the fact that further maturation happens during storage and longer storage time aids in stronger aroma forming. Flavour intensity was most pronounced in sample C (p=0.0430), this is due to the fact that further maturation happens during storage and as in the case of aroma, longer storage time also aids in stronger flavour forming. Kvass sample A, which was stored for the shortest time, was rated as having the most intense colour (p=0.008). Further maturation aid in the formation of sludge, yeast and protein residue which gives a hazy, muddy colour; the intensity of colour in sample C was the least pronounced.

Samples C and B (total production time 84 h) were rated as having the most pronounced acidity.

Because of further maturation happening during storage, yeasts left in kvass continue to ferment remaining sugars, resulting in increased acidity.

Conclusions

During production stages, changes in active acidity range from pH 3.77 to pH 4.08. Relative dry matter content reduced from 5.96% to 4.94%.

Sensory evaluation showed that the intensity of flavour, aroma and acidity was most pronounced in kvass sample C (total production time 156 h), however, colour was most pronounced in kvass sample A (total production time 36 h). Longer maturation process aids in the formation of more robust flavour as well as yeast and protein residue.

References

1. Arhipova, I., Bāliņa, S. (1999) *Statistika ar Microsoft Excel ikvienam* 1. daļa. Rīga: Datorzinību centrs, 168 p. (in Latvian)
2. Bidzāne, S. (2000) Ēdināšanas uzņēmumu aprīkojums. Jumava, 112 p.
3. Birch A.N., Petersen M.A., Arneborg N., Hansen A.S. (2013) Influence of commercial baker's yeasts on bread aroma profiles. *Food Research International*. Vol. 52, Iss. 1, p. 160–166.
4. Costa H.S., Albuquerque T.G., Sanches-Silva A., Vasilopoulou E., Trichopoulou A., D'Antuono L.F., Alexieva I., Boyko N., Costea C., Fedosova K., Hayran O., Karpenko D., Kilasonia Z., Finglas P. (2013) New nutritional composition data on selected traditional foods consumed in Black Sea Area countries. *Journal of the Science of Food and Agriculture*, Vol. 93, No. 14, p. 3524–3534.
5. Dlusskaya E., Jaensch A., Schwab C., Gaenzle M.G. (2008) Microbial and chemical analysis of a kvass fermentation. *European Food Research and Technology*, Vol. 227, No. 1, 261–266.
6. Feik P., Gerloff A., Singer V.M. (2010) Beer and its Non-Alcoholic Compounds: Role in Pancreatic Exocrine Secretion, Alcoholic Pancreatitis and Pancreatic Carcinoma, *International Journal of Environmental Research and Public Health*, Vol. 7, No. 3., p. 1093–1104.
7. Hugenholtz J. (2013) Traditional biotechnology for new foods and beverages. *Current Opinion in Biotechnology*, No. 24, p.155–159.
8. Lidums I. (2011) Raudzēta kvasa mikrobiāla drošība. (Microbiological safety of fermented kvass: scientific work for a professional master's degree in food hygiene). Latvia University of Agriculture. Faculty of Veterinary Medicine. Jelgava: 58 p. (in Latvian)
9. Næs T., Brockhoff P., Tomic O. (2011) Statistics for Sensory and Consumer Science. West Sussex: John Wiley & Sons, 300 p.
10. Sacher B. (2013) Production of fermented kvass malt. *Brauwelt International*, Vol. 31, No. 4, p. 202–210.
11. Zariņš, P. (1991) Mājas alus: alus gatavošana tehnoloģija. Tukums: Diēta, 17 lpp.
12. Дотарецкий, В.А. (1990) Производство конценратов, экстрактов и безалкогольных напитков. Киев: Урожай, 185 с.
13. Покровский, А.А (1976) Химический состав пищевых продуктов, Пищевая промышленность, Москва, 124 с.
14. Рудольф, В.В. (1982) Производство кваса. Легкая и пищевая промышленность. Москва, 89 с.

INFLUENCE OF FREEZING AND DRYING ON THE PHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF HORSERADISH AND LOVAGE

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Abstract

A perennial herb lovage (*Levesticum officinale* L.) and horseradish (*Armoracia rusticana* L.) contains biologically active substances and are cultivated in temperate regions of the world. The aim of this work was to study the effect of the technological processes (freezing and drying) on the polyphenol content and antioxidant activity of horseradish leaves and lovage leaves and stems. The samples were processed using freezing (-20 °C) and drying (at temperature of +24 °C, in a dark, till moisture content 10±2%), and for a comparison fresh samples were analysed. Lovage leaves and stems and horseradish leaves were extracted with ethanol using conventional extraction. Total phenols, total flavonoids, antioxidant activity were determined spectrophotometrically, and individual polyphenolics were identified using HPLC. Analysis of the phenolic compounds and antioxidant activity of lovage and horseradish showed differences depending on the technological processes applied. The predominant phenolic acid in lovage samples was caffeic acid, but horseradish leaves – chlorogenic acid, and the major flavonoid was rutin. Only sinapic acid in lovage stems were found to be higher in the dried samples, compared to the fresh and frozen samples. From analysed methods better results for preserving phenolic compounds and antioxidant activity of lovage leaves and stems and horseradish leaves proved to be freezing.

Keywords: antioxidant, horseradish, lovage, phenolic, treatment

Introduction

World scientists have been paid attention to preservation of biologically active substances in food. Plants provide abundant natural antioxidants, which are vitally important for human health (Naczk, Shahidi, 2006).

Horseradish (*Armoracia rusticana* L.) and lovage (*Levisticum officinale* L.) are perennial plants which belong to *Brassicaceae* and *Umbelliferae* families, respectively. Horseradish is with a particularly pungent flavour, rich in vitamin C (302 mg 100 g^{-1}) (Raghavan, 2000) All parts of lovage are strongly aromatic with a characteristic earthy, celery-like flavour and smell (Szebeni-Galambosi et al., 1992; Raghavan, 2000). Both plants contain compounds that can act as natural antioxidants (Raghavan, 2000). The antioxidant characteristics of plant derived materials can be attributed to their polyphenols.

Phenolic composition of plants is affected by different factors – variety, genotype, climate, harvest time, storage, processing, and treatment (Marrelli et al., 2012). Changes of the content of biologically active compounds in plants can cause climate, soil, vegetative stage of the plant and also technological processes applied – drying, freezing and other heat treatment methods, irradiation etc. (Angela, Meireles, 2009). Technological processes has important role in final product quality and especially to the antioxidant activity. Part of biologically active compounds are instable during technological processes applied, resulting in decrease of biological value of fruit and it is very important to find best methods for preserving their value.

Drying is widely used preservation method as the drying process inhibits enzymatic degradation and limits microbial growth (Ahrne et al., 2007) Drying method also influence the composition and biological activity of plant (Ahrne et al., 2007). Treatment at higher temperature decreases content of gallic acid (Orkoula et al., 2004), whereas during drying of sweet potatoes total phenolic content and antioxidant activity increases (Yang et al., 2010).

Results of investigations about influence of freezing and storage in frozen state to the changes of phenolic composition differ, and tendencies depend on food matrix used. Freezing has not significant influence to the content of ellagic acid and total phenols content in raspberries (Ancos et al., 2000), but in frozen blueberries total phenols content decreases (Rizzolo et al., 2003).

Irish scientists reported that after processing of six Lamiaceae herbs (rosemary, oregano, marjoram, sage, basil, thyme) content of rosmarinic acid increased (Hossain et al., 2010), similar trend also was observed for polyphenolic compounds in horseradish roots (Tomsone et al., 2013).

The aim of this work was to study the effect of the technological processes (freezing and drying) on the polyphenol content and antioxidant activity of horseradish leaves and lovage leaves and stems.

Materials and Methods

Chemicals

Gallic acid, Folin-Ciocalteu phenol reagent, and 2,2-diphenyl-1-picrylhydraziyl (DPPH˙) were purchased from Sigma-Aldrich (Switzerland). All other chemicals (Na_2CO_3, ethanol) used in the research were obtained from Acros Organic (USA).

Sample preparation

Fresh horseradish leaves (*Armoracia rusticana* L.) and lovage leaves and stems (*Levisticum officinale* L.) were collected in Latvia in June 2013.

The samples were processed using the following methods: a) freezing (-20 °C), b) drying (in a dark, temperature of +24 °C, till moisture content 10±2%).

Extraction procedure

The homogenized sample were extracted with ethanol in a conical flask with a magnetic stirrer (magnet 4.0×0.5 cm) at 700 rpm for 1 h at room temperature (20±1 °C). The extracts were then filtered (paper No. 89). The extraction process was done in triplicate.

Determination of total phenolic and total flavonoid compounds

The total phenolic content (TPC) of the plant extract was determined according to the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999) with some modifications. The absorbance was measured at 765 nm and total phenols were expressed as the gallic acid equivalents (GAE) 100 g^{-1} dry weight (DW) of plant material. The total flavonoid content (TFC) was measured by a colorimetric method (Kim et al., 2003) with minor modification. The absorbance was measured at 415 nm and total flavonoids were expressed as the catehin equivalents (CE) 100 g^{-1} DW of plant material.

Determination of antioxidant activity

Antioxidant activity of the plant extracts was measured on the basis of scavenging activities of the stable 2,2-diphenyl-1-picrylhydraziyl ($DPPH^{\bullet}$) radical as outlined by Yu et al. (2003). The absorbance was measured at 517 nm. The radical scavenging activity (RSA) of extract was also measured by 2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic) acid ($ABTS^{\bullet+}$) radical cation assay (Re et al., 1999). For the assessment of extracts, the $ABTS^{\bullet+}$ solution was diluted with a phosphate buffer solution to obtain the absorbance of 0.800±0.030 at 734 nm. The RSA was expressed as TE 100 g^{-1} DW of plant material. The higher the Trolox equivalent antioxidant capacity (TEAC) of a sample, the stronger the antioxidant activity. The reducing power can be determined by the method of Athukorala et al. (2006). The absorbance was measured at 700 nm and reducing power was expressed as the ascorbic acid equivalents (AAE) 100 g^{-1} DW of plant material.

Additionally for all horseradish leaves and lovage leaves and stems samples the moisture content was determined according to the standard ISO 6496:1999 and all results were expressed on dry basis.

Reversed phase high performance liquid chromatography (HPLC) analysis of the extracts

The analyses were carried out using a Shimadzu liquid chromatograph LC-20AD with the analytical column C18, photo diode array detector SPD M20A. As eluting solvents were used methanol (A, 20%), water (B, 78.4%), and acetic acid (C, 1.6%) using a gradient mode: 17.50 minutes – 40.3% A concentration, 58.5% B concentration, C concentration of 1.2%, 35th minute till end. The sample injection into the chromatograph was performed using an automatic sample injection system SIL-20AC. Eluent flow rate was 1.0 mL min^{-1}. Several wavelengths were used to define polyphenols. Using wavelength 253 nm 4-hydroxybenzoic acid and rutine were determined; 263 nm – gallic acid; 278 nm – catechin, caffeic acid, syringic acid; 298 nm – chlorogenic acid, epicatechine, coumaric acid, sinapic acid, and ferulic acid.

Statistical analysis

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

Results and Discussion

Total phenolics and flavonoids content

The TPC and TFC determined in horseradish leaves and lovage leaves and stems extracts depending on treatment are shown in Table 1. Results of multivariate dispersion analyses showed that both type of treatment and plant material are significant factors affecting TPC and TFC ($p<0.05$). The highest content of phenolic compounds was determined in horseradish leaves.

Table 1

Total phenolic and flavonoid content in plants depending on treatment

Plant material	Type of treatment	TPC, mg GAE 100 g^{-1} DW	TFC, mg CE 100 g^{-1} DW
Horseradish leaves	Fresh	2368.48±2.03^{b*}	5889.85±6.02^{b}
	Frozen	2722.13±2.03^{a}	6178.03±6.27^{a}
	Dried	123.60±0.17^{h}	287.79±0.28^{h}
Lovage leaves	Fresh	1593.61±1.09^{d}	2965.40±2.69^{d}
	Frozen	1601.87±1.47^{c}	3548.33±3.08^{c}
	Dried	359.75±0.37^{g}	551.01±0.55^{g}
Lovage stems	Fresh	381.15±0.39^{f}	1208.18±1.83^{f}
	Frozen	458.03±0.40^{e}	1259.94±1.51^{e}
	Dried	44.83±0.07^{i}	184.04±0.14^{i}

Processing technologies has similar influence to the content of bioactive compounds for all types of plant materials investigated. It is possible to observe that for all plant materials after freezing content of phenolic compounds increased and the highest increase in lovage stems was detected – 20%. Whereas in lovage leaves TFC in frozen samples are for 20% higher. The same tendency was also observed in investigations about fresh and frozen horseradish roots (Tomsone et al., 2013). Similar results that at lower temperature increase TPC also were reported in *Etlingera elatior* and *Morus alba* L. (Chan et al., 2013). Obtained results could be explained by fact that ice crystals formed within the plant matrix can rupture cell structure. This allows the exit of cellular components and access of solvent (Asam et al., 2003). Whereas in maringold after freezing TPC and TFC slightly decreased

(Siriamornpun et al., 2012), that are opposite tendency comparing to our results.

Drying is one of the oldest methods of food preservation that is used for extending plants availability throughout the year. Drying results in significant losses in phenolic compounds in all studied materials. For example in lovage leaves losses of TPC after drying is 23%, but TFC – 19% comparing to fresh leaves. The possible reason for this could be that the preliminary process resulted in severe damage and deterioration of the integrity of leaf tissue. The release of active enzyme could cause enzymatic degradation and lose extractable phenolics. The enzyme inactivation required additional energy and during the periods of drying, the enzymes were inactivated due to decreased water activity (Lin et al., 2012). According to the study of Hossain et al. (2010), drying makes the plant tissue more brittle, which leads to rapid cell wall breakdown during the extraction procedure. Several scientists reported that drying reduced TPC of plant materials, but changes in treatment in low temperatures are dependent on plant variety and genotype (Chan et al., 2009; Ahmad-Qasem et al., 2013). Oposite to our results in the studies about sweet potatoes (Yang et al., 2010) and onion (Arslan, Musa Özcan, 2010) fresh samples contained less TPC, but dried samples – the highest TPC. Our findings are similar to the data from a previous study by Miean and Mohamed (2001) and Erbay and Icier (2009) who reported decreased after thermal processing in holy basil and olive leaves, respectively. TFC proportion in the content of total phenols in analysed samples differed. In literature it was found that for fruit the proportion between TFC/TPC ranged from 0.15 to 0.56, but for vegetables 0.07–0.78 (Marinova et al., 2005). The highest raio between TFC/TPC was observed for frozen horseradish leaves and lovage leaves. In horseradish and lovage leaves ratio of flavonoids after freezing increases, and similar trend for frozen horseradish roots also were observed (Tomsone et al., 2013). Results showed that freezing results in release of flavonoids, that in fresh samples are linked with other compounds. The results showed that both methods of treatment could lead to significantly different outcomes.

Individual phenolic compounds

The distribution of individual phenolic compounds (presenting more than 5 mg 100 g^{-1} DW) as affected by treatment and plant material is presented in Table 2.

Table 2

Total phenolic and flavonoid content depending on treatment

Plant material	Type of treatment	Rutine	Catechin	Caffeic acid	Chlorogenic acid	Coumaric acid	Sinapic acid	Ferulic acid
Horseradish leaves	Fresh	2376.16±2.09^{h*}	3.19±0.72^{c}	1.22±0.11^{a}	10.16±0.91^{d}	1.14±0.09^{b}	3.41±0.27^{d}	0.24±0.05^{a,b}
	Frozen	954.08±1.49^{g}	1.91±0.32^{b}	0.93±0.02^{a}	2.15±0.12^{a,b}	0.17±0.02^{a}	1.20±0.33^{a,b,c}	0.84±0.02^{a,b,c}
	Dried	191.07±0.99^{f}	0.85±0.58^{a}	0.1±0.00^{a}	0.95±0.28^{a}	0.01±0.00^{a}	1.08±0.58^{a,b,c}	ND
Lovage leaves	Fresh	65.06±0.50^{e}	0.25±0.02^{a}	39.29±0.72^{c}	3.90±0.70^{c}	23.92±0.72^{g}	8.57±0.62^{f}	5.71±0.22^{e}
	Frozen	55.46±0.50^{d}	0.09±0.01^{a}	31.08±0.29^{b}	3.37±0.31^{b,c}	20.47±0.19^{f}	5.85±0.42^{e}	2.65±0.23^{d}
	Dried	15.24±0.21^{c}	0.03±0.00^{a}	0.71±0.58^{a}	1.19±0.09^{a}	0.02±0.00^{a}	0.26±0.01^{a}	0.09±0.01^{a}
Lovage stems	Fresh	67.72±0.72^{e}	12.19±0.36^{d}	95.03±0.78^{d}	85.91±0.95^{e}	8.5±0.06^{e}	1.54±0.07^{b,c}	5.91±0.39^{e}
	Frozen	8.42±0.90^{b}	ND	0.72±0.52^{a}	3.24±0.26^{b,c}	7.54±0.59^{d}	0.99±0.08^{a,b}	1.34±0.84^{c}
	Dried	3.08±0.32^{a}	0.47±0.04^{a}	1.01±0.08^{a}	10.46±0.09^{d}	2.87±0.23^{c}	2.01±0.18^{c}	1.09±0.09^{b,c}

* Each value is the mean (mg 100 g^{-1} DW) of three replications ± standard deviation; ** Mean values within the same column followed by different letters significantly differ according to the LSD test ($p<0.05$). ND – not detected.

Higher amounts of all phenolic compounds were found in fresh samples. The predominant phenolic acid in lovage samples was caffeic acid, but in horseradish leaves – chlorogenic acid. The highest content of chlorogenic acid was in the lovage stems. The major flavonoid was rutin, and highest content in fresh horseradish leaves were identified. Comparing results of TPC, TFC and individual phenolic compounds (Table 1 and Table 2) it is possible to conclude that horseradish and lovage leaves contain other flavonoids, and rutin forms only part of TFC. Also in horseradish roots rutin, caffeic acid and chlorogenic acid in significant amounts were identified (Tomsone et al., 2013). Only sinapic acid in lovage stems were found to be higher in the dried samples, compared to the fresh and frozen samples. In research about horseradish roots higher content of rutin and caffeic acid in frozen samples were detected (Tomsone et al., 2013). Also literature studies showed that each phenolic compound behave different depending on treatment method. Content of rosmarinic acid after freezing and drying decreases, but carnosic acid content in the same conditions increased (Mulinaccia et al., 2011). Caffeic acid and ferulic acid both are hydroxycinnamic acids, and depending on treatment they behave different. In hara leaves content of caffeic acid after freezing increased, but ferulic acid has opposite effect (Lin et al., 2012). In literature it is found that phenolic

compounds can fast degradates in high temperatures in oxygen presence (Igual et al., 2012). Spanish scientists reported that in red onions frozen with CO_2 and stored at temperature individual phenolic content decreases for 24% (Pérez-Gregorio et al., 2011). Also flavonoids content in rosmary leaves during freezing and drying decreased (Mulinaccia et al., 2011). Whereas in hara leaves better results were obtained for samples dried in room temperatures (Lin et al., 2012). Phenolic compounds may differ from one another with respect to their binding status, depending on specific aspects of their chemical structures. Thus, processes may differ in their effectiveness in liberating phenolic acids from plant tissues. Phenolic acids occur in plants as metabolic intermediates, and they also accumulate in the vacuoles (Chism, Haard, 1996). Thermal processing may release more bound phenolic acids due to the breakdown of cellular constituents. Although disruption of cell walls also releases the oxidative and hydrolytic enzymes that can destroy the antioxidants in fruits and vegetables (Chism, Haard, 1996), thermal processing deactivates these enzymes to avoid the loss of phenolic acids (Dewanto et al., 2002).

Antioxidant activity

Phenolic compounds are commonly found in plants and they have been reported to have strong antioxidant activities (Bors et al., 2001; Li et al., 2006). The antioxidant potential of phenolic compounds is dependent on the number and arrangement of the hydroxyl groups as well as the presence of the electron donating substitute in the ring structure (Elzaawely et al., 2007). It is considered that in plant material poor in vitamin C, main antioxidants are phenolic acids and flavonoids (Igual et al., 2012).

Results of multivariate dispersion analyses showed that both type of treatment and plant material are significant factors affecting ($p<0.05$) for antioxidant activity (Table 3). Secondary antioxidant activity ($DPPH^{\cdot}$ and $ABTS^{\cdot+}$) for all samples are similar tendencies.

Table 3

Analysis of antioxidant capacity depending on treatment

Plant material	Type of treatment	$DPPH^{\cdot}$,mM TE 100 g^{-1} DW	$ABTS^{\cdot+}$, mM TE 100 g^{-1} DW	Reducing power, mg AAE 100 g^{-1} DW
Horseradish leaves	Fresh	$22.84\pm0.07^{b*}$	154.36 ± 0.12^{a}	9573.38 ± 9.07^{d}
	Frozen	13.21 ± 0.04^{d}	88.62 ± 0.09^{d}	15994.88 ± 13.02^{c}
	Dried	8.87 ± 0.02^{h}	12.80 ± 0.03^{h}	2254.65 ± 2.71^{i}
Lovage leaves	Fresh	10.90 ± 0.02^{e}	98.66 ± 0.07^{c}	21225.22 ± 20.04^{b}
	Frozen	10.12 ± 0.02^{f}	99.61 ± 0.07^{b}	22857.82 ± 22.06^{a}
	Dried	8.72 ± 0.01^{i}	30.13 ± 0.05^{g}	4196.27 ± 4.08^{g}
Lovage stems	Fresh	21.25 ± 0.06^{c}	32.50 ± 0.05^{f}	4330.21 ± 4.04^{f}
	Frozen	35.06 ± 0.07^{a}	42.46 ± 0.06^{e}	6684.49 ± 5.94^{e}
	Dried	9.82 ± 0.03^{g}	3.98 ± 0.01^{i}	2389.65 ± 2.43^{h}

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

Frozen lovage stem scavenging activity increases for 65%, and similar tendencies also were reported for horseradish roots (Tomsone et al., 2013). In horseradish leaves opposite tendency were observed, and scavenging activity decreases for 43% comparing to fresh samples. Increase of antioxidant activity could be explained by fact that splitting of molecular structure covalent complex deliberates antioxidant compounds as flavonoids, carotenoids, lycopene etc. (Siriamornpun et al., 2012). Many antioxidant phenolic compounds in plants are most frequently present in a covalently bound form with insoluble polymers (Siriamornpun et al., 2012).

In dried lovage leaves remains 80% of antioxidant activity, comparing to fresh material. In hara leaves DPPH radical scavenging activity after freezing is lower, comparing to drying at room temperatures (Lin et al., 2012). Decrease of antioxidant activity due to thermal degradation of phenolic compounds could be explained by to loss of antioxidant enzyme activities and activity of degradative enzymes (Lim, Murtijaya, 2007).

Similar like phenolic content, also primary antioxidant activity (reducing power) for all frozen samples are higher, compared to fresh samples. The same tendency for horseradish roots is observed (Tomsone et al., 2013). The highest increase in horseradish leaves and lovage stems were observed, 67% and 54%, respectively. Whereas after drying the higher primary antioxidant activity was maintained in lovage stem samples. Other authors reported similar tendencies in changes of antioxidant activity and TPC (Chan et al., 2009; Ahmad-Qasem et al., 2013).

Correlation between phenolic content and antioxidant activity

Phenolic compounds have radical scavenging activity. Correlation analysis was performed to determine relationship between these parameters. The antiradical capacity of an extract is often related to its polyphenolic constituents. In our study all correlations between analysed samples are positive and the strongest correlation was for lovage stems (Table 4), but for horseradish and lovage leaves medium or weak correlations (data not shown).

Table 4

Pearson`s coefficients between total antioxidant capacity, total phenolic and flavonoid content for lovage stems

	TPC	TFC	DPPH	ABTS
TPC	1			
TFC	0.943**	1		
DPPH	0.999**	0.940**	1	
ABTS	0.979**	0.991**	0.977**	1
Reducing power	0.997**	0.966**	0.996**	0.992**

**Correlation is significant at the 0.01 level (2-tailed).

For horseradish roots correlation between TPC and TFC was strong (r=0.86), but between different antioxidant assays are medium or weak (Tomsone et al., 2013), that is similar to results of current research. Kubola and Siriamornpun (2008) studied bitter gourd (*Momordica charantia* L.) leaf, stem and fruit fraction and referred that correlation between TPC and antioxidant activity (DPPH) was moderate (r=0.7), and between TPC and primary antioxidant activity (Reducing Power) very strong (r=0.95), but antioxidant activity (DPPH) and primary antioxidant activity (Reducing Power) show moderate correlation (r=0.55). Statistical correlations between TPC and total antioxidant capacity of litchi seed extract were strong (r=0.98) (Prasad et al., 2009). Strong correlation between phenolic compounds and antiradical activity was also found in experiments about seabuckthorn (*Hippohae rhamnoides* L.) leaves (Kumar et al., 2011), lychee (*L. chinenesis* Sonn.) flowers (Liu et al., 2009) and canola meal (Hassas-Roudsari et al., 2009).

Conclusions

Analysis of the phenolic compounds and antioxidant activity of the horseradish leaves and lovage leaves and stems showed differences depending on the technological processes applied. In order to select the best treatment method criteria such as phenolic compounds and antioxidant activity should be considered. The predominant phenolic acid in lovage samples was caffeic acid, but horseradish leaves – chlorogenic acid, and the major flavonoid was rutin. Only sinapic acid in lovage stems were found to be higher in the dried samples, compared to the fresh and freeze samples. Based on the total phenolic and flavonoid content, one of the best traditional methods for preserving phenolic compounds and antioxidant activity of the horseradish leaves and lovage leaves and stems proved to be freezing.

References

1. Ahmad-Qasem M.H., Barrajón-Catalán E., Micol V., Mulet A., García-Pérez J.V. (2013) Influence of freezing and dehydration of olive leaves (var. Serrana) on extract composition and antioxidant potential. *Food Research International*, No. 50, p. 189–196.
2. Ahrne L.M., Pereira N.R., Staack N., Floberg P. (2007) Microwave convective drying of plant foods at constant and variable microwave power. *Drying Technology*, No. 25(7), p. 1149–1153.
3. Ancos B., Gonzales E.M., Cano P.M. (2000) Ellagic acid, vitamin C, and total phenolic contents and radical scavenging capacity affected by freezing and frozen storage in raspberry fruit. *Journal of Agricultural and Food Chemistry*, Vol. 48, No.10, p. 4565–4570.
4. Angela M., Meireles A. (2009). *Extracting Bioactive Compounds for Food Products Theory and Applications*. Boca Raton, EUA: CRC Press Taylor & Francis Group. 466 p.
5. Arslan D., Musa Özcan M. (2010) Study the effect of sun, oven and microwave drying on quality of onion slices. *Lebensmittel-Wissenschaft & Technologie*, No. 43, p. 1121–1127.
6. Asami D.K., Hong Y.J., Barrett D.M., Mitchell A.E. (2003) Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. *Journal of Agricultural and Food Chemistry*, No. 51, p. 1237–1241.
7. Athukorala Y., Kim K.N., Jeon Y.J. (2006) Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, *Ecklonia cava*. *Food and Chemical. Toxicology*, No. 44, p. 1065–1074.
8. Bors W., Michel C., Stettmaier K. (2001) Structure–activity relationships governing antioxidant capacities of plant polyphenols. *Methods in Enzymology*, No. 335, p. 166–180.
9. Chan E.W.C., Lim Y.Y., Wong S.K., Lim K.K., Tan S.P., Lianto F.S., Yong M.Y. (2009) Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species. *Food Chemistry*, No. 113, p. 166–172.
10. Chan E.W.C., Lye P.Y., Eng S.Y., Tan Y.P. (2013) Antioxidant properties of herbs with enhancement effects of drying treatments: A synopsis. *Free Radicals and Antioxidants*, No. 3, p. 2–6.
11. Chism G.W., Haard N.F. (1996) Characteristics of edible plant tissues. **In**: *Food Chemistry*. O.R. Fennema (eds). New York: Dekker, p. 943–1011.
12. Dewanto V., Wu X., Adom K.K., Liu R.H. (2002) Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry*, No. 50, p. 3010–3014.
13. Elzaawely A.A., Xuan T.D., Koyama H., Tawata S. (2007) Antioxidant activity and contents of essential oil and phenolic compounds in flowers and seeds of *Alpinia zerumbet* (Pers.) B. L. Burtt. and R.M. Sm. *Food Chemistry*, No. 104, p. 1648–1653.
14. Erbay Z., Icier F. (2009) Optimization of drying of olive leaves in a pilot-scale heat pump dryer. *Drying Technology*, No. 27(3), p. 416–427.
15. Hassas-Roudsari M., Chang P.R., Pegg R.B., Tyler R.T. (2009) Antioxidant capacity of bioactives extracted from canola meal by subcritical water, ethanolic and hot water extraction. *Food Chemistry*, No. 114, p. 717–726.
16. Hossain M.B., Barry-Ryan C., Martin-Diana A.B., Brunton N.P. (2010) Effect of drying method on the antioxidant capacity of six Lamiaceae herbs. *Food Chemistry*, No. 123, p. 85–91.
17. Igual M., García-Martínez E., Martín-Esparza M.E., Martínez-Navarrete N. (2012) Effect of processing on the drying kinetics and functional value of dried apricot. *Food Research International*, No. 47, p. 284–290.

18. Kim D., Jeong S.W., Lee C.Y. (2003) Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry*, No. 81, p. 321–326.
19. Kubola J., Siriamornpun S. (2008) Phenolic contents and antioxidant activities of bitter gourd (*Momordica charantia* L.) leaf, stem and fruit fraction extracts *in vitro*. *Food Chemistry*, No. 110 (4), p. 881–890.
20. Kumar M.S.Y., Dutta R., Prasad D., Misra K. (2011) Subcritical water extraction of antioxidant compounds from Seabuckthorn (*Hippophae rhamnoides*) leaves for the comparative evaluation of antioxidant activity. *Food Chemistry*, No. 127(3), p. 1309–1316.
21. Li B.B., Smith B., Hossain Md. M. (2006) Extraction of phenolics from citrus peels I. Solvent extraction method. *Separation and Purification Technology*, No. 48, p. 182–188.
22. Lim Y.Y., Murtijaya J. (2007) Antioxidant properties of Phyllanthus amarus extracts as affected by different drying methods. *LWT - Food Science and Technology*, No. 40, p. 1664–1669.
23. Lin L., Lei F., Sun D.-W., Dong Y., Yang B., Zhao M.(2012) Thermal inactivation kinetics of Rabdosia serra (Maxim.) Hara leaf peroxidase and polyphenol oxidase and comparative evaluation of drying methods on leaf phenolic profile and bioactivities. *Food Chemistry*, No. 134, p. 2021–2029. http://dx.doi.org/10.1016/j.foodchem. 2012.04.008
24. Liu S.C., Lin J.T., Wang C.K., Chen H.Y., Yang D.J. (2009) Antioxidant properties of various solvent extracts from lychee (*Litchi chinenesis* Sonn.) flowers. *Food Chemistry*, No. 114, p. 577–581.
25. Marinova D., Ribarova F., Atanassova M. (2005) Total phenolics and total flavonoids in Bulgaria fruits and vegetables. *Journal of the University of Chemical Technology and Metallurgy*, No. 40, p. 255–260.
26. Marrelli M., Menichini F., Statti G.A., Bonesi M., Duez P., Menichini F., Conforti F. (2012) Changes in the phenolic and lipophilic composition, in the enzyme inhibition and antiproliferative activity of *Ficuscarica* L. cultivar Dottato fruits during maturation. *Food and Chemical. Toxicology*, No. 50, p. 726–733.
27. Miean K. H., Mohamed H. (2001) Flavonoid (myricetin, quercetin, kaempferol, luteolin and apigenin) content of edible tropical plants. *Journal of Agricultural and Food Chemistry*, No. 49(6), p. 3106–3112.
28. Mulinaccia N., Innocenti M., Bellumori M., Giaccherini C., Martini V., Michelozzi M. (2011) Storage method, drying processes and extraction procedures strongly affect the phenolic fraction of rosemary leaves: An HPLC/DAD/MS study. *Talanta*, No. 85, p.167–176. doi:10.1016/j.talanta.2011.03.050
29. Naczk M., Shahidi F. (2006) Phenolic in cereals, fruit and vegetables: Occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis*, No. 41(5), p. 1523–1542.
30. Orkoula M.G., Kontoyannis C.G., Markopoulou C.K., Koundourellis J.E. (2004) Development of methodologies based on HPLC and Raman spectroscopy for monitoring the stability of lovastatin in solid state in the presence of gallic acid. *Journal of Pharmaceutical and Biomedical Analysis*, No. 35, p. 1011–1016. ISSN: 0731-7085 DOI: 10.1016/j.jpba.2004.03.006
31. Pérez-Gregorio M.R., Regueiro J., González-Barreiro C., Rial-Otero R., Simal-Gándara J. (2011) Changes in antioxidant flavonoids during freeze-drying of red onions and subsequent storage. *Food Control*, No. 22, p. 1108–1113. doi:10.1016/j.foodcont.2011.01.006
32. Prasad K.N., Yang B., Yang S., Chen Y., Zhao M., Ashraf M., Jiang Y. (2009) Identification of phenolic compounds and appraisal of antioxidant and antityrosinase activities from litchi (Litchi sinensis Sonn.) seeds. *Food Chemistry*, No. 116(1), p. 1–7.
33. Raghavan Uhl S. (2000) *Handbook of Spices, Seasonings, and Flavorings*. Boca Raton: Technomic Publishing, Lancaster, 352 p.
34. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, No. 26, p. 1231–1237.
35. Rizzolo A., Nani R.C., Viscardi D., Bertolo G., Torreggiani D. (2003) Modification of glass transition temperature through carbohydrates additions and anthocyanin and soluble phenol stability of frozen bluberry juices. J*ournal of Food Enginiering*, No. 56, p. 229–231.
36. Singleton V.L., Orthofer R., Lamuela-Raventos R.M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, No. 29, p. 152–178.
37. Siriamornpun S., Kaisoon O., Meeso N. (2012) Changes in colour, antioxidant activities and carotenoids (lycopene, b-carotene, lutein) of marigold flower (Tagetes erecta L.) resulting from different drying processes. *Journal of Functional Foods*, No. 4, p. 757–766.
38. Szebeni-Galambosi Z., Galambosi B., Holm Y. (1992) Growth, yield and essential oil of lovage grown in Finland. *Journal of Essential Oil Research*, No. 4, p. 375–380.
39. Tomsone L., Krūma Z., Galoburda R., Dimins F., Kreicbergs V. (2013) Influence of technological processes on the phenol content and antioxidant properties of horseradish roots (*Armoracia rusticana* L.). **In:** 2nd International Conference on Nutrition and Food Sciences ICNFS 2013" 27.-28.07.2013. Moscow, Russia, DOI:10.7763/IPCBEE. 2013. Vol.53 (2), p. 6–10.
40. Yang J., Chen J., Zhao Y., Mao L. (2010) Effects of Drying Processes on the Antioxidant Properties in Sweet Potatoes. *Agr. Sci. China*., No. 9(10), p. 1522–1529. http://dx.doi.org/10.1016/S1671-2927(09)60246-7
41. Yu L., Haley S., Perret J., Harris M., Wilson J., Haley S. (2003) Antioxidant properties of bran extracts from Akron wheat grown at different locations. *Journal of Agriculture and Food Chemistry*, No. 51, p. 1566–1570.

DETERMINATION OF MAJOR SUGARS IN FRESH AND DRIED SPICES AND VEGETABLES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract

Carbohydrates are one of the most important energy sources in plants synthesized during photosynthesis. They are important for plants to grown and produce. Polysaccharide content changes depending on plant type, growing location and weather conditions. The highest content differs in roots and leaves- photosynthesis starts in leaves, but to grow and produce mostly carbohydrates are localized in roots. It is important to follow up in differences between sugars changes in vegetable and spices. There are many researches focusing on sugar changes mostly in fruits and cereals, less in vegetables, herbs, and spices. The aim of this research was to determine major sugars in nine spices and vegetables- celery (*Apium graveolens var dulce*), parsley (*Petroselinum crispum*), dill (*Anethum graveolens*), leek (*Allium ampeloprasum* L.), garlic (*Allium sativum* L.), onion (*Allium cepa*), celery root (*Apium graveolens var. rapaceum*), pumpkin (*Curcubica maxima*), carrot (*Daucus carota*) grown in Latvia in 2013. Analyses were made using high performance liquid chromatography (HPLC) in the laboratories of Latvia University of Agriculture, Faculty of Food Technology for fresh samples and samples dried in convective dryer at 45±1 °C temperature. Fructose, glucose, sucrose and maltose content was determined using HPLC method, and moisture content in each sample was established by drying samples till constant weight. Results were expressed as grams per 100 grams of dry weight (DW). Analysing obtained results the highest total sugar content was found in vegetable roots (carrot), lowest- in spices' leaves. Convective drying at 45±1 °C process takes from 12 till 48 hours to reduce moisture content. After drying process in most cases individual sugar content increased significantly. During drying process water is removed from samples, sugars are changing, which may indicate possible Maillard reaction.

Keywords: spices, vegetables, drying, sugars, HPLC.

Introduction

By culinary definition vegetables are edible plant parts including stems and stalks, roots, tubers, bulbs, leaves, flowers, some fruits and seeds (Pennington, Fisher, 2009).

Vegetables are an important part of our diet. They provide not only the major dietary fibre component in our food, but also a range of micronutrients, including minerals, vitamins and antioxidant compounds, such as carotenoids and polyphenols (Singh et al., 2012). Most vegetables are commonly cooked before being consumed. Cooking induces significant changes in chemical composition, affecting the bio accessibility and the concentration of nutrients and health-promoting compounds (Mazzeo et al., 2011).

Health beneficial attributes of spices have the potential of a possible therapeutically exploitation in a variety of disease conditions. Spices do not contribute significantly to the nutrient makeup of food, especially because of their small quantities encountered in diet (Srinivasan, 2005). Spices can be added to foods in several forms: as whole spices, as ground spices, or as isolates from their extracts. Spices are aromatic and pungent food ingredients (Suhaj, 2006).

Carbohydrates are divided into following groups- monosaccharides (simple sugars that are the simplest members of carbohydrates, cannot be subjected to hydrolysis); oligosaccharides (consists of small number of monosaccharides, usually two to ten molecules, with disaccharides being the most common in foods; polysaccharides (consists of large amount of monosaccharides). They vary in their physical and chemical properties compared to monosaccharides. Most important polysacharides in food are starch, glycogen and cellulose (Tzia et al., 2012). The final products of carbohydrate digestion in the digestive tract are almost entirely glucose, fructose, and galactose. Because glucose is the only carbohydrate that can be oxidized in muscle, much of the fructose and all of galactose are transported to the liver, after absorption from the intestinal tract, and converted into glucose. The conversion of fructose and galactose occurs in the liver at relatively low rates (Campbell, 2013). Simple monosaccharides such as glucose can be directly absorbed by the host. Disaccharides such as maltose, lactose and sucrose can be hydrolyzed to their respective monosaccharides, but the ability to digest complex plant polysaccharides such as inulin, pectin and xylan is very limited (Ibrahim, Anishetty, 2012).

One of the oldest methods for food preservation is drying, which consists of removing water from the product in order to provide microbiological safety and the most popular drying method is air-drying in convective drier. In this method the drying agent supplies heat to the material and removes moisture from material at the same time (Nawirska et al., 2009). Major disadvantages of hot air drying of foods are low energy efficiency and long drying time during the falling rate period. Because of the low thermal conductivity of food materials in this period, heat transfers to the inner sections of foods during conventional heating is limited (Wang, Xi, 2005). The drying time of the convective technique can be shortened using higher temperatures which increase moisture diffusivity and by cutting the material into smaller pieces. Increased drying temperature entails higher costs and may cause biochemical changes that degrade the dried product quality (Figiel, 2009)

High temperatures can destroy enzymes, vitamins and determine the rancidity of fat-containing foods, but also can lead to the production of free radical scavenging substances, such as Maillard reaction products (Melanoidins and Amadori rearrangement products). Both reactions induced by the transformation can take place simultaneously and can influence each other (Dini et al., 2013). The Maillard reaction is a complex series of reactions that involve reducing sugars and proteins, giving multitude of end products that are known as Advanced Glycation End products. They can contribute to the pathogenesis of diabetes and neurological diseases such as Alzheimer's disease. And also play a major role in vascular stiffening, atherosclerosis, osteoarthritis, inflammatory arthritis and cataracts (Edeas et al, 2010)

The aim of this research was to determine major sugars (fructose, glucose, sucrose and maltose) in nine spices and vegetables – celery, parsley, dill, leek, garlic, onion, celery root, pumpkin, carrot grown in Latvia in 2013. From obtained results was determined drying impact on sugars content.

Materials and Methods

All samples were grown and harvested reaching full maturity in Latvia in 2013. Analyses were made in the laboratories of Latvia University of Agriculture, Faculty of Food Technology.

Fresh samples were washed and cut in equal small pieces. For drying samples were washed, cut in small pieces and dried in a convective dryer with fan (Memmert, Model 100-800) at 45±1 °C temperature till constant (not changing) mass.

For samples moisture content was determined according to AOAC (1995). Samples were dried at 105±1 °C: fresh samples for 2 hours and dried samples for 30 minutes. Analysis were triplicated. Moisture content was expressed as percentes. From moisture was calculated dry matter.

Sugars content (fructose, glucose, sucrose and maltose) was determined using high performance liquid chromatography (HPLC) method (Shimadzu LC 20 Prominence) (Rybak-Chmielewska, 2007; Dimins et al., 2008; Beitane et al., 2013). Method is based on chromatographic separation of sugars and their retention time. Sample extracts were made to extract sugars according to following procedure- samples were blended in small particles (using commercial blender for fresh samples – Bosch MSM 6700 or coffee mill for dried samples – Scarlett SL-1545 Silver Line), soaked in water and extracted in ultrasound bath (SELECTA P ULTRASONS) at 35±1 °C for 45 minutes. Solution was filtrated twice using filter paper and 0.45 μm Millipore membrane.

The analysis by HPLC were performed at 25±1 °C under isocratic conditions. The mobile phase consisted from A – acetonitrile; B – water (70:30). Flow rate was 1.00 mL min^{-1} and injection volume was 10 μL. The analytic column Alltech-NH_2 (4.6 mm×250 mm×5 μm) was used and refractive index detector (RID-10A) was used. Total time of analysis was up to 30 min. The identification of sugars in vegetables and spices was done by comparing retention times of individual sugars in the reference vs. tested solution (qualitative analysis). The quantitative assays were made by the following carbohydrates: fructose, glucose, sucrose and maltose. The content of those compounds were assessed based on the comparing peak areas obtained from the reference analysis. Analysis was done duplicate. Results were expressed as grams per 100 g of dry weight (g 100 g^{-1} DW).

Results and Discussion

The highest dry matter content among fresh samples was established in garlic, lowest – in onion. In dried samples dry matter content was higher than in fresh samples (Table 1).

Table 1

Dry mater content in fresh and dried vegetables and spices

Samples	Dry matter in fresh samples,%	Dry matter in dried samples,%
Celery	17.57±0.18	90.66±0.91
Parsley	25.30±0.25	91.22±0.91
Dill	18.40±0.18	93.04±0.93
Leek	11.07±0.11	87.33±0.87
Onion	6.20±0.06	78.92±0.79
Garlic	45.59±0.46	87.44±0.87
Celery root	12.96±0.13	90.46±0.90
Carrot	11.60±0.12	84.87±0.85
Pumpkin	8.21±0.08	89.14±0.89

Dried onion, leek, garlic, carrot and pumpkin are samples which can absorb water from air in short time during grinding process.

Fructose and glucose content increased after drying in all samples (Table 2). All results are expressed as grams per 100 g dried weight (g 100 g^{-1} DW).

Increasing can be explained with polysaccharide reducing in smaller monomers. Vegetables and spices contain a large amount of poly carbohydrates.

The highest fructose content was determined in dried onions, lowest in fresh garlics. After drying process in most cases fructose content increases significantly ($p<0.05$). Only pumpkins fructose content decreases. The highest glucose content was reached in dried celery roots, lowest was in fresh garlics. Glucose content in most cases increases significant ($p<0.05$). Glucose content in pumpkins decreases significant ($p<0.05$).

Comparing reached data to USDA Nutrient database, glucose content in carrots are 0.28 g 100 g^{-1} fresh weight (FW), in onions – 1.97 g 100 g^{-1} FW, in celery 0.55 g 100 g^{-1} FW, in dried parsley 2.76 g 100 g^{-1} DW, in dried onion 0.73 g 100 g^{-1} DW, in dried garlic 0.07 g 100 g^{-1} DW. Fructose content in carrots are 0.26 g 100 g^{-1} FW, in onions 1.26 g 100 g^{-1} FW, in

celery 0.51 g 100 g^{-1} FW, in dried parsley 0.42 g 100 g^{-1} DW, in dried onion 1.67 g 100 g^{-1} DW, in dried garlic 0.31 g 100 g^{-1} DW. According to Shanmugavelan et al. (2013), fructose content in onions are 27.74 g 100 g^{-1} DW, in carrots 11.24 g 100 g^{-1} DW. Glucose in onions 31.80 g 100 g^{-1} DW, in carrots 10.37 g 100 g^{-1} DW. According to Caruso et al. (2014) reached data glucose content in onions are 27.8 g 100 g^{-1} DW and fructose are 21.5 g 100 g^{-1} DW. Obtained results can be compared with literature data.

Table 2

Fructose and glucose content in fresh and dried vegetables and spices

Samples		Fructose, g 100 g^{-1} DW	Glucose, g 100 g^{-1} DW
Celery	A	1.035±0.104	4.619±0.462
	B	1.806±0.181	12.407±1.241
Parsley	A	3.527±0.353	9.219±0.922
	B	5.437±0.544	11.729±1.173
Dill	A	1.089±0.109	2.282±0.228
	B	3.245±0.325	4.411±0.441
Leek	A	12.647±1.265	10.838±1.084
	B	12.232±1.223	13.322±1.332
Onion	A	18.442±1.844	18.094±1.809
	B	26.891±2.689	23.209±2.321
Garlic	A	0.273±0.027	0.097±0.001
	B	1.143±0.011	0.317±0.032
Celery root	A	0.815±0.082	20.586±2.059
	B	1.200±0.120	25.059±2.506
Carrot	A	10.345±1.035	13.161±1.316
	B	14.135±0.141	24.559±1.456
Pumpkin	A	18.867±1.887	17.771±1.777
	B	13.492±1.349	12.837±0.128

A–fresh samples; B–dried samples

The highest sucrose content was determined in dried celery roots, the lowest in fresh dill. The highest maltose content was in dried pumpkins, but not detected in fresh carrots (Table 3).

The highest sucrose content was determined in vegetable roots, less in leaves. Maltose content has no differences between vegetable roots and vegetable leaves in most cases. Content of sucrose and maltose increases significantly ($p<0.05$) after drying.

Comparing reached data to USDA Nutrition database, sucrose content in carrots are 1.92 g 100 g^{-1} FW, in onions 0.99 g 100 g^{-1} FW, in celery 0.11 g 100 g^{-1} FW, in dried parsley 4.09 g 100 g^{-1} DW, in dried onion 3.87 g 100 g^{-1} DW, in dried garlic 2.05 g 100 g^{-1} DW. Maltose content in all previously described has not been detected. According to Shanmugavelan et. al. (2013), sucrose content in onions is 8.32 g 100 g^{-1} DW, in carrots 20.09 g 100 g^{-1} DW. According to Caruso et al. (2014), sucrose content in onions are 11.3 g 100 g^{-1} DW. Maltose content was not analysed in both researches. Obtained results can be compared with literature data.

Table 3

Sucrose and maltose content in fresh and dried vegetables and spices

Samples		Sucrose, g 100 g^{-1} DW	Maltose, g 100 g^{-1} DW
Celery	A	0.707±0.071	0.205±0.021
	B	0.746±0.075	0.712±0.071
Parsley	A	0.353±0.035	0.692±0.069
	B	1.040±0.104	1.275±0.128
Dill	A	0.011±0.001	0.812 ±0.081
	B	0.405±0.041	1.626±0.163
Leek	A	2.152±0.215	0.405±0.041
	B	9.411±0.941	0.888±0.089
Onion	A	1.123±0.112	0.429±0.043
	B	5.274±0.527	0.826±0.083
Garlic	A	2.255±0.226	0.254±0.025
	B	2.709±0.271	0.509±0.051
Celery root	A	11.247±1.125	0.664±0.066
	B	10.260±1.026	0.742±0.074
Carrot	A	23.911±2.391	n.d.
	B	18.019±1.802	1.556±0.156
Pumpkin	A	2.050±0.205	1.088±0.109
	B	6.394±0.639	1.915±0.192

A–fresh samples; B–dried samples; n.d.–not detected

Increasing monosaccharide and disaccharides could be explained by samples possible oligosaccharides division in more simple compounds. According to Yang et.al (2014), the pre-treatment and hydrolysis of celluloses and oligosaccharides can be generated from hydrothermal (steaming, blanching, sterilization, drying etc.) pre-treatment of the cellulose. Using hydrolysis of oligosaccharides fermentable sugars can be recovered. Hydrolysis can be done using acid, alkaline, enzymes and oxidative degradation.

In future research will be analysed polysaccharide changes in fresh and dried vegetables and spices and how they are associated with antioxidant enlargement or reducing.

Conclusions

During drying process fructose, glucose, sucrose and maltose content significantly increased. Increasing may be explained with oligosaccharide dividing in monomers and dimers or during possible Maillard reaction. Catalyst for the decomposition reaction is heat. Spices and vegetables contain lower sucrose and maltose content, but higher fructose and glucose content. Vegetables and spices can be used in diets with low calorie amount than fruits.

References

1. AOAC (1995) Official Methods of Analysis (15th ed.) Washington, DC: Association of Official Analytical Chemists.
2. Beitane I., Straumite E., Cinkmanis I. (2013) Nutritional value and sensory properties of yoghurt enriched with barley grains and malt extract. **In**: *Annual 19th International Scientific Conference Proceedings Research for Rural Development 2013*, Latvia, Jelgava, Vol. 1., p. 125–130.
3. Campbell B. (2013) Carbohydrate Metabolism. **In**: *Sports Nutrition. Enhancing Athletic Performance*. B. Campbell (eds). CRC Press, Tayloy&Francis Group, p.45– 74.
4. Caruso G., Conti S., Villari G., Borrelli C., Melchionna G., Minutolo M., Russo G., Amalfitano C. (2014) Effects of transplanting time and plant density on yield, quality and antioxidant content of onion (*Allium cepa* L.) in southern Italy. *Scientia Horticulturae*, 166, p.111- 120.
5. Dimins F., Kuka P., Cakste I. (2008) Content of carbohydrates and specific rotation angle of honey. **In**: *3rd Baltic Conference on Food Science and Technology. Conference Proceedings FOODBALT 2008*, Latvia, Jelgava, p. 121–125.
6. Dini I., Tenore G.C., Dini A. (2013) Effect of industrial and domestic processing on antioxidant properties of pumpkin pulp. *LTW-Food Science and Technology*, Vol. 53, Iss. 1., p. 382–385.
7. Edeas M., Attaf D., Mailfert A.S., Nasu M., Joubet R. (2010) Maillard Reaction, mitochondria and oxidative stress: Potential role of antioxidants. *Pathologie Biologie*, Vol. 58, p. 220–225.
8. Figiel A. (2009) Drying kinetics and quality of vacuum-microwave dehydrated garlic cloves and slices. *Journal of Food Engineering*, Vol. 94, p. 98–104.
9. Ibrahim M., Anishetty S. (2012) A *meta-metabolome* network of carbohydrate metabolism: Interactions between gut microbiota and host. *Biochemical and Biophysical Research Communications*, Vol. 428, p. 278–284.
10. Mazzeo T., N'Dri D., Chiavaro E., Visconti A., Fogliano V. (2011) Effect of two cooking procedures on phytochemical compounds, total antioxidant capacity and colour of selected frozen vegetables. *Food Chemistry*, Vol. 128, p. 627–633.
11. Nawirska A., Figiel A., Kucharska A.Z., Sokol-Letowska A., Biesiada A. (2009) Drying kinetics and quality parameters of pumpkin slices dehydrated using different methods. *Journal of Food Engineering*, Vol. 94, p. 14–20.
12. Pennington J.A.T., Fisher R.A. (2009) Classification of fruits and vegetables. *Journal of Food Composition and Analysis*, Vol. 225, p. 523–531.
13. Rybak-Chmielewska H. (2007) High Performance Liquid Chromatography (HPLC) study of sugar composition in some kinds of natural honey and winter stores processed by bees from starch syrup. *Journal of Apicultural Science*, Vol. 51, Iss. 1, p. 23–38.
14. Singh D.S., Beloy J., McInerney J.K., Day L. (2012) Impact of boron, calcium and genetic factors on vitamin C, carotenoids, phenolic acids, anthocyanins and antioxidant capacity of carrots (*Daucus carota*). *Food Chemistry*, Vol. 132, p. 1161–1170.
15. Shanmugavelan P., Yeon King S., Bong King J., Woong Kim H., Mok Cho S., Na Kim S., Young Kim S., Sook Cho Y., Ran Kim H. (2013) Evaluation of sugar content and composition in commonly consumed Korean vegetables, fruits, cereals, seed plants, and leaves by HPLC-ELSD. *Carbohydrate Research*, Vol. 380, p. 112–117.
16. Suhaj M. (2006) Spice antioxidants isolation and their antiradical activity: a review. *Journal of Food Composition and Analysis*, Vol.19, p. 531–537.
17. Srinivasan K. (2005) Spices as influencers of body metabolism: an overview of three decades of research. *Food Research International*, Vol.38, p. 77–86.
18. Tzia C., Giannou V., Lebesi D., Cranioti C. (2012) Chemistry and functional properties of carbohydrates and sugars (monosaccharides, disaccharides, and polysaccharides). **In**: *Sweeteners. Nutritional aspects, applications, and production technology*. T.Varzakas, A.Labopoulos, S.Anestis (eds). CRC Press, Taylor&Francis Group, p.11–45.
19. *USDA National Nutrient Database for Standard Reference*, Release 26. [accessed on 27.02.2014] Available : www.ndb.nal.usda.gov/ndb/search/list
20. Wang J., Xi Y.S. (2005) Drying characteristics and drying quality of carrot using two-stage microwave process. *Journal of Food Engineering*, Vol.68, p.505-511
21. Yang F., Zhang Q., Fan H.X., Li Y., Li G. (2014) Electrochemical control of the conversion of cellulose oligosaccharides into glucose. *Journal of Industrial and Engineering Chemistry*.

PORE SIZE DISTRIBUTION OF EGGPLANTS DRIED BY DIFFERENT DRYING METHODS

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Abstract

The main objective of this study is to investigate the effects of hot air drying and microwave-infrared combination drying on porous structure of eggplants. Hot air drying was performed in a tray dryer at 50°C with an air velocity of 1.5 m s^{-1}. In microwave-infrared combination oven, different microwave powers (30%, 40% and 50%) were combined with different infrared powers (10%, 20%). During drying process, initial moisture content decreased from 14 kg water kg^{-1} dry solid to approximately 0.13 kg water kg^{-1} dry solid for eggplants. Pore size distributions of dried samples were analyzed with mercury porosimetry. Pores in different samples were characterized by cumulative intrusion curves which showed total volume of mercury intruded the poresin any size range and the threshold diameter. Cumulative intrusion curves had a sharp rise that indicated existence of macro pores on the surface. Unlike microwave–infrared combination dried eggplants, hot air dried eggplants did not have pores above 200 µm size. Threshold diameter of eggplants dried in microwave-infrared combination oven were in the range of 48.86–73.42 µm which were greater than that of hot air dried eggplant, 42.47 µm. Microwave-infrared combination drying provided more porous structure than hot air dried ones due to higher internal pressure. As infrared and microwave power increased, threshold diameter increased and eggplants with more porous structure were obtained.

Keywords: microwave, infrared, drying, eggplant, porosity.

Introduction

Eggplant (*Solanum melongena* L.) is an important market vegetable of Asian and Mediterranean countries. It contains a variety of phytochemicals such as phenolics and flavonoids (Akanitapichat et al., 2010). It is ranked amongst the top ten vegetables in terms of antioxidant capacity due to the phenolic constituents (Cao et al., 1996). However, due to the higher moisture content, they have limited shelf life. In order to extend their shelf lives, it is common to dry eggplants. Dried eggplants can be used as an ingredient in different kinds of meals, instant soups and sauces.

Drying is a removal of moisture from the food materials to prevent the growth and reproduction of spoilage microorganisms and to slow down the action of enzymes and minimize many of the moisture mediated deteriorative reactions (Wu et al., 2007). Texture, appearance, colour, flavor, taste and nutritional value are affected from drying process conditions. Therefore, deciding suitable drying system is very important for food products. Although hot air drying method is used most commonly in industry, during hot air drying, food is exposed to heat for longer time. It causes problems related to quality parameters such as unacceptable color, flavor, texture, sensory characteristics, loss of nutrients, shrinkage, reduction in bulk density and rehydration capacity (Maskan, 2001). In order to eliminate these problems, microwave heating can be used for drying of foods. It provides high internal pressure that causes shorter drying time and more porous structure. In recent years, microwave drying has been used in drying of carrots (Arikan., 2012), potatos (Wang et al., 2004) andapples (Askari et al., 2006).However, due to the lower surrounding temperature, microwave heating causes condensation at the food surface. In order to eliminate this problem, Datta, Ni (2002) recommended the combination of infrared heating with microwave heating to dry food products. Infrared heating increases the surface temperature so excess surface moisture evaporates. Therefore, microwave-infrared combination drying has time saving advantage without the formation of sogginess.However, in literature, there are limited studies on microwave-infrared combination drying (Sumnu et al., 2005; Tireki et al., 2006). Sumnu et al. (2005) dried carrot by using microwave-infrared combination and hot air drying. It was stated that by using microwave-infrared combination drying, drying time was reduced to 98% of hot air drying time. Moreover, in the case of microwave-infrared combination drying, less color change and higher rehydration capacity of carrots were obtained as compared to conventional drying. Tireki et al. (2006) produced bread crumbs by conventional and microwave-infrared combination drying and it was stated that by using microwave-infrared combination drying, drying time was reduced to 96.8–98.6% of conventional drying.

One of the most important parameters that shows the effect of drying process on the quality of food is pore structure. More porous structure is an indication of less damage during drying. However, there is limited information about pore structure of dried fruits and vegetables. Schiffmann (1986) and Krokida, Maroulis (1999) analyzed porosity of microwave-dried apples, bananas, carrots and potatoes and concluded that microwave drying increased product porosity. Russo (2013) studied the effect of air temperature on microstructure of hot air dried eggplants and stated thatthe porosity increased with the air temperature.However, the effect of microwave-infrared combination drying on pore structure of eggplants has not been studied yet. Therefore, the objective of this study was to compare the effects of hot-air convectional drying and microwave-infrared combination drying on pore structure of eggplants.

Materials and Methods

Material

Eggplants (*Solanum melongena* L.) used in this study were obtained from the local market and stored in a refrigerator at 4 °C. Prior to drying, samples were washed and cut into slices, of a thickness 5 mm using a kitchen slicer. Diameters of the eggplants were 5.0±0.5 cm and the weight of each sample was 7.0±0.5 g. The initial moisture content of eggplant was found to be 14 ±0.314 kg water kg^{-1} dry solid by using moisture analyzer (MX-50 AND Moisture Analyzer, Tokyo, Japan).

Drying method

Hot air drying was performed in a tray dryer (Armfield Limited, D 27412, Ringwood Hampshire, England). The size of the tray was 18×30 cm^2. Drying experiments were carried out at 50 °C with an air velocity of 1.5 m s^{-1} until a moisture content of 0.13±0.002 kg water kg^{-1} dry solid was reached in almost 4 hour. In each experiment, 100–115 g of eggplants were dried and weight of samples was recorded at every 1 hour interval. That is, 1.85–2.13 kg eggplants were placed on one m^2 of tray for drying.

Microwave-infrared combination drying experiments were performed in microwave-infrared combination oven (Advantium oven™, General Electric Company, Louisville, KY, USA). In the experiments, powers of halogen lamps two at the top and one at the bottom which are sources of infrared power were 1500 W. Combinations of different microwave power levels (30%, 40% and 50%) and infrared powers (10% and 20%) were used in drying of eggplants. The power of the oven was determined as 630 W by using IMPI 2 liter test (Buffler, 1993). For both cases, 100–115 g of eggplant was dried until a final moisture content of approximately 0.13 kg water kg^{-1} dry solid was achieved in almost between 19–30 minutes. In every 2 minutes, weight of the samples was recorded by a digital balance (ARD-110 - Single Unit, China).

Pore-size distribution

Pore size distribution was determined by using mercury porosimeter (Poremaster 60, Quantichrome Corp., Boynton Beach, Florida, USA). About 0.5 g of dried eggplants was used for each experiment. Measurements were done at pressure range of 0–50 psia, and for calculations, surface tension and contact angle of mercury was taken as 480 erg cm^{-2} and mercury contact angle as 140°, respectively. Relation between applied pressure (P) and pore size was expressed by Washburn equation (Russo et al., 2013) which describes a linear relationship between the size of an intrudable circular pore and the applied mercury pressure in the mercury porosimeter.

$$P.r = -2\gamma\cos\theta \quad (1)$$

where r is the pore radius (μm), γ is the Hg surface tension (N/m), θ is the contact angle (°) and P is the absolute applied pressure (N).

Results and Discussion

The plot of cumulative volume of mercury intruded versus pore size or versus pressure is called as cumulative intrusion curve. From the cumulative intrusion curve, the total volume of mercury intruded the pore volume in any pore size range and the threshold diameter (the diameter above which comparatively low mercury intruded) can be determined (Aligizaki, 2006).

The cumulative volumes of mercury intruded as a function of pore size and pressure of dried eggplants using different combination of microwave and infrared powers were indicated through Figures 1 to 4. Figure 5 was given to show the cumulative intrusion curve of eggplant dried by hot air.

Initially, there was a sharp increase in intrusion volume and then a relatively constant region with increasing pressure in all graphs. Initial step rise indicated that there were macro pores on the surface and the same sized pores existed predominantly. This can be the result of less collapse in the structure due to short drying time. A gradual rise on the slope can be an indication of decreasing pore size through the sample (Rahman et al., 2002).

Pore size ranges for eggplants dried at 10% infrared and 30% microwave power, 20% infrared and 30% microwave power, 10% infrared and 50% microwave power and 20% infrared & 50% microwave power combinations were from 227.4 to 4.39 μm; from 201.3 to 4.28 μm; from 235.3 to 4.27 μm; 210.6 to 4.27 μm, respectively (Figures 1–4). When hot air dried eggplants were compared with microwave-infrared combination dried eggplants, pore size range of hot air dried eggplants was narrower. Unlike microwave-infrared combination dried eggplants, hot air dried eggplants did not have pores above 200 μm size (Figure 5).

Threshold pore size was the pore diameter where the vertical line was observed and the diameter above comparatively low mercury intruded. Threshold pore size of hot air dried eggplants was 42.47 μm which was lower than the threshold pore size of eggplants dried with microwave-infrared combination. This result could be related to higher shrinkage and lower porosity due to higher drying time of conventionally dried eggplants.

As infrared and microwave power increased, threshold pore size increased. Threshold pore sizes were 48.86 μm and 55.40μm for 10% infrared and 30% microwave power combination and 20% infrared and 30% microwave power combination, respectively. Also, the threshold pore sizes were 64.89 μm and 73.42 μm for 10% infrared and 50% microwave power combination and 20% infrared and 50% microwave power combinations, respectively. This can be attributed to the reduction of shrinkage due to the increased microwave and infrared power. Similar pattern was observed for spouted bed drying of wheat (Kahyaoglu, 2009). As the temperature increased, shrinkage was enhanced so threshold pore size decreased slightly.

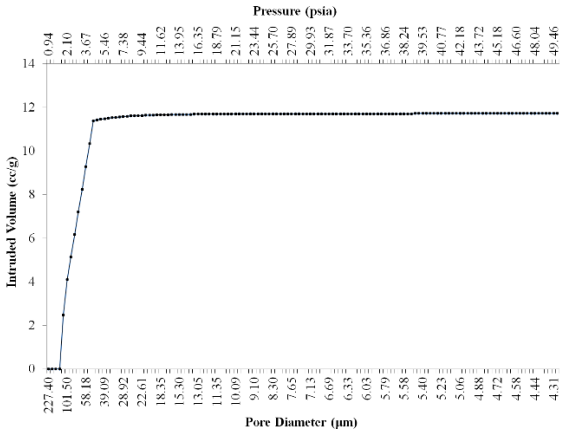

Figure 1. Cumulative intrusion curve for eggplants dried in microwave-infrared combination oven at 10% infrared and 30% microwave powers

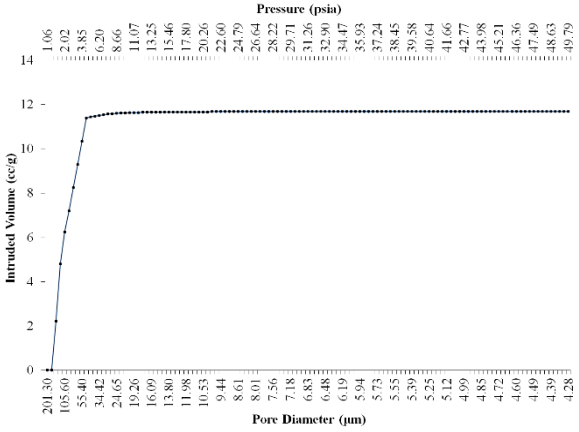

Figure 2. Cumulative intrusion curve for eggplants dried in microwave-infrared combination oven at 20% infrared and 30% microwave powers

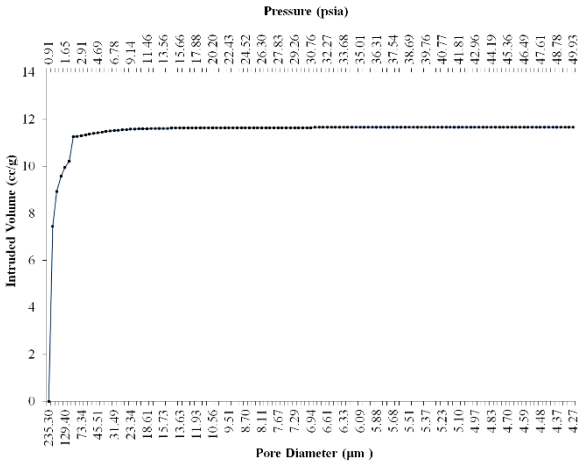

Figure 3. Cumulative intrusion curve for eggplants dried in microwave-infrared combination oven at 10% infrared and 50% microwave powers

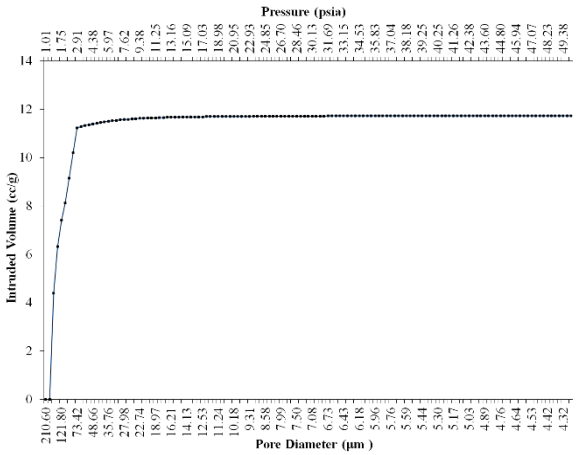

Figure 4. Cumulative intrusion curve for eggplants dried in microwave-infrared combination oven at 20% infrared and 50% microwave powers

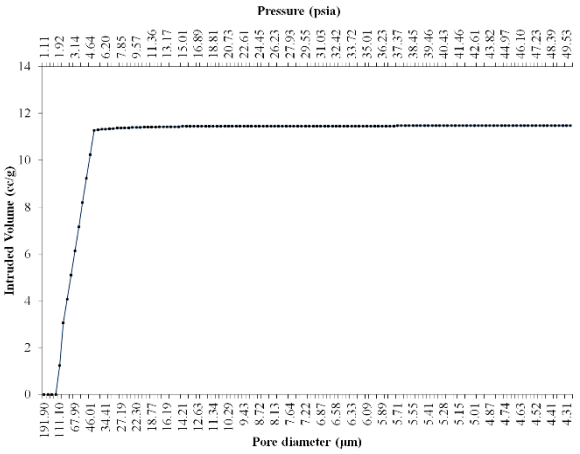

Figure 5. Cumulative intrusion curve for eggplants dried in hot air dryer

Conclusions

According to cumulative intrusion curves, hot air dried eggplants had less porous structure than the ones dried in microwave-infrared combination oven. In addition, increasing microwave and infrared power resulted in more porous structure. The optimum drying condition is 20% infrared power and 50 microwave power combination that provides more porous structure. Thus, microwave-infrared combination drying can be recommended to be used for drying of eggplants.

References

2. Akanitapichat P., Phraibung K., Nuchklang K., Prompitakkul S. (2010) Antioxidant and hepatoprotective activities of five eggplant varieties. *Food and Chemical Toxicology*, Vol. 48, p. 3017–3021.
3. Aligizaki K. K. (2006) *Mercury intrusion porosimetry, Pore Structure of Cement-Based Materials: Testing Interpretation and Requirements.* New York: Taylor & Francis.

4. Arikan M.F., Ayhan Z., Soysal Y., Esturk O. 2012 Drying characteristics and quality parameters of microwave-dried Grated Carrots. *Food Bioprocess Technology*, Vol. 5, p. 3217–3229.
5. Askari G. R., Emam-Djomeh Z., Mousavi S. M. (2006) Effects of combined coating and microwave assisted hot-air drying on the texture, microstructure and rehydration characteristics of apple slices. *Food Science and Technology International*, Vol. 12, p. 39–46.
6. Buffler C. (1993) *Microwave cooking and processing: Engineering fundamentals for the food scientist.* New York: Avi Book.
7. Cao G., Sofic E., Prior R. (1996) Antioxidant capacity of tea and common vegetables. *Journal of Agriculture and Food Chemistry*, Vol. 44, p. 3426–3431.
8. Datta A. K., Ni H. (2002) Infrared and hot air-assisted microwave heating of foods for control of surface moisture. *Journal of Food Engineering*, Vol. 51, p. 355–364.
9. Kahyaoglu L. N. (2009) *Usage of spouted and microwave assisted spouted in bulgur production*.
10. Krokida M. K., Maroulis Z. B. (1999) Effect of microwave drying on some quality properties of dehydrated products. *Drying Technology*,Vol. 17(3), p. 449–466.
11. Maskan M. (2001) Drying, shrinkage and rehydration characteristics of kiwifruits during hot air and microwave drying. *Journal of Food Engineering*, Vol. 48, p. 177–182.
12. Rahman M. S., Al-Amri, O. S., Al-Bulushi I. M. (2002) Pores and physico-chemical characteristics of dried tuna produced by different methods of drying. *Journal of Food Engineering*, Vol. 53, p. 301–313.
13. Russo P., Adiletta G., Matteo M. D. (2013) The influence of drying air temperature on the physical properties of dried and rehydrated eggplant. *Food and Bioproducts Processing*, Vol. 91, p. 249–256.
14. Schiffmann R. F. (1986) Food product development for microwave processing. *Food Technology*, p. 94–98.
15. Sumnu G., Turabi E., Oztop M. (2005) Drying of carrots in microwave and halogen lamp–microwave combination ovens. *LWT-Food Science and Technology*, Vol. 38, p. 549–553.
16. Tireki S., Şumnu G., Esin A. (2006) Production of bread crumbs by infrared-assisted microwave drying. *European Food Research and Technology*, Vol. 222, p. 8–14.
17. Wang J., Xiong Y.-S., Yu Y. (2004) Microwave drying characteristics of potato and the effect of different microwave powers on the dried quality of potato. *European Food Research and Technology*, Vol. 219, p. 500–506.
18. Wu L., Orikasa T., Ogawa Y., Tagawa A. (2007) Vacuum drying characteristics of eggplants. *Journal of Food Engineering*, Vol. 83, p. 422–429.

CAROTENOIDS AND TOTAL PHENOLIC CONTENT IN POTATOES WITH DIFFERENT FLESH COLOUR

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Abstract

Polyphenols are recognized as the most abundant antioxidants in our diet. Potatoes are a good source of these compounds. Phenolic compounds represent a large group of minor chemical constituents in potatoes, which play an important role in determining their organoleptic properties. Further, phenolics have a wide-array of health providing characteristics. The aim of this research was to determine the content of total phenolic and carotenoid (TPC) content in relationship with the colour of organically and conventionally cultivated potato varieties (*Solanum tuberosum* L.) with different flesh colour. In cooperation with the State Priekuli Plant Breeding Institute (Latvia), sixteen potato genotypes were studied. The highest TPC and carotenoid content was determined in potatoes of Purple Peru variety when conventionally cultivated. Correlation was found between TPC and colour L* ($r=-0.813$). The changes in carotenoid content and TPC of potatoes vary significantly according to the type of cultivation practise, depending on variety. There are common tendencies in the changes of TPC and carotenoid content – the variety was the most significant factor ($p<0.001$).

Keywords: potato, colour, TPC, carotenoids, organic and conventional.

Introduction

The research in potato chemistry has established the fact that there is a lot more in potatoes than starch. Natural colorant and antioxidant present in yellow-, purple- and red-flesh potatoes can be used for developing functional foods. Considering the large quantities in which potatoes are consumed throughout the world, potatoes could be a very good vehicle for addressing some health related problems (Ezekiel et al., 2013). Many of the compounds present in potatoes are important because of their beneficial effects on health, therefore, they are highly desirable in the human diet (Katan, De Roos, 2004).

Colour is an important food quality parameter. It affects consumer acceptance and can even evoke emotional feelings in humans (Crisosto et al., 2003; Ou et al., 2004). Coloured potatoes have attracted the attention of investigators as well as consumers because of their antioxidant activities, taste and appearance (Jansen, Flamme, 2006). The antioxidant activity in coloured potatoes is associated with the presence of polyphenols anthocyanins, flavonoids, carotenoids, ascorbic acid, tocopherols, alpha-lipoic acid and selenium (Lachman et al., 2005a). Therefore, coloured potatoes have the potential to be one of the richest sources of antioxidants in the human diet.

As food and life style choices have been increasingly recognised as useful approaches in prevention or delaying the onset of chronic diseases, more and more research and commercial development are focused on food phytochemicals such as polyphenolics and carotenoids (Maiani et al., 2009; Spencer, 2008; Minich, Bland, 2008; Hunter et al., 2008; Stevenson, Hurst, 2007).

Carotenoids are lipophilic compounds synthesized in plastids from isoprenoids which are widespread in nature and have broad range of functions, especially in relation to human health and their role as biological antioxidants (Dellapenna, Pogson, 2006; Fraser, Bramley, 2004). Because of their high carotenoids content potatoes are particularly beneficial for eye health (Tan et al., 2008).

Potato cultivars with white flesh contain less carotenoids as compared to cultivars with yellow or orange flesh. Total carotenoids content was reported in the range of 50–350 μg 100 g^{-1} FW and 800–2000 μg 100 g^{-1} FW, respectively, in white- and yellow-fleshed potato cultivars (Brown, 2008).

Polyphenols comprise over 8000 identified substances, which can be divided into groups according to their chemical structure, such as phenolic acids, stilbenes, coumarins, lignins and flavonoids (Ross, Kasum, 2002).

Phenolic compounds are considered to be health-promoting phytochemicals as they have shown in vitro antioxidant activity and have been reported to exhibit beneficial antibacterial, antiglycemic, antiviral, anticarcinogenic, anti-inflammatory and vasodilatory properties (Duthie et al., 2000; Mattila, Hellstrom, 2006). Potatoes are a good source of phenolic compounds. Phenolic compounds represent a large group of minor chemical constituents in potatoes, which play an important role in determining their organoleptic properties. Further, phenolics have a wide-array of health providing characteristics (Bravo, 1998), therefore, have potential for use as functional food for improving human health. The phenolic content of potatoes was reported to be high, and ranged from 530 to 1770 μg g^{-1} (Al-Saikhan et al., 1995). Potatoes were considered the third most important source of phenols after apples and oranges (Chun, 2005).

Potato quality varies depending on the growing area, cultivar and aspects of the chemical composition of main crop potato tubers have been shown to depend on the cultivation system as well. The improved qualitative value of organic vs. conventional produce, however, has not been ascertained (Dangour et al.,

2009; Moschella et al., 2005). Although nutrient content depends on a number of factors, the potato variety is thought to be among the most significant factors (Toledo, Burlingame, 2006).

The interest in organic agriculture and environmentally-friendly agricultural products is increasing, and in particular consumers have made potatoes one of their top organic purchases among fresh vegetables even though organic potatoes carry a price significantly higher than most other vegetables (Carillo et al., 2012). In this respect, it is not known whether and how different agriculture techniques and/or cultivation systems may affect the nutrients composition of the final product. Comparison of organic and conventional foods in terms of nutritional value, sensorial quality and food safety, has often highlighted controversial results. As a consequence, a clear link between cultivation system and nutritional profile of agricultural products is still missing (Bourn, Prescott, 2002).

The aim of this research was to determine the content of total phenolic and carotenoid content in relationship with the colour of organically and conventionally cultivated potato varieties (*Solanum tuberosum* L.) with different flesh colour.

Materials and Methods

The potatoes were planted in the middle of May and harvested in last decades of August or first days of September. Field trials were conducted in three replications. The certified potato seed material was used. Seed tubers were planted in rows; the distance between rows was 0.7 m and the distance between tubers 0.3 m.

Organic field. The soil type was sod podzolic (PVv), loamy sand. Organic matter content in soil was 25 mg kg^{-1}, pH_{KCl} was 6.3, the availability in soil of K was low and P was medium. The common agronomic practices were used during vegetation period.

Conventional field. The soil type in conventional field was sod-podzolic (PVv), sandy loam. Organic matter content in soil was 27 mg kg^{-1}, pH_{KCl} was 5.7, availability of K and P in soil was high. Fertilizer P – 55, K – 90 kg ha^{-1} and N – 60 kg ha^{-1} was used in conventional field. The common agronomic practices were used during vegetation period. Herbicides in field were used for weed control. The fungicides for restriction fungal diseases were used two times in July.

The haulm was cut in last decade of August and the tubers were harvested in the beginning of September.

Potatoes were stored at the State Priekuli Plant Breeding Institute at an air temperature of 4±1 °C and at a relative air humidity of 80±5%.

Plant material. In the experiment 16 potato (*Solanum tuberosum* L.) varieties with white, yellow and purple coloured flesh were evaluated, whose seed was obtained in the State Priekuli Plant Breeding Institute (SPPBI) (Latvia). Potatoes were grown in organic and conventional field and controlled by SPPBI. The characterization of potato varieties and the type of cultivation practise used per each potato variety is presented in Table 1.

Table 1

Description of potato varieties

Name and acronym* of potato variety	Flesh colour	Origin of variety	Cultivation practice applied**
Agrie Dzeltenie (Adz)	yellow	Latvia	O/C
Prelma (P)	yellow	Latvia	O/C
Imanta (I)	white	Latvia	O/C
Lenora (L)	yellow	Latvia	O/C
Brasla (Br)	yellow	Latvia	O/C
Bionica (Bi)	white	Netherlands	O/C
Anuschka (An)	yellow	Germany	O/C
Gundega (G)	yellow	Latvia	O/C
S04009-37 (S37)	yellow	Latvia	C
S03135-10 (S10)	white	Latvia	C
S99108-8 (S8)	light yellow	Latvia	C
Fenton (F)	purple	US	C
Purple Fiesta (PF)	purple	US	C
British Columbia Blue (BCB)	purple	US/Canada	C
Purple Peru (PP)	purple	Peru	C
Blue Congo (BC)	purple	Czech Republic	C

* Acronyms of potato varieties used throughout the paper presented in brackets

** Cultivation practice: O/C – cultivated organically and conventionally, C – cultivated conventionally

Sampling. For testing, a total of 10 kg (around 50–60 potato tubers) of table potato tubers per variety were selected into small piles, from ten different wooden boxes. Five potatoes were selected from several location points of each box (Murniece et al., 2011). All operations during sample preparation were performed very quickly so as to avoid deviations from the qualitatively obtained results. Potato tubers were peeled and analysed without a skin. In the analysis on total phenolic content (TPC) and carotenoids, the test and analysis were run in triplicate and averaged.

Carotenoids were analyzed by spectrophotometric method (with the UV/VIS spectrophotometer Jenway 6705) at 440 nm (Ермаков, 1987) and is expressed on mg 100 g^{-1} fresh weight (FW) of potatoes.

Total Phenolic Content (TPC). For extraction of phenolic compounds five grams of the homogenized sample were extracted with 50 mL of ethanol water solution (80%) in a conical flask with a magnetic stirrer (magnet 4.0×0.5 cm) at 700 rpm for 1 h at room temperature (20±1 °C). The potatoes extracts were then filtered via the paper with No 89.

The TPC of the extracts was determined according to the Folin-Ciocalteu spectrophotometric method

(Singleton et al., 1999) with some modifications. To 0.5 mL of extract 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times with water) and, after 3 minutes 2 mL of sodium carbonate (Na_2CO_3) (75 g l^{-1}) was added. The sample was mixed. After 30 minutes of incubation at room temperature, the absorbance was measured at 765 nm. Total phenols were expressed as gallic acid equivalents (GAE) mg 100 g^{-1} FW of potatoes.

Colour analysis. The colour of potato samples was measured by "Colour Tec-PCM" device. For evaluation of the colour of potato samples, potato slices were cut shortly before measurement in order to avoid formation of melanin pigments during non-enzymatic browning reaction which can affect the accuracy of colour measurement. Potato samples were covered by a transparent PP film ("Forpus"), thickness of 25 μm, to avoid direct contact between the equipment of the measuring device and the product. The colour was measured at least in seven various locations of the sample in order to obtain higher accuracy after calculation of the mean value. For data processing, "ColorSof QCW" software was used.

Statistical Analysis. For statistical analysis, the data were processed using the S-PLUS 6.1 Professional Edition software and XLSTAT 2014 program. Data are presented as a mean ± standard deviation (SD). The differences between independent groups were specified by one way and two way analysis of variance (ANOVA), and values of $p<0.05$ were regarded as statistically significant. In case of establishing statistically significant differences, homogeneous groups were determined by Tukey's multiple comparison test at the level of confidence $\alpha=0.05$. Relationships between carotenoid, total phenolic content and colour were made by Principal Component Analysis (PCA).

Results and Discussion

Carotenoid content in potatoes cultivated organically varied from 0.089 to 0.385 mg 100 g^{-1} FW while in conventionally cultivated potatoes – from 0.068 to 0.371 mg 100 g^{-1} FW (Fig. 1). Bonierbale et al. (2009) has found that carotenoid content might vary in potatoes from 0.103 to 2.135 mg 100 g^{-1} FW). The results of current research make the range much wider. Differences in this case might be influenced by several factors, for example variety and maturity stage of tubers (Murniece et al., 2011). It has been found that total carotenoid content is higher in immature tubers and it decreased with tuber maturity (Kotikova et al., 2007; Morris et al., 2004).

Comparing coarotenoid content between potato tuber varieties with white and yellow flesh, content was found to be the highest in Lenora variety when cultivated organically and Anuschka variety when cultivated conventionally.

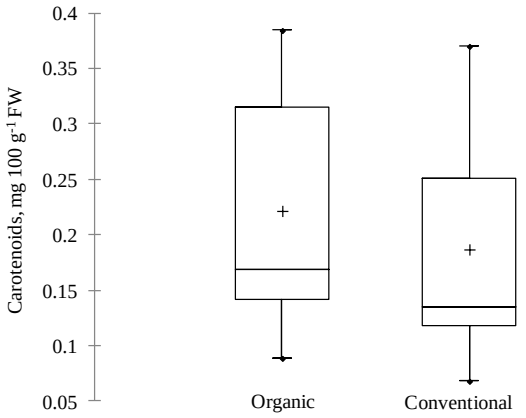

Figure 1. Carotenoid content in potato tubers with white and yellow flesh

In potato tubers with purple flesh carotenoid content was 78% lower than in potato tubers with white and yellow flesh and it varied from 0.012 to 0.084 mg 100 g^{-1} FW (Table 2).

In the evaluation of carotenoid content within the factor of variety and factor of cultivation practise, statistical results showed that significance was noticed between varieties ($p=0.019$) while no significant difference was noticed when potato tubers were cultivated organically or conventionally ($p=0.316$). The most significant difference in carotenoid content was determined between Imanta and Anuschka varieties.

Kotikova et al. (2007) have obtained the same result that the application of fertilizers did not bring any significant changes in carotenoids of potatoes.

The TPC might be affected during the development of the flesh colour (purple, violet, yellow) of potato tubers (Lachman et al., 2005b), due to the environmental conditions, such as longer days and cooler temperatures or fertilization (Kumar et al., 2004; Reyes et al., 2004).

TPC in potato tubers cultivated organically varied from 34.475 to 64.230 mg GAE 100 g^{-1} FW, in 25% of analysed potato tubers TPC was no higher than 36.937 mg GAE 100 g^{-1} FW while in 75% of analysed potato tubers TPC reached to 49.436 mg GAE 100 g^{-1} FW. In the case when potato tubers were cultivated conventionally TPC was in lower amount and it varied from 26.854 to 52.172 mg GAE 100 g^{-1} FW. In 25% of analysed potato tubers TPC was no more than 31.410 mg GAE 100 g^{-1} FW of while in 75% – 49.040 mg 100 g^{-1} FW (Fig. 2).

Comparing the obtained results with Faller and Failho (2009), also in this particular research, TPC significantly differed between varieties ($p=0.015$) while cultivation practise did not present significance ($p=0.164$). The most significant difference in TPC was determined for Anushcka variety when compared with Brasla, Imanta and Lenora varieties.

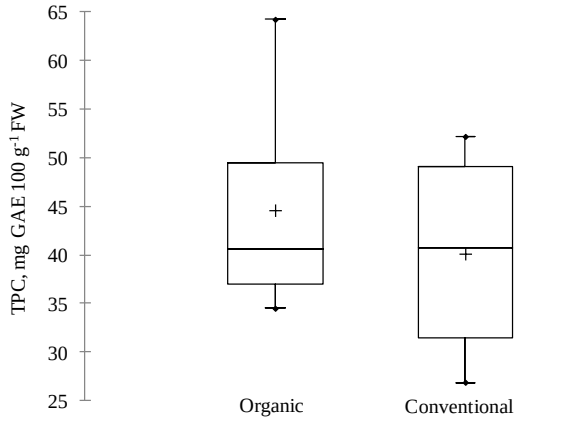

Figure 2. Total phenolic content in potato tubers with white and yellow flesh

Comparing TPC of potatoes with white and yellow flesh with purple fleshed it was 81% higher in potato tubers with purple flesh. The highest TPC between purple fleshed varieties was determined for Purple Peru variety while the lowest – in Purple Fiesta variety (Table 2).

Table 2

Total phenolic and carotenoid content in potato tubers with purple flesh

Name of potato variety	Carotenoids	TPC
	mg 100 g^{-1} FW	mg GAE 100 g^{-1} FW
Fenton	0.012±0.002	75.92±0.14
Purple Fiesta	0.070±0.002	69.54±0.56
British Colombia Blue	0.084±0.005	73.02±0.38
Purple Peru	0.029±0.002	86.72±0.25
Blue Congo	0.030±0.001	77.07±0.23

Results present means of triplicate determinations with ± standard deviation

To better understand the relationship between TPC and carotenoid content with colour of organically and conventionally cultivated potato varieties, PCA was applied. In Figure 3 the first and second principal components accounted for 73.47% of total variance (PC1 – 51.22% and PC2 – 22.25%). Projection of the samples in the space formed by PC1 and PC2 shows that colour intensity (L*) is low of potato tuber flesh of Imanta variety and for the same (i.e. Imanta variety) the difference in flesh colour was noticed when potatoes were cultivated organically and conventionally (Fig. 3). In addition, the projection of PCA presents the opposite side of potato varieties with yellowish flesh (b*) and with higher carotenoid content (carotenoids) of potato varieties projected in the same area. Potato varieties projected in the positive area of F1 and F2 are higher in TPC and colour flesh is much lighter than in the varieties projected in the positive area of F1 and negative of F2 i.e. of b* and carotenoids.

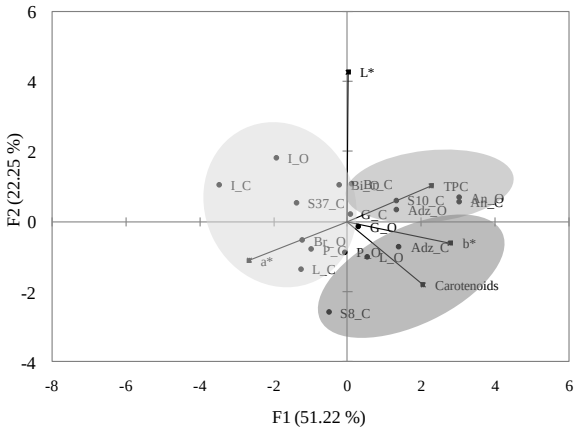

Figure 3. Principal component analysis, projection of potato varieties with white and yellow flesh cultivated organically and conventionally

The first letter indicates variety and the second – cultivation practice. An example: I_O – Imanta variety cultivated organically while I_C – Imanta variety cultivated conventionally. Acronyms are summarized in the Table 1.

When potatoes of purple flesh were analysed by applying PCA, the projection of factors was different from potatoes with white and yellow flesh. In the Figure 4 first and second principal components accounted 86.58% of total variance (PC1 – 55.51% and PC2 – 31.07%). Potato varieties which are projected in the negative area of F1 are higher in TPC and colour flesh is much darker (purplish to bluish) whereas in the positive area of F2 with the predominant factor L* Blue Congo variety cultivated conventionally comparing to other potatoes tubers with purple flesh was with lighter colour (Fig. 4).

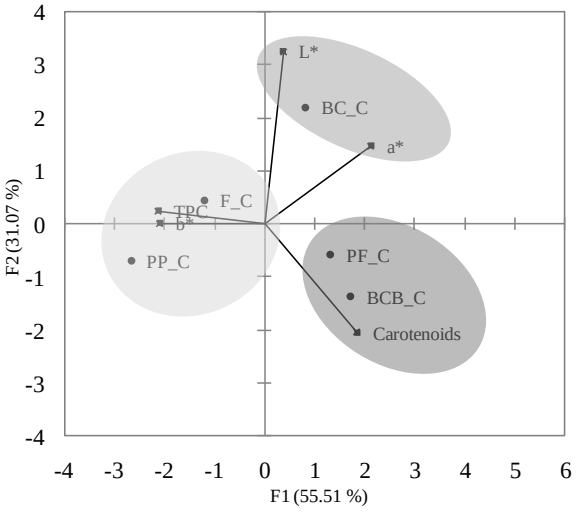

Figure 4. Principal component analysis, projection of potato varieties with purple flesh cultivated conventionally

First letter indicates variety and second – cultivation practise. An example: BC_C – Blue Congo variety cultivated conventionally. Acronyms are summarized in the Table 1.

In the correlation analysis (Fig. 5) of potato tubers with white and yellow flesh obtained results represented negative correlation between TPC with the colour of redness value a* (r=0.594), positive correlation was found between TPC and carotenoid content with the colour of yellowness value b* (r=0.490) and (r=0.591) respectively.

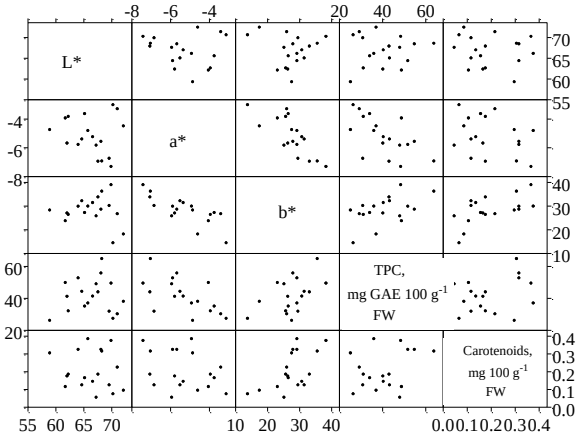

Figure 5. Relationship of total phenolic and carotenoid content with colour L* a* b*

When correlation analysis was applied to all potato varieties used in the research, between TPC and L* correlation was found (r=0.813).

In the colour analysis the obtained results showed that colour intensity within potato tuber varieties with white and yellow flesh significantly differed between Imanta and Prelma varieties ($p<0.05$). Significant difference was noticed on yellowness value b* ($p\leq0.001$) while factor a* which represents redness did not show significance on colour flesh between all potato varieties with white and yellow flesh ($p>0.05$). In the case of statistical analysis of potato tubers with purple flesh there were no significant affect on any of parameters determined in research ($p>0.05$).

Conclusions

From the nutritional point of view, apart from the macronutrient content, potatoes contain traces of nutrients which are proved to be beneficial for human health. From the results of this particular research it might be concluded that the colour of potato flesh could be one of the factors taken into account for modelling and predicting health promoting substances like carotenoid, anthocyanin content and also TPC. In future, much deeper investigation is required in this particular field of interest.

Acknowledgment

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References

1. Al-Saikhan M. S., Howard L. R., Miller J. C. (1995) Antioxidant activity and total phenolics in different genotypes of potato (*Solanum tuberosum* L.). *Journal of Food Science*, No. 60, p. 341–343.
2. Bonierbale M., Gruneberg W., Amoros W., Burgos G., Salas E., Porras E., Felde T. M. (2009) Total and individual carotenoid profiles in *Solanum phureja* cultivated potatoes: II. Development and application of near-infrared reflectance spectroscopy (NIRS) calibrations for germplasm characterization. *Journal of Food Composition and Analysis*, No. 22, p. 509–516.
3. Bourn D., Prescott J. (2002) A comparison of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods. *Critical Reviews in Food Science and Nutrition*, No. 42, p. 1–34.
4. Bravo L. (1998) Polyphenols: Chemistry, dietary sources, metabolism and nutritional significance. *Nutrition Reviews*,
No. 56, p. 317–333.
5. Brown C. R. (2008) Breeding for phytonutrient enhancement of potato. *American Journal of Potato Research*, No. 85, p. 298–307.
6. Carillo P., Cacace D., De Pascale S., Rapacciuolo M., Fuggi A. (2012) Organic vs. traditional potato powder. *Food Chemistry*, No. 133, p. 1264–1273.
7. Chun O. K., Kim D. O., Smith N., Schroeder D., Han J. T., Lee C. Y. (2005) Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. *Journal of the Science of Food and Agriculture*, No. 85, p. 1715–1724.
8. Crisosto C. H., Crisosto G. M., Metheney P. (2003) Consumer acceptance of 'Brooks' and 'Bing' cherries is mainly dependent on fruit SSC and visual skin color. *Postharvest Biology and Technology*, No. 28, p. 159–167.
9. Dangour A. D., Dodhia S. K., Hayter A., Allen E., Lock K., Uauy R. (2009) Nutritional quality of organic foods: A systematic review. *The American Journal of Clinical Nutrition*, No. 90, p. 680–685.
10. Dellapenna D., Pogson B. J. (2006) Vitamin synthesis in plants: Tocopherols and carotenoids. *Annuual Review of Plant Biology*, No. 57, p. 711–738.
11. Duthie, G., Duthie, S., Kyle, J. (2000) Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. Nutrition Research Review, Vol. 13, p. 79–106.
12. Ezekiel R., Singh N., Sharma S., Kaur A. (2013) Beneficial phytochemicals in potato – A review. *Food Research International*, Vol. 50, p. 487–496.
13. Faller A. L. K., Fialho E. (2009) The antioxidant capacity and polyphenol content of organic and conventional retail vegetables after domestic cooking. *Food Research International*, No. 42, p. 210–215.
14. Fraser P., Bramley P. (2004) The biosynthesis and nutritional uses of carotenoids. *Progress in Lipid Research*, No. 43, p. 228–265.
15. Hunter D. C., Skinner M. A., Lister C. E. (2008) Impact of phytochemicals on maintaining bone and joint health. *Nutrition*, Vol. 24, Issue 4, p. 390–392.
16. Jansen G., Flamme W. (2006) Coloured potatoes (*Solanum tuberosum* L.) – anthocyanin content and tuber quality. *Genetic Resources and Crop Evolution*, No. 53, p. 1321–1331.
17. Katan M. B., De Roos N. M. (2004) Promises and problems of functional foods. *Critical Reviews in Food Science and Nutrition*, No. 44, p. 369–377.

18. Kotikova Z., Hejtmankova A., Lachman J., Hamouz K., Trnkova E., Dvorak P. (2007) Effect of selected factors on total carotenoid content in potato tubers (*Solanum tuberosum* L.). *Plant Soil Environment*, No. 53, p. 355–360.
19. Kumar P., Pandey S. K., Singh S.V., Rawal S., Kumar D. (2004) Effect of potassium fertilization on processing grade tuber yield and quality parameters in potato (*Solanum tuberosum* L.). *The Indian Journal of Agricultural Science*s, No. 74, p. 177–179.
20. Lachman J., Hamouz K., Orsaká M. (2005a) Red and purple coloured potatoes—a significant antioxidant source in human nutrition. *Chemické Listy*, No. 99, p. 474–482.
21. Lachman J., Hamouz K., Orsaká M., Pivec V., Dvořák P. (2005b) The influence of flesh colour and growing locality on polyphenolic content and antioxidant activity in potatoes. *Scientia Horticulturae*, No. 117, p. 109–114.
22. Maiani G., Caston M. J., Catasta G., Toti E., Cambrodon I. G., Bysted A. et al. (2009) Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. *Molecular Nutrition and Food Research*, Vol. 53, Issue 2, p. 194–218.
23. Mattila P., Hellstrom J. (2006) Phenolic acids in potatoes, vegetables, and some of their products. *Journal of Food Composition and Analysis*, Vol. 20, p. 152–160.
24. Minich D. M., Bland J. S. (2008) Dietary management of the metabolic syndrome beyond macronutrients. *Nutrition Reviews*, Vol. 66, Issue 8, p. 429–444.
25. Morris W. L., Ducreux L., Griffiths D. W., Stewart D., Davies H. V., Taylor M. A. (2004) Carotenogenesis during tuber development and storage in potato. *Journal of Experimental Botany*, No. 55, p. 975–982.
26. Moschella A., Camin F., Miselli F., Parisi B., Versini G., Ranalli P. et al. (2005) Markers of characterization of agricultural regime and geographical origin in potato. *Agroindustria*, Vol. 4, Issue 3, p. 325–332.
27. Murniece I., Karklina D., Galoburda R., Santare D., Skrabule I., Costa H. S. (2011) Nutritional composition of freshly harvested and stored Latvian potato (*Solanum tuberosum* L.) varieties depending on traditional cooking methods. *Journal of Food Composition and Analysis*, Vol. 24, p. 699–710.
28. Ou L.-C., Luo M. R., Woodcock A., Wright A. (2004) A study of emotion and color preference. Part I. Color emotions for single colors. *Color Research and Application*, No. 29, p. 232–240.
29. Reyes L. F., Miller J. C., Cisneros-Zevallos L. (2004) Environmental conditions influence the content and yield of anthocyanins and total phenolics in purple- and red-flesh potatoes during tuber development. *American Journal of Potato Research*, No. 81, p. 187–193.
30. Ross J. A., Kasum C.M. (2002) Diertary flavonoids: Bioavailabiligy metabolic effects, and safety. *Annual Review on Nutrition*, No. 22, p. 19–34.
31. Singleton V. L., Orthofer R., Lamuela-Raventos R. M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, No. 29, p. 152–178.
32. Spencer J. P. (2008) Flavonoids: Modulators of brain function? *British Journal of Nutrition*, Vol. 99, Issue 1, p. 60–77.
33. Stevenson D.E., Hurst R.D. (2007) Polyphenolic phytochemicals – Just antioxidants or much more? *Cellular and Molecular Life Sciences*, Vol. 64, Issue 22, p. 2900–2916.
34. Tan J. S., Wang J. J., Flood V., Rochtchina E., Smith W., Mitchell P. (2008) Dietary antioxidants and the long-term incidence of age-related macular degeneration: The Blue Mountains Eye Study. *Ophthalmology*, No. 115, p. 334–341.
35. Toledo A., Burlingame B. (2006) Biodiversity and nutrition: a common path toward global food security and sustainable development. *Journal of Food Composition and Analysis*, Vol. 19, Issue 6–7, p. 477–483.
36. Ермаков А. И. (1987) Методы биохимического исследования растений. Под ред. Ермакова А. И. Ленинград: ВО «Агропромиздат», с. 112–113.

THE INFLUENCE OF PRE-TREATMENT METHOD ON THE FAT CONTENT DECREASE IN FRENCH FRIES

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Abstract

French fries are very popular product in many countries. But this product together with potato chips is included in the group of unhealthy products due to high content of fats, acrylamid, and peroxide value. Therefore the aim of this research was to evaluate the pre-treatment methods for reducing the fat content, and improving other quality parameters.

Before frying the potato strips were blanched in hot water at 85 °C, and dried in microwave-vacuum dryer for 3, 6 and 8 min, and in conventional dryer with air ventilation for 15 min at 60 and 80 °C, and 10 min at 100 °C temperature. The frying was done at 170 °C for 1.5–2 min. The colour (in CIE L*a*b*), texture (with texture analyser), the moisture content, the total fat content, and the ascorbic acid content were analysed for all prepared samples.

The best preliminary drying technology was drying in the conventional dryer at 100 °C temperature for 10 min. The product prepared with this technology had the lowest total fat content (3.4%) and the lowest hardness (lowest maximal cutting force: 11.3 N). The lowest moisture content was to sample dried in in microwave-vacuum dryer for 8 min (36.84%). This sample was also the hardest one (maximal cutting force: 52.41 N). There were no significant differences ($p>0.05$) in colour (L* a* b*) values between control samples and samples dried in conventional dryer but the samples dried in microwave-vacuum dryer became darker than control samples (lower L* value 57.37).

Keywords: French fries, preliminary drying, total fat content.

Introduction

French fries are very popular product in many countries. But this product together with potato chips is included in the group of unhealthy products due to high content of fats, acrylamid, and peroxide value.

High oil content is a major factor affecting consumer acceptance of oil-fried products today and the low fat food products are becoming more popular (Bunger, Moyano, Rioseco, 2003; Mai Tran et al., 2006). Oil consumption especially saturated fat is considered a major factor increasing health risks such as coronary heart disease (CHD), cancer, diabetes and hypertension, and even linked to increased causes of deaths. Fried foods contribute a significant proportion of the total fat consumed in the Western world. The fat content in French fries produced by different companies can vary between 3.4 and 20.9% (USDA National Nutrient Database for Standard Reference). That proves the influence of production technology and other factors to the total fat content of this product.

Knowledge of optimal frying conditions is important to produce French fries with low fat content, both under industrial conditions and at home.

During deep-fat frying, water in the crust will evaporate and move out of the food. In order for the flow of vapour to continue, sufficient water has to be able to migrate from the core of the food to the crust and the crust has to remain permeable. The fact that the vapour leaves voids for the fat to enter later, is the reason why fat uptake is largely determined by the moisture content of the food (Mehta, Swinburn, 2001). Similarly, sections of the food with more moisture loss also show higher uptake of fat. Some even argue that the total volume of fat will equal the total volume of water removed (mass balance) (Pinthus et al., 1993). An increase in the density of potato tuber also leads to a decrease in fat absorption. In one study of fried potato chips, tubers with a higher density (1.103 g cm^{-3}) yielded chips with lower fat content (42.1%) than slices with lower density of 1.093 g cm^{-3} (48.8% fat) (Ufheil, Escher, 1996). The thickness of the potato strips is an important factor affecting the overall fat content of French fries. Thick-cut strips (12 mm or bigger) absorb less fat than thin-cut strips (Blumentahl, 1991).

In the preparation of French fries, several processes are involved. The most common sequence is cutting – blanching – drying – pre-frying – freezing / chilling – packing – thawing. Each step may be important for the final product quality. The fat uptake varies with the pre-treatment of the potato. Blanching reduces the subsequent cooking time. Blanching also makes the colour more uniform after frying and it forms a layer of gelatinized starch that limits oil absorption and improves texture (Moreira, 1999).

Low temperature blanching enhances pectin methyl esterase (PME) activity and is another option proposed that affects both textural quality and oil uptake (Aguilar et al., 1999). Drying the surface of the potato strips before frying reduces the fat uptake in French fries. In the experiments of Lamberg et al. (1990) drying reduced the fat uptake by 7 to 29% (for 1 min frying) and by 15 to 40% (for 5 min frying). Drying causes a 'skin' to form on the surface of the potato strip and this reduces vapour transport through the surface layer. Raising the temperature of the fat higher than 185 °C causes the fat to break down more rapidly due to an increased rate of oxidation and polymer formation in the fat. When frying at 200 °C or above, excess energy in the fat is converted into cross-links leading to case-hardening of food. This results in a brown surface forming on the food before the inside is completely cooked and immersing the food for a longer time to cook the inside properly may cause burning on

the outside (Blumenthal, Stier, 1991). In some countries maximum frying temperature is set at 180 °C. On the other hand, if French fries are cooked at lower temperature, or the 'boiling action' on the surface ceases due to a lower heat input, the crust does not form on the surface. This allows extra fat to penetrate into the core of the French fries. About 40% more fat is absorbed when the fat temperature is 10 °C lower than the recommended cooking temperature of 180 °C to 185 °C (Mehta, Swinburn, 2001).

The reducing of fat content in fried products is became as very actual topic of the research but there is still not a uniform opinion on the best solutions for reducing the fat content in fried French fries. There is a little information also about the influence of different pre-drying methods to the possible of the reducing of oil content in French fries. Therefore the aim of this research was to evaluate the pre-treatment methods for reducing the fat content, and improving other quality parameters.

Materials and Methods

The experiments were carried out in the laboratories of the Faculty of Food Technology, Latvia University of Agriculture. The potatoes of cultivar 'Laura' were used. The samples were cut into straws manually by hands 5×5 mm wide. Before frying the potato straws were blanched in hot water at 85 °C for 8 min, and dried in microwave-vacuum dryer MYCCOH-1 for 3, 6 and 8 min, and in conventional dryer with air ventilation for 15 min at 80 °C, and 10 min at 100 °C temperature. The frying was done at 170 °C for 2 min. The colour (in CIE L* a* b*), texture (with texture analyser), the moisture content, the total fat content, and the ascorbic acid content were analysed for all prepared samples.

Determination of textural properties

The textural properties of potato straws were measured in terms of the cutting force. A Texture Analyzer TA.XTplus (Stable Microsystems, UK) was used for cutting force determination. HDP/BSW blade set with Warner Blatzer was used for detection of cutting force to potato straws, moving at a test speed of 1.5 mm s^{-1} over a distance of 5.0 mm. The numerical results were expressed in N.

Total fat content

Determination of total fat content was occurred by using the SoxCapTM2047 in combination with Soxtec extraction systems. The total fat content was tested to samples, which were treated with oil, and with oil and water, but was not tested to samples treated only with water because of too low fat content.

Colour measurements

Colour of samples was measured in CIE L*a*b* colour system using Tristimulus Colorimeter, measuring Hunter colour parameters by Colour Tec PCM/PSM. Colour values were recorded as L* (brightness) – the vertical co-ordinate runs from L* = 0 (black) through grey to L* = 100 (white); a* (-a, greenness, +a, redness) – the horizontal co-ordinate, that runs from -a* (green) through grey to +a* (red) and b* (-b, blueness, +b, yellowness) – another horizontal co-ordinate, that runs from -b* (blue) through grey to +b* (yellow) (Coultate, 2002). The measurements were repeated on different randomly selected locations at the surface of each sample.

Total colour difference (ΔE*) between control sample and samples dried in different conditions was calculated using the following equation (1):

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

where ΔE* – total colour difference; L*, a* and b* are the lightness (L), greenness (a) and blueness (b) values for the pre-dried samples; L_0*, a_0* and b_0* are the corresponding colour values for the control sample. The difference L*-L_0* is difference of lightness, a*-a_0* – difference of green and red colour and b*-b_0* – difference of blue and yellow colour.

The moisture content

Moisture content was determined using standard method ISO 6496:1999 by verified balance KERN (Germany) with precision ±0.001 g; mass loss calculation (%) were determined by weighing samples on the electronic scales, by standard LVS ISO 1442:1997.

The content of ascorbic acid

The content of ascorbic acid was determined by titration with 0.05-M iodine solution (Jansons, 2006). The French fries sample (25 g) were poured with 6% solution of oxalic acid and homogenized. Then the sample was filtered. 2 mL of 1% solution of starch was added to 10 mL of filtrate and the filtrate was titrated until the colour changed which does not disappeared during a 30 sec interval. For standard solution of ascorbic acid 20 mg of ascorbic acid were dissolved in 100 mL of the oxalic acid solution. Two mL of the starch solution was added to 25 mL of the standard-solution and the mixture was titrated. The content of vitamin C (ascorbic acid) mg per 100 g of the product dry matter was calculated with the following equation (2):

$$C = 5000 \cdot \frac{V_{sample}}{m \cdot V_{standard}}, \quad (2)$$

where V_{sample} – volume of the iodine solution titrated in a sample, mL;

$V_{standard}$ – volume of the iodine solution titrated in a standard solution, mL;

m – the amount of sample, g.

Statistical analysis

The results were processed by mathematical and statistical methods. One-way analyses of variance ($p \leq 0.05$) were used to determine significance of differences between samples prepared by different drying methods in the main quality parameters: hardness, total fat content, colour (L* a* b*) and ascorbic acid content.

Results and Discussion

Textural properties

There were significant differences ($p<0.001$) in maximal cutting force between potato samples prepared with different pre-treatment methods. The samples dried in conventional dryer were with more similar textural properties comparing to control sample than samples dried in microwave-vacuum dryer (Fig. 1).

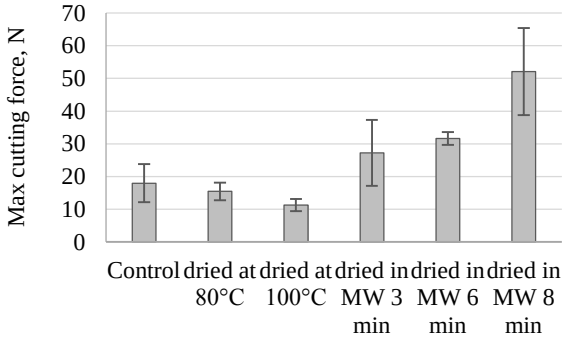

Figure 1. The texture of French fries pre-treated with different methods

The highest maximal cutting force was to French fries sample dried before frying in microwave-vacuum dryer for 8 minutes (in average 52.13 N) but the lowest maximal cutting force was observed to sample dried in conventional dryer at 100 °C temperature.

Total fat content

As the aim of these investigations was to find the method for production of French fries with the reduced oil content then total fat content of samples is very important quality parameter.

The total fat content of control sample (French fries blanched at 85 °C for 7 min, and just fried without drying) was in average 10.15% but in all samples, which were dried after blanching, the fat content was significantly lower ($p=0.028$) and didn't reach 8% (Fig. 2).

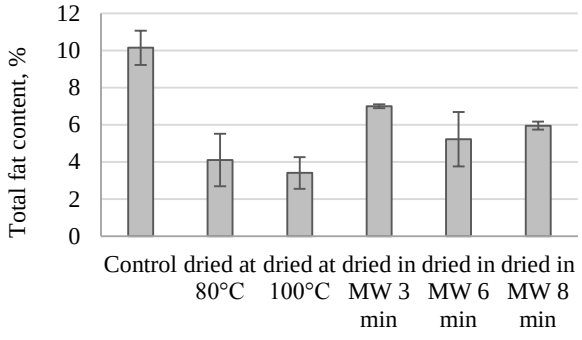

Figure 2. The total fat content of French fries pre-treated with different methods

The lowest total fat content was detected in samples dried in conventional dryer: in average 4.10% in samples dried at 80 °C, and 3.40% in samples dried at 100 °C. The samples dried in microwave-vacuum dryer contained higher amount of oil than conventionally dried samples. The effect of pre-drying to the oil content of French fries was found also by some other authors (Krokida et al., 2001). According to the results of these authors pre-drying pre-treatment had a significant effect on oil uptake and moisture loss, as well as structural properties of French fries. The use of air drying caused decreasing of the oil and moisture contents of French fries, while the porosity increased.

The colour L a* b* of different French fries samples*

Colour is another important factor in the qualified processing of French fries. The brown colour of potato chips is formed by a reaction between reducing sugars and amino acids. Brown colour of potato chips is formed when the moisture content decreases to below 6–12% (Mai Tran et al., 2006). Similarly the brown colour in French fries starts to form after rapid decreasing of moisture content in outside layers of potato strips. There were no significant differences found between samples in colour value a* ($p=0.057$), and value b* ($p=0.338$) but the brightness (L* value) significantly differed between samples ($p<0.001$) (Fig. 3).

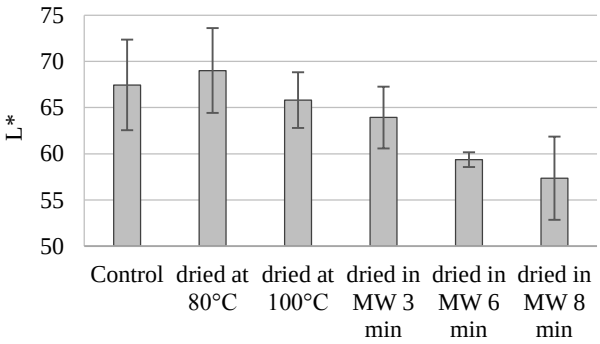

Figure 3. The brightness (L* value) of French fries pre-treated with different methods

The brightest was the sample dried at 80 °C temperature in conventional dryer (L*=69.01) but the darkest was the sample dried in microwave-vacuum dryer 8 min (L*=57.37).

In comparison with control sample the lowest total colour difference ΔE was observed also to sample dried at 80 °C temperature in conventional dryer but the highest colour difference ΔE was to sample dried in microwave-vacuum dryer 8 min (Fig. 4). Drying in conventional dryer does not change the colour of French fries while after drying in microwave-vacuum dryer the colour becomes darker. It could be explained with faster decreasing of moisture from the inside cells of potato strips. The authors Song et al., 2007 in the experiments with vacuum-fried potato chips with microwave-vacuum pre-drying also found that the vacuum-microwave pre-drying had a negative effect on colour of potato chips, which decreased the L* value of potato chips and increased Hunter a* and b* values.

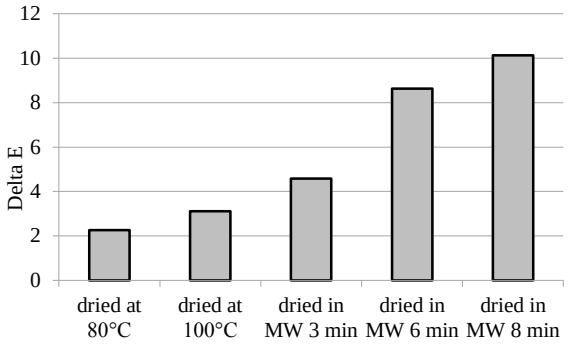

Figure 4. The total colour difference of French fries pre-treated with different methods

The moisture content

The moisture content of all French fries samples was similar, and there were no significant differences (p=0.21) found between samples pre-treated with various methods. But still there was tendency that samples dried in microwave-vacuum dryer for longer time contained lower moisture content than control sample.

The ascorbic acid content

The ascorbic acid content significantly differed between French fries samples (p=0.007). The highest ascorbic acid content was detected in sample dried in microwave-vacuum dryer 6 min. It was similar to control sample (in average 50.7 mg 100 g^{-1} of dry weight). The higher ascorbic acid content in microwave-vacuum dried potato products was detected also by other authors (Khraisheh, 2004). Our investigations with different fruit and vegetables showed higher retention of ascorbic acid after microwave-vacuum drying process compared to conventional air drying, too (Galoburda et al., 2012; Sne, Kampuse, 2011; Dorofejeva et al., 2011). The sample with the lowest ascorbic acid content was French fries dried in conventional dryer at 100 °C (in average 38.09 mg 100 g^{-1} of dry weight) (Fig. 5).

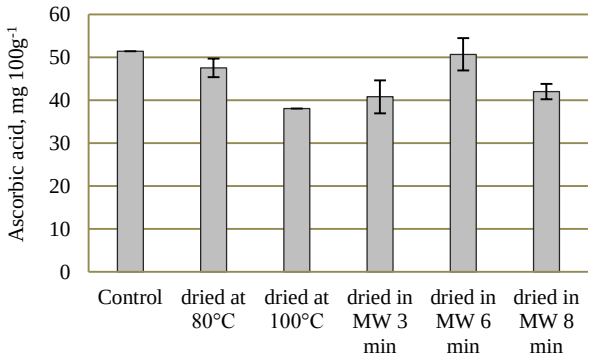

Figure 5. The ascorbic acid content of French fries pre-treated with different methods

With lowering the drying temperature in conventional dryer it was possible to save higher amounts of ascorbic acid – the French fries dried at 80 °C contained in average 47.56 mg 100 g^{-1} of dry weight ascorbic acid. The influence of temperature to the content of ascorbic acid in conventionally dried cranberries was proved also by investigations of Dorofejeva et al. (2010) where ascorbic acid content in cranberries rapidly decreased by rising temperature. But in experiments with red pepper scientists found that both vitamin C content and the total phenolic content decreased as air-drying temperature decreased (Vega-Gálvez et al., 2009). It means that processes of vitamin C degradation during drying can be influenced by the type of product and many other factors.

Conclusions

The drying in conventional dryer provided better quality parameters in the end product than drying in microwave-vacuum dryer. The lowest total fat content in French fries can be reached by drying in conventional dryer at 100 °C, but the lowest colour difference ΔE and the most similar cutting force to the control sample can be provided by drying in conventional dryer at 80 °C temperature.

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References

1. Aguilar C.N., Anzaldúa-Morales A., Talamás R., Gastélum G. (1997) Low-temperature blanch improves textural quality of French fries. *Journal of Food Science*, No. 62, p. 568–571.
2. Blumenthal M.M. (1991) A new look at the chemistry and physics of deep fat frying. *Food Technology*, No. 45, p. 68–71.
3. Blumenthal M.M., Stier R.F. (1991) Optimisation of deep fat frying operations. A review. *Trends in Food Science and Technology*, No. 2, p. 144–148.
4. Bunger A., Moyano P., Rioseco V. (2003) NaCl soaking treatment for improving the quality of French-fried potatoes. *Food Research International*, No. 36, p. 161–166.
5. Coultate T. P. (2002) *Food: the chemistry of its components*. Cambridge, UK: RSC Paperbacks, p. 213–217.
6. Dorofejeva K., Rakcejeva T., Galoburda R., Dukalska L., Kviesis J. (2011) Vitamin C content in Latvian cranberries dried in convective and microwave vacuum driers. **In**: *Proceedings of 11th International Congress on Engineering and Food, Food Process Engineering in a Changing World*, Athens, Greece. Vol. 2, p. 1199–1200.
7. Dorofejeva K., Rakcejeva T., Skudra L., Dimins F., Kviesis J. (2010) Changes in Physically-Chemical and Microbiological Parameters of Latvian Wild Cranberries

During Convective Drying. **In:** *Proceedings Annual 16th International Scientific Conference Research for Rural Development*, Jelgava, Latvia, p. 132-137.
8. Galoburda R., Kruma Z., Ruse K. (2012) Effect of Pretreatment Method on the Content of Phenolic Compounds, Vitamin C and Antioxidant Activity of Dried Dill. *World Academy of Science, Engineering and Technology*, No. 64, p. 1075-1079.
9. Jansons E. (2006) *Analītiskās ķīmijas teorētiskie pamati (Basics in Analytical Chemistry*), Rīga: LU Akadēmiskais apgāds, 307 p.
10. Khraisheh M.A.M., McMinn W.A.M, Magee T.R.A. (2004) Quality and structural changes in starchy foods during microwave and convective drying. *Food Research International*, No. 37, Iss. 5, p. 497–503.
11. Krokid M.K., Oreopoulou V, Maroulis Z.B., Marinos-Kouris D. (2001) Effect of pre-drying on quality of french fries. *Journal of Food Engineering*, No. 49, Iss. 4, p. 347–354.
12. Lamberg I., Hallstrom B., Olsson H. (1990) Fat uptake in a potato drying/frying process. *Lebesmittel Wissenschaft und Technologie*, No. 23, p. 295–300.
13. Mai Tran T.T., Dong C. X., Southern C. (2006) Reducing oil content of fried potato crisps considerably using a 'sweet' pre-treatment technique. *Elsevier Ltd*. All rights reserved.doi:10.1016/j.jfoodeng.2006.06.031.
14. Mehta U., Swinburn B. (2001) A review of factors affecting fat absorption in hot chips. *Critical Reviews in Food Science and Nutrition*, No.41, p.133–154.
15. Moreira R.G., Castell-Perez M.E., Barrufet M.A. (1999) *Deep-fat frying: fundamentals and applications.* Aspen Publishers, Gaithensburg, MA, p.6, 98, 179, 202.
16. Pinthus E.J., Weinberg P., Saguy I.S. (1993) Criterion for oil uptake during deep-fat frying. *Journal of Food Science*, No. 58, p. 204.
17. Sne E., Kampuse S. (2011) Bioactive compounds in different highbush blueberry cultivars grown in Latvia. In*: Food and Nutrition / Toit ja toitumine XVIII. Book of Proceedings of the 5th Baltic Conference on food science and technology FOODBALT-2010*. Tallin University of Technology, p. 70–75.
18. Song X., Zhan M., Mujumdar A.S. (2007) Effect of Vacuum-Microwave Predrying on Quality of Vacuum-Fried Potato Chips. *Drying Technology: An International Journal*, No. 25, Iss. 12, p. 2021–2026.
19. Ufheil G., Escher F. (1996) Dynamics of oil uptake during deep-fat frying of potato slices. *Lebensmittel Wissenschaft und Technologie*, 29: 640-643.
20. *USDA National Nutrient Database for Standard Reference*. Release 22. [accessed on 23.03.2014.]. Available: http://www.nal.usda.gov/fnic/foodcomp/search/.
21. Vega-Gálvez A., Di Scala K., Rodríguez K., Lemus-Mondaca R., Miranda M., López J., Perez-Won M. (2009) Effect of air-drying temperature on physico-chemical properties, antioxidant capacity, colour and total phenolic content of red pepper (*Capsicum annuum* L. var. Hungarian). *Food Chemistry*, No. 117, Iss. 4, p. 647–653.

PACKAGING MATERIAL AND STORAGE-INDUCED QUALITY CHANGES IN FLEXIBLE RETORT POUCH POTATOES' PRODUCE

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Abstract

Experiments have been carried out at the Latvia University of Agriculture Department of Food Technology. The aim of this work was to apprize the quality changes of potato produce in dry butter and mushroom dressing which was thermally treated in vacuum packed soft retort pouches and stored for 12 months at ambient temperature +18±2 °C. A retort pouch is a heat-resistant bag made of laminated plastic films or foil. It is then heat-sealed and sterilized by pressure cooking in a retort. Two laminated polymers with barrier properties were used: transparent polyamide/polyethylene (PA/PE) and lightproof polyethylene terephthalate/aluminium/ polyamide/polypropylene (PET/ALU/PA/PP). Samples of potato products in packaging were analysed immediately after retort thermal treatment and after 1, 2, 3, 4, 6, 8, 10 and 12 months of storage. The quality changes of potato produce during storage was characterized by measuring such parameters as structure, pH, colour and microbiological parameters. Data obtained emphasize the importance of selected laminate PET/ALU/PA/PP with aluminium layer preventing light transparency which could provide stable quality of ready to eat potato produce thermally treated in retort pouch for long-duration up to 12 months. The aim of this work was to apprize the quality changes of potato produce in dry butter and mushroom dressing which was thermally treated in vacuum packed soft retort pouches and stored for 12 months at ambient temperature +18±2 °C.

Keywords: retort pouch, quality, storage, potatoes.

Introduction

Nowadays, potatoes are popular for human consumption around the world and in more than 100 countries potatoes are staple food for many (Singh, Kaur, 2009). Raw potatoes are difficult to digest, therefore several types of preparation, such as cooking, baking, braising, grilling, and microwave processing, are used to improve the structure of potatoes and make them more accessible to the diet (Carini et al., 2013). The main factors that affect potato quality among consumers are potato size, colour and texture after cooking. When purchasing processed potato products the focus is on the appearance, taste, texture and nutritional value of the product (Suryawanshi, 2008). Today, food market offers a wide range of potato varieties; most of them are classified according to organoleptic characteristics, quality and end-use (Lopez-Vizcón, Ortega, 2012). The demand for vacuum-packed, healthy, cut potatoes is ever increasing (Lante, Zocca, 2010). With increasing consumer demand for convenience and high quality products, potato processing companies constantly look for new and innovative ways to use potatoes effectively (Abu-Ghannam, Crowley, 2006). Popular processed potato products include potato crisps, French fries (chips), potato powder, potato cubes, slices and potato starch (Suryawanshi, 2008). During the recent years, development of new food products has been receiving increased attention. It is mainly because the demand for safe food with extended shelf life has been increasing in both consumer and merchant groups (Valceschini, 2006). Heat treatment is a common food preservation method. Based on the intensity of the treatment, it can be divided into two main groups' – pasteurization and sterilization. Each of these treatments has its own specific features (intensity, suitability for the type of product), which determined the shelf life of food. Heat treatment can also affect the quality of food. The optimal treatment regime needs to be selected for each type of food in order to minimize unwanted quality losses and increase the desired quality, while ensuring microbiological safety (Lammens et al., 2013). Sterilization is one of the most important processes to maintain packaged food quality (Marra, Romano, 2003). Currently, treatment of products in packaging is one of the leading processing technologies that impart long-term storage at room temperatures (Ito et al., 2014).

Using this method, the product can maintain most of its original texture, as well as minimal loss of nutrients can be ensured, in addition to obtaining a longer shelf life. This technology provides a number of advantages; it allows to effectively managing the heat from the steam or water to the product through the packaging, preventing recontamination of the product. It also excludes off-flavour formation and oxidation of the product, preventing moisture and nutrient losses during evaporation and loss of volatile compounds during cooking, as well as preventing development of aerobic microorganisms in the product. Heat treatment of vegetables and fruits in packing is recognized as an excellent way to maintain their structure without losing such valuable nutrients as vitamins and minerals, which can significantly decrease when vegetables and fruits are boiled in water. This high nutrient retention contributes to the persistence of flavour and colour in such vegetables as turnips, swedes, carrots etc. (Sansone et al., 2012).

Packaging is essential for products intended for long-term storage because storage time and temperature significantly affect the appearance, aroma, taste and structure (Clark et al., 2002). Thermal treatment of food products in soft packaging is becoming more widely used and it serves as an alternative to metal cans. Packaging bags intended for heat treatment usually consist of up to three layers: polypropylene (PP), aluminium foil and polyethylene

terephthalate (PET). Aluminium foil and polyethylene terephthalate are primarily used to provide the desired barrier properties. Polypropylene provides an excellent sealing of packaging. Polypropylene layer protects aluminium foil from the inside and promotes overall strength (Byun et al., 2010, Srinivasa Gopal et al., 2001).

Suitable and qualitative packaging has a major role in maintaining the quality of the product and also in the convenience of customer use (Ahvenainen, 2003). The quality of products must be maintained during storage; therefore food companies need to constantly work on improving recipes, readjusting packaging materials and technology in order to reduce the initial product quality changes during storage. In turn, scientists need to look for more innovative ideas to provide entrepreneur, producer, and customer satisfaction (Puligundla et al., 2012).

Materials and Methods

Research was carried out during the period from November 2011 to January 2014. Product samples were prepared at vegetable processing company in Latvia. Physical and chemical analysis (colour, hardness, pH) were performed at the Packaging material property testing laboratory at the Department of Food Technology (Latvia University of Agriculture). Microbiological and nutrition analyses and corresponding to them expiry date were defined at the laboratory of Food and Environmental Investigations at Institute of Food Safety, Animal Health and Environment *BIOR***.**

In total, three different types of samples were prepared: control sample of cut potatoes without dressing, and two samples of potatoes with butter and mushroom *(Agaricus bisporus)* dressing. Raw potatoes were pealed and cut into equal-sized 8 mm wide slices. After dressing addition, potato products were dosed in soft retort pouches made out of two different packaging materials, portion mass in each pouch was 500±0.05 g. Two laminated polymers with barrier properties were used: transparent polyamide/polyethylene (PA/PE) and lightproof polyethyleneterephtalate/aluminium/polyamide/polypropylene (PET/ALU/PA/PP) films. Size of each pouch was 200×250 mm, thickness of film 80 µm. Potato products were placed in soft retort pouches, vacuum-sealed using a two-chamber vacuum packaging machine Falcon 2-70; hermetic sealing mode – vacuum, 20 MPa, sealing time 3.8 s for PE/PA pouches and 5.3 s for PET/ALU/PA/PP pouches. A retort pouch is a heat-resistant bag made of laminated plastic films or foil. It is then heat-sealed and sterilized by pressure cooking in a retort "Lagarde", sterilizing temperature +121±2 °C, cooling temperature +20±2 °C. Samples were stored for 12 months at ambient temperature +18±2 °C. Physical and chemical properties as well as microbiological testing of products were carried out on the production day (day 0) and after 1, 2, 3, 4, 6, 8, 10 and 12 months of storage. Samples were encrypted with letters and numbers (Fig. 1), samples in PE/PA packaging were marked as P1 (control sample), P2 (sample with butter dressing) and P3 (sample with mushroom dressing), while samples in PET/ALU/PA/PP packaging were marked as A1 (control sample), A2 (sample with butter dressing) and A3 (sample with mushroom dressing).

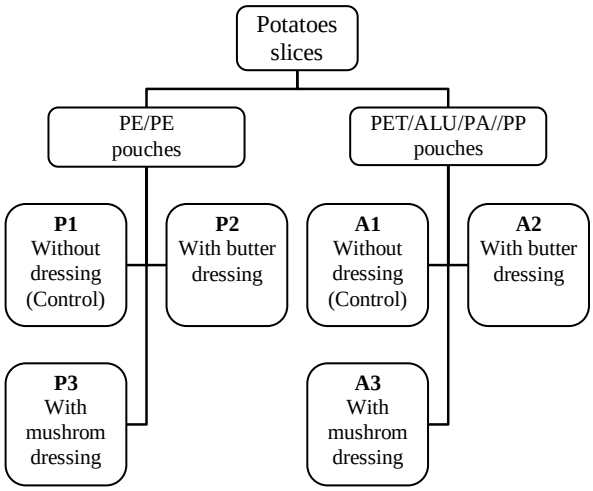

Figure 1. Research structure

Colour analysis

Colour of potato product samples were determined using *Colour Tec PCM / PSM* with CIE L* a* b* colour system. Measurements were completed in tenfold repetition for each sample for more precise calculations of the mean value and standard deviation. Measurements were recorded using data program *Colour Soft QCW*. Total colour difference (ΔE*) of potatoes' products between initial value and after storage was calculated using the following equation 1: (MacDougall, 2002).

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

where ΔE* – total colour difference of the product during storage, L* – colour intensity value at the final product storage day, L_0* – colour intensity value at day 0, a* – value of colour component green – red at the final product storage day, a_0* – value of colour component green – red at day 0, b* – value of colour component blue – yellow at the final product storage day, b_0* – value of colour component blue – yellow at day 0.

Determination of nutritional value

Nutritional value of developed products was determined using the following methods: protein content (BS EN 12135:2001 standard), carbohydrates were determined by difference, fat content (GOST 8756.21-89 p.2 method), crude fibre content (ISO 5498:1981), ash content (GOST 25555.4-91 p.2 method), moisture content (GOST 28561-90 p.2

method). Energy value was calculated according to the results of analysis above. Nutritional values testing of products were carried out on the production day.

Determination of hardness

The hardness of potato products was determined with *TA.XT. Plus Texture Analyser, Blade set with knife (Stable Microsystems, UK*). Data collection and analysis was carried out with program *Texture EXPONENT 32*. Blade movement speed during the test mode was 1 mm s^{-1} (forwards) and 10 mm s^{-1} (backwards). The force at which data collecting started was 0.04903 N. Measurements were completed tenfold for each sample for more precise calculations of the mean value and standard deviation.

Determination of pH

pH of potato products was determined using pH-meter *JENWAY 3510* with electrodes *JENWAY 3 mol / KCl* (standard method ISO 1132:2001).

Microbiological analysis

Total plate count (TPC) was determined according to the standard LVS EN ISO 4833:2003. To determine sulphite-reducing *Clostridia* in the product (*Clostridium* spp.), GOST 29185-91*, standard method was used.

Data analysis

The obtained data were processed using SPSS 16.0 software package; arithmetic mean value and standard deviation were calculated. The results were analysed using univariate analysis of variance (ANOVA). Factors were rated as significant if p-value< $\alpha_{0.05}$. For the interpretation of the results it was assumed that α=0.05 with 95% confidence.

Results and Discussion

Nutrition is an important factor for consumers when making a food choice. More attention is paid to the composition of ingredients and nutritional value of products because the number of food-related diseases is ever increasing (Patras et al., 2009). Therefore, nutritional value of potato products was determined and the results are given in Table 1.

The results show the energy value of control sample – 301 kJ 100 g^{-1}, which mainly constitutes of potato carbohydrates (15.7%), followed by protein (1.8%) and a small proportion of total fat (0.1%).

Potatoes contain about 20% of dry matter; the rest is water (Ek et al., 2012). Total water content in the control sample is 81%. The addition of dressing mixtures to potatoes changes and affects their nutritional value. Nutrition changes are mainly related to the dressing mixture composition. Energy value of potatoes with butter dressing is 346 kJ 100 g^{-1}, which is by 45 kJ more than of the control sample. The sample with butter dressing contains 16.8% carbohydrates, which is 1.1% more than in the control sample. Potatoes with butter dressing contain about 0.2% more protein and 0.9% more fats than the control sample; moisture content is for 2% lower.

Energy value of potatoes with mushroom dressing is 341 kJ 100 g^{-1}. This sample contains fewer kJ than potatoes with butter dressing. Total carbohydrate content in potatoes with mushroom dressing is 17.7%, which is by 2.0% and 0.9% more than in the control sample and potatoes with butter dressing, respectively. Protein content in potatoes with mushroom dressing is 2.4%, which is by 0.6% higher and 0.4% lower than in the control sample and potatoes with butter dressing, respectively. Moisture content in potatoes with mushroom dressing is 78.2%, which is by 3.2% and 0.8% lower than in the control sample and potatoes with butter dressing, respectively. The results show that addition of dry dressing mixtures does not significantly change energy value of potato products.

Table 1

Nutritional value of potato products

Parameters	Content per 100 g of product		
	control sample	sample with butter dressing	sample with mushroom dressing
Energy value	71 kcal / 301 kJ	82 kcal / 346 kJ	80 kcal / 341 kJ
Protein, %	1.8	2.0	2.4
Carbohydrates, %	15.7	16.8	17.7
Fat, %	0.1	1.0	0.3
Crude fibre,%	0.6	0.6	0.7
Moisture, %	81.0	79.0	78.2
Ash content, %	0.8	1.2	1.4

Colour is one of the main factors which the consumer perceives as the quality indicator of the product (Suryawanshi, 2008). Generally, the colour may be affected by genetic factors and technological parameters of production process. Technological parameters include packaging material, light exposure during storage and the type of preparation (Murcia et al., 2003). Total colour changes of potato products during the storage are characterized by the total colour difference ΔE^* which is calculated from measured L*, a* and b* values.

There are significant disparity ($p<0.05$) between the total colour difference ΔE^* of potato products arising during storage in studied PE/PA pouches ($p<0.05$) and PET/ALU/PA/PP packaging (Fig. 2), which can be explained by light transmission of packaging material. After the comparison of the samples packaged and stored in both packaging materials for 12 months, it can be concluded that PET/ALU/PA/PP packaging ensures a significantly ($p<0.05$) lower product colour changes than PE/PA packaging.

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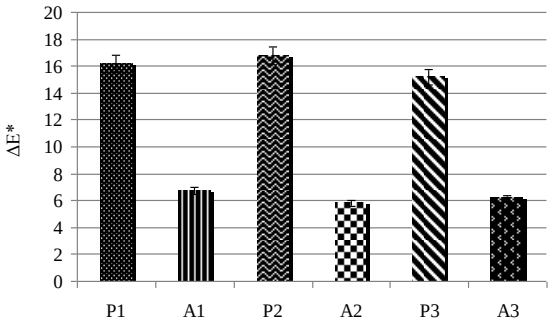

Figure 2. The total colour difference ΔE* of potato products after 12 months of storage

P1 – control sample in PE/PA packaging, A1 – control sample in PET/ALU/PA/PP packaging, P2 – potatoes with butter dressing in PE/PA packaging, A2 – potatoes with butter dressing in PET/ALU/PA/PP packaging, P3 – potatoes with mushroom dressing in PE/PA packaging, A3 – potatoes with mushroom dressing in PET/ALU/PA/PP packaging

An important quality indicator of thermally-treated potatoes in packaging is structure which indicates the hardness of the product; therefore changes in potato hardness after thermal treatment are mainly due to the changes in the structure and chemical composition (Abu- Ghannam, Crowley, 2006).

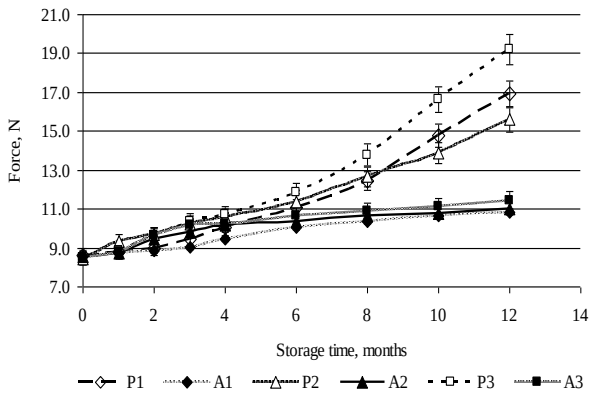

Figure 3. Changes in hardness of potato products during storage

P1 – control sample in PE/PA packaging, A1 – control sample in PET/ALU/PA/PP packaging, P2 – potatoes with butter dressing in PE/PA packaging, A2 – potatoes with butter dressing in PET/ALU/PA/PP packaging, P3 – potatoes with mushroom dressing in PE/PA packaging, A3 – potatoes with mushroom dressing in PET/ALU/PA/PP packaging

In order to evaluate potato hardness during 12 month storage period, samples of potato products in both packaging materials, which were stored at a constant temperature +18±2 °C, were compared (Fig. 3).

During the six month period of storage, hardness of potato products has risen evenly in both packaging materials from 8.5 to 10.8–11.8 N; during the first half of storage (6 months) the studied packaging materials evenly affected the changes in potato hardness. However, a significant increase in hardness ($p<0.05$) was observed after 12 months of storage in PE/PA packaged potato products from 11.8 to 15.6–19.2 N. These significant changes occurred because PE/PA packaging has higher water vapour permeability; hence it cannot maintain initial quality of potato products during storage. Changes in structure of heat-treated products in packaging are usually caused by water loss during storage (Alonso et al., 2013).

pH is an important factor as it determines which microorganisms can proliferate in the product (Suryawanshi, 2008). Initial pH for potatoes without dressing was 5.84, for potato products with dressing – 5.75 (Fig. 4).

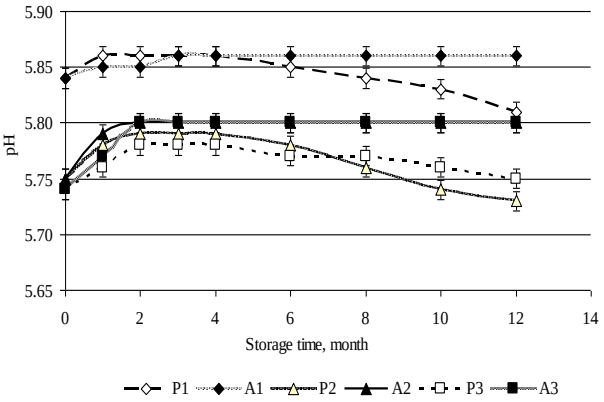

Figure 4. Changes in pH of potato products during storage

P1 – control sample in PE/PA packaging, A1 – control sample in PET/ALU/PA/PP packaging, P2 – potatoes with butter dressing in PE/PA packaging, A2 – potatoes with butter dressing in PET/ALU/PA/PP packaging, P3 – potatoes with mushroom dressing in PE/PA packaging, A3 – potatoes with mushroom dressing in PET/ALU/PA/PP packaging

Stability in pH during storage of heat-treated products in packaging is an important quality indicator. Potato products in PE/PA packaging presented slight decrease in pH during storage; pH in the control sample reduced to 5.81, in the sample with butter dressing to 5.73 and in the sample with mushroom dressing till 5.75, these changes were not significant ($p>0.05$).

Changes in pH of potato products in PET/ALU/PA/PP packaging were not significant ($p>0.05$) as well, while pH in the control sample rose to 5.86 and in samples with dressing pH rose or dropped to a small extent.

It can be concluded that both PE/PA packaging and PET/ALU/PA/PP packaging are able to provide practically constant pH during storage. This is an important indicator when assessing the possible development of microorganisms in thermally treated products in packing during storage.

Consumer demand for food depends on the quality of the product, convenience of use, and safety of the product (Chen et al., 2012). One of the most common types of food contamination is biological

contamination. It is relevant to emphasize the importance microbiological contamination has on public health, because direct contact and other transfer factors help microorganisms to get and proliferate in a variety of foodstuffs and the digestive tract of animals and humans. Microbiological contamination of food is one of the most common types of food contamination not only in countries where there are problems with provision of food hygiene requirements, but also in the European Union countries and the United States where a high level of consumer protection is ensured (Food and Veterinary Service Research Centre, 2004).

Total plate count and the presence of sulphite-reducing Clostridia were determined immediately after thermal treatment and during 12 month storage period. The total plate count (number of microorganisms) in potato products in both types of packaging materials during storage did not exceed 10 CFU g^{-1}. It clearly demonstrates that the selected treatment regime and the choice of packaging material can ensure product safety. Potatoes and potato products are recognized as one of the main foods that may cause a significant risk of becoming infected with *Clostridium* species bacteria (Barker et al., 2005). *Clostridium* species are anaerobic spore-producing bacteria, so it is necessary to ensure effective heat treatment process to eliminate the risk of infection. Sulphite-reducing *Clostridia* were not present in any of the samples during storage which indicates the effectiveness of the technology used and the ability to ensure the security and safety of the product during storage.

Microbiological indicators are one of the main criteria that determine shelf life of the product and its safe use in the diet. According to the results of the total plate count, absence of sulphite-reducing *Clostridia*, as well as such quality characteristics as colour, hardness and pH of potato products, the optimal shelf life for thermally treated potato products in PE/PA packing stored at the temperature of +18±2 °C could be three months but thermally treated potato products in PET/ALU/PA/PP pouches can be used up to 12 months.

Conclusions

Changes in the total colour difference ΔE* of potato products during 12 month storage packed in PET/ALU/PA/PP are not significant ($p>0.05$), while significant colour changes have been observed in potato products packed in PE/PA ($p<0.05$).

A steady increase in potato hardness from 8.5 N to 10.8–11.8 N was observed during the six month storage in both packaging materials. After 12 month storage, a significant ($p<0.05$) increase in hardness was observed in potato products stored in PE/PA packages (from 11.8 N to 15.6–19.2 N).

The selected thermal treatment in vacuum packed soft retort pouches and both types of packaging materials provide microbiological safety of potato products during 12 month storage at +18±2 °C temperature, but only laminated lightproof PET/ALU/PA/PP film provides constant product quality during storage. Potato products in PET/ALU/PA/PP packaging are safe for human consumption up to 12 months of storage.

References

1. Abu-Ghannam N., Crowley H. (2006) The effect of low temperature blanching on the texture of whole processed new potatoes. *Journal of Food Engineering*, Vol. 74, p. 335–344.
2. Ahvendinen R. (2003) Novel *Food Packaging Techniques.* Cambridge England: CRC Press Woodhead publishing limited. 590 p.
3. Alonso A.A., Méndez A.A., Balsa-Canto E., García M., Molina J., Vilas C., Villafín M. (2013) Real time optimization for quality control of batch thermal sterilization of prepackaged food. *Food Control*, Vol. 32, p. 392–403.
4. Barker G.C., Malakar P.K., Torre M.D., Stecchini M.L., Peck M.W. (2005) Probabilistic representation of the exposure of consumers to *Clostridium botulinum* neurotoxin in a minimally processed potato product. *International Journal of Food Microbiology*, Vol. 100, p. 345–357.
5. Byun Y., Hong S.I., Mangalassary S., Bae H.J., Cooksey K., Park H.J., Whiteside S. (2010) The performance of organic and inorganic coated retort pouch materials on the shelf life of ready-to-eat rice products. *LWT – Food Science and Technology*, Vol. 43, p. 862–866.
6. Carini E., Curti E., Littardi P., Luzzini M., Vittadini E. (2013) Water Dynamics of ready to eat shelf stabule pasta meals during storage. *Innovative Food Science & Emerging Technologies*, Vol. 17, p. 163–168.
7. Chen Q., Anders S., An H. (2013) Measuring Consumer Resistance to New food technology. *Food Quality and Preference*, Vol. 28, p. 419–428.
8. Clark S., Warner H., Rodríguez J.J., Olivas G.I., Sepúlveda D., Bruins R., Barbosa-Cánovas G.V. (2002) Residual gas and storage conditions affect sensory quality of diced pears in flexible retortable pouches. *Food Quality and Preference*, Vol. 13, p. 153–162.
9. Ek K. L., Brand-Miller J., Copeland L. (2012) Glycemic effect of potatoes. *Food Chemistry*, No.133, p. 1230–1240.
10. Ito R., Fukuoka M., Hamada-Sato N. (2014) Innovative food processing technology using ohmic heating and aseptic packaging for meat. *Meat Science*, Vol. 96, p. 675–681.
11. Lante A., Zocca F. (2010) Effect of β-cyclodextrin addition on quality of precooked vacuum packed potatoes. *LWT – Food Science and technology*, Vol. 43, p. 409–414.
12. Lemmens L., Colle I., Knockaert G., Buggenhout S.V., Loey A.V., Hendrickx M. (2013) Influence of pilot scale in pack pasteurization and sterilization treatments on nutritional and textural characteristics of carrot pieces. *Food Research International*, Vol. 50, p. 526–533.
13. Lopez-Vizcón C., Ortega F. (2012) Detection of mislabeling in the fresh potato retail market employing microsatellite markers. *Food Control*, Vol. 26, p. 575–579.
14. Marra F., Romano V. (2003) A mathematical model to study the influence of wireless temperature sensor during assessment of canned food sterilization. *Journal of Food Engineering*, Vol. 59, p. 245–252.

15. Murcia M.A., Martínez-Tomé M., Nicolás M.C., Vera A.M. (2003) Extending the shelf-life and proximate compositions stability of ready to eat foods in vacuum or modified atmosphere packaging. *Food Microbiology*, Vol. 20, p. 671–679.
16. Patras A., Tiwari B.K., Brunton N.P., Butler F. (2009) Modelling the effect of different sterilisation treatments on antioxidants activity and colour of carrot slices during storage. *Food Chemistry*, Vol. 114, p. 484–491.
17. Pārtikas un veterinārā dienesta Pētniec. centrs, Pārtikas piesārņojums. Rīga: 2004, 64 lpp.
18. Puligundla P., Jung J., Ko S. (2012) Carbon dioxide sensors for intelligent food packaging applications. *Food Control*, Vol. 25, p. 328–333.
19. Sansone L., Aldi A., Musto E.D., Amendola E., Mensitieri G. (2012) Assesing the suitability of polylactic acid flexible films for high pressure pasteurization and sterilization of packaged foodstuff. *Journal of Food Engineering*, Vol. 111, p. 34–45.
20. Singh J., Kaur L. (2009) *Advances in Potato Chemistry and Technology*. Academic Press, Chapter 7, p. 163–219.
21. Srinivasa Gopal T.K., Vijayan P.K, Balachandran K.K, Madhavan P., Iyer T.S.G (2001) Traditional Kerala style fish curry in indigenous retort pouch. *Food Control*, Vol. 12, p. 523–527.
22. Suryawanshi M.V. (2008) Minimal processing and packaging studies in potato. Departament of horticulture. College of Agriculture, Dharwad University of Agricultural Sciences Dharwad, p. 82.
23. Valceschini E. (2006) Poultry meat trends and consumer attitudes, *Report of National Institute of Agronomic Research (France)*, p. 1–10.

PHYTASE ACTIVE YEASTS ISOLATED FROM BAKERY SOURDOUGHS

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Abstract

Nowadays, consumption of whole-grain bread is gaining popularity across the world due to its many health promoting effects. Despite that whole grains contain 20–70% of the daily requirements of the minerals, their absorption in human gut are very low due to antinutritional phytate. To increase the bioavailability of minerals, enzymatic degradation of phytate is needed. The aim of this study was to isolate and identify phytase active yeasts from sourdoughs. The use of selected microorganisms with high phytase activity could find use as starter cultures to improve mineral bioavailability in whole-grain bread fermented in a short time as alternative to sourdough bread. In addition, purification and characterization of phytase produced by *Saccharomyces cerevisiae* was done. The screening of phytase-positive strains was carried out at temperature of 30 °C and pH 5.5, conditions optimal for leavening of bread dough. To investigate whether the yeasts produce phytases selective defined minimal medium was developed. Phytase activity was determined colorimetrically by measuring the inorganic phosphate released by enzyme. Specific extracellular phytase activities of yeasts were at least 20-fold higher than the intracellular activities. The highest activities were observed in two *Saccharomyces cerevisiae* isolates, i.e. L1.12 (10.6 U $(10^{10}$ CFU$)^{-1}$) and L6.06 (8.2 U $(10^{10}$ CFU$)^{-1}$). The purified phytase produced by *S. cerevisiae* was most active at pH 4.0 and 35°C. This phytase is quite stable at pH range from 3.5 to 5.5 and temperature range from 25 °C to 40 °C.

Keywords: phytase, sourdough, yeast, *Saccharomyces cerevisiae.*

Introduction

Major epidemiological studies have shown that consumption of whole-grain foods is protective against the development of several chronic diseases. Whole-grains have been found to help regulation of blood glucose and protect against type 2 diabetes (de Munter et al., 2007) as well as reduce the risk of obesity (van de Vijver et al., 2009). The importance of whole-grain foods intake on blood glucose and weight regulation is considered as a result of the content of dietary fibre and magnesium in whole grains. Other studies concern the contribution of dietary fibre, folate and, to a certain extent, vitamin B_6 and magnesium from whole-grain foods as protective components against cardiovascular diseases and certain type of cancer (Slavin, 2004).

Besides dietary fibre, the whole-grain foods are also important source of minerals. It was shown that 100 g of whole grains contain 20–70% of the daily requirements of the minerals, such as iron, zinc, magnesium, potassium, phosphorus and manganese (Cordain, 1999). However, most of the minerals in cereals are complexly bound to phytic acid as phytate, consequently reducing their bioavailability (Kumar et al., 2010).

Iron deficiency is the most common nutrient deficiency, widespread both in western and developing countries (WHO, 2002). Zinc represents the other nutritionally significant mineral that has been associated with phytate inhibition effect.

To increase the bioavailability of minerals from a given food, enzymatic degradation of phytate and its dephosphorylated isomer IP5 (inositol pentaphosphate) is needed (Sandberg et al., 1999). Efficient reduction of phytate can be achieved by enzymatic degradation during food processing, either by increasing the activity of endogenous phytase, or by addition of phytase-active yeast, lactic acid bacteria, or other microorganisms.

Cereals exhibit high phytase activities with values ranging from approx. 900 to 2900 U kg^{-1} dry matter in wheat grain, and from 4100 to 6100 U kg^{-1} dry matter in rye grain (Eeckhout, Depaepe, 1994; Greiner, Egli, 2003). However, the activities in wheat were considered insufficient to notably improve the mineral bioavailability in whole-grain wheat bread (Türk, Sandberg, 1992). Several studies were carried out on phytase activity from baker's yeast (Andlid et al., 2004; Türk, Sandberg, 1992). They showed that only marginal amount of phytate was degraded during 3 h fermentation.

From an industrial point of view, the extracellular phytase activity would be more important for bread making than the intracellular phytase activity, because cells of the yeast should be intact in dough in order to ensure a good fermentation. In this case, the intracellular phytase will not have access to phytate in the dough. So far, no high phytase-active yeasts were identified for bread industry in order to increase mineral bioavailability.

The aim of this study was therefore to isolate and identify yeasts from different commercial sourdoughs and to study their phytase activity under conditions optimal for leavening of wheat bread dough (30 °C and pH 5.5) in order to identify species and/or strains with high phytase activities that might be used in baking industry. Further, purification and characterization of high-active extracellular phytase produced by generally recognized as safe (GRAS) yeast *Saccharomyces cerevisiae* was done.

Materials and Methods

Microorganisms. Yeasts tested for phytase activity were isolated from seven Lithuanian sourdoughs, further purified and identified (Nuobariene et al., 2012). *Saccharomyces cerevisiae* L1.12 isolated from rye sourdough and identified as a phytase-positive

yeast strain was used for further phytase production and purification.

Growth test for phytase-active yeasts. To check the ability of yeasts to synthesize phytase, yeast strains were cultivated on Defined Minimal Medium (Delft) plates supplemented with phytic acid dipotassium salt (P5681, Sigma-Aldrich, Broendby, Denmark) as the only phosphorus source (Delft+Phy). As control, yeast strains were cultivated on phosphate-containing (Delft+P, positive control) and phosphate-free (Delft-P, negative control) medium (Nuobariene et al., 2012). Growth tests were performed both on solid and in liquid medium.

Phytase assay. Extracellular phytase extracts were prepared according to Nuobariene et al. (2011). Phytase activities were determined as described previously (Nuobariene et al., 2012). Briefly, 10 µl of enzyme extract was added into pre-incubated 40 µl 3 mM phytic acid dipotassium salt solution, gently mixed by inverting tube and incubated at 30 °C. Samples were taken every 15 min (0, 15, 30, and 45 min) during assaying. The reaction was stopped by adding 50 µl 10% TCA (trichloroacetic acid solution). A separate enzyme blank was prepared from sodium acetate/HCl buffer mixed with enzyme extract and TCA. Substrate blank was prepared from substrate solution mixed with TCA. To determinate the content of liberated inorganic phosphate during phytase activity freshly prepared acid molybdate solution was used in colorimetric analysis.

One unit of phytase activity was defined as the amount of phytase that liberates 1 µmol inorganic phosphate per minute from 3 mM phytic acid dipotassium salt at pH 5.5 and at 30 °C.

Phytase purification. The extracellular phytase produced by *S. cerevisiae* L1.12 was purified using ultrafiltration and affinity chromatography. All purification steps were performed at 4 °C.

After cultivation, the yeast cells from the fermented broth were removed by centrifugation (5000 *g* for 10 min, 4 °C); culture supernatant with extracellular protein fractions was collected and filtered (0.2 µm Minisart filters; Bie & Berntsen, Herlev, Denmark). 100 mL of prepared extracellular protein extract, as described above, was desalted and concentrated to 40 mL using Centriprep 3K centrifugal filter device with Ultracel YM membrane (the nominal molecular weight limit 3000).

The present of glycan on the yeast phytase protein (Segueilha et al., 1992) facilitated further purification of the desalted extract. Concanavalin A (Con A) sepharose, a lectin-conjugate resin that binds high glucose and mannose groups, was used for affinity chromatography. The sample was loaded at flow rate of 0.1 mL min^{-1} onto Con A sepharose column (20 mm diameter×30 mm) equilibrated with buffer A (0.02 M Tris-HCl and 0.5 M NaCl, pH 7.2) and, afterwards, washed extensively (3 column volumes) to remove unbound proteins. Bound glycoproteins were competitively eluted with 0.25 M methyl-α-D-mannopyranoside solubilized in buffer A. Aliquots of eluent (1 mL) were applied to the column at interval of 30 min. Collected fractions (1 mL each) which show phytase activities were combined as a single fraction and assayed for phytase activity.

Protein quantification. Protein concentration was determined by measuring the absorbance of enzyme extract at 600 nm using the Bio-Rad Colorimetric Protein assay, Kit II (Bio-Rad Laboratories Inc). BSA (bovine serum albumin) was used as protein concentration standard.

Effects of pH and temperature on phytase activity and stability. The effect of pH on the phytase activity was determined by incubating the enzyme in phytic acid dipotassium solution at various pH (pH 3.0 to 7.0) using the standard assay conditions. The buffers used for reaction were 0.2 M sodium acetate / HCL (pH 3.0–6.0) or 0.2 M Tris-HCl (pH 6.0–7.0). The maximum activity was taken as 100% and percentage relative activity plotted against different pH values.

The effect of pH on phytase stability was studied by incubating the purified enzyme at 30°C for 1, 2 and 4 h at various pH values ranging from 3.0 to 7.0 followed by measuring the residual activity under standard assay conditions.

The phytase assays for determination of temperature optimum were performed as earlier outlined, except for the variation of the incubation temperature. For this purpose, phytase activity was assayed over a temperature range of 25 °C to 60 °C at 5 °C intervals. The maximum activity was taken as 100% and percentage relative activity were plotted against different temperatures.

Thermostability of the phytase was tested by incubating enzyme extract without substrate addition at a temperature range of 25 °C to 65 °C for 2 h and samples were taken every 10 min. Residual activities where then measured as indicated above, at a fixed temperature of 30 °C and pH 5.5.

Results and Discussion

Phytase-active yeasts from sourdough. The number of yeasts in all tested sourdoughs was about the same and ranged from 6.7 to 7.9 log CFU (colony forming units) g^{-1} sample. In total, 140 yeast colonies were isolated, purified and subjected to the rep-PCR analysis. Based on results from cluster analysis, isolates were grouped according to their fingerprint pattern (Nuobariene et al., 2012). For a representative number of isolates within each group; i.e. 11 isolates in total, the identification to species level were further done by sequencing of the D1/D2-region of the 26S rRNA gene. Based on results from sequencing, two yeast species isolated from seven different sourdoughs were identified, i.e. *S. cerevisiae* and *Pichia kudriavzevii* (formerly named as *Issatchenkia orientalis*, anamorph *Candida krusei*). A pure culture of *S. cerevisiae* was found in five out of seven tested sourdoughs with fermentation temperature of 30–35 °C, while *P. kudriavzevii* was the dominant

species in other two sourdoughs with fermentation temperature of 24–26 °C.

To check the ability of identified 11 yeast strains to produce phytase they were cultivated on/in solid/liquid minimal Delft medium supplemented with phytic acid dipotassium salt as the only phosphor source. All tested strains grew very well on/in Delft+Phy medium which indicates that tested strains produce phytase (Figure 1).

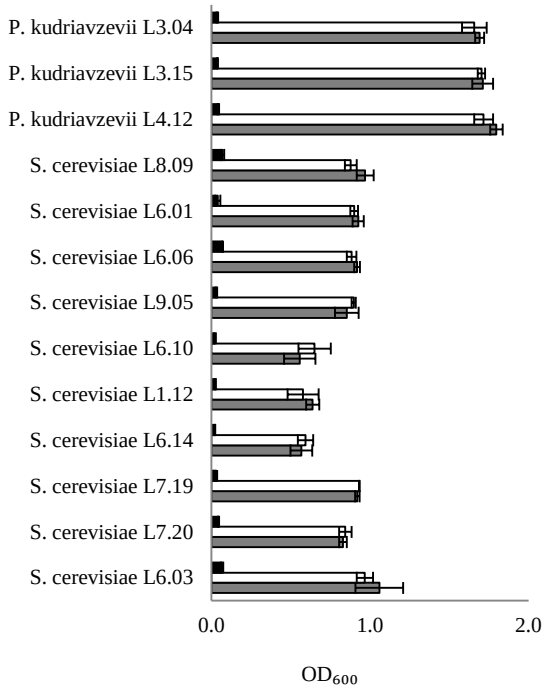

Figure 1. Optical density values of yeast cell growth at 30 °C in experimental media after 48 h incubation

Black bars-Delft-P medium, white bars – Delft+P medium, grey bars – Delft+Phy medium

However, from growth test, based on monitoring of cell growth in phytate rich environment, it is impossible to predict which yeast strains will have higher or lower activities (Nuobariene et al., 2011). Therefore, it is essential also to perform phytase activity assay on each strain.

The highest specific extracellular activity of all isolates was observed in *S. cerevisiae* L1.12 with a value of 10.6 U $(10^{10}$ CFU$)^{-1}$, followed by *S. cerevisiae* L 6.06 with a value of 8.2 U $(10^{10}$ CFU$)^{-1}$ (Figure 2). Extracellular specific activities among other *S. cerevisiae* strains differed dramatically and ranged from 0.9 to 7.3 U $(10^{10}$ CFU$)^{-1}$. The specific extracellular phytase activity values within three *P. kudriavzevii* strains varied between 1.1 and 4.6 U $(10^{10}$ CFU$)^{-1}$.

Cultivation of *S. cerevisiae* L1.12 for phytase production. The activity of phytase in response to phytic acid dipotassium salt and the growth of *S. cerevisiae* L1.12 during 72 h of cultivation are shown in Figure 3. The yeast cells reached their stationary growth phase after 42 h of cultivation. Increase in phytase activity during the exponential growth phase was very slow.

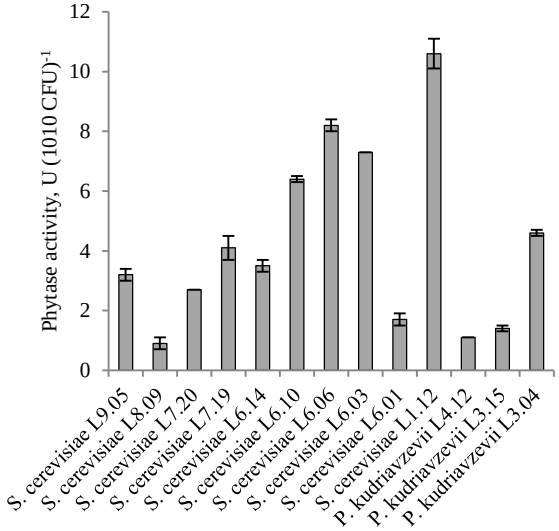

Figure 2. Specific extracellular phytase activity from yeast strains of *S. cerevisiae* and *P. kudriavzevii*

However, rapid increase in phytase activity was observed in the late exponential growth phase (36–42 h of cultivation) and was continuously increasing during early stationary growth phase. It can be seen from Figure 3 that the maximum extracellular volumetric phytase activity (0.062±0.005 U mL^{-1}) was obtained when yeast cells reached the stationary phase and remained almost constant for the next 12 h of cultivation. Afterwards, slight decrease in phytase activity was observed in late stationary growth phase. This finding is in agreement with Quan et al. (2001) findings which showed that phytase production by *Candida krusei* occurred in the late stage of exponential growth phase and that phytase activity increased gradually with increasing incubation time.

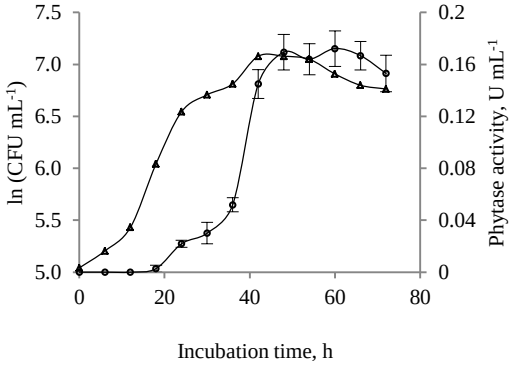

Figure 3. Phytase activity and yeast growth at different time points

Circles – phytase activity, U mL^{-1}; triangles – yeast growth, ln (CFU mL^{-1})

Phytase purification. Up to now, there have been only few reports on the yeast extracellular phytases purification and enzymatic properties. During the last decade, extracellular phytases from yeast, including the wild strain of *Schwanniomyces castellii* (Segueilha et al., 1992), marine yeast *Kodamaea ohmeri* (Li et al., 2008), and *S. cerevisiae* isolated from the mash of traditional Korean wine (In et al., 2009) have been purified to homogeneity.

However, phytase produced by yeast species, mentioned above mostly are attributed as potential for commercial application in the feed industry. Therefore, purification and characterization of *S. cerevisiae* L1.12 isolated from sourdough which may be an ideal candidate for improving mineral bioavailability in whole grain is of importance.

Table 1

Purification of the extracellular phytase from *S. cerevisiae* L1.12

Purification steps	Total activity	Total protein	Specific activity	Yield
	U	mg	U mg^{-1}	%
Culture supernatant	24.6	4.62	5.3	100
Ultrafiltration	24.5	4.12	6.0	99.7
Con-A Sepharose	0.9	0.02	42.9	3.7

The results of phytase produced by *S. cerevisiae* L1.12 purification are summarized in table 1. The first purification step, ultrafiltration, leads to an increase in purity of only 1.1-fold but recover nearly all the protein from the original extract. Second purification step, a Con-A affinity chromatography, took advantage of the presence of high glucose and mannose side chains in phytase. This resulted in separation of phytase from major non-glycosylated proteins. The phytase was purified 8.1-fold, with a yield of 3.7% to a maximum specific activity of 42.9 U mg^{-1}.

Effect of pH and temperature on phytase activity. Figure 4 demonstrate the effect of pH variation on phytase activity produced by *S. cerevisiae* L1.12 at 30 °C (relative activities).

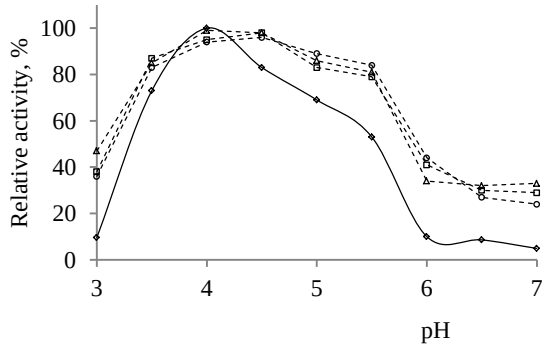

Figure 4. Effect of pH on the relative activity (solid line) and stability (dashed lines) of extracellular phytase from *S. cerevisiae* L1.12

The optimum pH at 4.0 for purified phytase is in agreement with Vohra and Satyanarayana (2001). In et al. (2009) reported that yeast phytase had sharp decline in activity as the pH value moved towards the neutral range 0.5-units from optimum. In contrast, we observed less than 15% decrease in relative phytase activity when the pH value was moved 0.5-units from optimum towards the neutral or acidic range. This can be explained by the fact that the pH of rye dough is about 4.3–4.6, except for the sourdough where pH is around 3.7 (Nielsen et al., 2007) and that phytase purified from rye sourdough yeast is well adapted for current conditions. However, in the whole wheat sourdough where pH varies between 5.3 and 5.5 the activity of yeast phytase seems to be only about 50–70% of optimum activity.

The effect of pH on the enzyme stability indicates that the extracellular phytase produced by *S. cerevisiae* isolated from rye sourdough is active in the pH range 3.5–5.5, with more than 80% of the initial activity remaining. However, sharp decline in activity was observed when the enzyme was incubated at pH 6.0 as well as in more neutral pH.

Figure 5 shows the effect of temperature variation on purified phytase activity at pH 5.5 (relative activities). Maximum purified extracellular phytase activity was exhibited at 35 °C with 98% and 78% of residual activity at temperature of 30 °C and 40°C, respectively. The residual activity at 55 °C was found to be only 7%. Comparison of the optimal temperature of phytase suggested that the phytase produced by *S. cerevisiae* L1.12 isolated from rye sourdough had slightly lower optimal temperature than those of *S. cerevisiae* isolated from the mash of traditional Korean yakju (40 °C) (In et al., 2009).

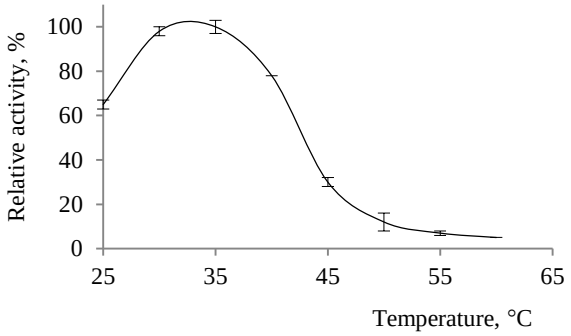

Figure 5. Effect of temperature on the relative activity of extracellular phytase from *S. cerevisiae* L1.12

Effect of temperature on the stability of purified phytase during different incubation time is summarized in table 2. During an incubation period of 2 h, no loss of phytase activity was observed at temperature below 40 °C, while about 8% of the activity was maintained after 15 min incubation at 55 °C. No phytase activity was detected when enzyme was incubated at 65 °C (Table 2). Although phytase produced by *S. castellii* and *Pichia anomala* have been reported to be

thermostable at temperature above 70 °C (Segueilhaet al., 1992; Vohra, Satyanarayana, 2001), our findings are in agreement with those of Quan et al. (2002). They observed that phytase produced by *C. krusei* is easily inactivated at temperature above 50 °C. Further, our tested phytase at 45 °C displays an increasing trend in activity after 2 h of incubation. Even though the phytase of *S. cerevisiae* L1.12 has a low activity at 45 °C, seems that it is very stable at this temperature.

Table 2

Effect of temperature on the stability of extracellular phytase from *S. cerevisiae* L1.12

Temp., °C	Time in minutes							
	15	**30**	**45**	**60**	**75**	**90**	**105**	**120**
25	96	100	115	136	133	129	115	98
30	100	98	96	124	131	112	101	105
35	99	97	100	98	115	100	99	103
40	82	87	96	112	110	98	103	99
45	34	31	37	61	66	57	58	58
55	10	9	9	8	12	7	7	6
65	nd*	nd	nd	nd	nd	nd	nd	nd

*nd – not detected

Conclusions

The highest extracellular phytase activities were observed in two *S. cerevisiae* strains; i.e. L1.12 and L6.06 and therefore they may be the best candidates for improving bioavailability in whole-grain bread.

Concanavalin A chromatography process was successfully implemented to purify phytase from *S. cerevisiae* L1.12 which resulted in 8.1-fold purification with a yield of 3.6% to a maximum activity of 42.9 U mg^{-1}. The optimum pH and temperature values for purified phytase were found to be 4.0 and 35 °C, respectively.

Acknowledgment

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References

1. Andlid T. A., Veide J., Sandberg A. S. (2004) Metabolism of extracellular inositol hexaphosphate (phytate) by Saccharomyces cerevisiae. *International Journal of Food Microbiology*, Vol. 97(2), p. 157–169.
2. Cordain L. (1999) Cereal grains: humanity's double edged sword. *World Review of Nutrition and Dietetics*, Vol. 84 p. 19–73.
3. de Munter J. S. L., Hu F. B., Spiegelman D., Franz M., Van Dam R. M. (2007) Whole grain, bran, and germ intake and risk of type 2 diabetes: A prospective cohort study and systematic review. *Plos Medicine*, Vol. 4(8), p. 1385–1395.
4. Eeckhout W., Depaepe M. (1994) Total Phosphorus, Phytate-Phosphorus and Phytase Activity in Plant Feedstuffs. *Animal Feed Science and Technology*, Vol. 47 (1–2), p. 19–29.
5. Greiner R., Egli I. (2003) Determination of the activity of acidic phytate-degrading enzymes in cereal seeds. *Journal of Agricultural and Food Chemistry*, Vol. 51(4), p. 847–850.
6. In M. J., Seo S. W., Kim D. C., Oh N. S. (2009) Purification and biochemical properties of an extracellular acid phytase produced by the Saccharomyces cerevisiae CY strain. *Process Biochemistry*, Vol. 44(1), p. 122–126.
7. Kumar V., Sinha A. K., Makkar H. P. S., Becker K. (2010) Dietary roles of phytate and phytase in human nutrition: A review. *Food Chemistry*, Vol. 120(4), p. 945–959.
8. Li X. Y., Chi Z. M., Liu Z. A., Li J., Wang X. H., Hirimuthugoda N. Y. (2008) Purification and characterization of extracellular phytase from a marine yeast Kodamaea ohmeri BG3. *Marine Biotechnology*, Vol. 10(2), p. 190–197.
9. Nielsen M. M., Damstrup M. L., Dal Thomsen A., Rasmussen S. K., Hansen A. (2007) Phytase activity and degradation of phytic acid during rye bread making. *European Food Research and Technology*, Vol. 225(2), p. 173–181.
10. Nuobariene L., Hansen A. S., Arneborg N. (2012) Isolation and identification of phytase-active yeasts from sourdoughs. *Lwt-Food Science and Technology*, Vol. 48(2), p. 19–196.
11. Nuobariene L., Hansen A. S., Jespersen L., Arneborg N. (2011) Phytase-active yeasts from grain-based food and beer. *Journal of Applied Microbiology*, Vol. 110(6), p. 137–1380.
12. Quan C. S., Fan S. D., Mang L. H., Wang Y. J., Ohta Y. (2002) Purification and properties of a phytase from Candida krusei WZ-001. *Journal of Bioscience and Bioengineering*, Vol. 94(5), p. 41–425.
13. Quan C. S., Zhang L. H., Wang Y. J., Ohta Y. Y. (2001) Production of Phytase in a Low Phosphate Medium by a Novel Yeast Candida Krusei. *Journal of Bioscience and Bioengineering*, Vol. 92(2), p. 15–160.
14. Sandberg A. S., Brune M., Carlsson N. G., Hallberg L., Skoglund E., Rossander-Hulthen L. (1999) Inositol phosphates with different numbers of phosphate groups influence iron absorption in humans. *American Journal of Clinical Nutrition*, Vol. 70(2), p. 24–246.
15. Segueilha L., Lambrechts C., Boze H., Moulin G., Galzy P. (1992) Purification and Properties of the Phytase from Schwanniomyces-Castellii. *Journal of Fermentation and Bioengineering*, Vol. 74(1), p. 7–11.
16. Slavin J. (2004) Whole grains and human health. *Nutrition Research Reviews*, Vol. 17(1), p. 9–110.
17. Türk M., Sandberg A. S. (1992) Phytate Degradation During Breadmaking - Effect of Phytase Addition. *Journal of Cereal Science*, Vol. 15(3), p. 28–294.
18. van de Vijver L. P. L., van den Bosch L. M. C., van den Brandt P. A., Goldbohm R. A. (2009) Whole-grain consumption, dietary fibre intake and body mass index in the Netherlands cohort study. *European Journal of Clinical Nutrition*, Vol. 63(1), p. 31–38.
19. Vohra A., Satyanarayana T. (2001) Phytase production by the yeast, Pichia anomala. *Biotechnology Letters*, Vol. 23(7), p. 551–554.
20. WHO (2002) The World Health Report. Reducing Risks, promoting Health. [accessed on 27.02.2014]. Available: http://www.who.int/whr/2002/en/whr02_en.pdf?ua=1

THE INFLUENCE OF BAKING TEMPERATURE ON THE QUALITY OF TRITICALE BREAD

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Abstract

During the baking, there occur different changes in dough depending on time and temperature – starch gelatinization, protein denaturation, enzyme inactivation, gas expansion, evaporation of moisture from the crumb and crust. Varying the baking time and temperature the bread physical-chemical parameters and volatile compounds profile change significantly. The research was accomplished on triticale flour blend, which was made from whole grain triticale, rye, hull-less barley flour and rice, maize flour. The aim of the research was to investigate the influence of baking temperature on the physical-chemical properties and volatile compounds profile of triticale bread. Triticale bread was baked 45 minutes at 160±10 °C, 200±10 °C and 240±10 °C temperature. Hardness, stickiness, colour (L* a* b*), moisture, water activity, pH and titrable acidity of triticale bread crumb using standard methods were evaluated, but only colour (L* a* b*) of bread crust was evaluated during research. The volatile compounds profile was identified in bread crumb and crust separately. During research it was established that increasing baking temperature the bread crumb moisture, water activity, stickiness and pH decreased, but hardness and acidity increased. Variation of the baking temperature has significant influence ($p=0.02$) on bread crumb and crust colour. In the bread crumb and crust totally 26 volatile compounds were detected. There were identified 13 volatile compounds – common in all bread crumb and crust samples. All 26 volatile compounds belong to alcohols, aldehydes, acids, terpenes and ketones. Therefore it was ascertained that the baking temperature has significant influence ($p<0.05$) on the quality of triticale bread.

Keywords: triticale bread, quality, volatile compounds.

Introduction

Triticale (*Triticosecale wittmack*) is the first man-made cereal produced by crossing wheat (*Triticum* spp.) and rye (*Secale ceral* L.). The nutrition value of triticale is close to that of wheat and rye (Salmon et al., 2002). In order to extend the product assortment and improve their nutrition value, there can be used triticale, hull-less barley, buckwheat, hull-less oat and other grain flour that are used elsewhere in the world (Taketa et al., 2004). For expanding the range of bakery production in the world there are being developed various recipes for product enriching with fibre, 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 (Sabovics, Straumite, 2012).

In bread making, baking is a key step in which the raw dough piece is transformed into a light, porous, readily digestible and flavourful product, under the influence of heat. During baking, the most apparent interactions are volume expansion, crust formation, inactivation of yeast and enzymatic activities, protein coagulation and partial gelatinisation of starch in dough (Therdthai et al., 2002). The quality of baked product is affected by the time and temperature of the baking process (Mondal, Datta, 2008).

The baking temperature can affect the bread texture and surface colour. The formation of colour in bakery products during baking is widely known as browning. Browning is the result of non-enzymatic chemical reactions which produce coloured compounds during the baking process: such reactions are the Maillard reaction and caramelization (Purlis, 2010).

Taste, smell and the flavour are undoubtedly the most important attributes determining the quality of bread or baked cereals in general and one of the most important attributes influencing the acceptance of the consumer (Hansen, Schieberle, 2005). Flavour is usually the result of the presence, within complex matrices, of many volatile and non-volatile components possessing diverse physical-chemical properties. Whereas, the non-volatile compounds contribute mainly to the taste, the volatile ones influence both – taste and aroma. A vast array of compounds may be responsible for the aroma of the food products, such as alcohols, aldehydes, esters, dicarbonyls, short to medium-chain free fatty acids, methyl ketones, lactones, phenolic compounds and sulphur compounds (Urbach, 1997).

For volatile compound investigation in bread samples can be used a solid-phase microextraction (SPME). Solid-phase microextraction is a relatively new sample preparation technique (Pawliszyn, 1997) before analysis through gas chromatography (Hook et al., 2002). SPME shows some advantages over other widely used techniques: solvent-free and rapid sampling, low cost, and higher sensitivity (Pawliszyn, 1997).

The aim of the research was to investigate the influence of baking temperature on the physical-chemical properties and volatile compounds profile of triticale bread.

Materials and Methods

Experiments were carried out at the Department of Food Technology in the Latvia University of Agriculture.

Triticale, rye and hull-less barley crops cultivated at the Priekuli Plant Breeding Institute (Latvia), rice and maize flour purchased from Joint Stock Company Ustuniu Malunas (Lithuania) were used in the current study.

Triticale, rye and hull-less barley were ground in a laboratory mill Hawos (Hawos Kornmühlen GmbH, Germany) obtaining whole grain fine flour.

Flour blend was made from 60% of whole grain triticale, 15% of whole grain rye, 15% of whole grain hull-less barley, 5% of rice and 5% of maize flour. Ingredients, such as sugar, salt, water and yeast were included in dough formulation in order to improve sensory properties and keep quality of bread.

In the research two dough samples using sugar, salt, dried yeast and water, mixed for 8 and 10 min (dough temperature 25 °C) were prepared. Dough samples were fermented 30 min at temperature 35 °C. For baking process three temperatures – 160±10, 200±10 and 240±10 °C were chosen using one baking time 45 min. The following physical parameters were analysed in obtained triticale bread after cooling it to room temperature 21±2 °C.

- *Moisture content* of bread crumb was determined using air-oven method (AACC method 44-15.02).
- *pH* of bread crumb was measured by JENWAY 3510 pH-meter (Bibby Scientific Ltd., UK).
- *Titratable acidity* of bread crumb was determined by titration where the sample (5 g) was dissolved and dispersed in 50 mL distilled water. Phenolphthalein indicator 0.5 mL 1% was added and titrated with standardized 0.1N NaOH until a faint pink colour endures for 30 seconds.
- *Hardness and stickiness* (N) of bread crumb samples were determined on the Texture Analyser – TA.XT.plus Texture Analyser (Stable Micro Systems Ltd., UK), using a modified version of AACC method 74-09.01.
- *Water activity* of bread crumb was determined by standard ISO 21807:2004, AquaLab LITE device (Decagon Devices, Inc., USA).
- *Colour* of bread crumb and crust samples were measured in CIE L* a* b* colour system using Tristimulus Colorimeter (ColorTec Associates, Inc., USA**)**. Colour Tec PCM/PSM colour values were recorded as L* (brightness) – the vertical co-ordinate runs from L* = 0 (black) through grey to L* = 100 (white); a* (-a, greenness, +a, redness) – the horizontal co-ordinate, that runs from -a* (green) through grey to +a* (red) and b* (-b, blueness, +b, yellowness) – another horizontal co-ordinate, that runs from -b* (blue) through grey to +b* (yellow) (Coultate, 2009). The measurements were repeated on different randomly selected locations at the surface of each crumb and crust sample (n=10).

Volatile compounds in bread crumb and crust were extracted using solid-phase microextration (SPME) in the combination with gas chromatography/mass spectrometry. Fiber was coated with a thin polymer film – Carboxen/Polydimethylsiloxane (CAR/PDMS). The film thickness is 85 μm with bipolar polarity (Supelco, Inc., USA). Five grams of sample were placed in 20 mL vial (small glass bottle). Extration time is 65 min at 40 °C (incl., pre-incubation without the fibre for 15 min, 40 °C). Volatile compounds from fibre were thermally desorbed in the injector of gas chromatograph PerkinElmer 500 GC/MS with a capillary column Elite-Wax ETR (60 m×0.25 mm i.d.; DF 0.25 μm). The details of the program used in GC/MS analysis are following: the initial temperature was 40 °C, held for 7 min, then ramped from 40 °C to 160 °C at a rate of 6 °C min^{-1} and from 160 °C to 210 °C at a rate of 10 °C min^{-1} with the holding time for 15 min. Mass spectrometer in Electron impact ionization mode was set on 70 eV as the electron energies, while the ion source temperature was set to 250 °C and inlet line temperature was set to 250 °C. Helium (he) was used as carrier gas at a constant flow of 1 mL min^{-1}. Acquisition parameters in full scan mode: scaned m/z 40–300. Compounds were identified by comparing their mass spectra with mass spectral library Nist98.

The results (mean, standard deviation, p value) were processed by mathematical and statistical methods. Data were subjected to one-way analysis of variance (ANOVA) by Microsoft Office Excel 2007; significance was defined at $p<0.05$. Principal component analyses were processed by MultiBase2014.

Results and Discussion

The comparison of baking temperature effect on changes of physical-chemical parameters and volatile compounds between the samples was done in the research. The triticale bread moisture and water activity are summarised in Table 1. In the baking time occurs evaporation of moisture and as well as decrease of the water activity.

Table 1

Moisture and water activity of triticale bread

Sample	Moisture, %	Water activity
M8-F[1]	47.08±0.07	0.961±0.002
M8-C45-T160[3]	44.76±0.09	0.960±0.002
M8-C45-T200[3]	43.74±0.07	0.959±0.001
M8-C45-T240[3]	42.04±0.10	0.954±0.001
M10-F[2]	47.03±0.04	0.961±0.002
M10-C45-T160[3]	44.57±0.09	0.960±0.001
M10-C45-T200[3]	43.72±0.08	0.960±0.002
M10-C45-T240[3]	42.12±0.03	0.953±0.001

[1] moisture of fermented dough before baking, dough mixed 8 min; [2] moisture of fermented dough before baking, dough mixed 10 min; [3] M8 means dough mixed 8 min, C45 – baking time (45 min), T160 – baking temperature (160 °C).

Smaller moisture losses comparing to the fermented dough before baking were found in sample M8-C45-T160 (2.32%) and M10-C45-T160 (2.46%), but the highest losses of moisture were in samples M8-C45-T240 and M10-C45-T240, as a result the moisture of samples decreased by 5.04% and 4.91%,

respectively. After obtained data evaluating, it could be concluded that not significant moisture evaporation for 8 min mixed dough, baked for 45 min at 160 °C, was found comparing with the sample M10-C45-T160. If fermented semi-finished product was baked at temperature 240 °C, moisture evaporation was the most intensive in M8-C45-T240 sample comparing with M10-C45-T240 sample. The water activity decreases with the increase of baking temperature. The changes in water activity basically paralleled the changes in moisture content, which was in agreement with study of Mathlouthi (2001) and Jakubczyk et al. (2008).
Increasing the baking temperature also increases the acidity of bread, but pH decreases (Table 2).

Table 2

Titratable acidity and pH of triticale bread

Sample	Acidity, °	pH
M8-F[1]	3.27±0.06	5.224±0.006
M8-C45-T160[3]	3.30±0.04	5.211±0.008
M8-C45-T200[3]	3.32±0.04	5.196±0.007
M8-C45-T240[3]	3.40±0.05	5.099±0.004
M10-F[2]	3.30±0.04	5.189±0.006
M10-C45-T160[3]	3.34±0.02	5.166±0.003
M10-C45-T200[3]	3.40±0.01	5.097±0.004
M10-C45-T240[3]	3.47±0.03	5.007±0.004

[1] moisture of fermented dough before baking, dough mixed 8 min; [2] moisture of fermented dough before baking, dough mixed 10 min; [3] M8 means dough mixed 8 min, C45 – baking time (45 min), T160 – baking temperature (160 °C).

The highest increase of titratable acidity was found in M8-C45-T240 and M10-C45-T240 samples, comparing with acidity in the fermented dough. Evaluating the data of moisture, water activity, acidity and pH, it can be seen that the baking temperature has significant influence ($p<0.05$) on changes of bread moisture, water activity, acidity and pH parameters.
After bread cooling the bread crumb hardness and stickiness were analysed (Table 3). As evidence by the experimental results, the baking temperature has significant effect ($p<0.05$) on the crumb hardness and stickiness.
The softest (7.35±0.95 N and 7.79±0.82 N) bread crumb was acquired for triticale bread baked for 45 min at the temperature 160 °C, but the hardest bread crumb – for bread baked for 45 min at the temperature 240 °C. Comparing M8-C45-T160 and M8-C45-T240 samples, it can be seen that the the bread crumb was harder by 12.01 N. As a result the mixing time does not affect the bread crumb hardness, but bread hardness mainly depends on the baking temperature. Das et al. (2012) in their research found that by increasing the baking temperature increases the hardness of the bread, losing part of the moisture in baking process.

Table 3

The hardness and stickiness of bread crumb

Sample[1]	Crumb hardness, N	Crumb stickiness, N
M8-C45-T160	7.35±0.95	-0.82±0.06
M8-C45-T200	14.53±0.83	-0.56±0.04
M8-C45-T240	19.36±0.92	-0.38±0.03
M10-C45-T160	7.79±0.82	-0.79±0.06
M10-C45-T200	14.92±0.51	-0.51±0.03
M10-C45-T240	19.81±0.25	-0.31±0.04

[1] Sample description: M8 means dough mixed (8 min), C45 – baking time (45 min), T160 – baking temperature (160 °C).

Evaluating triticale bread crumb stickiness after baking, it can be concluded that more sticky crumb was for M8-C45-T160 and M10-C45-T160 samples, presenting the negative force value -0.82±0.06 N and -0.79±0.06 N, respectively. Increasing the baking temperature the stickiness of the bread crumb decreases, where M8-C45-T240 (-0.38±0.03 N) and M10-C45-T240 (-0.31±0.04 N) showed lower stickiness of the bread crumb. The correlation analysis shows that the triticale flour blend crumb hardness ($r=-0.983$) and stickiness ($r=-0.981$) have strong negative correlation on the moisture content of the bread. Thus, it can be concluded that the moisture content in bread affects the crumb hardness and stickiness.
The triticale bread crumb and crust colour was measured after baking, taking into account the baking temperature. The baking temperature has significant influence ($p<0.05$) on bread crumb and crust colour changes.
Triticale bread crumb L* colour component value (Fig. 1) at different baking temperatures of all baked samples was significantly different ($p<0.05$).

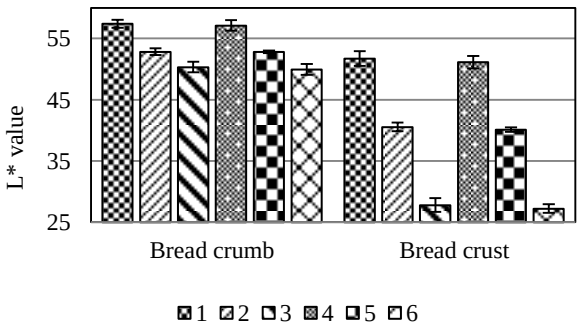

Figure 1. The changes of bread crumb and crust L* colour component value in different baking temperatures
1 – M8-C45-T160, 2 – M8-C45-T200, 3 – M8-C45-T240, 4 – M10-C45-T160, 5 – M10-C45-T200, 6 – M10-C45-T240

The bread crumb becomes darker increasing the baking temperature. The dough mixing time did not have significant affect ($p>0.05$) on crumb colour component

L* value. For example, the crumb L* colour component value of sample M8-C45-T160 is 57.40±0.66 and of sample M10-C45-T160 – 57.12±0.87 was detected. Triticale bread baked for 45 min at 240 °C, apart from the previous dough mixing time, the colour of crumb and crust was obtained comparing to the other samples. After 45 min of baking at 240 °C the crust colour component L* value was 1.8 times lower in comparison with the crust colour of M8-C45-T160 and M10-C45-T160 samples. The baking temperature has no significant impact (p=0.06) on bread crumb dark/light shades, but significantly impacts (p=0.02) crust colour of all samples. Differences among the bread crumb and crust colour was obtained during research, as a result the colour of crust becomes darker increasing the baking temperature. Such interconnection was observed, because at higher temperature caramelization and Maillard reaction occurs faster (Martins et al., 2000).
The value of colour compound a* of bread crumb was in range from 0.50 to 0.76 (Fig. 2), but of bread crust – from 2.91 to 5.41. Increasing the baking temperature, there are no significant (p=0.06) changes of crumb colour a* value, but the baking temperature significantly affects (p=0.03) the crust colour. The colour value of the crust colour component a* decreases by about 2.27±0.14 from sample M8-C45-T160 to M8-C45-T240.

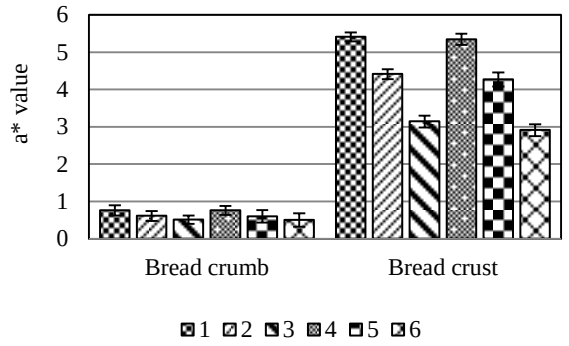

Figure 2. The changes of bread crumb and crust colour compound a* value during baking
1 – M8-C45-T160, 2 – M8-C45-T200, 3 – M8-C45-T240, 4 – M10-C45-T160, 5 – M10-C45-T200, 6 – M10-C45-T240

The triticale bread crumb colour component b* value was in the range from 24.92±0.66 (M10-C45-T240) to 21.12±0.75 (M8-C45-T160) (Fig. 3) and was not significantly affected (p=0.07) by dough mixing time and bread baking temperature. Baking temperature has significant influence on crust colour component b* value changes. The highest intensity of yellow colour was found for the crumb samples M8-C45-T160 (21.12±0.75) and M10-C45-T160 (21.75±0.79), but the lowest intensity of yellow colour was found for the crust samples M8-C45-T240 and M10-C45-T240, as 8.14±0.80 and 7.94±0.31 respectively. Increasing the baking temperature, component b* value in crumb samples increases, but in crust – decreases. It means that the bread crumb at higher baking temperature becomes yellowish. The yellow colour in the crust decreases increasing the baking temperature and this is mainly due to the fact that the rate of brown pigment formation increases with temperature (Das et al., 2012).

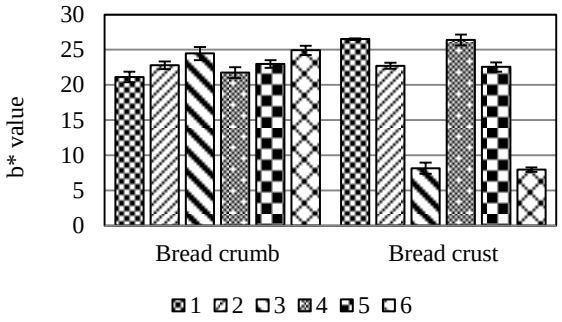

Figure 3. The changes of bread crumb and crust b* colour compound value during baking
1 – M8-C45-T160, 2 – M8-C45-T200, 3 – M8-C45-T240, 4 – M10-C45-T160, 5 – M10-C45-T200, 6 – M10-C45-T240

The L* a* b* colour values change similarly in all samples, what can be explained by moisture loses and brown colour formation during baking.
The volatile profile of different bread types has been widely investigated during the past years. These studies demonstrate that bread flavour is composed by different volatile compounds, belonging to several chemical classes, mainly heterocyclic compounds, alcohols, aldehydes, ketones, etc. Depending on the characteristic of each kind of bread, volatile compounds are present in well-defined ratios (Rehman et al., 2006; Bianchi et al., 2008).
Analysing of the volatile compounds in bread crumb and crust after baking overall there were identified 26 volatile compounds. All bread samples, which were baked at three different temperatures, contain 13 volatile compounds in common, identified both in crumb and crust. Among identified volatile compounds seven were alcohols (cyclobutanol, 4-penten-2-ol, 1-hexanol, 3-methyl-1-butanol, 3-methyl-2-hexanol, phenylethyl alcohol and maltol), two aldehydes (hexanal and heptanal), two acids (acetic acid and hexanoic acid), one ketone (3-hydroxy-2-butanone) and one terpene (carvone).
From all identified volatile compounds in bread crumb and crust samples the highest peak areas were for three alcohols – 4-penten-2-ol, 3-methyl-1-butanol and 1-hexanol, their peak areas are shown in Table 4. The peak areas of alcohols (4-penten-2-ol, 1-hexanol and 3-methyl-1-butanol) are higher in bread crumb than in crust. But there can be observed that, the peak areas of alcohols decrease in bread crumb and crust increasing baking temperature, which is related to volatility of alcohols. According to Schieberle (1996) the amount of flavour compounds formed in bread can be affected by yeast amount and activity, fermentation and baking time and temperature.

Table 4

Peak areas of main alcohols in the triticale bread crumb and crust

Samples		Peak areas of alcohols (PAU×10^6)		
		4-penten-2-ol	3-methyl-1-butanol	1-hexanol
M8-C45-T160	MC*	328.31±3.00	121.07±0.59	59.85±0.59
M8-C45-T160	GC**	187.95±3.04	61.77±2.29	22.07±0.77
M8-C45-T200	MC	214.97±2.80	103.47±0.75	51.78±0.54
M8-C45-T200	GC	171.68±2.37	43.72±1.17	17.76±0.70
M8-C45-T240	MC	98.21±3.96	72.91±0.30	21.62±1.64
M8-C45-T240	GC	51.96±2.11	16.47±2.90	4.13±0.85
M10-C45-T160	MC	329.86±4.96	121.66±0.98	68.38±0.35
M10-C45-T160	GC	190.83±2.20	83.13±0.07	32.13±0.88
M10-C45-T200	MC	254.25±4.97	108.37±0.24	60.80±0.88
M10-C45-T200	GC	175.67±2.50	47.17±0.06	20.17±0.32
M10-C45-T240	MC	108.29±3.65	85.76±0.03	30.71±0.46
M10-C45-T240	GC	56.89±2.24	24.55±0.12	10.46±0.51

*MC – crumb; **GC – crust

The volatility of alcohols is dependent on the temperature in which it starts disappearing (boiling point), such as 4-penten-2-ol volatility intensively occurs in 134–137 °C, 3-methyl-1-butanol – 131 °C, but 1-hexanol from sample starts disappear at 155–159 °C (Janežič et al., 2006). It also explains why peak areas in the bread crust of 4-penten-2-ol and 3-methyl-1-butanol are more loss than in crumb increasing the baking temperature.

The highest peak areas in bread crumb and crust of 4-penten-2-ol are identified in samples M8-C45-T160 and M10-C45-T160, but smaller ones – M8-C45-T240 and M10-C45-T240. The same regularities are observed for 3-methyl-1-butanol and 1-hexanol. The peak areas of 4-penten-2-ol in the sample M8-C45-T160 of crumb and crust are 3.0 to 3.3 times higher than in the sample M8-C45-T240. The peak areas of 3-methyl-1-butanol in bread crumb are 2.1 to 4.4 times higher than in crust. Volatile compounds distribution in the triticale flour blend bread crumb and crust, depending on baking temperature is shown in Figure 4. Evaluating the principal component analysis of the data, the distribution of volatile compounds depending on baking temperature explained 61.4% and 19.2% of variation on PC1 and PC2, respectively. Variation of PC1 shows bread crumb and crust samples, while PC2 represents the volatile compounds dispersion in bread crumb and crust samples.

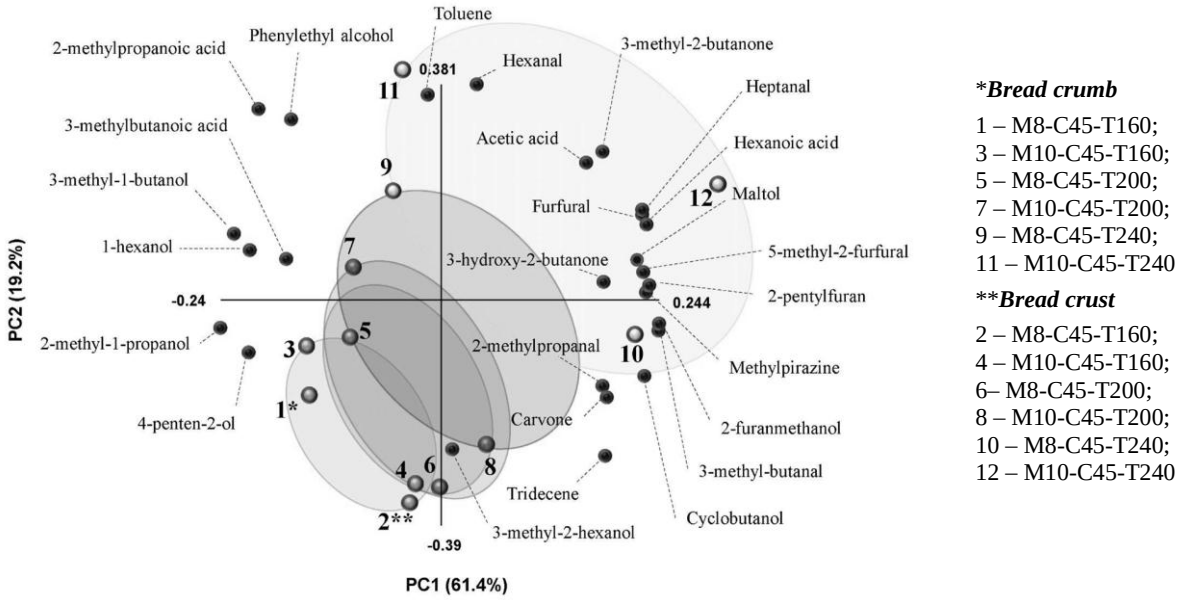

Figure 4. Distribution of volatile compounds in the crumb and crust of the triticale bread samples

The baking time has significant impact on volatile compound formation, because $F=26.50>F_{crit}=1.53$ with a probability of $p<0.05$ (95%). In the bread crust samples (mixed 8 and 10 min and baked 45 min at 240 °C) were detected the most pronounced volatile odour. In this samples more volatile compounds with higher peak areas are such as methylpirazine, 2-pentylfuran, hexanoic acid, maltol, furfural, 5-methyl-2-furfural, 2-furanmethanol, acetic acid, 3-hydroxy-2-butanone, and heptanal. Some of these volatile compounds are forming in fermentation time, but most of them in caramelization and Maillard reaction. Volatile compounds detected in triticale bread crumb forms bread aroma profile, which includes fruit (4-penten-2-ol), malty (3-methyl-1-butanol and 2-methyl-1-propanol), honey (phenylethyl alcohol), green (hexanal, 1-hexanol), caramel (maltol), sweet (hexanoic acid, 3-methyl butanoic acid) aroma. But volatile compounds identified in bread crust supplement aroma profile with green bean (2-pentylfuran), bread (furfural) and butter (2-pentylfuran, 3-hydroxy-2-butanone) notes.

Conclusions

Baking temperature has significant influence ($p<0.05$) on changes of bread moisture, water activity, acidity and pH parameters, as well as hardness of bread crumb. Baking temperature significantly influences triticale bread crust colour and volatile compounds formation in the crumb and crust.

Totally in all samples baked at different temperature were identified 26 volatile compounds. Detected volatile compounds in bread crumb form bread aroma profile, which includes fruit, malty, honey, green, caramel, sweet aroma, but volatile compounds identified in bread crust supplement aroma profile with green bean, butter and bread notes.

Acknowledgment

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References

1. Bianchi F., Careri M., Chiavaro E., Musci M., Vittadini E. (2008) Gas chromatographic-mass spectrometric characterisation of the Italian Protected Designation of Origin ''Altamura" bread volatile profile. *Food Chemistry*, Vol. 110, p. 787–793.
2. Coultate T. (2009) *Food: the chemistry of its components*, Cambridge: RSC Publishing, Royal Society of Chemistry. 501 p.
3. Das L., Raychaudhuri U., Chakraborty R. (2012) Effect of baking conditions on the physical properties of herbal bread using RMS. *Journal of Food, Agriculture and Veterinary Science*, Vol. 2, No. 2, p. 106–114.
4. Hansen A., Schieberle P. (2005) Generation of aroma compounds during sourdough fermentation: applied and fundamental aspects. *Trends in Food Science and Technology*, vol. 16, p. 85–94.
5. Hook G.L., Kimm G.L., Hall T., Smith P.A. (2002) Solid-phase microextraction (SPME) for rapid field sampling and analysis by gas chromatography-mass spectrometry (GC-MS). *Trends in Analytical Chemistry*, Vol. 21, No. 8, p. 534–543.
6. Jakubczyk E., Marzec A., Lewicki P.P. (2008) Relationship between water activity of crisp bread and its mechanical properties and structure. *Polish Journal of Food and Nutrition Science*, Vol. 58, No. 1, p. 45–51.
7. Janežič D., Lučić B., Nikoloć S., Miličević A., Trinajstić N. (2006) Boiling Points of Alcohols – A Comparative QSPR Study. *Internet Electronic Journal of Molecular Design*, Vol. 5, p. 192–200.
8. Martins S.I.F.S., Jongen W.M.F., Boekel M.A.J.S. (2000) A review of Maillard reaction in food and implication to kinetic modelling. *Trends in Food Science and Technology*, Vol. 11, p. 364–373.
9. Mathlouthi M. (2001) Water content, water activity, water structure and the stability of foodstuffs. *Food Control*, Vol. 12, p. 409–417.
10. Mondal A., Datta A.K. (2008) Bread baking – A review. *Journal of Food Engineering*, No. 86, p. 465–474.
11. Pawliszyn J. (1997) *Solid Phase Microextraction: Theory and Practice*, New Jersey: Wiley and Sons, Inc., 264 p.
12. Purlis E. (2010) Browning development in bakery products – A review. *Journal of Food Engeneering*, No. 99, p. 239–249.
13. Rehman S., Peterson A., Piggot J.R. (2006) Flavour in sourdough breads: A review. *Trends in Food Science and Technology*, Vol. 17, No. 10, p 557–566.
14. Sabovics M., Straumite E. (2012) Rheological properties of triticale (*Triticosecale Wittmack*) flour blends dough. **In:** *International Scientific Conferences „Research for Rural Development 2012";* Annual 18th International Scientific Conference Proceedings, Latvia, Jelgava, Vol. 1, p. 143–148.
15. Salmon D., Temelli F., Spence S. (2002) Chemical composition of Western Canadian triticale varieties. **In:** *Proceedings of the 5th International Triticale Symposium*, Poland, Radzików, Vol. 2, p. 445–450.
16. Schieberle P. (1996) Intense aroma compounds-useful tools to monitor the influence of processing and storage on bread aroma. *Advances in Food Science*, Vol. 18, p. 237–244.
17. Taketa S., Kikuchi S., Awayama T., Yamamoto S., Ichii M., Kawasaki S. (2004) Monophyletic origin of naked barley inferred from molecular analyses of a marker closely linked to the naked caryopsis gene (nud). *Theoretical and Applied Genetics*, Vol. 108, p. 1236–1242.
18. Therdthai N., Zhou W., Adamczak T. (2002) Optimisation of the temperature profile in bread baking. *Journal of Food Engineering*, No. 55, p. 41–48.
19. Urbach G. (1997) The flavour of milk and dairy products: II. Cheese: Contribution of volatile compounds. *International Journal of Dairy Technology*, Vol. 50, No. 3, p. 79–89.

SENSORY, COLOUR AND STRUCTURAL PROPERTIES OF PANCAKES PREPARED WITH PEA AND BUCKWHEAT FLOURS

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Abstract

Pea and buckwheat flours, that are gluten free, possess nutritionally important amino acids, therefore they are useful for people suffering from celiac disease. The treatment of celiac disease is gluten free diet, thus it is important to produce gluten free products with high nutritional value. The aim of the research was to evaluate the effect of pea and buckwheat flour on colour, sensory and structural properties in pancakes. The results showed that during and after frying process pancakes kept their shape when 100% of pea flour was used. The structure of pancakes was changed from harder to the softer and was floury due to the pea flour replacement to the buckwheat flour. When the pancake was prepared with 100% of buckwheat flour, the pancakes fell apart – did not keep the shape properly-during frying. The sensory analysis results showed that pea and buckwheat flour affected pancake colour and taste. When higher amount of buckwheat flour was used in pancake preparation, the colour of pancakes changed to darker, the structure was softer and buckwheat flavour was predominant. The experienced panellists indicated that pancakes with 40% and 60% supplemented buckwheat flour were the most pleasant during sensory analysis. Similar tendency was obtained with results of colour (CIE Lab system) and texture analysis. L* and b* colour indices of pancakes had decreasing tendency when the amount of buckwheat flour was increased.

Keywords: pea flour, buckwheat flour, sensory properties, texture, colour.

Introduction

Celiac disease is one of the most common lifelong disorders, affecting 1% of the population worldwide (Catassi, Yachha, 2009). It is a digestive tract disease that damages the small intestine and interferes with the absorption of nutrients from food (De la Hera et al., 2013).

Gluten is a major protein component of wheat flour which is responsible from dough forming characteristics in bakery industry (Torbica et al., 2010), affects elastic properties of dough and contributes to the overall appearance and crumb structure of many baked products (Torbica et al., 2012). However, gluten must be eliminated from the diet of patients with celiac disease. Therefore it is important to produce gluten free products with high nutritional value. Many researchers have studied the substitution of gluten by ingredients with the similar functional properties (Sciarini et al., 2008). Most of the gluten free products are on starch or rice basis with addition of different types of hydrocolloids (Torbica, 2010).

Legumes have an important place from the nutritive point of view (Kohajdováet al., 2013), they are as good sources of proteins, carbohydrates, several water soluble vitamins (Sreerama et al., 2012) and minerals such as calcium, iron, zinc and magnesium (Kaur et al., 2007; Amarakoon et al., 2012). Legumes have been identified as low glycaemic index foods (Bornet et al., 1997), which proteins can be successfully used in baked products to obtain a protein-enriched product with improved amino acids balance (Mohammed et al., 2012). Legume flours were successfully applied as functional ingredients in cereal based foods such as bread (Hefnawy et al., 2012), cake (Gómez et al., 2008), cracker biscuits (Kohajdová et al., 2013) and spaghetti (Arab et al., 2010).

Field pea is one of the oldest domesticated food crops, which is grown widely as a cool season grain legume (Wang et al., 2011) and which is an excellent source of complex carbohydrates, protein, dietary fibre, vitamins and mineral micronutrients (Wang et al., 2010; Amarakoon et al., 2012). The starch and protein content of peas range between 30–50% and 20–25% of the dry matter, respectively (Simsek et al., 2009; Piecyk et al., 2012). Kohajdová et al. (2013) indicated that inclusion of pea flour to wheat dough resulted in increased water absorption capacity and dough development time, in addition incorporation of higher levels of pea flour (20% and 30%) into crackers significantly reduced volume index, width to spread ratio of final products.

Buckwheat is recognised as an important functional food in some countries such as China, Japan and Taiwan and can be used to replace rice or potatoes in the regular meal (Lin et al., 2009). Buckwheat proteins consisted mainly of globulins (up to 50%) and albumins (about 25%) (Choi et al., 2006), they are known for their well-balanced amino acid content (Ikeda, Asami, 2000) and are particularly rich with essential amino acids as lysine and arginine (Choy et al., 2013) as well as they are gluten free which makes them useful for patients suffering from celiac disease (Ikeda, 2002). In addition, buckwheat was found to be a prebiotic, because the research of Prestamo et al. (2003) showed that buckwheat could increase lactic acid bacteria in rat intestine. In general buckwheat, which is added to food as a supplement, can provide beneficial health effects and prevent food from oxidation during processing (Lin et al., 2009).

Pea and buckwheat flours, that are gluten free, possess nutritionally important amino acids, therefore they are useful for people suffering from celiac disease. Choy et al. (2013) incorporated buckwheat flour into noodle formulation instead of wheat flour in order to analyse

the colour of final products. They found that the brightness of noodles was low that can be regard to brownish colour. Lin et al. (2009) indicated that buckwheat enhanced wheat bread exhibited lower brightness and higher redness and yellowness.

The above mentioned research findings showed that pea and buckwheat possess unique nutritional properties and in the development of new products, the addition of pea and buckwheat flour would enrich nutritional composition of the product and thus would be beneficial for our health. However, replacement of wheat flour with pea and buckwheat flour would lead to some changes on rheological and sensory properties. Therefore, the aim of the research was to evaluate the effect of pea and buckwheat flour on colour, sensory and structural properties of pancakes.

Materials and Methods

Materials

Pea flour (PF) and buckwheat flour (BF) were purchased from Fasma, Lithuania. Sugar, baking powder, milk with fat content 2.5% and refined rapeseed oil for dough preparation of pancakes were obtained from the local food market.

The procedure of pancake preparation

At the beginning of pancake preparation, 100% of pea flour (PF) was used and then pea flour was replaced by buckwheat flour (BF) in following PF : BF ratios: 80 : 20, 60 : 40, 40 : 60 and 20 : 80. Because of radical changes in structural properties of the final product, pancakes prepared with 100% of buckwheat flour were excluded in this research.

In the preparation of the dough, all ingredients were mixed to reach smooth dough consistency; then dough was kept for 20 min (resting time) then fried for 8±1 min on frying pan where oil temperature was adjusted to 150±2 °C. Pancakes were served at 65 ±2 °C to determine sensory properties.

According to the added amount of pea and buckwheat flour, the following abbreviations for the samples in current research were used:

- 100% PF – pancakes with 100% of pea flour;
- 80% PF+20% BF – pancakes with 80% of pea and 20% of buckwheat flour;
- 60% PF+40% BF – pancakes with 60% of pea and 40% of buckwheat flour;
- 40% PF+60% BF – pancake with 40% of pea and 60% of buckwheat flour;
- 20% PF+80% BF – pancake with 20% of pea and 80% of buckwheat flour.

Sensory evaluation of pancakes

In order to evaluate differences among pancakes prepared with different amount of buckwheat flour, sensory assessment was performed. Eight experienced staff members from Latvia University of Agriculture Faculty of Food Technology (females between 33–62 years old) were selected. They were selected according to their willingness, availability, motivation and previously demonstrated capability to work as a member of a sensory panel.

Five sensory properties – aroma, taste, colour, hardness and structure were analyzed using a line scale (ISO 4121:2003). Pea and buckwheat aroma, pea and buckwheat taste, yellow and grey colour was followed from ‘not perceptible’ to ‘strongly perceptible’. Hardness was defined from ‘soft’ to ‘strong’ and structure expressed from ‘porous’ to ‘dense’.

In the second part of sensory analysis, the ranking test (ISO 8587:2006) was used with the aim of ranking pancake samples in the following order: the most pleasant – the worst.

Colour measurement

Colour measurements of pancakes were carried out in quintuple using CIE Lab system. The obtained results were expressed in terms of CIE L*, a* and b* values. L* indicates brightness, *a** represents red to green coordinates and b* represents blue to yellow coordinates of a product.

Structure analysis

Texture analyzer “TA.XT.plus Texture Analyser” (Stable Micro Systems Ltd., Surrey, UK) with a Warner–Bratzler shear blade with guillotine probe was used. The cut was performed perpendicularly to the main axis of the pancake until completely breaking. The peak force obtained was taken to be the result from the test (N). The cutting parameters: pre-test speed 1 mm s^{-1}; test speed 3 mm s^{-1}; post test speed 10 mm s^{-1}; cutting distance of 25 mm into the pancake sample. The maximum force required for sample cutting was calculated as an average of six measurements. Obtained data were processed in the Texture Exponent 32 programme.

Statistical analysis

The results were analyzed using one way and two way analysis of variance (ANOVA). T-test was applied to compare the mean values, and p-value at 0.05 was used to determine the significant differences. The correlation analysis was performed in order to determine the relationship between results of sensory analysis and measurements of colour and structure.

Results and Discussion

It is known that consumer behaviour about food choice is determined not only by nutritional value and healthy properties but also sensory evaluation of particular food product. Good quality pancake should possess pleasant pea or buckwheat taste and aroma as well as soft and porous structure. The changes of sensory properties of pancakes with pea flour to buckwheat flour ratios were shown in Figure 1.

The changes of sensory properties of pancakes were affected by the amount of buckwheat flour replaced with the pea flour. By increasing the amount of buckwheat flour the structure of pancakes became softer and denser, colour became darker and buckwheat taste as well as aroma – predominant.

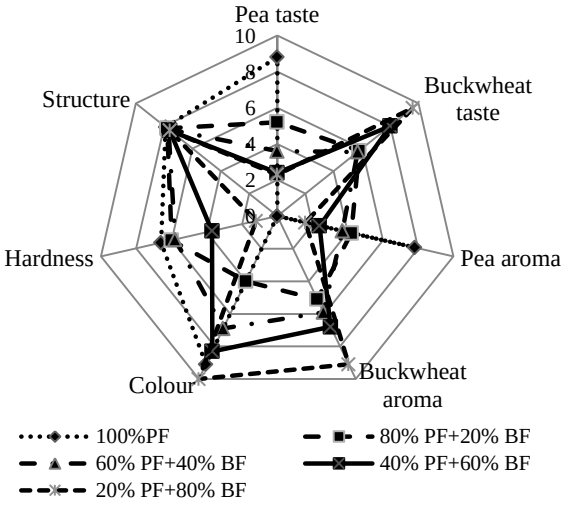

Figure 1. Intensity of sensory properties of pancakes prepared with different ratios of pea flour to buckwheat flour

Evaluation of sensory properties of pancakes with pea and buckwheat flour showed that there were significant differences in intensity of pea and buckwheat taste and aroma, in addition to colour and hardness ($p<0.05$), but there was no significant difference in their structures ($p>0.05$). Pancake samples prepared with 100% PF had significant difference in terms of intensity of pea taste and aroma ($p<0.05$) when compared to other pancakes. The intensities of buckwheat taste and aroma, colour and hardness exhibited significant differences ($p<0.05$) when the pancakes produced with the highest amount of buckwheat flour (20% PF+80% BF). The structure of the same sample was softer and colour much darker when compared to the lightest grey colour obtained with the sample of 80% PF+20% BF.

Focusing on the results of structure, pancake sample having 40% PF+60% BF ratio had significant difference ($p<0.05$) when compared to other samples in term of its soft consistence.

Sensory analysis showed that buckwheat flour addition into the pancake formula caused decreasing of pea taste and aroma, in other words, predominance of pea taste and aroma felt by experts was not so acceptable. Similar conclusions were reported by Kohajdová et al. (2013) in which higher levels of pea flour caused significant reduction in taste, odour and overall acceptance of final crackers due to higher intensity of leguminous taste and odour.

The sensory analysis of ranking showed that the most pleasant pancake samples were obtained with 40% PF+60% BF and 60% PF+40% BF and they were significantly different from other pancake samples ($p<0.05$).

In evaluation of the colour changes in pancake samples with different buckwheat flour supplementation ratio, sensory experienced staff determined similar results. When the buckwheat flour addition was increased in pancake preparation by replacing pea flour, the brightness (L*) of pancake decreased apparently (Fig.2). Similar results were obtained in the study of Lin at al. (2009) by substituting 15% of wheat flour with buckwheat flour in wheat bread preparation. It was the fact that buckwheat flour exhibited beige colour. Choy et al. (2013) and Chillo et al. (2008) reported that the L* values of instant noodles and spaghetti containing buckwheat flour significantly decreased when compared with control samples.

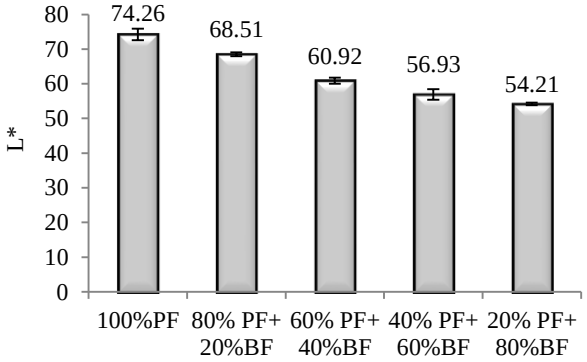

Figure 2. Lightness of pancakes prepared with different ratios of pea flour to buckwheat flour

Decreasing of lightness in 20% PF+80% BF pancake preparation was more than 25% when compared with 100% PF pancake formulation. Pancake samples became much darker when buckwheat flour incorporation increased. On the other hand, Lin et al. (2009) reported that the dark colour of buckwheat incorporated wheat bread might be noticeable to attract consumer's attention.

The correlation was found as very close ($r=-0.995$) between the lightness measurements and experts assessments related to colour intensity in pancake samples.

Buckwheat flour incorporation in pancake preparation resulted an increase in a* values from -3.58 to 2.27 (Fig. 3).

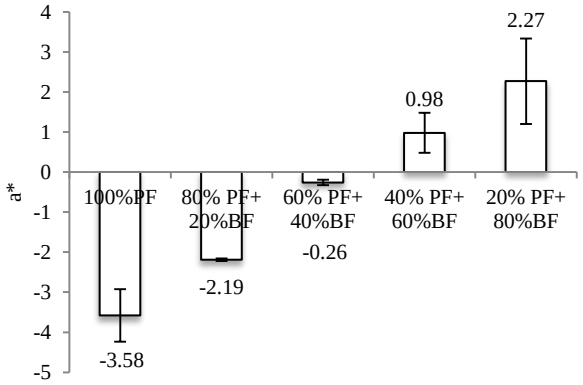

Figure 3. The a* value intensity of pancakes prepared with different ratios of pea flour to buckwheat flour

Similar findings were reported by Choy et al. (2013) where a* value changed from negative to positive value in instant noodles by increasing the buckwheat

amount. Lin et al. (2009) determined similar results like an increase of redness value in wheat bread with the addition of buckwheat flour. In addition, differences were determined between unhusked and husked buckwheat supplemented wheat bread.

The correlation analysis showed that there was close correlation (r=0.998) between measurements of a* value and results of sensory analysis.

In evaluation of the b* value intensity in pancake samples, there was concluded that buckwheat flour significantly affected b* value by decreasing yellowness value in pancakes (Fig. 4). Similar conclusions were indicated by Torbica et al. (2012). In their research related to cookie production with rice and buckwheat flour, yellowness values slightly decreased by increasing the amount of buckwheat flour in cookie formulation.

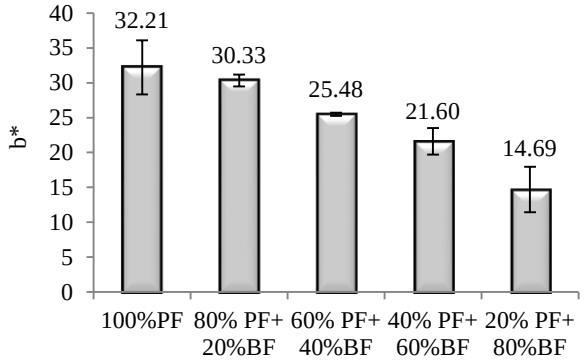

Figure 4. The b* value intensity of pancakes prepared with different ratios of pea flour to buckwheat flour

When the 100% PF pancake sample was compared with the 20% PF+80% BF pancake sample, an increased amount of buckwheat flour caused more than 50% decrease on yellowness. Furthermore, close correlation (r=-0.974) was determined between measurement of b* value and expert's assessment of colour intensity for pancakes prepared with pea and buckwheat flour.

The results of colour measurements showed that the increased amount of buckwheat incorporation in pancake formulation significantly ($p<0.05$) affected L*, a* and b* values.

The hardness results of pancakes prepared with different ratios of pea flour to buckwheat flour were given in Figure 5. The adding of buckwheat in concentrations of 60% and 80% significantly decreased hardness values of pancake samples when compared to other samples. It was observed that an increase of buckwheat flour incorporation caused a decrease on hardness values of pancake samples. Choy et al. (2013) indicated that incorporating buckwheat flour significantly affected hardness of instant noodles which became softer.

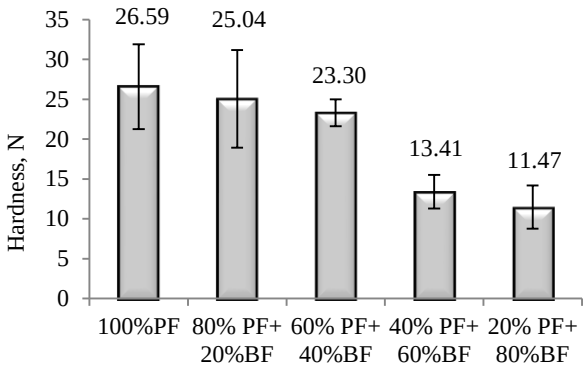

Figure 5. Hardness of pancakes prepared with different ratios of pea flour to buckwheat flour

Whereas Kahajdová et al. (2013) established that addition of pea flour concluded in increased hardness of crackers. Therefore, it could be concluded that addition of buckwheat flour provided softer structure of pancakes. These changes were observed by experts too. The results of sensory analysis and structure analysis exhibited close correlation (r = 0.951).

Conclusions

The sensory properties were affected by the amount of added buckwheat flour in pea flour pancakes. There were significant differences in intensity of pea and buckwheat taste and aroma, colour and hardness ($p<0.05$), but there was no significant difference in structure ($p>0.05$). Sensory analysis showed that buckwheat flour incorporation into the pancake formulation caused decreasing of pea taste and aroma – predominance of pea taste and aroma was not acceptable according to the evaluation of sensory panelists. The amount of added buckwheat in pancake formula significantly ($p<0.05$) affected L*, a* and b* values as well as resulted softer structure on pancakes.

References

1. Amarakoon D., Thavarajah D., McPhee K., Thavarajah P. (2012) Iron-, zinc- and magnesium-rich field peas (*Pisum sativum* L.) with naturally low phytic acid: A potential food-based solution to global micronutrient. *Journal of Food Composition and Analysis*, Vol. 27, p. 8–13.
2. Arab E.A.A., Helmy I.M.F., Barch G.F. (2010) Nutritional evaluation of functional properties of chickpea (*Cicer arietinum* L.) flour and the improvement of spaghetti produced from its. *Journal of American Science*, Vol. 6(1), p. 1055–1072.
3. Bornet F.R., Billaux M.S., Messing B. (1997) Glycaemic index concept and metabolic diseases. *International Journal of Biology Macromolecules*, Vol. 21, p. 207-219.
4. Catassi C., Yachha S.K. (2009) The epidemiology of celiac disease. **In:** *The science of gluten-free foods and beverages*. E.K. Arendt, F. Dal Bello (eds). Minnesota, USA: Academic Press, p. 1–13.
5. Chillo S., Laverse J., Falcone P.M., Protopapa A., Del Nobile M.A. (2008) Influence of the addition of buckwheat flour and durum wheat bran on spaghetti quality. *Journal of Cereal Science*, Vol. 47, p. 144–152.

6. Choi S.M., Mine Y., Ma C.Y. (2006) Characterization of heat-induced aggregates of globulin from common buckwheat (*Fagopyrum esculentum Moench*). *Journal of Biological Macromolecules*, Vol 39, p. 201–209.
7. Choy A-L., Morrison P.D., Hughes J.G., Marriott Ph.J., Small D.M. (2013) Quality and antioxidant properties of instant noodles enhanced with common buckwheat flour. *Journal of Cereal Science*, Vol. 57, p. 281–287.
8. De la Hera E., Martinez M., Gómez M. (2013) Influence of flour particle size on quality of gluten-free rice bread. *LWT – Food Science and Technology*, Vol. 54, p. 199–206.
9. Gómez M., Oliete B., Rosell C.M., Pando V., Fernández E. (2008) Studies on cake quality made of wheat-chickpea flour blends. *LWT – Food Science and Technology*, Vol. 41, p. 1701–1709.
10. Hefnawy T.M.H., El-Shourbagy G.A., Ramadan M.F. (2012) Impact of adding chickpea (*Cicer arietinum* L.) flour to wheat flour on the rheological properties of toast bread. *International Food Research Journal*, Vol. 19(2), p. 521–525.
11. Ikeda K. (2002) Buckwheat: Composition, chemistry and processing. *Advances in Food and Nutrition Research*, Vol. 44, p. 395–434.
12. Ikeda K., Asami Y. (2000) Mechanical characteristics of buckwheat noodles. *Fagopyrum*, Vol. 17, p. 67–72.
13. Kaur M., Sandhu K.S., Singh N. (2007) Comparative study of the functional, thermal and pasting properties of flours from different field pea (*Pisum sativum* L.) and pigeon pea (*Cajanus cajan* L.). *Food Chemistry*, Vol. 104(3), p. 259–267.
14. Kohajdová Z., Karovičová J., Magala M. (2013) Rheological and qualitative characteristics of pea flour incorporated cracker biscuits. *Croatian Journal of Food Science and Technology*, Vol. 5(1), p. 11–17.
15. Lin L-Y., Liu H-M., Yu Y-W., Lin S-D., Mau J-L. (2009) Quality and antioxidant property of buckwheat enhanced wheat bread. *Food Chemistry*, Vol. 112, p. 987–991.
16. Mohammed I., Ahmed A.R., Senge B. (2012) Dough rheology and bread quality of wheat-chickpea flour blends. *Industrial Crops and Products*, Vol. 36, p. 196–202.
17. Piecyk M., Wołosiak R., Drużynska B., Worobiej E. (2012) Chemical composition and starch digestibility in flours from Polish processed legume seeds. *Food Chemistry*, Vol. 135, p. 1057–1064.
18. Prestamo G., Pedrazuela A., Penes E., Lasuncion M.A., Arroyo G. (2003) Role of buckwheat diet on rats as prebiotic and healthy food. *Nutrition Research*, Vol. 23, p. 803–814.
19. Sciarini S.L., Ribotta D.P., León E.A., Pérez T.G. (2008) Influence of gluten-free flours and their mixtures on batter properties and bread quality. *Food and Bioprocess Technology*. doi: 10.1007/s11947-008-0098-2
20. Simsek S., Tulbek M.C., Yao Y., Schatz B. (2009) Starch characteristics of dry peas (*Pisum sativum* L.) grown in the USA. *Food Chemistry*, Vol. 115, p. 832–838.
21. Sreerama Y.N., Sashikala V.B., Pratape V.M., Singh V. (2012) Nutrients and antinutrients in cowpea and horse gram flours in comparison to chickpea flour: Evaluation of their flour functionality. *Food Chemistry*, Vol. 131(2), p. 462–468.
22. Torbica A., Hadnadev M., Dapčević T. (2010) Rheological, textural and sensory properties of gluten-free bread formulations based on rice and buckwheat flour. *Food Hydrocolloids*, Vol. 24, p. 626–632.
23. Torbica A., Hadnadev M., Hadnadev T.D. (2012) Rice and buckwheat flour characterisation and its relation to cookie quality. *Food Research International*, Vol. 48, p. 277–283.
24. Wang N., Hatcher D.W., Warkentin T.D., Toews R. (2010) Effect of cultivar and environment on physicochemical and cooking characteristics of field pea (*Pisum sativum* L.). *Food Chemistry*, Vol. 118, p. 109–115.
21. Wang S., Sharp P., Copeland L. (2011) Structural and functional properties of starches from field peas. *Food Chemistry*, Vol. 126, p. 1546–1552.

GLUTEN-FREE SOURDOUGH BREAD PREPARED WITH CHESTNUT AND RICE FLOUR

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Abstract

In order to meet the rising demand of celiac patients for high quality and healthier products, the old biotechnological process, sourdough technology, was applied in gluten-free bread-making. In present study, the effect of addition of different amounts of sourdough (0%, 20% and 40%, % flour basis) on the rheological behaviour of the gluten-free chestnut-rice dough formulations prepared with chestnut and rice flours as well as on the quality parameters (pH, total titratable acidity (TTA), firmness and volume) of chestnut-rice breads was pointed out. The increasing levels of sourdough addition reduced pH and increased TTA values of samples. The addition of sourdough decreased complex modulus (G*) of dough samples showing softening of dough. The highest specific volume and the lowest firmness values were obtained from gluten-free breads with the addition of 20% sourdough. However, higher level (40%) of sourdough addition had detrimental effect on volume and firmness of breads. The results of present study showed that addition of 20% of sourdough can be used to improve the quality of gluten-free chestnut-rice breads.

Keywords: chestnut flour, gluten-free bread, rice flour, sourdough.

Introduction

Celiac disease, which is an autoimmune disorder, occurs in genetically vulnerable individuals by exposure to cereal gluten proteins. Since gluten creates a continuous protein network which helps to retain gas produced from yeast fermentation and oven rise, lots of the gluten-free baked products on the market are of poor quality with low volume, and poor texture. For these reasons, gluten replacement is still remaining to be one of the most trivial tasks for cereal technologist and scientists (Moore et al., 2008; Demirkesen et al., 2010). Sourdough, a mixture of flour and water fermented with lactic acid bacteria and/or yeast, are used to improve the quality of wheat breads (Moroni et al., 2009). In order to meet the rising demand of celiac patients for high quality and healthier products, this old biotechnological process has lately been applied as a new approach in gluten-free bread-making.

Chestnut flour contains high quality proteins with essential amino acids (4–7%), relatively high amount of sugar (20–32%), starch (50–60%), dietary fibre (4–10%), and low amount of fat (2–4%) (Demirkesen et al., 2010). It also includes some important vitamins such as vitamins E, C, B group and minerals such as potassium, phosphorous, magnesium, calcium, copper, iron, manganese and sulphur (Chenlo et al., 2007; Demirkesen et al., 2010). In addition, it has some important phenolics (gallic and ellagic acid) that have various positive health effects (Blaiotta et al., 2012). It has been stated that gluten-free breads prepared using chestnut-rice flour mixtures had higher volume and softer texture compared with those prepared with only chestnut or rice flour (Demirkesen et al., 2010; 2011; 2013a). In a recent study, it has also been observed that the staling of gluten-free rice breads was delayed by the partial replacement of rice flour with chestnut flour (Demirkesen et al., 2013b). The main objective of the present study was to investigate the effect of the addition of different amounts of sourdough (0%, 20% and 40%, % flour basis) on the rheological behaviour of gluten-free chestnut-rice dough formulations. In addition, the quality parameters (pH, total titratable acidity (TTA), firmness and volume) of chestnut-rice breads were determined.

Materials and Methods

Characterisation of ingredients

Chestnut flour was supplied by Kafkas Pasta Şekerleme San. & Tic. A.Ş. (Karacabey, Bursa, Turkey). Bob's Red Mill Organic Brown Rice Flour (Milwaukie, OR, USA) was obtained from a local market. Sugar (sucrose), salt, vegetable oil (MarketPantry® vegetable oil, MN, USA) and instant yeast (RedStarYeast & Products, Milwaukee, WI, USA) were purchased from local markets. A gluten-free commercial starter named brown rice sourdough starter (*L. plantarum*) specifically designed for use in gluten-free sourdough breads was used. Emulsifier DATEM (diacetyltartaric acid esters of monoglycerides) (E) was obtained from Danisco Co., (Copenhagen, Denmark). Xanthan (X) and guar (G) were obtained from Sigma–Aldrich (St. Louis, MO, USA).

Sourdough (SD) preparation

The starter stain (0.6 g) was added to water (100 g), rice flour (70 g) and chestnut flour (30 g) mixture thereby giving a dough yield of 200 (Table 1). The resultant dough was mixed thoroughly by hand for 2 min, poured into large beakers, covered and then incubated in proofer (InterMetro Industries Co., Wilkes-Barre, PA) at 30 °C and 85% relative humidity (RH) for 72 hours. Then, different amount of the sourdough (20% and 40% flour basis) was added to bread formulations.

Bread making procedure

Gluten-free bread formulations used in this study are presented in Table 1. Percentages of ingredients were based on 100 g of flour amount.

Table 1

Formulations used to prepare different gluten–free bread types containing different amount of sourdough

Ingredients (% flour basis)	Control	20% SD*	40% SD
Chestnut flour	30	24	18
Rice flour	70	56	42
SD	-	20	40
Water	163	143	123
Sugar	8	8	8
Salt	2	2	2
Oil	8	8	8
Yeast	2	2	2
X-G gum blend	0.5	0.5	0.5
** E	0.5	0.5	0.5

* SD – sourdough
** E – emulsifier DATEM

During dough preparation, dry ingredients (chestnut flour, rice flour, instant yeast, sugar, salt and emulsifier) were mixed thoroughly, and then the melted shortening was added. Finally gum suspension and water were added slowly and mixed for 3 min at 85 rpm and then 2 min at 140 rpm using a mixer (Kitchen Aid, 5K45SS, ELKGROVE Village, USA). After complete mixing, fermentation was done at 35 °C and 85% RH in a proofer (InterMetro Industries Co., Wilkes-Barre, USA) for 40 min. Following fermentation, gluten-free bread samples were baked in a rotatory electric oven (National Mfg. Co., Lincoln, USA) and breads samples (200 g each) were baked at 200 °C for 30 min. Gluten-free breads prepared with 30% of chestnut and 70% rice flour and without addition of sourdough were used as controls.

Rheological measurements

Dough samples for the rheological tests were prepared without adding any yeast to the formulation to avoid interference of bubble formation. The rheological measurements were conducted using a rheometer (ARG-2 Model, from TA Instruments, Newcastle, DE, USA) with a parallel plate geometry (40 mm diameter and 2 mm gap) at 25 °C. For the relaxation of the residual stresses, the dough was rested at room temperature for 20 minutes before testing. Frequency sweep experiments were carried out at 0.5% strain rate between 0.1 to 10 Hz. Results were expressed in terms of complex modulus (G*) values. All the rheological experiments were performed at least twice and their averages were reported in the study.

pH and TTA (total titratable acidity) in gluten-free sourdoughs and breads

The total titratable acidity (TTA) and pH values were determined in bread dough and breads. The pH values were measured from an aliquot of 10 g of sourdough, bread dough and bread crumb suspended with 100 mL distillated water. For the determination of TTA, these suspensions were titrated to against 0.1 N NaOH to a final pH valve of 8.5. TTA was expressed as the amount of 0.1 N NaOH 10 g^{-1} consumed in mL. Each analysis was done in triplicate and their averages were reported in the study.

Firmness

Baked loaves were allowed to cool for 1 h before obtaining measurements using the TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA). Samples in cubic shapes having dimensions of 25×25×25 mm were taken from the centre of bread and were compressed to 25% of its thickness with a cylindrical probe of diameter 25.4 mm (approved method 74–09, AACC 2000). The firmness, F (the force required to compress the sample) of the crumb was calculated from a force–distance graph. The measurements were done in duplicate.

Specific bulk volume

To determine specific volume, weight of loaves (g) was measured and bread volume (cm^3) was determined by the rapeseed displacement method 20 min after cooling at 25 °C. Then, specific volume was calculated as the volume/mass ratio (cm^3 g^{-1}) of bread according to (approved method 10–05, AACC 2000). The measurements were done in duplicate.

Statistical analysis

One-way ANOVA was used to determine whether gluten-free formulations affected firmness and specific volume of breads significantly ($p \leq 0.05$). If significant difference was found, means were compared by using the Tukey multiple comparison test ($p \leq 0.05$) by using MINITAB (Version 16) software.

Results and Discussion

pH and TTA of sourdough, dough and bread

Data on acidification of gluten-free bread dough and breads prepared with/without the addition of different amounts of sourdough was compared in Table 2. In the lack of sourdough, control dough and bread samples had the highest pH and the lowest TTA values. The increasing levels of sourdough addition reduced pH and increased TTA values of samples. The similar findings have been reported by Crowley et al., 2002 and Moroni et al., 2011.

Table 2

pH and TTA values of dough's and breads prepared with the addition of different amount of sourdough

Sample	Dough		Bread	
	pH	TTA	pH	TTA
Control	6.41±0.08	2.82±0.02	6.44±0.02	2.97±0.04
20% SD	5.32±0.21	5.41±0.01	5.42±0.06	5.23±0.07
40% SD	4.87±0.01	7.37±0.05	4.94±0.04	6.97±0.09

Rheological measurements

The complex modulus (G*) values of dough samples prepared with/without the addition of different amounts of sourdough can be seen in Figure 1.

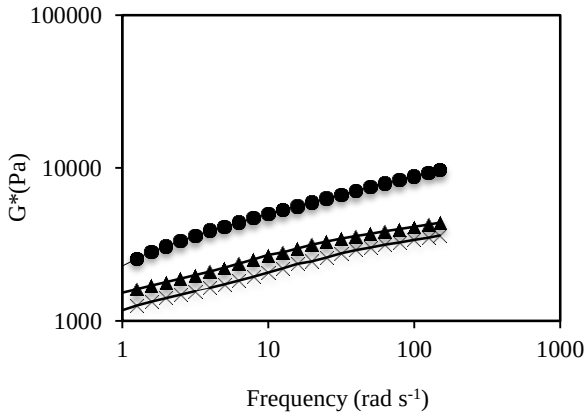

Figure 1. Complex modulus (G*) of gluten-free dough samples prepared with/without the addition of different amounts of sourdough

(●) – control chestnut-rice dough, (▲) –dough prepared with addition of 20% sourdough, (×) – dough prepared with addition of 40% sourdough

The highest complex modulus over the whole frequency range was obtained from control chestnut-rice dough samples. The addition of sourdough decreased complex modulus (G*) of dough samples. Further decreases in the complex modulus (G*) were observed with the addition of higher amount of sourdough (40%). The decreases in complex modulus (G*) of dough samples showed that the addition of sourdough promoted softening of dough. Protein breakdown and the consequent release of small polypeptide during fermentation have also been identified as the major cause for the weakening of the protein-protein and protein-starch interactions resulting in impairing of elasticity of dough (Houben et al., 2010). Consequently, the decreases of complex modulus in gluten-free dough samples might be related to reduced water holding capacity of hydrolyzed proteins, which was induced by organic acids and enzymes released during the sourdough fermentation. Thus, the lack of sourdough fermentation, higher complex modulus (G*) values were obtained as compared to control dough samples. Drastic decreases in the degree of elasticity of dough by sourdough fermentation were also shown in different studies (Clarke et al., 2002; Houben et al., 2010; Galle et al., 2012).

Firmness and specific volume of breads

Firmness and specific volume of breads prepared with/without the addition of different amount of sourdough can be seen in Fig. 2–3.

The addition of sourdough improved the texture of gluten-free breads, beyond a certain amount. Therefore, the lowest firmness values were obtained from breads with the addition of 20% sourdough.

Figure 2. Firmness values of gluten-free dough samples prepared with/without the addition of different amounts of sourdough

Control: control chestnut-rice bread, 20% SD: bread prepared with addition of 20% sourdough, 40% SD: bread prepared with addition of 40% sourdough.

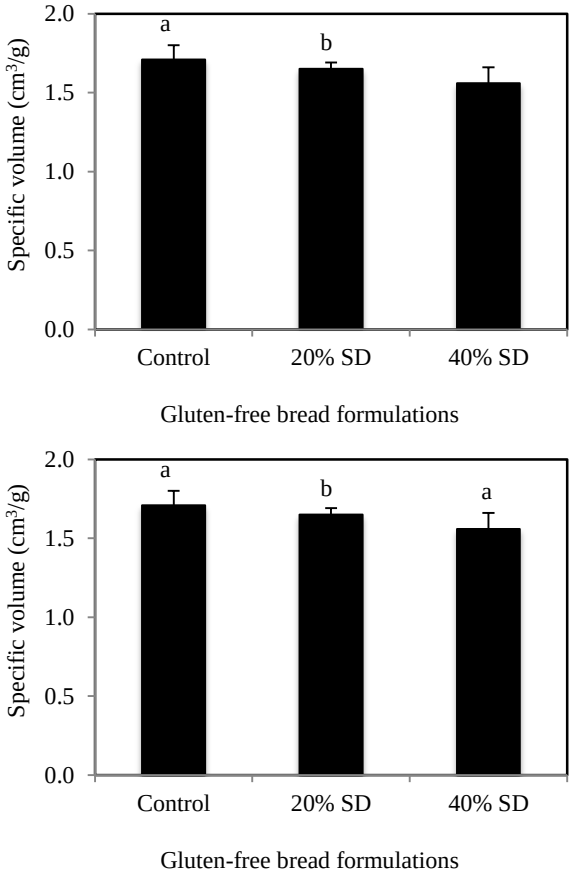

Figure 3. Specific volume values of gluten-free dough samples prepared with/without the addition of different amounts of sourdough

Control – control chestnut-rice bread, 20% SD – bread prepared with addition of 20% sourdough, 40% SD – bread prepared with addition of 40% sourdough.

The volume of gluten-free breads prepared with by the addition of 20% sourdough had comparable specific volume with control breads. However, further addition of sourdough deteriorated the quality of gluten-free

breads. Softer dough formation with the addition of sourdough might have facilitated greater bubble expansion upon fermentation process. Thus, the enhancement of specific volume and texture of gluten-free breads by the addition of certain amount of sourdough (20%) might be related to the improvement of the capacity of dough to retain CO_2. On the other hand, addition of higher level of sourdough (40%) had detrimental effect on volume and texture of breads since viscosity and viscoelastic properties of dough were not sufficient enough to allow bubble capture. Several previous studies also reported that sourdough addition might improve specific volume and texture of breads as long as it is used at certain amount (Crowley et al., 2002; Novotni et al., 2012).

Conclusion

Gluten-free breads formulated with the addition of different amounts of sourdough (0%, 20% and 40%) were evaluated using rheological and baking measurements. Rheological measurements showed that the addition of sourdough led to decreases in complex modulus (G*) of dough samples promoting softening of dough. According to baking tests, the breads prepared with the addition of 20% sourdough had the best quality parameters. However, higher level of sourdough addition led to some deterioration in quality parameters. Therefore, the results of the present study showed that the quality of gluten-free chestnut-rice breads can be successfully improved by the addition of 20% sourdough.

References

1. AACC (2000). Approved Methods of the American Association of Cereal Chemists (10th ed.) Methods 74–09, 10–05. The Association, St. Paul, Minnesota.
2. Blaiotta G., Di Capua M., Coppola R., Aponte M. (2012) Production of fermented chestnut purees by lactic acid bacteria. *International Journal of Food Microbiology*, Vol. 158, p. 195–202.
3. Chenlo F., Moreira R., Pereira G., Silva C. C. (2007) Evaluation of the rheological behaviour of chestnut (castanea sativa mill) flour pastes as function of water content and temperature. *Electronic Journal of Environmental Agriculture and Food Chemistry*, Vol. 6, p. 1794–1802.
4. Clarke C. I., Schober T. J., Arendt E. K. (2002) Effect of single strain and traditional mixed strain starter cultures on rheological properties of wheat dough and bread quality. *Cereal Chemistry*, Vol. 79, p. 640–647.
5. Crowley P., Schober T. J., Clarke C. I., Arendt E. K. (2002) The effect of storage time on textural and crumb grain characteristics of sourdough wheat bread. *European Food Research Technology*, Vol. 214, p. 489–496.
6. Demirkesen I., Campanella O. H., Sumnu G., Sahin, S., Hamaker, B. R. (2013b) A study on staling characteristics of gluten free breads prepared with chestnut and rice flours. *Food Bioprocess and Technology*, Vol. 7, p. 806–820.
7. Demirkesen I., Mert B., Sumnu G., Sahin S. (2010) Utilization of chestnut flour in gluten-free bread formulations. *Journal of Food Engineering*, Vol. 101, p. 329−336.
8. Demirkesen I., Sumnu G., Sahin S., Uysal N. (2011) Optimization of formulations and infrared-microwave combination baking conditions of chestnut-rice breads. I*nternational Journal of Food Science and Technology*, Vol. 46, p. 1809−1815.
9. Demirkesen I., Sumnu G., Sahin S. (2013a) Image analysis of gluten-free breads prepared with chestnut and rice flour and baked in different ovens. *Food Bioprocess and Technology*, Vol. 6, p. 1749–1758.
10. Galle S., Schwab C., Dal Bello F., Coffey A., Ganze M. G., Arendt E. K. (2012) Influence of in-situ synthetized exopolysaccharides on the quality of gluten-free sorghum sourdough bread. *International Journal of Food Microbiology*, Vol. 155, p. 105–112.
11. Houben A., Götz H., Mitzscherling M., Becker T (2010) Modification of the rheological behavior of amaranth (Amaranthushypochonriacus) dough. *Journal of Cereal Science*, Vol. 51, p. 350–256.
12. Moore M. M, Dal Bello F., Arendt E. K. (2008) Sourdough fermented by Lactobacillus plantarum FST 1.7 improves the quality and shelf life of gluten-free bread. *European Food Research Technology*, Vol. 226, p. 1309–1316.
13. Moroni A. V. Dal Bello F., Arendt E. K. (2009) Sourdough in gluten-free bread-making: An ancient technology to solve a novel issue? *Food Microbiology*, Vol. 26, p. 676–684.
14. Novotni D., Cukelj N., Smerdel B., Bituh M., Dujmic F., Curic D. (2012) Glycemic index and firming kinetics of partially baked frozen gluten-free bread with sourdough. *Journal of Cereal Science*, Vol. 55, p. 120–125.

THE EFFECT OF SPRAY DRYING PROCESSING CONDITIONS ON PHYSICAL PROPERTIES OF SPRAY DRIED MALTODEXTRIN

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Abstract

Maltodextrins have wide applications particularly in the food industry. They have many functionalities including usage as wall material, dispersing aid, flavor carrier, bulking agent, viscosifier or fat re-placer, and they exhibit only a slightly sweet taste. Maltodextrin was subjected to spray drying to determine the effect of spray drying conditions on moisture content, particle properties (particle size distribution and particle density) and bulk properties (bulk and tapped densities and porosity) of the powder product. Experiments have been performed in a lab scafle spray-dryer (BüchiLabortechnik AG, Flawil, Switzerland) using a full-factorial design to provide data and correlations that predict the powder properties as a function of the main operational variables of the spray-dryer. The inlet (140 and 200 °C) and feed temperatures (10 and 50 °C), feed flow rate (2.1×10^{-4} and 9.6×10^{-4} kg s^{-1}) and atomizing air flow (1.3×10^{-4} and 1.9×10^{-4} m^3 s^{-1}) were investigated as spray drying process variables. The effect of spray drying conditions on physical properties of powders was expressed with three dimensional response surface and perturbation graphs. Perturbation graphs revealed that atomizing air flow and inlet air temperature had more effect than feed temperature and feed flow rate on the physical properties of maltodextrin powder. The results showed that the Sauter mean diameter ($D_{3,2}$) was between 3.503 and 6.045 μm for maltodextrin powders.

Keywords: maltodextrin, spray drying, particle and bulk properties, particle size distribution.

Introduction

Spray drying is a well-established and widely used method for transforming a wide range of liquid food products into powder form. Spray-dried powders can be stored at ambient temperature for prolonged periods without compromising the powder stability. They are also cheaper to transport and easier to handle in manufacturing plants (Koç et al., 2010; Koç et al., 2011). However, caking or stickiness as one of the major degradation problems hindered the development of powders (Adhikari et al., 2007). The problem is mainly due to the existence of low molecular weight sugars with low glass transition temperatures. To produce food powders, using maltodextrins as a drying carrier is a popular method nowadays (Bhandari et al., 1997). Maltodextrin can significantly increase the glass transition temperature and reduce the hygroscopicity of dried products (Goula, Adamopoulos, 2008). Maltodextrin, a common encapsulating material used in the food industry, is made by the hydrolysis of starch, and comes in the form of a white powder and has a sweet taste. Maltodextrins have particularly wide applications in the food industry especially in spray drying. They have many functionalities including usage as wall material, dispersing aid, flavor carrier and bulking agent. They are mainly used in materials that are difficult to dry -such as fruit juices, flavorings, and sweeteners- and to reduce stickiness, thereby improving the product stability (Bhandari et al., 1993; Bhandari et al., 1997; Roos, Karel, 1991). For this reason, spray drying conditions and physical properties of maltodextrin should be determined.

Physical properties of food powders including the particle shape, density and porosity, surface characteristics, diameter, and size (Kurozawa et al., 2009) can be affected by the spray drying temperatures and the type of atomizer, that are important in the storage, handling and final application of powder product (e.g. particle and bulk properties).

Particle properties are directly related to physical properties of powder food products (Schubert, 1987). It is known that complex changes in the particles properties (size, shape, density and appearance) of droplets occur during spray drying and that the protection of these properties is related to the porosity and integrity of the microcapsules. With respect to morphology, the particles produced by spray drying generally show a smooth surface and are spherical in shape, have lowest surface-to-volume ratio (aroma retention), highest bulk densities (best packing) and best flowability (Kurozawaet et al., 2009).

One of the most important physical parameters of powders with regard to handling is particle size. Particle size can influence flow out of storage bins, the blending of different components, compaction, and the segregation of a mixture, in which smaller particles stay distributed on the bottom and larger particles on top. In addition, these properties significantly influence the essential properties of food products such as smell, texture, and appearance. As particle size decreases, the increase in the particle surface area causes higher affinity with moisture and higher ability to agglomerate during the drying process. The knowledge of food density is of fundamental use for material properties studies and for industrial processes in adjusting storage, processing, packaging, and distribution conditions. Bulk density includes the volumes of the solid and liquid materials and all pores and is generally used to characterize a final product obtained by drying (Kurozawa et al., 2009).

In this study, it was aimed to determine the influence of spray drying process, in terms of inlet air and feed temperatures, feed flow rate and atomizing air flow on moisture content, water activity, particle properties (particle size distribution and particle density) and bulk properties (bulk and tapped densities, porosity,

flowability) of maltodextrin.

Materials and Methods

Maltodextrin (DE=8), used as the test material was supplied from Çağdaş Kimya, Turkey. Maltodextrin was dissolved in the distilled water and the solution containing 25% maltodextrin was used in the experiments.

Spray Drying

Experiments were conducted in a lab scale spray dryer (BüchiLabortechnik AG, Flawil, Switzerland). Maltodextrin solution was atomized from 0.7 mm nozzle into vertical, co-current drying chamber, 0.16 m diameter and 0.5 m height, using a full-factorial design to provide data and correlations that predict the powder properties as a function of the main operational variables of the spray-dryer. The inlet air (T_{inlet}) (140 and 200 °C) and feed (T_{feed}) (10 and 50 °C) temperatures, feed flow rate (V_{feed}) (2.1E-04 and 9.6E-04 kg s^{-1}) and atomizing air flow ($V_{air\text{-}flow}$) (1,3 E-0.4 and 1.9E-0.4 m^3 s^{-1}) were investigated as spray drying process variables.

Moisture Content

The moisture content (MC) of maltodextrin was measured with a halogen moisture analyzer (Ohaus MB45, Switzerland) which was correlated well with the oven method, drying at 110 °C for 2 h.

Particle Properties

Particle size distribution: The particle size distribution of the maltodextrin was measured using a laser light diffraction particle size analyzer (MasterSizer model S 2000, Malvern Instruments Ltd., Worcestershire, U.K.) in which a small quantity of the powder was dispersed in water and the particle distribution was monitored during five successive trials. The particle size was expressed as mean area size $D_{3,2}$ (Sauter mean diameter), and was calculated as follows:

$$d_{3,2} = \frac{\sum_{i}^{N} n_i d_i^3}{\sum_{i}^{N} n_i d_i^2} \quad (1)$$

Where n_i is the number of particles of diameter d_i. The particle size distribution of the powder was measured as the span which is defined as;

$$span = \frac{d_{90} - d_{10}}{d_{50}} \quad (2)$$

Where d_{90}, d_{10}, and d_{50} are the equivalent volume diameters at 90%, 10%, and 50% cumulative volume, respectively.

Particle density: Particle density (ρ_p) of the powder samples was analyzed according to a study by Barbosa-Cánovas et al. (2005). The liquid (petroleum ether) pycnometry was used to determine particle density depending on the volume of pycnometer bottle used.

Bulk Properties

Bulk and tapped densities: The bulk density (ρ_b) of the maltodextrin was determined by measuring the weight of the powder and the corresponding volume. Approximately 20 g of powder sample was placed in a 100 mL graduated cylinder. The bulk density was calculated by dividing the mass of the powder by the volume occupied in the cylinder. For the tapped density (ρ_t), the cylinder was tapped vigorously by hand until no further change in volume occurred (Jinapong et al., 2008).

Porosity: Porosity (ε) of the powder samples was calculated using the relationship between the tapped (ρ_t) and particle (ρ_p) densities of the powders as shown below (Jinapong et al., 2008):

$$\varepsilon = \frac{(\rho_p - \rho_t)}{\rho_t} \times 100 \quad (3)$$

Statistical Analysis

All samples were analyzed in triplicate. The analysis of variance (ANOVA) at a confidence level of 95% was performed. All the results that were obtained were analyzed using Design Expert–version 7.0 software (Statease Inc., MI, USA).

Results and Discussion

$D_{3,2}$ (Sauter mean diameter) and span values of maltodextrin with respect to the inlet air and feed temperatures, feed flow rate and atomizing air flow are given in Table 1.

Table 1

Sauter mean diameter and span values of spray dried maltodextrin under 16 different experimental spray drying conditions

No	T_{feed}, °C	T_{inlet}, °C	V_{feed}, kg s^{-1}	$V_{air\text{-}flow}$, m^3 s^{-1}	$D_{3,2}$, µm	Span
1	10	140	2.1E-04	1.3 E-0.4	4.720	2.841
2	10	200	2.1E-04	1.3 E-0.4	4.644	2.119
3	50	140	2.1E-04	1.3 E-0.4	5.158	2.489
4	50	200	2.1E-04	1.3 E-0.4	4.691	1.906
5	10	140	9.6E-04	1.3 E-0.4	5.797	3.439
6	10	200	9.6E-04	1.3 E-0.4	4.019	1.768
7	50	140	9.6E-04	1.3 E-0.4	5.029	2.640
8	50	200	9.6E-04	1.3 E-0.4	6.045	3.022
9	10	140	2.1E-04	1.9E-0.4	3.815	1.860
10	10	200	2.1E-04	1.9E-0.4	3.919	1.642
11	50	140	2.1E-04	1.9E-0.4	4.184	1.954
12	50	200	2.1E-04	1.9E-0.4	3.904	1.905
13	10	140	9.6E-04	1.9E-0.4	4.006	2.279
14	10	200	9.6E-04	1.9E-0.4	3.503	1.997
15	50	140	9.6E-04	1.9E-0.4	5.586	3.653
16	50	200	9.6E-04	1.9E-0.4	4.003	2.058

Spray-dried products are usually nonhomogeneous. For nonhomogeneous systems, with particle size distribution, the voids between big particles are filled with smaller particles, which cause an increase of the bulk density (Schubert, 1987). The Sauter mean

diameter ($D_{3,2}$) of a sphere, which gives an indication of the diameter corresponding to the average particle volume of the particle size distribution, and the span values varied between 1.642 and 3.653. The span-value expressing the width of the size distribution was also in the same order of magnitude. The particle size distribution of spray dried maltodextrin in 16 different experiments was shown in Figure 1-a and Figure 1-b. It can be seen that spray dried maltodextrin had a narrower particle size range with a relatively uniform distribution. The particle size distribution showed that all samples ranged from 3.503 and 6.045 μm. These results show that spray drying does not produce large particles. The mean particle size of a material may greatly influence its reactivity and the quality of the end product (Baranauskiene et al., 2006). Perturbation plots (Figure 2) showed that the Sauter mean diameter ($D_{3,2}$) of the maltodextrin powder was significantly affected by the atomizing air flow and inlet air temperature.

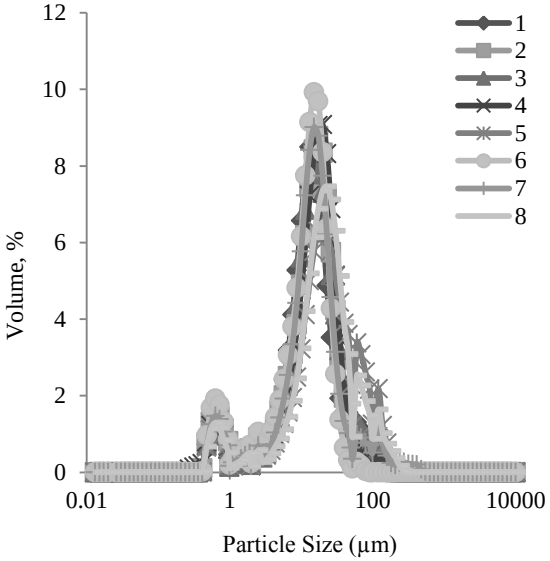

Figure 1-a. Particle size distribution of spray dried maltodextrin under 16 different experimental spray drying conditions (first 8 experiments)

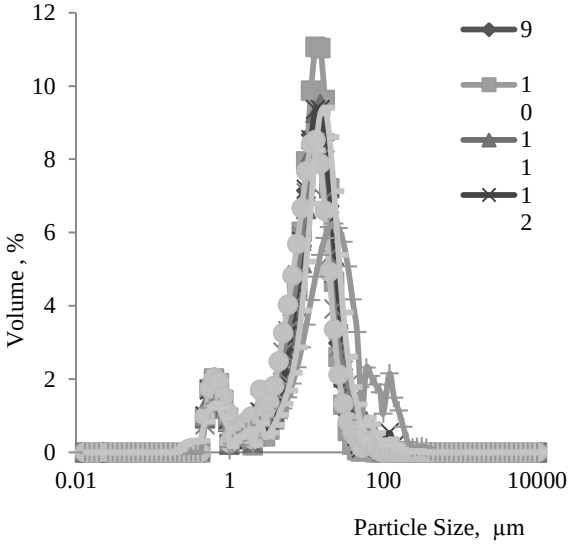

Figure 1-b. Particle size distribution of spray dried maltodextrin under 16 different experimental spray drying conditions (last 8 experiments)

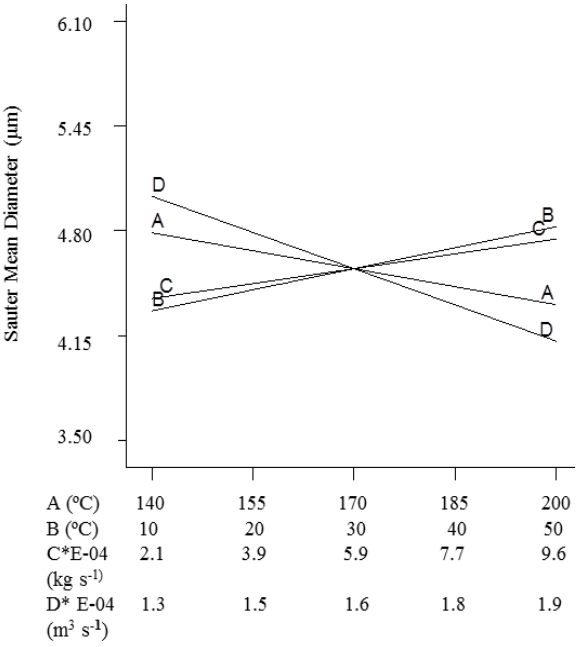

Figure 2. Perturbation plot of Sauter mean diameter ($D_{3,2}$)

A – Inlet air temperature, B – Feed temperature, C – Feed flow rate, D – Atomizing air flow

Table 2

Bulk and particle properties and moisture content of spray dried maltodextrin under 16 different experimental spray drying conditions

No	MC, %, wb	ρ_b, kg m^{-3}	ρ_t, kg m^{-3}	ρ_p, kg m^{-3}	ε
1	5.857	287.9	510.5	1094	53.34
2	4.863	253.5	426.8	924.9	53.86
3	5.477	293.1	439.7	1088	59.57
4	4.600	262.6	409.9	1180	64.27
5	8.880	329.7	572.9	1061	45.98
6	4.267	254.0	402.4	1110	63.73
7	6.353	299.4	455.6	1304	65.05
8	4.633	331.6	538.9	1190	54.70
9	4.300	279.0	484.3	1034	53.14
10	3.813	245.2	417.6	993.8	57.98
11	11.13	297.8	489.5	1165	57.97
12	6.750	306.9	455.5	1077	56.72
13	6.743	313.0	536.5	1127	52.39
14	6.263	263.0	445.6	1089	59.09
15	10.91	349.1	518.4	1263	58.97
16	6.097	302.7	489.3	1057	53.72

The moisture content (%, wet basis), bulk and particle properties of spray dried maltodextrin obtained at

different experimental spray drying conditions is presented in Table 2.

Moisture content values varied between 3.81 and 11.13%, wet basis. Maximum moisture content (11.13%, wb) value was recorded at inlet air temperature of 140 °C, feed temperature of 50 °C, feed flow rate 2.1E-04 kg s^{-1} and atomizing air flow 1.9E-0.4 m^3 s^{-1} (Table 2). According to perturbation graphs (Figure 3), moisture content was affected significantly by the inlet air temperature.

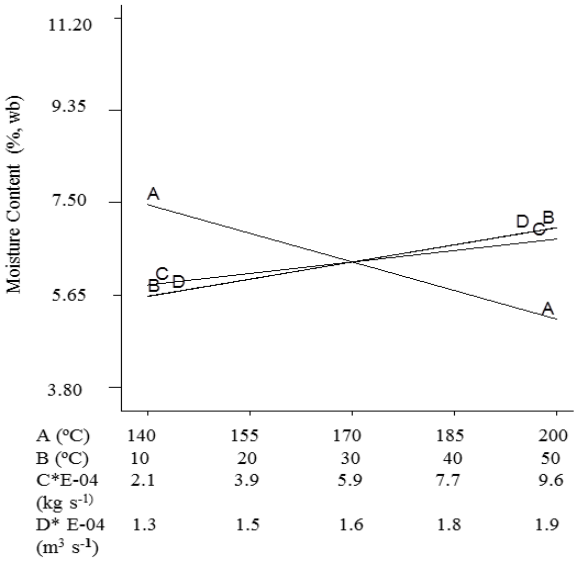

Figure 3. Perturbation plot of moisture content
A – Inlet air temperature, B – Feed temperature, C – Feed flow rate, D – Atomizing air flow

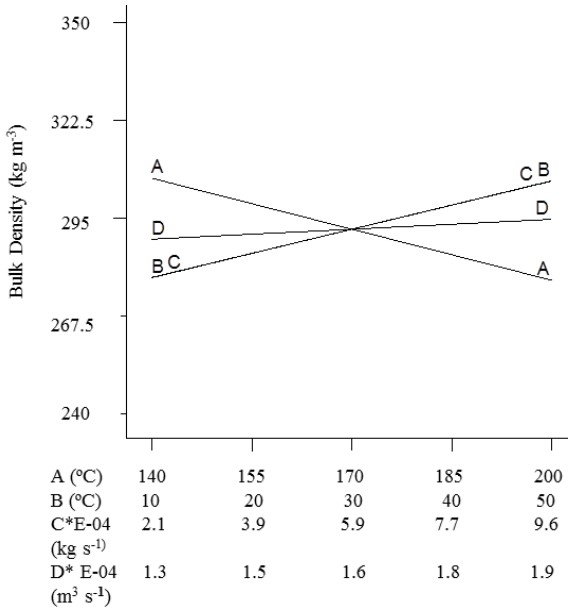

Figure 4. Perturbation plot of bulk density
A – Inlet air temperature, B – Feed temperature, C – Feed flow rate, D – Atomizing air flow

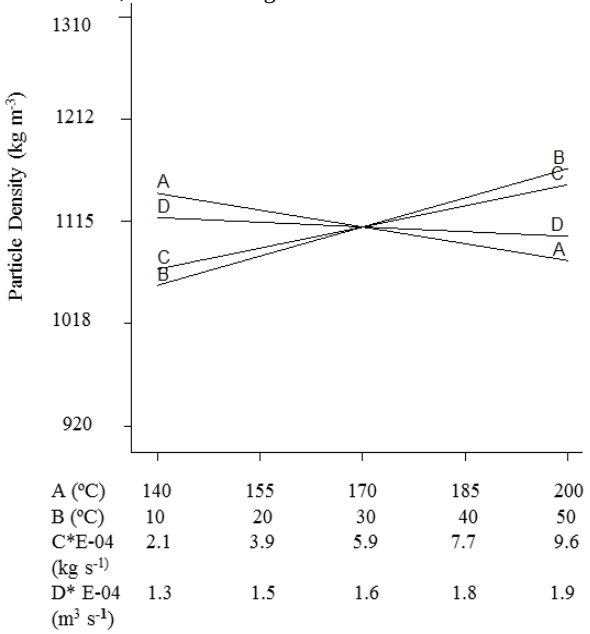

Figure 5. Perturbation plot of particle density
A – Inlet air temperature, B – Feed temperature, C – Feed flow rate, D – Atomizing air flow

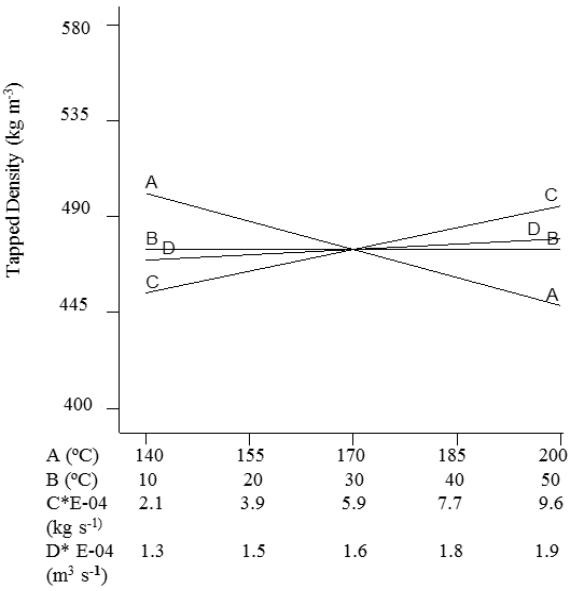

Figure 6. Perturbation plot of tapped density
A – Inlet air temperature, B – Feed temperature, C – Feed flow rate, D – Atomizing air flow

The bulk properties (bulk and tapped densities and porosity) of a food powder are highly dependent on particle size and its distribution (Barbosa-Cánovas et al., 2005). Lower bulk densities of a product are not desirable, resulting in a greater volume of package. Moreover, lower the bulk density, more occluded air within the powders would be and a greater possibility for product oxidation resulting in reduced storage stability (Kurozawa et al., 2009). The bulk densities of samples were changed in the range of 245 and

349 kg m^{-3} (Table 2). Bulk density and particle density were affected by all the independent variables except atomizing air flow (Figure 4 and 5) whereas tapped density was affected by only inlet air temperature and feed flow rate, respectively (Figure 6).

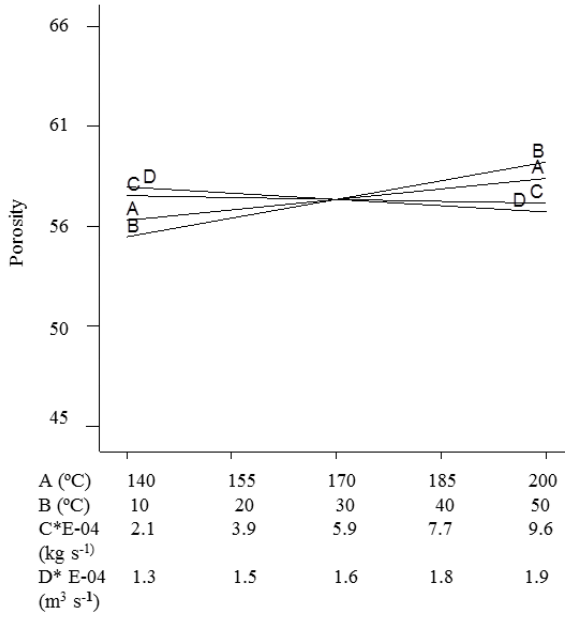

Figure 7. Perturbation plot of porosity

A – Inlet air temperature, B – Feed temperature, C – Feed flow rate, D – Atomizing air flow

Maximum porosity value (65.05) was recorded at inlet air temperature of 140 °C, feed temperature of 50 °C, feed flow rate 9.6E-04 kg $s^{-1}$and atomizing air flow 1.3 E-0.4 m^3 s^{-1} (Table 2). Spherical particles were packed in the best and thus, have the highest bulk densities and porosity (Reineccius 2004). Porosity was influenced by the feed temperature and atomization air flow (Figure 7).

Conclusions

In this study, physical properties of maltodextrin powders were investigated. The results showed that spray drying does not produce larger particles. The bulk, tapped and particle densities of spray dried maltodextrin were (<350), (<575) and (<1310) kg m^{-3}, respectively. These values are an indication that maltodextrin has fair porosity (65). For purposes of understanding the particle formation process, predicting product quality and evaluating the behavior of spray-dried maltodextrin during microencapsulation, this information will provide a helpful approach. Perturbation graphs revealed that atomizing air flow and inlet air temperature had more effect than feed temperature and feed flow rate on the physical properties of spray dried maltodextrin.

References

1. Adhikari B., Howes T., Shrestha A. K., Bhandari B.R. (2007) Development of stickiness of whey protein isolate and lactose droplets during convective drying. *Chemical Engineering and Processing*, Vol. 46, p. 420–428.
2. Baranauskiene R., Venskutonis P. R., Dewettinck K., Verhe R. (2006) Properties of oregano (*Origanum vulgare* L.), citronella (*Cymbopogon nardus* G.) and marjoram (*Majorana hortensis* L.) flavors encapsulated into milk protein-based matrices, *Food Research International*, Vol. 39, p. 413–425.
3. Barbosa-Canovas G.V., Ortega-Rivas E., Juliano P., Yan H. (2005) *Food Powders: Physical Properties, Processing, and Functionality*, Kluwer Academic/Plenum Publishers, New York.
4. Bhandari B.R., Datta N., Howes T. (1997) Problems associated with spray drying of sugar-rich foods. *Drying Technology*, Vol. 15 (20), p. 671–684.
5. Bhandari B.R., Snoussi A., Dumoulin E.D., Lebert A. (1993) Spray drying of concentrated fruit juices. *Drying Technology*, Vol. 11 (5), p. 1081–1092.
6. Goula A.M., Adamopoulos K.G. (2008) Effect of maltodextrin addition during spray drying of tomato pulp in dehumidified air: ii. Powder properties. *Drying Technology*, Vol. 26(6), p. 726–737.
7. Jinapong N., Suphantharika M., Jamnong P. (2008) Production of instant soymilk powders by ultrafiltration, spray drying and fluidized bed agglomeration, *Journal of Food Engineering*, Vol. 84, p. 194–205.
8. Koc B., Sakin M., Balkir P., Kaymak-Ertekin F. (2010) Spray drying of yoghurt: optimization of process conditions for improving viability and other quality attributes. *Drying Technology,* Vol. 28, p. 495–507.
9. Koc M., Koc B., Susyal G., SakinYılmazer M., KaymakErtekin F., Bağdatlıoğlu N. (2011) Functional and physicochemical properties of whole egg powder: effect of spray drying conditions. *Journal of Food Science and Technology*, Vol. 48(2), p. 141–149.
10. Kurozawa L. E., Morassi A. G., Vanzo A.A ., Park K. J., Hubinger M. D. (2009) Influence of spray drying conditions on physicochemical properties of chicken meat powder. *Drying Technology*, Vol. 27(11), p. 1248–1257.
11. Reineccius G. A. (2004) The spray drying of food flavors. *Drying Technology*, Vol. 22(6), p. 1289–1324.
12. Roos Y., Karel M. (1991) Applying state diagrams to food processing and development. *Food Technology*, Vol. 45, p. 66–71.
13. Schubert H. (1987) Food particle technology. Part I: properties of particles and particulate food systems. *Journal of Food Engineering*, Vol. 6, p. 1–32.

CHEMICAL COMPOSITION OF LATVIAN WILD EDIBLE MUSHROOM *CANTHARELLUS CIBARIUS*

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Abstract

The aim of this study was to investigate the chemical composition of widely used wild edible mushroom *Cantharellus cibarius*. Mushrooms *Cantharellus cibarius* were collected in Jelgava region (Latvia) in late summer 2011. Mushrooms were freeze-dried and used to determine chemical composition.

Ash amount was determined by incineration at 550 °C; protein content was determined by Lowry method; total content of phenolic compounds was determined by Folin-Ciocalteu assay. The concentrations of β-carotene and lycopene were determined spectrophotometrically in methanol extract. Protein content in dry matter of *Cantharellus cibarius* was 190 mg g^{-1}. Electrical conductivity (2550 μs cm^{-1}), titratable acidity (0.238 mmol of NaOH per g of dry matter) and formol number (0.163 mmol NaOH per g of mushrooms dry matter) were measured in water extract. The total content of phenolic compounds was 5.09 mg of gallic acid equivalents per 1 g of mushrooms dry matter. The content of β-carotene was 4 times higher than the content of lycopene for *Cantharellus cibarius*, but 4.6 times less than for *Boletus edulis f. beticola*. Using gas chromatography-mass spectrometry (GC-MS) volatile compounds for the first time were determined in both fresh and freeze-dried samples of mushroom *Cantharellus cibarius* and the dominant compound was found to be oct-1-en-3-ol. Results of the chemical composition of Latvian mushroom *Cantharellus cibarius* were compared to previously published chemical composition of *Cantharellus cibarius* collected in other European regions and to our previous results of the chemical composition of Latvian mushroom *Boletus edulis*. Although the amount of substances tested is slightly higher in mushroom *Boletus edulis*, Latvian mushroom *Cantharellus cibarius* is a rich source of biocompounds and mineral substances.

Keywords: chemical composition, mushroom *Cantharellus cibarius*.

Introduction

Wild edible mushrooms have been widely used for centuries as a source of food and food-flavoring material in many countries. Mushrooms are valued due to their aroma and flavour (Guedes de Pinho et al., 2008a), rich content of carbohydrates and fibres (Mattila et al., 2000), vitamins and minerals, proteins and unsaturated fatty acids (Ribeiro et al., 2009). Edible mushrooms are often regarded as a food with antimicrobial, cholesterol-lowering, anticancer and antioxidant properties (Ribeiro et al., 2006; Barros et al., 2007a; Aryantha et al., 2010). These mushrooms also have prophylactic properties against coronary heart disease and hypertension (Matilla et al., 2000).

Various studies have been carried out on the chemical composition of the European edible wild mushrooms from several countries like Finland (Mattila et al., 2002), Poland (Bernas, Jaworska, 2010), Spain (Diez, Alvarez, 2001), Portugal (Barros et al., 2006, 2007a, 2007b, 2008), Italy (Manzi et al., 2001, 2004), Macedonia (Bauer-Petrovska, 2001), Greece (Ouzouni et al., 2007), Turkey (Ayaz et al., 2011) and Croatia (Beluhan, Ranogajec, 2011).

Carbohydrates and crude proteins are the two main components, thus *Cantharellus cibarius* contains crude protein 53.7%, carbohydrates 31.9% and lipids 2.9% of the dry matter (Barros et al., 2008). Dry matter of mushrooms usually is in the range of 60–140 g kg^{-1} (Kalač, 2009). Relatively low content of dry matter and lipids result in the low energy value of mushrooms, that for *Cantharellus cibarius* is 118 kJ 100 g^{-1} of fresh mushrooms (Barros et al., 2008). The analysis of the obtained profiles of fatty acids showed that oleic, linoleic and, to a lesser extent, palmitic and stearic acids were the main fatty acids in the studied *Cantharellus cibarius* (Ribeiro et al., 2009). The profile of organic acids consisted of citric, malic, fumaric, shikimic and ascorbic acids (Valentao et al., 2005). The content of free amino acids in mushrooms is low, only about 1% of the dry matter. The major amino acids found in *Cantharellus cibarius* were glutaminic acid, lysine, alanine, and threonine (Beluhan, Ranogajec, 2011; Surinrut et al., 1987).

Phenolic compounds have usually attracted attention due to their antioxidant properties. When the individual profile of phenolic compounds in edible mushrooms was studied, *Boletus edulis* was found to present the highest total content of phenolic compounds (Palacios et al., 2011) and total content of flavonoids (Palacios et al., 2011; Robaszkiewicz et al., 2010). *Cantharellus cibarius* was found to contain catechin, pyrogallol, myricetin, phenolic acids such as caffeic, ferulic, gallic, p-hydroxybenzoic, gentisic, homogentisic and protocatechuic acids. Content of caffeic acid and catechin in *Cantharellus cibarius* was higher than in other studied species, including *Boletus edulis*. Although the total content of phenolic compounds is lower, the total antioxidant activity in *Cantharellus cibarius* is higher than in any other mushroom studied, indicating that the increased content of caffeic acid and catechin is responsible for greater antioxidative power (Palacios et al., 2011). Valentao et al. (2005) have identified also the presence of 3-, 4- and 5-O-caffeoilquinic acids and rutin in *Cantharellus cibarius*.

Mushrooms are also characterized by a high level of well assimilable mineral constituents. Potassium, magnesium, and phosphorus containing compounds are

the most abundant in *Cantharellus cibarius* (Falandysz et al., 2012; Konuk et al., 2006). Portuguese scientists have investigated some volatile components in *Chantharellus cibariu*s (Guedes de Pinho et al., 2008b), but no information is available on the changes in the content of volatile compounds after freeze-drying.

The aim of the present study was to investigate the chemical composition of widely used wild edible mushroom *Cantharellus cibarius* collected in Latvia.

Materials and Methods

Samples of *Cantharellus cibarius* were collected in Jelgava region (Latvia) in late summer 2011. After collection, mushrooms were freeze-dried (Christ Freeze Dryer Alpha 1-2 LD plus, Germany). All dried mushroom samples were grounded in a blender and then stored in air-tight bags at the room temperature until analysis.

Results of analysis were standardized by dry matter of samples. The ash content of mushrooms was determined by incineration at 550 °C (Manjunathan, Kaviyarasan, 2011). The protein amount in mushroom dry matter was determined by Lowry procedure (Lowry et al., 1951) using albumin as a standard.

Water extract was prepared as follows: 1 g of powdered mushrooms 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 and used for determination of total phenolic content, formol number, titratable acidity, electrical coductivity.

The total content of phenolic compounds in water extract 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 using UV/Vis spectrophotometer Jenway UV 6405. The total content of phenolic compounds was expressed as milligrams of gallic acid equivalents (GAE) per gram of mushroom dry matter (Barros et al., 2007b).

In water extract titratable acidity was determined by potentiometric titration as described previously (Tanner, 1987) and calculated as mmol of NaOH per 1 g of mushroom dry matter.

The formol number was determined in water extract by potentiometric titration as described previously (Tanner, 1987). Formol number was calculated as mmol of NaOH per 1 g of mushroom dry matter.

Electrical conductivity in water extract was determined to characterise the total content of mineral substances using electrode TetraCon 325 connected to conductometer inoLab pH/Cond 720 (WTW, Germany).

Mushroom powder sample (1 g) was extracted with 50 mL of methanol at 25 °C for 24 h (Ribeiro et al., 2006; Barros et al., 2007a) and used for analysis of β-carotene and lycopene.

The content of the 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 and 663 nm. The content of β-carotene (mg 100 mL^{-1}) and lycopene (mg 100 mL^{-1}) was calculated according to the following equations (Barros et al., 2007b):

$$Lyc = -0.0458A_{663} + 0.372A_{505} - 0.0806A_{453}, \quad (1)$$

where Lyc – lycopene content, mg 100 mL^{-1};
A_{663} – absorbance at 663 nm;
A_{505} – absorbance at 505 nm;
A_{453} – absorbance at 453 nm.

$$\beta\text{-carotene} = 0.216A_{663} - 0.304A_{505} + 0.452A_{453}, \quad (2)$$

where β-carotene – β-carotene content, mg 100 mL^{-1}.

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

Volatiles from mushrooms were extracted using solid phase microextraction (SPME). 0.5 g of freeze dried mushrooms was weighed into a vial. Extraction and injection was performed manually. A 85 μm carboxen/ polydimethylsiloxane (Car/PDMS) fiber (Supelco Inc., Bellefonte, PA, USA) was used for headspace SPME sampling. SPME parameters were: equilibration time 10 min, extraction temperature 40±1 °C, extraction duration 30 min, desorption 15 min, 250 °C. For the analysis of the volatile compounds a Perkin Elmer Clarus 500 GC/MS equipped with a capillary column Elite-Wax ETR (60 m×0.25 mm i.d.; DF 0.25 μm) was used. Operating conditions were: injector temperature 250 °C; oven temperature start at 40 °C, hold 7 min, programmed from 40 to 160 °C at 6 °C min^{-1}, hold 10 min, and from 160 to 210 °C at 15 °C min^{-1}, hold 15 min; carrier gas (He) 2 mL min^{-1}; split 1 : 2; ionization EI 70 eV; acquisition parameters in full scan mode: scanned m/z 50–300. Identification of compounds was achieved by comparing the mass spectra present in the NIST98 MS Library Database.

The results are presented as the mean ± standard deviation of three measurements.

Results and Discussion

The content of dry matter of *Cantharellus cibarius* was 9.5±0.5% and was similar to our previous results of mushroom *Boletus edulis* (Kuka, Cakste, 2011). The content of dry matter of *Cantharellus cibarius* from Croatia was 14.2±0.2% (Beluhan, Ranogajec, 2011), while in study by Bernaś et al. (2006) the content of dry matter was found to be 7–12 g per 100 g of fresh matter and similar results were obtained in our study.

Ash amount was determined to characterize the content of mineral substances. Ash amount of *Cantharellus cibarius* was 7.76±0.02% of mushroom dry matter, more than 25% higher than for *Boletus edulis*. Similar tendency was observed when ash content was compared in *Cantharellus cibarius* (8.8±0.05 g 100 g^{-1}) and *Boletus edulis* (5.3±0.87 g 100 g^{-1}) mushrooms collected in Croatia (Beluhan, Ranogajec, 2011).

Electrical conductivity in water extract of *Cantharellus cibarius* was 2550±8 μS cm^{-1}. The obtained result also indicates that the amount of strong electrolytes in *Cantharellus cibarius* is high. Titratable acidity, that characterises the total amount of acids, is shown in Figure 1. Titratable acidity for *Cantharellus cibarius* was similar to the *Boletus edulis f. pinicol*a and *Boletus edulis f. beticola* (0.26 and 0.22 mmol NaOH g^{-1} respectively (Kuka, Cakste, 2011).

The formol number, which characterises the concentration of free amino acids, is shown in Figure 1. The concentration of free amino acids was almost negligible in *Cantharellus cibarius*, while the formol number for *Boletus edulis f. beticola* was 4.6 times higher (Kuka, Cakste, 2011).

The protein amount in mushroom *Cantharellus cibarius* was 190±2 mg g^{-1}, 1.7 times and 2.8 times lower than for *Boletus edulis f. beticola* and *Boletus edulis f. pinicol*a, respectively (Kuka, Cakste, 2011). Content of protein in *Cantharellus cibarius* collected in Latvia was approximately 20%, while in several other studies the amount of protein was shown to vary from 10% (99 g kg^{-1}) (Danell, Eaker, 1992) to 53.7% of dry matter of *Cantharellus cibarius* (Kalač, 2009).

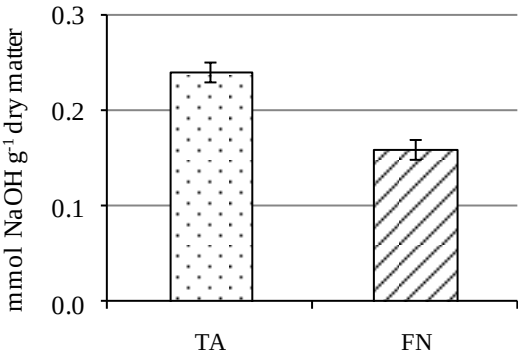

Figure 1. Titratable acidity (TA) and formol number (FN) expressed of *Cantharellus cibarius* dry matter

The content of total phenolic compounds was determined in water extract of *Cantharellus cibarius* and characterised using gallic acid equivalents (GAE) per 1 g of mushroom dry matter and was found to be 5.09±0.01 mg GAE g^{-1} of mushroom dry matter. That is 2.2 times lower than for *Boletus edulis f. beticola* and 2.5 times lower than for *Boletus edulis f. pinicol*a (Kuka, Cakste, 2011). *Cantharellus cibarius* from Poland and Portugal was shown to contain phenolic compounds 2.39±0.23 mg GAE g^{-1} (Robaszkiewicz et al., 2010) and 1.75±0.50 mg GAE g^{-1} (Barros et al., 2008) of dry matter, respectively, while *Cantharellus cibarius* from Latvia contained approximately 2-fold more phenolic compounds calculated as mg GAE g^{-1}.

The content of β-carotene and lycopene was determined using methanol extract of mushroom dry matter (Figure 2). The content of β-carotene was 4 times higher than the content of lycopene for *Cantharellus cibarius*, but 4.6 times less than for *Boletus edulis f. beticola* (Kuka, Cakste, 2011). *Cantharellus cibarius* collected in Portugal contained 5.77±0.42 μg g^{-1} of β-carotene and 1.95±0.28 μg g^{-1} of lycopene (Barros et al., 2008) and comparable amounts of β-carotene and lycopene were also found in mushrooms collected in Latvia.

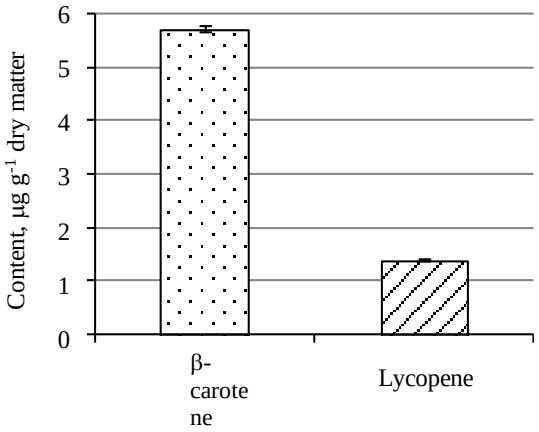

Figure 2. The content of β-carotene and lycopene of mushroom *Cantharellus cibarius* dry matter

Using gas chromatography-mass spectrometry (GC-MS) volatile compounds were identified in both fresh and freeze-dried mushrooms *Cantharellus cibarius*. Oct-1-en-3-ol, (*Z*)-oct-2-en-1-ol, hexanal, hept-1-en-3-one, ethyldeca-2,4-dienoate, (*E*)-oct-2-enal, octan-3-one were identified in fresh mushroom (Figure 3). The dominant compound was found to be oct-1-en-3-ol. The study of Du et al. (2010) similarly to the current research revealed that most abundant aroma compounds are alcohols, aldehydes, and ketones. Among other important compounds were identified terpenes, eight carbon compounds and their derivatives. According to the research of Cheng et al. (2012) oct-1-en-3-ol gives a unique earthy taste and sweetness to mushroom aroma. As show previous studies alcohols and ketones, mainly C8 aliphatic compounds, like octan-3-ol, oct-2-en-1-ol, octan-1-ol and octan-3-one were the main compounds responsible for characteristic mushroom-like aroma emitted by some of mushrooms (Malheiro et al., 2013).

In the current study great losses of volatile compounds were found in freeze-dried mushrooms. The total sum of peak areas in fresh *Cantharellus cibarius* mushroom was 166.08×10^6, which decreased as a result of drying till 93.8×10^6. Content of oct-1-en-3-ol was decreased more than 5 times suggesting significant change of mushroom-like aroma. The loss of the noted alcohol could be due to is high volatility. (*Z*)-oct-2-en-1-ol, hept-1-en-3-one, ethyldeca-2,4-dienoate, (*E*)-oct-2-enal and octan-3-one were not identified in freeze-dried samples.

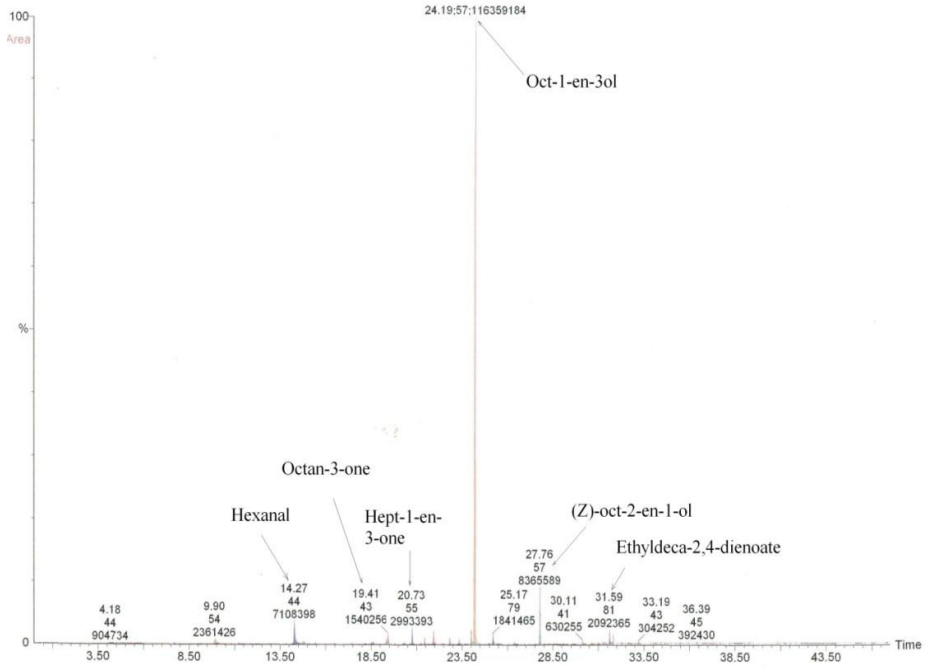

Figure 3. Chromatogram of volatile compounds of fresh mushroom *Cantharellus cibarius*

Conclusions

Although the amount of substances tested is slightly higher in mushroom *Boletus edulis*, mushroom *Cantharellus cibarius* is a rich source of biocompounds and mineral substances.

Freeze-drying significantly reduces the amount of volatile compounds and could affect flavour of mushrooms.

Together, Latvian *Cantharellus cibarius* have similar dry matter, ash and protein content, amount of β-carotene and lycopene, but two fold higher amount of phenolic compounds than *Cantharellus cibarius* collected in other regions of Europe.

References

1. Aryantha I.N.P., Kusmaningati A.B., Sutjiatmo A.B., Sumartini Y., Nursidah A., Narvikasari S. (2010) The effect of *Laetiporus* sp. (Bull. ex. Fr.) bond. et sing. (polyporaceae) extract on total blood cholesterol. *Biotechnology*, Vol. 9, p. P. 312–318.
2. Ayaz F.A., Torun H., Özel A., Col M., Duran C., Sesli E., Colak A. (2011) Nutritional value of some wild edible mushrooms from Black Sea Region (Turkey). *Turkish Journal of Biochemistry*, Vol. 36, p. 213–221.
3. Barros L., Calhelha C., Vaz J., Ferreira I., Baptista P. Estevinho L. (2007a) Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms methanolic extracts. *European Food Research and Technology*, Vol. 225, p. 151–156.
4. Barros L., Ferreira M., Ferreira I., Queiros B., Baptista P. (2007b) Total phenols, ascorbic acid, β-carotene, and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chemistry*, Vol. 103, p. 413–419.
5. Barros L., Venturini B.A., Baptista P., Estevinho L.M., Ferreira I.C.F.R. (2008) Chemical composition and biological properties of Portuguese wild mushrooms: A comprehensive study. *Journal of Agricultural and Food Chemistry*, Vol. 56, p. 3856–3862.
6. Bauer-Petrovska B. (2001) Protein fractions in edible Macedonian mushrooms. *European Food Research and Technology*, Vol. 212, p. 469–472.
7. Beluhan S., Ranogajec A. (2011) Chemical composition and non-valatile components of Croatian wild edible mushrooms. *Food Chemistry*, Vol. 124, p. 1076–1082.
8. Bernaś E., Jaworska G. (2010) Comparison of amino acids content in frozen *P. Ostreatus* and *A. Bisporus* mushrooms. *Acta Scientarum Polonorum, Technologia Alimentaria*, Vol. 9, p. 295–303.
9. Bernaś E., Jaworska G., Lisiewska Z. (2006) Edible mushrooms as a source of valuable nutritive constituents. *Acta Scientarum Polonorum, Technologia Alimentaria*, Vol. 5(1), p. 5–20.
10. Cheng Y., Sun J., Ye X.Q., Lv B.B., Chu Y., Chen J.C. (2012) Advances on flavor substances of edible mushrooms. *Science and Technology of Food Industry*, Vol. 10, p. 412–414.
11. Diez A.A., Alvarez A. (2001) Compositional and nutritional studies on two wild edible mushrooms from northwest Spain. *Food Chemistry*, Vol. 75, p. 417–422.
12. Danell E., Eaker D. (1992) Amino acid and total protein content of the edible mushroom *Cantharellus cibarius* (Frees). *Journal of Science of Food and Agriculture*, Vol. 60, p. 333–337.
13. Du P., Zhang X., He S.F., Sun H. (2010) Flavor and elementary analysis of the wild *Tremellodon gelatinosum* from Yunnan. *Chemistry and Industry of Forest Products*, Vol. 3, p. 97–102.
14. Falandysz J., Drewnowska M., Jarzyńska G., Zhang D., Zhang Y., Wang J. (2012) Mineral constituents in common chanterelles and soils collected from a high mountain and lowland sites in Poland. *Journal of Mountain Science*, Vol. 9, p. 697–705.
15. Guedes de Pinho P., Ribeiro B., Goncalves R.F., Baptista P., Valentao P., Seabra R.M., Andrade P.B. (2008a) Correlation between the pattern volatiles and

overall aroma of wild edible mushrooms. *Journal of Agricultural and Food Chemistry*, Vol. 56, p. 1704–1712.
16. Guedes de Pinho P., Ribeiro B., Gonçalves R.F., Baptista P., Valentão P., Seabra R.M., Andrade P.B. (2008b) Aroma Compounds in Eleven Edible Mushroom Species: Relationship Between Volatile Profile and Sensorial Characteristics. **In:** *Expression of Multidisciplinary Flavour Science.* Proceedings of the 12th Weurman Symposium. (Blank I, Wüst M, Yeretzian C eds) Zürich University of Applied Sciences, Wädenswil, Switzerland p. 467–471.
17. Kalač P. (2009) Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry*, Vol. 113, p. 9–16.
18. Konuk M., Afyon A., Yagiz D. (2006) Chemical composition of some naturally growing and edible mushrooms. *Pakistan Journal of Botany*, Vol. 38, p. 799–804.
19. Kuka M., Cakste I. (2011) Bioactive Compounds in Latvian Edible Mushroom *Boletus edulis*. **In:** *The 6th Baltic Conference on Food Science and Technology "Innovations fot food science and production" Foodbalt 2011*; Conference Proceedings, Latvia, Jelgava, p. 116–120.
20. Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193(1), p. 265–275.
21. Malheiro R., Guedes de Pinho P., Soares S., Ferreira A.C.S., Baptista P. (2013) Volatile biomarkers for wild mushrooms species discrimination. *Food Research International*, Vol. 54, p. 186–194.
22. Manjunathan J., Kaviyarasan V. (2011) Nutrient composition in wild and cultivated edible mushroom, *Lentinus tuberregium* (Fr.) Tamil Nadu., India. *International Food Research Journal*, Vol. 18, p. 809–811.
23. Manzi P., Aguzzi A., Pizzoferrato L. (2001) Nutritional value of mushrooms widely consumed in Italy. *Food Chemistry*, Vol. 73, p. 321–325.
24. Manzi P., Marconi P., Aguzzi A., Pizzoferrato L. (2004) Commercial mushrooms: Nutritional quality and effect of cooking. *Food Chemistry*, Vol. 84, p. 201–206.
25. Mattila P., Suonpaa K., Piironen V. (2000) Functional properties of edible mushrooms. *Nutrition*, Vol. 16, p. 694–696.
26. Mattila P., Salo-Väänänen P., Könkö K., Aro H., Jalava T. (2002) Basic composition and amino acid contents of mushrooms cultivated in Finland. *Journal of Agricultural and Food Chemistry*, Vol. 50, p. 6419–6422.
27. Ouzouni P.K., Riganakos K.A. (2007) Nutritional value and metal content of Greek wild edible fungi. *Acta Alimentarius*, Vol. 36, p. 99–110.
28. Palacios I., Lozano M., Moro C., Arrigo D., Rostagno M.A., Martinez J.A., Garcia-Lafuente A., Quillamon E., Villares A. (2011) Antioxidant properties of phenolic compounds occuring in edible mushrooms. *Food Chemistry*, Vol. 128, p. 674–678.
29. Ribeiro B., Andrade P., Silva B., Baptista P., Seabra R., Valentao P. (2006) Contents of carboxylic acids and two phenolics and antioxidant activity of dried Portuguese wild edible mushrooms. *Journal of Agricultural and Food Chemistry*, Vol. 54, p. 8530–8537.
30. Ribeiro B., Guedes de Pinho P., Andrade P.B., Baptista P., Valentao P. (2009) Fatty acid composition of wild edible mushrooms species: A comparative study. *Microchemical Journal*, 9 Vol. 3, p. 29–35.
31. Robaszkiewicz A., Bartosz G., Lawrynowicz M., Soszynski M. (2010) The Role of Polyphenols, β-carotene, and Lycopene in the Antioxidative Action of the Extracts of Dried, Edible Mushrooms. *Journal of Nutriton and Metabolism*, ID 173274, 9 p.
32. Surinrut P., Julshamn K., Njaa L. (1987) Protein, amino acids and some major and trace elements in Thai and Norwegian mushrooms. *Plant Foods for Human Nutrition*, Vol. 37, p. 117–125.
33. Tanner H., Brunner H.R. (1987) *Getränke – Analytik. Untersuchungsmethoden für die Labor und Betriebspraxis.* – Schwäbisch Hall: Heller Chemie – Verwaltungs GmbH, S. 39–41, S. 95–96.
34. Valentao P., Andrade P.B., Rangel J., Ribeiro B., Silva B.M., Baptista P. Seabra R.M. (2005) Effect of the conservation Procedure on the Contents of Phenolic Compunds and Organic Acids in Chanterelle (*Cantharellus cibarius*) Mushroom. *Journal of Agricultural and Food Chemistry*, Vol. 53, p. 4925–4931.

SOLVENT EXTRACTION OF EGG OIL FROM LIQUID EGG YOLK

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Abstract

The egg yolk lipids have a very high nutritional value. Due to the fatty acid profile, high oil soluble vitamin and lecithin content egg yolk oil can be used as a very good additive to a human nutrition. There are several methods for oil extraction from egg yolks known, but in this manuscript solvent extraction of egg yolk oil from the liquid egg yolk was studied as a most economically reasonable method. The aim of this study was to compare two different solvent mixtures ethanol/chloroform (30/70 by volume) and 2-propanol/hexane (30/70 by volume) for oil extraction from liquid egg yolk and determine quality and nutritional properties of extracted oils. As a liquid egg yolk was used for extraction of oil, the choice of the solvents was based on solvents polarities. Also volatility and toxicity of the solvents were taken in account. The yield of extracted crude oil, water content, solvent residue, fatty acid profile and β-carotene content were determined and compared. The results show that extraction with 2-propanol/hexane gave higher yield of the crude oil than ethanol/chloroform, 28.90±0.27% and 26.37±1.04%, respectively. High water content and solvent residue in both oils mean that there is a purification of solvent extracted egg yolk oil needed. There was no significant difference in fatty acid profile in both oils, but β-carotene content was higher in oil extracted with 2-propanol/hexane 81.02±0.37 mg kg^{-1}, than in ethanol/chloroform extract 73.16±1.53 mg kg^{-1}.

Keywords: egg oil, solvent extraction method.

Introduction

The egg yolk lipids have a very high nutritional value. Thanks to the fatty acid profile, high oil soluble vitamin and lecithin content egg yolk oil can be used as a very good additive to a human nutrition (Lewis et al., 2000).

Nutritional properties in eggs can be affected through the laying hen feed (Surai et al., 2008; Stibilj et al., 1999). There are good ways known how to enrich egg yolk oil with nutrients, but how to extract them as much as possible and keep them in their natural condition after extraction remains the question.

There are several methods for oil extraction from egg yolks known, but in this manuscript solvent extraction of egg yolk oil from the liquid egg yolk was studied as a most economically reasonable method.

In case of solvent extraction high technological and safety requirements must be taken in account when choosing solvents for extraction. Solvents must extract as much as possible lipids from raw material, must have a low boiling temperature, for easier and economical removing from the product, and must be as less as possible toxic.

The difference of oil extraction from egg or egg yolk powder is that liquid egg yolk contains a lot of water and extraction with non-polar solvents is not efficient due to difference in solvent and egg yolk polarities. Polar solvents, such as lower alcohols, denature egg yolk proteins destroying hydrogen bonds or electrostatic interaction in protein structure opening the way to the neutral lipids, what makes extraction with non-polar solvent possible. Without protein denaturation, polar solvents will extract polar membrane-associated lipids from the egg yolk.

The combination of polar and non-polar solvents for better lipid extraction from liquid egg yolk can be chosen. The 2-propanol/hexane mixture can be a good solvent for extraction of egg oil from liquid egg yolk (Ahn et al., 2006).

2-propanol / hexane solvent

2-propanol, a quite polar solvent, can denature egg yolk proteins and extract polar lipids, but it is also non-polar enough to be soluble in non-polar solvent as hexane. 2-propanol is relatively low toxic and is accepted as a solvent in the processing of foodstuff (EFSA, 2005). Main disadvantages of 2-propanol is its boiling temperature which is 82.6 °C, but boiling temperature can be decreased by using vacuum in evaporation procedure.

Hexane is a most popular solvent for lipid extraction for food application. It is widely used in production of vegetable oils. In comparison with 2-propanol, hexane will extract mainly simple triglycerides – neutral lipids. Hexane boiling temperature is 69 °C, which makes it very economical in solvent extracted oil production. Taking in account all before mentioned advantages the choice of non-polar solvent was quite simple.

Because two solvents was mixed together to make extraction mixture, the ratio 30 : 70 between 2-propanol and hexane was chosen. Too much of 2-propanol will make mixture too polar to extract neutral lipids as much as possible.

Ethanol/chloroform solvent

One of the main tasks of oil extraction is the high yield of oil. The process where total lipid extraction is needed is an analysis of the fat content in food products. For fat analysis in egg containing food products methanol/chloroform is used as a solvent (Boselli et al., 2001).

Methanol usage for food grade oil extraction is not safe due to the toxicity of this solvent. For that reason methanol was replaced with ethanol. Ethanol is a lower alcohol, same as 2-propanol, it denatures egg yolk proteins and as a polar solvent extracts polar lipids.

Chloroform is widely used in defatting of biological materials. The process of lipid extraction from tissues with methanol/chloroform first was mentioned in 1957 (Folch et al., 1957). The so-called Folch procedure requires addition of water to solvent mixture, but in

case of extraction of liquid egg yolk, water is already present in the egg yolk. Chloroform / methanol mixture still is used in laboratories for fat content determination in food products (Cizkova et al., 2004). But it is must be admitted that chloroform is toxic and even suspected of causing cancer (WHO, 2004).

The aim of this study was to compare two different solvent mixtures – ethanol/chloroform (30/70 by volume) and 2-propanol / hexane (30/70 by volume) for oil extraction from liquid egg yolk and determine quality and nutritional properties of extracted oils.

The quality of egg yolk oil during the storage can be decreased by lipid oxidation. In this case water content in the oil can affect oxidation process. Egg yolk contains approximately 50% of water and extraction of egg yolk lipids with polar solvents, without extraction of polar lipids, can also bring some water to the extract.

After oil extraction with organic solvents they must be removed from the final product. The solvent residue in extracted oil is an important quality parameter. All solvents used in food processing are strictly controlled by food safety regulators on its presence in food products (Directive 2009/32/EC, 2009).

The main nutritional value of egg yolk oil is unsaturated fatty acids. The extraction method can be evaluated as acceptable if it does not affect the fatty acid profile of egg oil.

β-carotene, as a vitamin A precursor, is a representative of oil soluble vitamins in egg yolk. Content of β-carotene in egg yolk oil can reflect the efficiency of extraction process. The very attractive colour of egg yolk oil is related to β-carotene and therefore its concentration can be easily detected by a simple photometric method.

Materials and Methods

Materials

Commercially available homogenized, pasteurized and chilled liquid egg yolk was obtained from JSC Balticovo (Iecava area, Latvia). Liquid yolk was stored at +4 °C and used with the same temperature for extraction. All solvents (ethanol, 2-propanol, chloroform, hexane) used in egg yolk oil extraction were with the analytical grade from Sigma Aldrich, Germany.

Egg yolk oil extraction

For egg yolk oil extraction two solvent mixtures - ethanol/chloroform (30/70) and 2-propanol/hexane (30/70) were used. For each extraction process solvents were mixed by volume and poured in beaker. Liquid egg yolk was added to solvent mixture with a thin squirt vigorously mixing. The ratio 2 : 1 between solvent mixture and egg yolk was used. Extraction was done at +21 °C temperature vigorously mixing for 30 minutes. Extracts were filtered using vacuum filtration and collected into a clean container. The oil was recovered by evaporation of the solvent mixture using rotary evaporator IKA RV 10 Control (IKA-Werke GmbH & Co. KG) at +70 °C for ethanol / chloroform extract and +80 °C for 2-propanol / hexane extract under the vacuum.

Analyses

The yield of egg oil after extraction with different solvent mixtures was expressed as a weight of total lipids extracted from liquid egg yolk in percent.

Fatty acid profile was determined in accordance with standard methods ISO 12966-2 and ISO 5508, GC-FID (gas chromatography with flame ionization detector). Shimadzu GC 2010 Plus gas chromatograph with flame ionization detector (Shimadzu Corporation, Japan) was used.

Water content in egg yolk oil was measured with Moisture balance MOC-120H scale from Shimadzu (Shimadzu Corporation, Japan). Water evaporated at 120°C until difference in the sample weight was less than 0.05 percent.

β-carotene content was determined in accordance with standard method ISO 17932:2012 using UV spectrophotometer UV-1800 (Shimadzu Corporation, Japan).

Solvent residue in both oil samples was detected by combination of headspace and GC-FID (gas chromatography with flame ionization detector) methods (Restek, 2000; ISO 9832:2002; Stenerson, Verma, 2011; Tiscione et al., 2011).

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

Results and Discussion

Extraction process

It needs to be admitted that in the extraction process the particle size of denatured egg yolk proteins was different. In ethanol/chloroform extraction protein fraction had smaller aggregates and, due to the high density of chloroform, protein fraction was positioned in the top layer. Vacuum filtration of ethanol/chloroform extract requires a longer period of time comparing to 2-propanol / hexane. In case of 2-propanole / hexane extraction, yolk proteins had a bigger particle size and proteins were deposited in bottom layer. This information is important for choosing filtration equipment for egg yolk oil in large scale production.

The results presented in Table 1 show quality parameters of both extracted egg yolk oils. The yield of crude egg yolk oil extracted from liquid egg yolk with 2-propanol / hexane solvent was significantly higher than from ethanol / chloroform extraction ($p<0.05$). The determined water content was high in both oils. This can be explained by the fact that liquid egg yolk contains approximately 50% of water and extraction with mixture of polar and non-polar solvent causes the phase separation where egg yolk phospholipids dissolve in polar phase and start to swell absorbing the water from the egg yolk. The presence of water in egg yolk oil can affect quality of egg oil during storage

resulting in rancidity. Egg oil needs to be purified to increase its storage time.

After solvent removal from the extracts by means of evaporation under the vacuum, crude egg yolk oil was analyzed for solvent residues (Table 1). Ethanol, 2-propanol and hexane, can be used as extraction solvents in food processing in compliance with good manufacturing practice, but anyway these solvent residues in food product are specified by a maximum residue limits in extracted foodstuff or food ingredient, where 2-propanol is limited to 10 mg kg^{-1} and hexane to 1 mg kg^{-1} (Directive 2009/32/EC, 2009; EFSA, 2005). The residue of 2-propanol and hexane in egg oil exceeds the limits allowed in food products and it means that the solvent recovery process must be optimized for better solvent removal. The allowed daily intake of ethanol varies, but its residue in ethanol/chloroform extracted egg yolk oil must not exceed the dangerous limits for human health. Chloroform is a very toxic chemical and its presence in food products even in a very small concentration can have influence on human health therefore chloroform containing food is prohibited under any circumstance in most countries.

Table 1

Egg yolk oil quality parameters

Quality parameters	Extraction solvent	
	2-propanol / hexane	ethanol / chloroform
Yield, %	28.90±0.27	26.37±1.04
Water content, %	14.75±1.05	12.76±2.05
2-propanol, mg kg^{-1}	264.14±7.18	–
Hexane, mg kg^{-1}	2.03±0.02	–
Ethanol, mg kg^{-1}	–	86.08±12.38
Chloroform, mg kg^{-1}	–	22.04±0.77

β-carotene content

β-carotene content in egg oil extracted with 2-propanol/hexane solvent was 81.02±0.37 mg kg^{-1} and 73.16±1.53 mg kg^{-1} in ethanol / chloroform extracted oil. The higher content of β-carotene in 2-propanol / hexane extracted egg yolk oil can be explained by usage of particularly non-polar solvent hexane. β-carotene is a non-polar compound and it dissolves better in non-polar solvents such hexane. Hexane is usually used for extraction of β-carotene from carotenoid containing products.

Fatty acid profile

The fatty acid profile of 2-propanol / hexane and ethanol / chloroform extracted egg yolk oils presented in Table 2. The results show that there is no difference in fatty acid content for both oils. Both 2-propanol / hexane and ethanol/chloroform gave the same fatty acid profile of egg yolk oil.

Table 2

Fatty acid profile of egg yolk oil

Fatty Acid	Extraction solvent	
	2-propanol / hexane g 100 g^{-1}	ethanol /chloroform g 100 g^{-1}
(C14:0)	0.14±0.02	0.09±0.01
(C14:1)	0.04±0.01	0.03±0.01
(C15:0)	0.08±0.01	0.09±0.01
(C16:0)	22.72±0.04	22.27±0.03
(C16:1)	0.28±0.02	0.29±0.01
(C17:0)	0.19±0.02	0.21±0.02
(C17:1)	0.12±0.01	0.11±0.01
(C18:0)	6.20±0.21	6.10±0.03
(C18:1)	52.61±0.06	53.21±0.13
(C18:2)	13.67±0.03	13.65±0.01
(C18:3)	1.72±0.01	1.77±0.01
(C20:1)	0.23±0.01	0.22±0.01
(C20:2)	0.01	0.01
(C20:3)	0.19±0.02	0.15±0.01
(C22:1)	0.03±0.01	0.03±0.01
(C20:4)	0.07±0.01	0.08±0.02
(C20:5)	0.03±0.01	0.03±0.01
(C24:1)	0.02	0.02
(C22:4)	0.08±0.02	0.09±0.01
(C22:5)	0.05±0.01	0.06±0.01
(C22:6)	1.02±0.02	0.95±0.02
Other	0.46±0.01	0.50±0.01
SFA	29.37	28.80
MUFA	53.33	53.91
PUFA	16.84	16.79

Egg yolk for an egg yolk oil extraction was obtained from laying hens which were fed with supplementation of canola oil and can be considered as a product enriched with unsaturated fatty acids. In comparison with other researchers (Stibilj et al., 1999; Souza et al., 2008) our extracted oil contains more oleic acid, but less palmitic and linoleic acids. But the difference in fatty acid content must be related more to the hen feed than to the extraction method or used solvents. Anyway extracted egg yolk oil due to the high content of monounsaturated and polyunsaturated fatty acids is a high value product which after purification can be used as an additive to a human nutrition providing the health benefits.

Figure 1 shows the content of main fatty acids yolk and total content of saturated, monounsaturated and polyunsaturated fatty acids in egg yolk oil and egg.

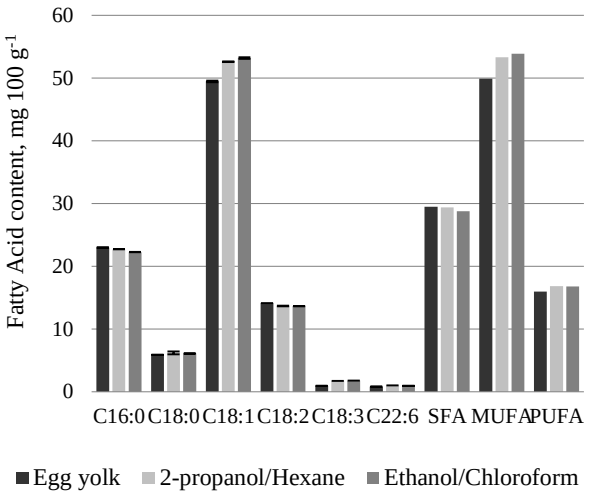

Figure 1. Fatty acid content in egg yolk and egg yolk oils extracted with 2-propanole / hexane and ethanol / chloroform

C16:0 – Palmitic Acid, C18:0 – Stearic Acid, C18:1 – Oleic Acid, C18:2 – Linoleic Acid, C18:3 – α-linolenic acid, C22:6 – Docosahexaenoic acid, SFA – Saturated Fatty Acids, MUFA – Monounsaturated Fatty Acids, PUFA – Polyunsaturated Fatty Acid

Conclusions

Solvent extraction of egg yolk oil from liquid egg yolk can be applied with combination of a polar solvent, as lower alcohols, and a non-polar solvent. As a result egg yolk oil rich in monounsaturated and polyunsaturated fatty acids and β-carotene can be obtained.

Better extraction results were achieved with 2-propanol / hexane solvent which gave higher yield of oil and β-carotene content. Extraction of egg yolk oil from liquid egg yolk with ethanol / chloroform also is possible, but for the health safety reasons usage of chloroform must be prohibited.

Both 2-propanol / hexane and ethanol / chloroform extracted oils have the same fatty acid profile.

Due to the high water content and solvent residue crude egg yolk oil must be purified.

References

1. Ahn D.U., Lee S.H., Singam H., Lee E.J., Kim J.C. (2006) Sequential separation of main components from chicken egg yolk. *Food Science and Biotechnology*, Vol. 15, No. 2, p. 189–195
2. Boselli E., Velazco V., Caboni M.F., Lercker G. (2001) Pressurized liquid extraction of lipids for the determination of oxysterols in egg-contained food. *Journal of Chromatography A*, No .917, p. 239–244
3. Cizkova V., Prokoratova V., Voldrich M., Kvasnicka F., Soukupova V. (2004): Determination of egg content in pasta. *Czech J. Food Sci.*, No. 22, p. 197–203.
4. Directive 2009/32/EC (2009), *Official Journal of European Union, L141*, Vol. 52, [accessed on 17.03.2014.]. Available: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:141:FULL:EN:PDF
5. EFSA (2005) *Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to Propan-2-ol as a carrier solvent for Flavourings*, No. 202, p. 1-10. [accessed on 17.02.2014.]. Available: http://www.efsa.eu.int/science/afc/catindex_en.html
6. Folch J., Lees M., Sloane Stanley G.H. (1957) A simple method for isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, No. 226, p. 497–509
7. ISO 12966-2 (2011) Animal and vegetable fats and oils – Gas chromatography of fatty acid methyl esters – Part 2: Preparation of methyl esters of fatty acids
8. ISO 17932 (2011) Palm oil – determination of the deterioration of bleachability index (DOBI) and carotene content.
9. ISO 5508 (1990) Animal and vegetable fats and oils – Analysis by gas chromatography of methyl esters of fatty acids.
10. ISO 9832 (2002) Animal and vegetable fats and oils – Determination of residual technical hexane content.
11. Lewis N.M., Seburg S, Flanagan N.L. (2000) Enriched eggs as a source of N-3 polyunsaturated fatty acids for humans, *Poultry Science*, No. 79, p. 971–974
12. Restek (2000) A Technical Guide for Static Headspace Analysis Using GC. *Restek Technical Guide, Lit.Cat. No. 59895B*. [accessed on 03.03.2014.]. Available: http://www.restek.com/pdfs/59895B.pdf
13. Souza J.G., Costa F.G.P., Queiroga R.C.R.E., Silva J.H.V., Schuler A.R.P., Goulart C.C. (2008) Fatty Acid Profile of Eggs of Semi-Heavy Layers Fed Feeds containing Linseed Oil, *Brazilian Journal of Poultry Science*, No.10 (1), p. 37-44. [accessed on 12.02.2014.]. Available: http://www.scielo.br/pdf/rbca/v10n1/a06v10n1.pdf
14. Stenerson K.K., Verma S. (2011) The Utility of Headspace Grade Solvents in the Analysis of Organic Volatile Impurities. *Supelco Publication T411031*. [accessed on 25.03.2014.]. Available: http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Supelco/Posters/1/t411031h.pdf
15. Stibilj V., Koman Rajšp M., Holcman A. (1999) Fatty acid composition of eggs enriched with omega-3 fatty acids on the market. *Zootehnika*, No. 74, [accessed on 03.03.2014.]. Available at: http://aas.bf.uni-lj.si/zootehnika/74-1999/PDF/74-1999-2-27-36.pdf
16. Surai P.F., Papazyan T.T., Sparks N.H.C., Speake B.K. (2008) Simultaneous Enrichment of Eggs with PUFAs and Antioxidants. The Columbus Concept, *Wild-Type Food in Health Promotion and Disease Prevention*, p. 139-153
17. Tiscione N.B., Alford I., Yeatman D.T., Shan X. (2011) Ethanol Analysis by Headspace Gas Chromatography with Simultaneous Flame-Ionization and Mass Spectrometry Detection. *Journal of Analytical Toxicology*, No. 35, p. 501–511
18. World Health Organization (2004) *Chloroform*, Concise International Chemical Assessment Document 58. [accessed on 4.03.2014]. Available: http://www.who.int/ipcs/publications/cicad/en/cicad58.pdf

STORAGE STABILITY OF MICROENCAPSULATED EXTRA VIRGIN OLIVE OIL POWDER

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Abstract

Microencapsulation is the process that aims to increase the stability of food components (oil, pigments, aroma, etc.) during the processing and storage. Microencapsulation is used to convert extra virgin olive oil to powder form maintaining high oxidation stability, phenolic content and antioxidant capacity along the storage. In this study, the storage stability of microencapsulated extra virgin olive oil powder coated with maltodextrin and whey protein isolate as wall materials was investigated by evaluating microencapsulation efficiency, peroxide value, phenolic content and antioxidant activity during storage in aluminium polyethylene pouches at 25 °C and 50% relative humidity (RH) for 180 days. The results showed that microencapsulation efficiency (ME), phenolic content and antioxidant activity of microencapsulated extra virgin olive oil powder (MEVOP) during storage decreased 16%, 44.6% and 58.6%, respectively as compared to initial value, whereas the peroxide value of sample increased from 11.6 meq O_2 kg^{-1} oil to 35.6 meq O_2 kg^{-1} oil in the 180 days of storage. During storage, the degradation of phenolic content, antioxidant activity and the increase in the peroxide value of microencapsulated extra virgin olive oil were found to fit the first-order kinetic model. Although microencapsulation efficiency of samples changed slightly, phenolic content and antioxidant activity drastically reduced and peroxide value of samples increased sharply during the storage. This is possibly caused by small particle size and/or huge surface area of olive oil powder.

Keywords: microencapsulation, extra virgin olive oil, storage, oxidation.

Introduction

Extra virgin olive oil is obtained mechanically from ripe fruit of the olive tree without any chemical treatment. Extra virgin olive oil is an important vegetable oil with unique taste and odour which can be consumed naturally (Gögüş et al., 2009). Among the different categories of olive oil, the extra virgin olive oil is outstanding in gastronomic, nutritional, therapeutic and economic importance (Mendez, Falque, 2007). Studies conducted by other researchers attributed these benefits were due to its mono unsaturated fatty acid content (Fernández-Jarne et al., 2002; Martínez-González et al., 2002; Tripoli et al., 2005). However, recent research indicates that minor constituents appear to prevent several diseases. The phenolic fraction has importance regarding to antioxidant activities (Bendini et al., 2010) and the changes in the phenolic compounds over time could be an important quality control parameter of extra virgin olive oil.

The environmental conditions including oxygen, light, moisture and temperature make olive oil susceptible to oxidation due to high amount of monounsaturated fatty acid (oleic acid) content (Calvo et al., 2010; O'Brien, 2004). Oxidation is the main cause of olive oil quality deterioration and its reaction rate determines the shelf life of oil (Gomez-Alonso et al., 2007). Oxidation that occurs in edible oils relates to the loss of minor components and formation of new compounds, causing nutritional loss as well as the development of rancid and other off-flavours (Velasco et al., 2003; Sun-Waterhouse et al., 2011, Cicerale et al., 2013).

Storage conditions are considered as critical variables that affect the quality of olive oil and its shelf life which is attributed to lipid oxidation mechanism which leads to rancidity (Vacca et al., 2006). Because olive oil is produced in a limited period of time, but consumed throughout the year, it must be stored, and this storage period determines the commercial life of the olive oil (Hrncirik, Fritsche, 2005; Kiritsakis, Dugan, 1984; Zanoni et al., 2005). Many studies on oxidation of virgin olive oils were based on accelerated test measurements (Aparicio et al., 1999; Baldioli, 1996; Gutierrez et al., 2002; Krichene et al., 2010). In these studies, good correlations between changes in various components and stability were found.

Microencapsulation can be used to protect fragrances or other active agents of olive oil from oxidation caused by heat, light, moisture, from contact with other substances over a long shelf life, to prevent evaporation of volatile compounds (Ghosh, 2006; Soest, 2007). Microencapsulation is a process of coating individual particles or droplets with a continuous film to produce capsules in a micrometer to milimeter in size (Tyagi et al., 2011). Microencapsulation process provides protection of reactive substances from the environment, converting liquid active component into a dry solid system and separation incompatible components for functional reasons. The surface oil content or microencapsulation efficiency of microencapsulated oil powder determines the storage stability. While converting olive oil to powder form, the surface area of olive oil expands that makes it more sensitive to oxidation.

The aim of this study was to evaluate the storage stability of microencapsulated extra virgin olive oil powder by analysing microencapsulation efficiency, peroxide value, total phenol content and antioxidant activity during 180 days storage.

Materials and Methods

Material

Extra virgin olive oil (ρ=910 kg m^{-3}, μ=0.083 Pa·s at 20 ºC) was purchased from a local grocery store in

Turkey. The whey protein isolate (WPI) supplied by Ak Gıda San. Tic. A.Ş. and maltodextrin (MD, DE19) provided by Qinhuangdao Starch Co Ltd., were used as wall materials in this study. Tween 20 (Merck, Darmstadt, Germany) was used to stabilize the olive oil-water emulsion.

Preparation of olive oil in water emulsion

Maltodextrin (128.8 g) was dissolved in water (200 g) and kept in the dark for one night. Whey protein isolate (9.8 g) was mixed with water (100 g) using mechanical homogenizer (Ultra Turrax T25) at 0.167 kHz for 60 s. Tween 20 was added to olive oil in concentration 1% (w/w) as a stabilizer. Mixture of olive oil and Tween 20 was poured drop wise into the WPI solution using the mechanical homogenizer at 0.167 kHz for 60 s for pre-mixing. In order to obtain stable olive oil in water emulsions (o/w), WPI, olive oil and Tween 20 containing solution was mixed with MD solution and the whole mixture were homogenized using a mechanical homogenizer (Ultra Turrax T25) at 0.291 kHz for 300 s. Prepared emulsion contains 92% (db) maltodextrin and 7% (db) whey protein isolate as wall material. The total solids concentration of feeding emulsion is 40%. The olive oil concentration of the emulsion is 30% in dry basis.

Microencapsulation of olive oil by spray drying and storage of powder

The prepared emulsions were dried in a laboratory scale spray drier (Buchi, B-290, Switzerland) equipment with 0.7 mm diameter nozzle. The spray dryer conditions; the inlet air temperature 200°C, pump rate 22%, aspiration ratio 0.0067 $m^3 s^{-1}$ and air flow rate 0.00013 $m^3 s^{-1}$ were kept constant during the experiments The feeding emulsions at 25 °C were fed to spray dryer and the obtained powder was packed in hermetically sealed aluminium laminated polyethylene (ALPE) pouches.

Microencapsulated olive oil powders were stored at 25 °C and 50% RH for 180 days. In the first 90 days of storage period, the analyses were be carried out in 15 days periods and then 30 days period was followed.

Total oil content

Total oil content of samples was determined according to method of Folch et al. (1957) with some modification. The microencapsulated extra virgin olive oil powder (4 g) was put into an extraction thimble that contained chloroform/methanol (80 mL; 3 : 2 v/v). The obtained mixture was homogenized at 0.0026 kHz for 900 s. Then water (16 mL) was added, and the mixture was shaken vigorously to facilitate the transfer of oil into the chloroform and other products into the water-methanol layer. The chloroform layer was then separated via a separation funnel and collected. The extraction steps were repeated two times. The obtained chloroform layers were combined and evaporated using a rotary evaporator at 65 °C, for 20–30 min. Then the trace amount of chloroform was evaporated in drying oven (at 102 °C temperature for 4 hours). After oven drying remaining is the amount of total oil of MEVOP.

Surface oil content

To determine surface oil, 5 g encapsulated beads were weighed into flaks and 25 mL petroleum ether was added. The mixture was extracted for 5min at room temperature in the dark. The mixture was filtered and petroleum ether was evaporated. Then trace amount of petroleum ether was evaporated in drying oven (at 102 °C temperature for 4 hours) (Folch et al., 1957). After oven drying remaining is the amount of surface oil of MEVOP.

Microencapsulation efficiency (ME)

Microencapsulation efficiency of extra virgin olive oil powder was calculated using the following formula:

$$\mathrm{ME} = \frac{\text{Total oil content} - \text{Surface oil content}}{\text{Total oil content}} \times 100 \quad (1)$$

Peroxide value

The peroxide value of extracted olive oil was expressed as peroxide milliequivalent per kg oil. Extracted olive oil (0.5 g) was dissolved in acetic acid-chloroform solution (3 mL, 3 : 2 v/v). After saturated potassium iodide KI solution was added, the mixture was left to stand for 1 min with occasional shaking. An aliquot (3 mL) of Milli-Q water was added. The mixture was titrated with 0.01 N standardized sodium thiosulphate solution until the yellow iodine colour just disappeared. Starch indicator solution (0.2 mL, 10 kg m^{-3}) was added. The titration continued until the blue colour derived from the iodine just disappeared. A blank sample as reagent control was set up and carried through all the steps (AOCS, 1998).

Phenol Extraction from Olive Oil and Total Phenol Analysis

For phenolic components analysis, firstly the cartridge (Maxi-Clean™ SPE 300mg Hi-Cap C18 cartridge) was conditioned by using dietylether / n-hexane (98 : 2 ratio). Then, 5 g extracted olive oil from microencapsules was weighed and 5 mL hexane was added. This mixture was filtered through the cartridge and cartridge was washed by using 2 mL hexane. Finally the 1.5 mL methanol was filtered from cartridge and the phenolic components were extracted from olive oil (Pellegrini et al., 2001). From the last extract, 50 µL sample was taken and 250 µL Folin-Ciocalteau reactive was added. After 5 min of holdingin dark at room temperature, 750 µL 7% Na_2CO_3 solution was added and the mixture was completed to 5mL by using distilled water. After 2 hours waited at room temperature in dark, the absorbance of the extracts was determined at 765 nm by using spectrometer. All these steps were done for blank. The standard curve was plotted by using caffeic acid as a standard (Singleton, Rossi, 1965; Singleton et al., 1999).

Total Antioxidant Activity Measurement-ABTS Method

Firstly ABTS radical was prepared as, 7mM ABTS water solutions and 2.45 mM potassium persulphate (1 : 0.5 ratio, v : v) were mixed and diluted by ethanol till the absorbance (730 nm) was 0.70. Then, 1 mL

ABTS radical was added into 20μL sample (0.1 g oil 1 mL chloroform). The absorbance was read at 730 nm. Finally, standard curve was drawn by using Trolox (1 mM to 5 mM). The results are given as Trolox equivalent (Pellegrini et al., 2001).

Kinetic analysis of the given quality parameters during storage

The obtained data were subjected to the kinetic modelling in order to determine the degradation kinetics of some quality characteristics (peroxide value, phenolic content and antioxidant activity) during storage. Reaction rate and the relevant coefficients were determined with Eq. (2) using SPSS version 13.0 Windows program (SPSS Inc., Chicago, IL).

$$-\frac{dC}{dt} = k \times C \qquad (2)$$

where: C is the given quality parameter values, t is time and the k is the kinetic constant.

Results and Discussion

The amount of surface oil content of encapsulated product is important for ensuring the storage stability (Anandaraman, Reineccius, 1987). The microencapsulated products, which have high surface oil content, could be more sensitive to oxidation. The change in total oil content, surface oil content and microencapsulation efficiency of microencapsulated extra virgin olive oil powder during storage were given in Table 1. Along the storage of microencapsulated extra virgin olive oil powder, surface oil content increased without a change in total oil content. Therefore, microencapsulation efficiency decreased with increasing storage time. This indicates that, microcapsules were broken and oil in the capsules moves to the surface.

Peroxide value (PV) is an indicator of the oxidation level of oils and fats during processing and storage (Sun-Waterhouse et al., 2011). In general, the PVs of oils increased when the storage proceeded. Initial peroxide value (11.59 meq O_2 kg^{-1} oil) of microencapsulated extra virgin olive oil powder is coherent with the study of Sun-Waterhouse et al. (2011). Peroxide value of our samples increased approximately three times during the storage period of 180 days, comparing to the initial peroxide value (Table 2). This circumstance was possibly related with high oil content at the surface and large surface area of samples. Surface oil content increased during the storage, so that more oil was exposed to oxidation.

The changes in total phenolic content and antioxidant activity of microencapsulated extra virgin olive oil powder during the storage period were given in Table 2.

Olive oil matrix (Fakourelis et al., 1987; Gutfinger 1981; Tripoli et al., 2005), storage temperature and period, oil extraction method and encapsulation materials (Naczk, Shahidi, 2004; Perez-Jimenez, Saura-Calixto, 2006; Reichardt, 1988; Yang et al., 2007) are the factors affecting the total phenolic content. Total phenolic content of microencapsulated extra virgin olive oil powder decreased 44.6% during storage as compared to initial value. Sharp decrease in the total phenol content of extra virgin olive oil was observed in the study of Mendez and Falque (2007), during storage.

Table 1

Total and surface oil content and microencapsulation efficiency values of MEVOP during storage

Time, days	Total oil, %	Surface oil, %	ME,%
0	27.28±0.22	3.63±0.30	86.69±1.91
15	26.45±0.40	4.19±0.11	84.15±1.91
30	27.05±0.41	4.70±0.56	82.63±1.76
45	27.54±0.47	5.10±0.25	81.45±1.18
60	28.02±0.34	5.36±0.71	80.84±0.82
75	27.34±0.27	5.44±0.13	80.12±1.09
90	28.39±0.30	5.73±0.14	79.83±0.70
120	27.11±0.50	6.68±0.68	75.35±2.41
150	27.97±0.88	8.15±0.54	73.49±1.59
180	28.39±0.50	6.77±0.99	72.80±3.46

Table 2

Peroxide, total phenolic content and antioxidant activity values of MEVOP during storage

Time, days	Peroxide value, meq O_2 kg^{-1} oil	Antioxidant activity, ppm	Total phenolic content, ppm
0	11.59±0.75	681.54±5.44	106.69±0.47
15	13.09±0.22	599.07±1.70	97.26±1.40
30	21.27±1.56	583.56±3.94	95.56±0.43
45	21.89±0.47	547.64±5.71	93.85±0.57
60	24.01±0.31	502.65±1.24	90.81±0.08
75	27.42±0.32	393.26±6.81	87.59±0.60
90	28.58±0.31	357.32±4.40	82.27±1.46
120	30.37±0.53	302.82±2.01	77.95±1.16
150	31.05±0.76	293.54±1.77	59.32±2.79
180	35.56±1.73	282.50±1.35	59.13±3.15

Extra virgin olive oil is the only common food rich in a potent antioxidant called hydroxytyrosol. Antioxidants in the olive oil absorb free radicals and appear to have a positive impact on cardiovascular and cancer ailments, as attributed to the Mediterranean diet. For this reason, antioxidant capacity of olive oil must be protected during the conversion to the powder form and the storage period. Antioxidant activity decreased slightly in the early months of storage, but after the second months of storage decrease in the antioxidant activity has accelerated.

Table 3

Estimated reaction rate constants and R^2 values

	k day^{-1}	R^2
Peroxide value	0.004	0.818
Total phenolic content	-0.003	0.945
Antioxidant activity	-0.006	0.952

During storage, the degradation kinetics of phenolic compounds and antioxidant activity and the oxidation kinetics using peroxide value of microencapsulated extra virgin olive oil powder were determined using the first-order reaction kinetic model (Eq 2). Estimated reaction rate constants and R^2 values were given in Table 3.

Conclusions

The present work showed that microencapsulation efficiency of the extra virgin olive oil powder decreased with respect to storage time. Peroxide value of sample increased from 11.6 meq O_2 kg^{-1} oil to 35.6 meq O_2 kg^{-1} oil at the end of storage period. Natural or synthetic antioxidants could be used to prevent lipid oxidation. During 180 days of storage period at 25 °C, antioxidant activity and total phenolic content values of microencapsulated extra virgin olive oil powder decreased 58.6% and 44.6%, respectively. First order reaction kinetic model was found to be suitable to describe the degradation kinetics for total phenol content and antioxidant activity of samples and the increase in peroxide value of samples.

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References

1. Anandaraman S., Reineccius G.A. (1987) Analysis of encapsulated orange peel oil. *Perfum-Flavor*, Vol. 12, p. 33–39.
2. Aparicio R., Roda L., Albi M.A., Gutierrez F. (1999) Effect of various compounds on virgin olive oil stability measured by Rancimat. *Journal of Agricultural and Food Chemistry*, Vol. 47, p. 4150–4155.
3. Baldioli M., Servili M., Perretti G., Montedoro G. F. (1996) Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. *Journal of the American Oil Chemists' Society*, Vol. 73, p. 1589–1593.
4. Bendini A., Cerretani L., Vecchi S., Carrasco Pancorbo A., Lercker G. (2006) Protective effects of extra virgin olive oil phenolics on oxidative stability in the presence or absence of copper ions. *Journal of Agricultural and Food Chemistry*, Vol. 54, p. 4880–4887.
5. Calvo P., Hernandez T., Lozano Mand Gonzalez-Gomez D. (2010) Microencapsulation of extra-virgin olive oil by spray-drying: Influence of Wall material and olive quality. *European Journal of Lipid Science and Technology*, Vol. 112, p. 852–858.
6. Cicerale S., Conlan X.A., Barnett N.W., Keast R.S.J. (2013) Storage of extra virgin olive oil and its effect on the biological activity and concentration of oleocanthal. *Food Research International*, Vol. 50, p. 597–602.
7. Fakourelis N., Lee E. C., Min D. B. (1987) Effects of chlorophyll and beta-carotene on the oxidation stability of olive oil. *Journal of Food Science*, Vol. 52, p. 234–235.
8. Fernández-Jarne E., Martínez-Losa E., Prado-Santamaría M., Brugarolas-Brufau C., Serrano-Martínez M., Martínez-González M. Á (2002) Risk of first non-fatal myocardial infarction negatively associated with olive oil consumption: a case control study in Spain. *International Journal of Epidemiology*, Vol. 31, p. 474–480.
9. Folch J., Lees M., Sloane Stanley G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, Vol. 226, p. 497–509.
10. Ghosh S.K. (2006) *Functional coatings and microencapsulation: a general perspective*, in: Swapan Kumar Ghosh (Ed.) Functional Coatings, Wiley-VCHVerlag GmbH & Co. KGaA.
11. Gomez-Alonso S., Manebo-Campos V., Desamparados Salvador M., Fregapane G. (2007) Evolution of major and minor components and oxidation indices of virgin olive oil during 21months storage at room temperature. *Food Chemistry*, Vol. 100, p. 36–42.
12. Göğüş F., Özkaya M.T., Ötleş S. (2009) *Zeytinyağı,* Ankara: Eflatun Yayınevi.
13. Gutierrez F., Villafranca M. J., Castellano J.M. (2002) Changes in the main components and quality indices of virgin olive oil during oxidation. *Journal of the American Oil Chemists' Society*, Vol. 79, p. 669–676.
14. Gutfinger T. (1981). Polyphenols in olive oil. *Journal of the American Oil Chemists' Society*, Vol. 58, p. 966–968.
15. Hrncirik K., Fritsche S. (2005) Relation between the endogenous antioxidant system and the quality of extra virgin olive oil under accelerated storage conditions. *Journal of Agricultural and Food Chemistry*, Vol. *53*, p. 2103–2110.
16. Kiritsakis A. K., Dugan L. R. (1984) Effect of selected storage conditions and packaging materials on olive oil quality. *Journal of the American Oil Chemists Society*, Vol. 61, p. 1868–1870.
17. Krichene D., Allalout A., Mancebo-Campos V., Salvador M. D., Zarrouk M., Fregapane G. (2010) Stability of virgin olive oil and behaviour of its natural antioxidants under medium temperature accelerated storage conditions. *Food Chemistry*, Vol. 121, p. 171–177.
18. Martínez-González M. A., Fernández-Jarne E., Serrano-Martínez M., Marti A., Martinez J. A., Martín-Moreno J. M. (2002). Mediterranean diet and reduction in the risk of a first acute myocardial infarction: an operational healthy dietary score. *European Journal of Nutrition*, Vol. 41, p. 153–60.
19. Mendez A.I., Falque E. (2007) Effect of storage time and container type on quality of extra virgin olive oil. *Food Control*, Vol. 18, p. 521–529.
20. Naczk M., Shahidi F. (2004) Extraction and analysis of phenolics in food. *Journal of ChromatographyA*, Vol. 1054, p. 95–111.
21. O'Brien R. D. (2004) *Fats and oils formulating and processing for applications* (2nd ed.). CRC Press.
22. Perez-Jimenez J., Saura-Calixto F. (2006) Effect of solvent and certain food constituents on different

antioxidant capacity assays. *Food Research International*, Vol. 39, p. 791–800.
23. Pellegrini N., Serafini M., Colombi B., Del Rio D., Salvatore S., Bianchi M., Brighenti F. (2001) Total Antioxidant Capacity of Plant Foods, Beverages and Oils Consumed in Italy Assessed by Three Different In Vitro Assays, *American Society for Nutritional Sciences*, p. 2812–2819.
24. Reichardt C. (1988) *Solvents and solvent effects in organic chemistry*. New York, USA: VCH Publishers.
25. Singleton V. L., Rossi J. A. (1965) Colorimetry of Total Phenolics with Phosphomolybdic-phosphotungstic Asit Reagents, *American Journal of Enology and Viticulture*, V. 16, p. 144–158.
26. Singleton V. L., Orthofer R., Lamuela-Raventos R. M. (1999) Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteau Reagent, *In Methods in Enzymology*, Vol. 299, p. 152–178.
27. Soest J.J.G. (2007) Encapsulation of fragrances and flavours: a way to control odour and aroma in consumer products. **In:** R.G. Berger (Ed.) *Flavours and Fragrances – Chemistry, Bioprocessing and Sustainability*, Springer, Germany, p. 439.
28. Sun-Waterhouse D., Zhou J., Miskelly G. M., Wibisono R., Wadhwaa S.S. (2010) Stability of encapsulated olive oil in the presence of caffeic acid. *Food Chemistry*, Vol. 126, p. 1049–1056.
29. Tripoli E., Giammanco M., Tabacchi G., Di Majo D., Giammanco S., La Guardia M. (2005) The phenolic compounds of olive oil: Structure, biological activity and beneficial effects on human health. *Nutrition Research Reviews*, Vol.18, p. 98–112.
30. Tyagi V.V., Kaushik S.C., Tyagi S.K., Akiyama T. (2011) Development of phase change materials based microencapsulated technology for buildings: a review. *Renewable and Sustainable Energy Reviews*, Vol. 15, p. 1373–1391.
31. Vacca V., Del Caro A., Poiana M., Piga A. (2006) Effect of storage period and exposure conditions on the quality of Bosana extra-virgin olive oil. *Journal of Food Quality*, Vol. 29(2), p. 139–150.
32. Velasco J., Dobarganes C. (2002) Oxidative stability of virgin olive oil. *European Journal of Lipid Science and Technology*, Vol. 104, p. 661–676.
33. Yang J., Paulino R., Janke-Stedronsky S., Abawi F. (2007) Free-radical scavenging activity and total phenols of noni (*Morinda citrifolia* L.) juice and powder in processing and storage. *Food Chemistry*, Vol. 102, p. 302–308
34. Zanoni B., Bertucciolo Rovellini P., Marotta F., Mattei A. (2005) A preliminary approach to predictive modelling of extra virgin olive oil stability. *Journal of the Science of Food and Agriculture*, Vol. 85, p. 1492–1498.

LEAF VEGETABLES AS SOURCE OF PHYTOCHEMICALS

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Abstract

Different vegetables are considered as sources of human health promoting components. Leaf vegetables are widely used in human diet, they are low in calories and fat, but high in dietary fibres, content of minerals, such as iron and calcium and very high in phytochemicals such as vitamin C, carotenoids, lutein and others. On the one hand phytochemicals are a plant's way of protecting itself. They help shield tender buds and sprouts from predators, the toxic elements, and pollution. On the other hand they have beneficial effect on human health. Genotype along with growing and management conditions can affect the content and the composition of phytochemicals in plants. The aim of research is to determine the content of phytochemicals (chlorophyll a and b, total carotenoids, lutein, vitamin C) in leaf vegetables. The lettuce (*Lactuca sativa*), spinach (*Spinacia oleracea*), green and violet basil (*Ocinum basilicum*), mustard (*Brassica juncea*) and common nettles young sprouts (*Urtica dioica*) were grown in greenhouse or in an open field. The content of plant pigments were determined spectrophotometrically, the titrimetric method was used for determination of vitamin C content.It was found out that young nettles sprouts and spinach are the good source of chlorophyll, basil (green and violet) contains more than 0.3 mg 100 g^{-1} total carotenoids, but for correcting the deficiency of vitamin C it is useful to use mustard, young nettles sprouts as well as leaves of basil.

Keywords: leaf vegetables, phytochemicals.

Introduction

Vegetables play an important role in human diets, as they support the normal functioning of the different body systems. They provide our cells with vitamins, minerals, fibber, essential oils and phytonutrients. Vegetables contain low amounts of fat and calories (Banerjee et al., 2012).

Leaf vegetables came from very wide variety of plants and they are plants with edible leaves. Each of us knows lettuce and spinach, as well as mustard, but also early springtime nettles are valuable source of vitamin C. Green leafy vegetables are popularly used for food, being a rich source of β-carotene, ascorbic acid, minerals and dietary fiber. One of the most popular vegetable is lettuce. Lettuce is cultivated worldwide, and is one the most consumed green leafy vegetables in the raw form for its taste and high nutritive value, being regarded as an important source of phytochemicals, including carotenoids, in the diet (Chang et al., 2013).

Nettles are known as widely used as food in early spring. Stinging nettle young leaves added to soups or salads and dried for winter use (Hojnik et al., 2007).

Spinach contains number of different phytochemicals which have a high nutritional value. It is a rich source of antioxidants, especially high in vitamins A, C, E, K, β-carotene, selenium and omega-3 fatty acids as well as rich in lutein and zeaxanthin. Spinach contains microelements such as potassium, calcium, magnesium, manganese, zinc and others. Spinach extracts have several beneficial effects, such as anticancer, antiaging and protecting of central nervous system (Lomnitski et al., 2003).

Sweet basil is a popular herbal crop grown for fresh or dry leaf or essential oils. The leaves of basil are a good source of vitamin P and ascorbic acid, contain microelements zinc and manganese. It have been found, that basil contains high amount of xantophyll (Calucci et al., 2003).

Mustard, like spinach, contains many phyto-nutrients that have health promotional and disease prevention properties. Mustards are very low in calories and fats, but are supposed to be one of the highest among leafy vegetables, which provide vitamin K. It is a rich source of flavonoids, carotenes, lutein and zea-xanthin. Moreover fresh mustard leaves are an excellent source of vitamin C, several essential minerals such as calcium, iron, magnesium, potassium, zinc, selenium, and manganese (Banerjee et al., 2012).

Knowledge on different phytochemical presence and its content in vegetables is important for an appropriate choice of products according to the physiological needs. Phytochemicals are a large group of plant derived compounds, the plant`s way of protecting itself. In addition they appear to have significant physiological effects in the human body. There are more than thousand known phytochemicals. They are acting as antioxidants, stimulating enzymes, interfering with DNA replication, destroying bacteria, as well as they seem to act to reduce the onset of diseases such as cancer and heart diseases (Krishnaswamy, Raghuramulu, 1998).

The leaves of plants contain number of colour pigments generally falling into two categories chlorophylls and carotenoids. Chlorophylls a and b are the pigments that make plants look green. Chlorophyll is often referred to as the green blood of plants due to the identical molecular structure with hemoglobin with only difference in centre atom (iron or magnesium). This similarity makes chlorophyll so important to our health, it improve digestive, immune and detoxification systems of human body (Kopsell et al., 2005).

Carotenoids are natural pigments that provide the natural yellow, orange or red colours of vegetables and fruits. These colours are a result of the presence of

conjugated double bonds, also providing carotenoids with antioxidant properties. Therefore the interest of carotenoids, which are found in vegetables, is not only due to their provitamin A activity but also to their antioxidant action by scavenging oxygen radicals and reducing oxidative stress in the organism (Rao, Honglei, 2002). They are thought to provide health benefits in decreasing the risk of disease, particularly certain cancers and eye disease. Besides beta-carotene, which is the best-known carotenoid, this group includes alpha-carotene, lycopene, lutein, zeaxanthin and cryptoxanthin. It is known that the largest contribution of vitamin A intake comes from the provitamin A carotenoids in plant food, which may contribute up to 82% of the total vitamin A intake (Van den Berg et al., 2000), therefore the green leafy vegetables are good sources of carotenoids. Carotenoids serve many functions in plants including light harvesting, structure stabilization and excess energy dissipation, they protect plants from free radicals, such as triplet excited chlorophyll (^{3}Chl) and singlet oxygen (^{1}O), produced when light intensity exceeds photosynthetic capacity (Mortensen et al., 2001).

Vitamin C (ascorbic acid) is a major antioxidant in the human body. This water-soluble vitamin is involved in many biological processes. Its biological significance is based on the ability to participate in enzymatic and hydroxylation reactions, participates in the oxidation - reduction processes. Vitamin C promotes the absorption of microelements iron and copper, participates in trace element metabolism, and protects cells from damage caused by free radicals, toxins and environmental pollution. It is directly related to protein exchange. Different fruit, vegetables and berries are rich in vitamin C, especially red pepper, black currents and the content of vitamin C may vary depending on the environmental and stress factors such as light intensity, temperature, humidity conditions, air pollution, etc. (Singh et al., 2012).

The aim of study was to determine the content of phytochemicals in leaf vegetables growing and harvested in Latvia.

Materials and Methods

Investigations were carried out at the Latvian University of Agriculture, Institute of Soil and Plant Sciences and Pure Horticultural Research Centre.

Plant material

Six types of leafy vegetables were selected for analysis. There were lettuce (*Lactuca sativa*) variety 'Rīga', spinach (*Spinacia oleracea*) variety "Matador", green basil (*Ocinum basilicum*) variety 'Genovese', violet basil (*Ocinum basilicum*) variety 'Aromat', leaf mustard (*Brassica juncea*) and common nettles (*Urtica dioica*). Seedlings of lettuce, spinach, basil and mustard were grown in 2 L pots with peat substratum in the polycarbonate greenhouse without artificial illumination. Temperature in green house was maintained between 8 °C (min at night) and 30 °C (max at day time). Vegetation time 2 months.

Nettles were grown in an open field. All samples were harvested in one day, when shoots was 10–15 cm long. Samples were cooled, packed and transported to the laboratory ensuring low temperature. In the same day the samples for biochemical analysis were prepared.

Phytochemical extraction and determination

All the reagents used were with the analytical grade from Sigma Aldrich, Germany. UV spectrophotometer UV-1800 (Shimadzu Corporation, Japan) was used for the absorbance measurements.

The chlorophylls, carotenoides and xantophylls were extracted with ethanol according to the methods described by Kukric et al. (2012) and Chang et al. (2013) with some modifications. For extraction a representative portion of sample (0.1±0.001 g) (mass) was accurately weighted, grinded and quantitavely transmit in a glass test tube. Then ethanol was added till 5 mL to it and the test tubes were held in dark for 15 min with occasional shaking at room temperature and finally centrifuged.

The chlorophylls, carotenoids and xantophylls content were analyzed spectrophotometrically by absorption measurements (A) at 350 to 700 nm with 1 nm interval and calculated according to the following equations:

$$\text{Chlorophyll a (mg g}^{-1}) = \frac{13.7A_{665} - 5.76A_{649}}{mass \cdot 200} \quad (1)$$

$$\text{Chlorophyll b (mg g}^{-1}) = \frac{25.8A_{649} - 7.6A_{665}}{mass \cdot 200} \quad (2)$$

$$\text{Carotenoids (mg g}^{-1}) = \frac{4.7A_{440} - 0.263\ c_{chla+chlb}}{mass \cdot 200} \quad (3)$$

$$\text{Xantophylls (lutein) (mg g}^{-1}) = \frac{11.51A_{480} - 20.61A_{495}}{mass \cdot 200} \quad (4)$$

The content of vitamin C was determined titrimetrically using 2.6- dichlorphenolindophenol (AOAC, 1990). For determination 2±0.001 g of sample was accurately weighted, grinded in porcelain mortar, than quantitatively transfer in 100 mL tubes, added 50 mL of 1% HCl and 5% HPO_3 mixture (v : v=1 : 1) and mix thoroughly. After 30 minutes solution was filtered through a filter paper No. 89th. For determination 10 mL (V_{anal}) of filtrate was titrated with 0.0005 molar solution of 2.6 dichlorphenolindophenol (V_{titr}).

The content of vitamin C was calculating according to the equation:

$$\text{Vitamin C (mg 100 g}^{-1}) = \frac{V_{titr} \cdot 0.044 \cdot V_{total} \cdot 100}{V_{anal} \cdot mass} \quad (5)$$

Experiments were done in 6 replicates and obtained data was elaborated by ANOVA.

Results and Discussion

Genotype along with growing conditions can affect the content and the composition of phytochemicals in

plant. In the present work it was evaluated the content of chlorophylls, total carotenoids, xantophylls (lutein) and vitamin C in the fresh leaf vegetables grown in Latvia. The content of chlorophylls determined in the analyzed samples is characterized in Table 1.

Table 1

Content of chlorophylls in leaf vegetables

Leafy vegetable	Chlorophyll a, mg g^{-1}	Chlorophyll b, mg g^{-1}	Total chlorophylls, mg g^{-1}
Lettuce	0.144±0.012	0.044 ±0.016	0.188±0.015
Spinach	1.043±0.069	0.461 ±0.035	1.503±0.102
Green basil	1.101±0.15	0.330±0.17	1.421±0.05
Violet basil	1.079±0.098	0.360±0.054	1.439±0.152
Mustard	0.687±0.105	0.155±0.026	0.824±0.129
Nettles	1.534±0.26	0.537±0.032	2.070±0.288

Obtained results showed that the richest source of chlorophyll is leaves of young nettle sprouts – the content of total chlorophylls was 10 times higher comparing with lettuce and for 2.5 times higher comparing with leafy mustard. The obtained results confirm the results mentioned in scientific literature (Banerjee et al., 2012, Kukric et al., 2012, Cruz et al, 2012). Hojnik et al. (2007) reported that nettle leaves contain significant amount of chlorophylls till 2.5 mg g^{-1} fresh weight. Beside other varieties of sweet basil, Kosell et al. (2005) characterized green basil variety Genovese. They reported that content of chlorophyll a is in range from 0.758 till 1.794 mg g^{-1}, chlorophyll b – from 0.149 till 0.344 mg g^{-1} depending on growing conditions. These results also confirm with our investigations. Taking into account the benefits of chlorophyll it is recommended to expand the young nettle leaves use as food ingredients, especially in springtime, when the intake of biologically active substances is difficult.

The results of research (Fig.1) showed significantly higher content of carotenoids and xantophylls in young nettle leaves comparing with other analyzed leave vegetables. The content of carotenoids in leaves of nettles was 0.834±0.072 mg g^{-1}, comparatively in leaves of lettuce the content of carotenoids was only 0.072±0.009 mg g^{-1}. For comparison, Kukric et al. (2012) established that content of carotenoids in leaves of young nettles grown in Serbia ranged from 0.216 to 0.323 mg g^{-1}. The content of carotenoids in analyzed samples of spinach, mustard and basil leaves ranged from 0.255 to 0.398 mg g^{-1}.

Similar tendency was observed analyzing the content of lutein. The leave vegetables which are rich in chlorophyll are also a good source of xantophylls. In the current research the content of lutein was determined in six leave vegetables and they can be arranged as follows (starting from vegetable with less lutein content): lettuce<spinach<mustard<basil violet<basil green<nettle.

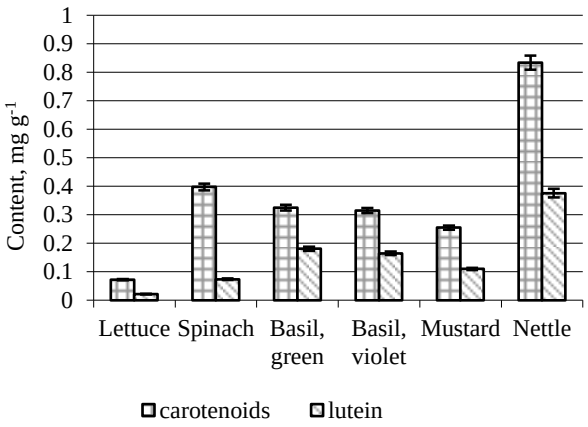

Figure 1. Content of total carotenoids and lutein in leaf vegetables

The obtained experimental results are slightly higher than reported in literature. Krumbein et al. (2005) noted that content of lutein in leaf mustard range from 0.034 till 0.089 mg g^{-1}, but Kopsell et al. (2005) reported that lutein content in sweet basil variety Genovese range from 0.04 till 0.07 mg g^{-1} depending on growing conditions.

Ascorbic acid is considered as one of the most important water soluble vitamins with different important biological functions. Significant amount of vitamin C was determined in mustard and leaves of young nettles sprouts (Fig.2).

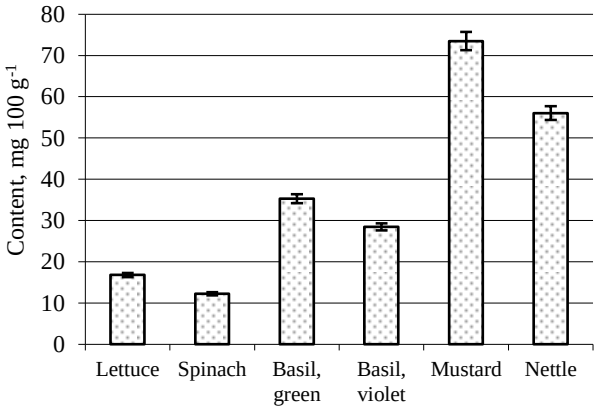

Figure 2. Content of ascorbic acid in leaf vegetables

Ascorbic acid content in leaves can be over a wide range depending on cultivar as well as due to the influence of different factors including environmental and growing conditions. Therefore data in literature regarding ascorbic acid content will be very different. The obtained results confirm with investigations of Staugaitis and Viškelis (2001) done in Lithuania as well as Krumbein et al. (2005) reported that leaves of mustard had relatively high content of ascorbic acid till 89.1 mg 100 g^{-1}. In comparison Banerjee et al. (2012) studies found that content of ascorbic acid in

different mustard varieties range between 12.1 till 19.6 mg 100 g^{-1}.

Summarizing the obtained results the conclusion can be drawn that young nettle sprouts and leaves of mustard are very suitable as sources of human health promoting components due to high content of chlorophyll, carotenoids, lutein and ascorbic acid. For example, increased intake of lutein and β-carotene has been associated with decreased risks of cancer and other chronic deceases, especially age-related eyes diseases (Sommerburg et al., 1999), but sufficient amount of vitamin C ensuring a good health due to it antioxidative properties (Martin, 2003). The Regulations No. 988 of Cabinet of Ministers of Latvia, 2009 September 1 determined that the recommended daily intake for adults is 80 mg of vitamin C, but it will achieve 150 mg (Levine et al., 1999). Unfortunately it should be noted that lettuce which is one of the most popular and consumed green leafy vegetables in the raw form especially in spring time contains less phytochemicals comparing with previous mentioned leafy vegetables. Therefore it will be recommended to popularize and increase the consumption of not so traditional leafy vegetables such as leaf mustard and young sprouts of nettles.

Conclusions

The content of total chlorophylls in leaves of young nettle sprouts was 10 times higher comparing with lettuce and for 2.5 times higher comparing with leafy mustard.

Basil (green and violet) contains more than 0.3 mg 100 g^{-1} of total carotenoids, but the richest source of carotenoids and lutein is young sprouts of nettles.

For correcting the deficiency of vitamin C it is useful to use leaves of mustard, young nettles sprouts and basil.

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References

1. AOAC (1990) Official methods of analysis of the Association of Official Analytical Chemists**.** Fifteen edition. Arlington VA, Association of Official Analytical Chemists, p. 1058–1059.
2. Banerjee A., Datta J.K., Mondal N.K. (2012) Biochemical changes in leaves of mustard under the influence of different fertilizers and cycocel. *Journal of Agricultural Technology*, Vol. 8(4), p.1397–1411.
3. Calucci L., Pinzino C., Zandomeneghi M., Capocchi A., Ghiringhelli S., Saviozzi F., Tozzi S., Galleschi I. (2003) Effects of irradiation on the free radical and antioxidant contents in nine aromatic herbs and species. *Journal of Agricultural and Food Science*, Vol. 51 (4), p.927–934.
4. Chang S.K., Nagendra Prased K., Amin I. (2013) Carotenoids retention in leafy vegetables based on cooking methods. *International Food Research Journal*, Vol. 20 (1), p.457–465.
5. Cruz R., Baptista P., Cunha S., Pereira J.A., Casal S. (2012) Carotenoids of Lettuce (Lactuca sativaL.) grown on Soil Enriched with Spent Coffee Grounds. *Molecules*, No.17, p. 1535–1547.
6. Hojnik M., Škerget M., Knez Ž (2007) Isolation of chlorophylls from stinging nettle (*Urticadioica* L.). *Separation and Purification Technology*, Vol. 57, Issue 1, p. 37–46.
7. Kopsell D.A., Kopsell D.E., Curran-Celentano J. (2005) Carotenoid and chlorophyll pigments in sweet basil grown in the field and greenhouse. *HortScience*, Vol. 40 (5), p. 1230–1233.
8. Krishnaswamy K., Raghuramulu N. (1998) Bioactive phytochemicals with emphasis on dietary practices. *Indian Journal of Medical Research*, No. 108, p. 167–181
9. Krumbein A., Schonhof I., Schreiner M. (2005) Composition and content of phytochemicals (glucosinolates, carotenoids and chlorophylls) and ascorbic acid in selected Brassica species (B.juncea, B.rapa subsp. nipposinica var. chinoleifera, B.rapa subsp. chinesis and B.rapa subsp. rapa). *Journal of Applied Botany and Food Quality*, No. 79 (3), p. 168–174.
10. Kukric Z.Z., Topalic-Trivunovic L.N., Kukavica B.M., Matoš S.B., Pavičic S.S., Boroja M.M., Savic A.V. (2012) Characterization of antioxidant and microbial activities of nettle leaves (*Urtica dioica L.*). *Acta Periodica Technologica*, Issue 43, p. 257–272.
11. Levine M., Rumsey S.C., Daruwala R., Park J.B., Wang Y.H. (1999). Criteria and recommendations for vitamin C intake. *The Journal of the American Medical Association*. Vol. 281, p. 1415–1423.
12. Lomnitski L., Bergman M., Nyska A., Ben-Shaul V., Grossman S. (2003) Composition, efficiency and safety of spinach extracts. *Nutrition and cancer*, Vol. 46 (2), p. 222–231.
13. Martin A. (2003) Antioxidant vitamins E and C and risk of Alzheimer`s disease. *Nutrition Reviews*, No. 61, p. 69–79.
14. Mortensen A., Skibsted L.H., Truscott T.G. (2001) The interaction of dietary carotenoids with radical species. *Archives of Biochemistry and Biophysics*, Vol. 385, p. 13–19.
15. Rao A.V., Honglei S. (2002) Effect of low dose lycopene intake on lycopene bioavailability and oxidative stress. *Nutrition Research*, Vol. 10, p. 1125–1131.
16. Singh D.P., Beloy J., McInerney J.K., Day L. (2012) Impact of boron, calcium and genetic factors on vitamin C, carotenoids, phenolic acids, anthocyanuns and antioxidant capacity of carrots. *Food Chemistry*, Vol. 132, p. 1161–1170.
17. Sommerburg O., Siems W.G., Hurst J.S., Lewis J.W., Kliger D.S., Kuijk F.J. Lutein and zeaxanthin are associated with photoreceptors in the human retina. *Current Eue Research*, Issue 19(6), p. 491–495.
18. Staugaitis G., Viškelis P. (2001) Quality assessment of leaf vegetables, vitamin C and nitrates. *Sodininkyste ir Dazininkyste*, Lithuanian Institute of Horticulture, Vol. 20 (4), p. 61–69.
19. Van den Berg H., Faulks R., Granado H.F., Hirschberg J., Olmedilla B., Sandmann G., Southon S., Stahl W. (2000) The potencial for the improvement of carotenoid levels in foods and the likely systemic effects. *Journal of the Science of Food and Agriculture*, Vol. 80, p. 880–912.

THE INFLUENCE OF FERMENTATION TEMPERATURE ON THE DEVELOPMENT OF EXOPOLYSACCHARIDES IN YOGHURT PRODUCTION

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Abstract

Exopolysaccharides (EPS) have potential for development and exploitation as functional ingredients with health and economic benefits in dairy industry. The information on the biosynthesis, molecular organisation and fermentation conditions of EPS is rather scarce and the kinetics of EPS formation are poorly described. This study was designed to evaluate the effect of fermentation temperature on the development of lactic acid bacteria (LAB) starters EPS production potential. The commercial starters (Harmony 1.0, TWIST 1.0 and YF-L902, Chr.Hansen, Denmark) are used for yoghurt production. Milk samples were incubated at 38° C, 40° C and 43° C for 7, 6 and 5 hours, respectively, reaching pH 4.5. EPS and lactic acid concentration, LAB colony forming units (CFU) were measured in yoghurt samples using an appropriate analytical technique or standard procedures.

The production of intracellularly synthesized EPS varies roughly from 25.28 to 440.81 mg L^{-1} during fermentation. The fermentation temperature significantly contributes to EPS concentration because the increased rate of fermentation temperature is attributed to increased metabolic activity of LAB. Thermophilic strains produce maximal amounts of EPS under conditions optimal for growth. There isn't established the correlation between the amount of EPS and CFU of LAB in samples fermented at different temperatures, but there is found stable CFU concentration in samples with higher initial EPS concentration during the shelf-life of yoghurt. EPS in their natural environment are thought to play a role in the protection of the microbial cell against desiccation to phagocytosis and phage's attack, osmotic stress, adhesion to solid surfaces and biofilm formation. The fermentation temperature has a crucial role for the development of yoghurt quality and functional properties.

Keywords: exopolysaccharides, lactic acid bacteria, starters, yoghurt, fermentation.

Introduction

Lactic acid bacteria (LAB) have been extensively studied for their economic importance in food fermentation. LAB secrete exopolysaccharides, which play a significant role in the protection of microbial cell against phagocytosis, phage attacks, antibiotics, toxic compounds, osmotic stress and bacteriocins (De Vuyst, Degeest, 1999; Ruas-Madiedo et al., 2001; O'Connor et al., 2007), and in assuring the proper consistency and texture of fermented dairy products and cheese (Petry et al., 2000; Folkenberg et al., 2006). Additionally, some of EPS have prebiotic properties that could find important applications in functional foods (Ruas-Madiedo et al., 2010; Hidalgo-Cantabrana et al., 2012).

EPS are long-chain polysaccharides consisting of branched, repeating units of sugars or sugar derivatives. These sugar units are mainly glucose, galactose and rhamnose in different ratios. EPS are secreted into cells surroundings during growth and are not attached permanently to the surface of the microbial cell. This distinguishes them from the structurally similar capsular polysaccharides, which do remain permanently attached to the surface of the cell (Cerning, 1990; De Vuyst et al., 2001).

The total yield of EPS produced by LAB depends on the composition of the medium and the conditions in which the strains grow, i.e. temperature, pH and incubation time. The production of intracellularly synthesized EPS varies roughly from 0.045 to 0.350 g L^{-1} when the bacteria are grown under non-optimized culture conditions. Optimal culture conditions result in EPS yields from 0.150 to 0.600 g L^{-1}, depending on the strain (Cerning, 1990; Ruas-Madiedo et al., 2010). Optimal conditions of temperature, pH and incubation time result in improved EPS yields. Several reports show that low temperatures markedly induce slime production of LAB (De Vuyst, Degeest, 1999; Cerning et al., 1992). This effect has been explained, based on information for EPS production from Gram-negative bacteria, by the fact that slowly growing cells exhibit much slower cell wall polymers biosynthesis, making more isoprenoid lipid carrier molecules available for EPS biosynthesis (Petry et al., 2000; De Vuyst et al., 2001). However, several investigators find higher EPS production by LAB strains at higher cultivation temperature (De Vuyst, Degeest, 1999; Degeest et al., 2001; Grobben et al., 1995) and under conditions optimal for growth, for instance, with respect to pH (De Vuyst and Degeest, 1999; Degeest et al., 2001; Mozzi et al., 1994; Mozzi et al., 1996; Degeest, De Vuyst, 1998; Grobben et al., 1998) and oxygen tension (Degeest et al., 2001; Degeest, De Vuyst, 1998; Grobben et al., 1998; Behare et al., 2009). Whereas mesophilic strains seem to produce maximal amounts of EPS under conditions not optimal for growth at low temperatures; EPS production from thermophilic lactic acid bacteria appears to be growth-associated, i.e. maximal production under conditions optimal for growth is observed (De Vuyst et al., 1998). In the case of growth-associated production, EPS biosynthesis generally starts almost simultaneously with growth of LAB, shows a maximum rate when the culture is in its exponential growth phase and reaches a maximum towards at the end of the active growth (De Vuyst et al., 1998). It means that during storage time of fermented dairy products EPS biosynthesis would be

continued with the different speed due to stationary growth phase of LAB.

EPS degradation often takes place upon prolonged incubation (De Vuyst and Degeest, 1999; Mozzi et al., 1996; De Vuyst et al., 1998; Gassem et al., 1997) and further biochemical reactions during the shelf-life of fermented dairy products. This may be due to glycohydrolase activity of LAB (De Vuyst, Degeest, 1999; Cerning et al., 1992; Gancel, Novel, 1994; Cerning et al., 1990). However, a marked reduction in the EPS yield upon prolonged fermentation seems to be dependent on the strain used and both chemical and physical conditions (temperature, pH, etc.) (De Vuyst, Degeest, 1999; De Vuyst et al., 1998; Gancel, Novel, 1994).

This study was designed to evaluate the effect of fermentation temperature on the development of lactic acid bacteria starters EPS production potential.

Materials and Methods

Preparation of samples

Pasteurized and cooled milk samples were inoculated with different starters (given in Table 1) and incubated at 38 °C, 40 °C and 43 °C for 7, 6 and 5 hours, respectively, until pH of coagulum reached 4.5. The amount of starters was added based on the recommendations of starters' manufacturer Chr. Hansen (Denmark).

Table 1

The characteristic of commercial starters for yoghurt production used in the study

Starter code	Composition	Producer
Harmony 1.0	*Streptococcus thermophilus, Lactobacillus delbrueckii subsp.bulgaricus, Lactobacillus fermentum*	
TWIST 1.0	*Streptococcus thermophilus, Lactobacillus johnsonii, Lactobacillus delbrueckii subsp. bulgaricus*	Chr. Hansen, Denmark
YF-L902	*Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus*	

Fermented milk samples were stirred and maturated at 4–6 °C for 10-12 hours and stored at 4–6 °C for 7 days.

Determination of pH, CFU of LAB and EPS

An amount of EPS synthesized by starters' cultures, pH and colony forming units (CFU) of lactic acid bacteria were detected in yoghurt samples after maturation and at the end of the shelf-life of yoghurts. MRS medium (de Man Rogosa & Sharpe; Scharlau, Spain) was used for analysing CFU of LAB. pH of samples was measured by pH-meter Jenway 3520.

EPS were determined in yoghurt samples according to below given methodology (Ruas-Madiedo, de los Reyes-Gavilán, 2005).

Isolation of EPS

300–500 mL of fermented sample were put into the laboratory flask and boiled in water bath at 100 °C for 30 minutes. After cooling samples were centrifuged at 8000 min^{-1} for 10 minutes and 17 mL of 85% trichloracetic acid were added to 100 mL of sample. Samples were cooled up to 4 °C and again centrifuged at 8000 rpm for 10 minutes. Precipitation of EPS from samples was provided using cold ethanol (-20 °C, 1:3). Samples were stood in the fridge for 48 h and late centrifuged (4 °C, 8000 min^{-1}, for 10 minutes), dissolved precipitation in dH_2O and defined EPS.

Quantification of EPS

5% phenol solution in water (dissolve 5 g fresh phenol in dH_2O and fill up to 100 mL into the flask) was prepared. Also 1 mg mL^{-1} glucose solution (dissolve 250 mg glucose in dH_2O and fill up to 250 mL into the flask) was prepared. For obtaining of calibration line, glucose solutions prepared in different proportions in 6 eppendorfs were used.

400 µL of sample was put into a glass tube and added 400 µL of 5% phenol solution in water. For controlling, 400 µL dH_2O + 400 µL of 5% phenol solution in water was used.

After that, 2 mL of concentrated sulphuric acid were added sharply into the solution in tube. Let the samples stood for 10 minutes, then stirred and let them stood for 10 minutes at 30 °C. Samples at 490 nm in quartz cuvettes were measured and compared with the control sample. The amount of EPS (mg) was calculated using glucose calibration line.

Statistical analysis

Descriptive statistics was carried out to determine the differences of produced EPS concentration in different samples by Microsoft Windows for SPSS 14.0 software. Correlation analysis was used for determination of the differences between EPS concentration and CFU of LAB in yoghurt samples.

Results and Discussion

Exopolysaccharides production of LAB is an important attribute for the fermented dairy products (Jolly et al., 2002; Welman, Maddox, 2003; Ruas-Madiedo et al., 2010). Many of the thermophilic microorganisms produce exopolysaccharides and are of great technological importance. Thermophilic lactic acid bacteria starters show an optimum temperature ranging around 40–43 °C. By providing the microorganisms in their optimum temperature range, incubation time of approximately 5 hours was achieved. It was generally accepted that the lower fermentation temperature the longer it takes to reach a certain pH and the firmness of the final products. Milk acidification was carried out for all yoghurts until pH 4.5. Then, they were rapidly cooled and stirred before storage at 4–6 °C. For better understanding the influence of different fermentation temperatures on the functional properties of yoghurt samples, especially EPS concentration and colony

forming units of LAB, pH dynamics of samples during fermentation was studied.

pH dynamics is quite similar during fermentation of samples in the same temperature. At the end of fermentation the pH of yoghurt samples ranged from 4.50 to 4.57 in the analysed samples using different starters and fermentation temperatures. The residual acidification activity was observed for all samples during the shelf-life with stable pH values ranging from 4.25 to 4.28 (Harmony 1.0), from 4.16 to 4.20 (YF-L902) and from 4.30 to 4.35 (TWIST 1.0) depending on conditions for fermentation. However no significant effect of EPS concentration was observed on yoghurt postacidification during the self-life and according to the findings of Doleyres and co-authors (2005) no significant effect of EPS was observed on yoghurt postacidification.

The production of intracellularly synthesized EPS in yoghurt samples varies roughly from 25.28 to 440.81 mg L^{-1} during fermentation. Our results showed that starters TWIST 1.0 and YF-L902 produced greater quantities of EPS, while Harmony 1.0 starter lower amounts of EPS (Table 2).

Table 2

EPS concentration in yoghurt samples depending on chosen commercial starters and fermentation temperature, mg L^{-1}

Starter	Fermentation temperature, °C		
	38 °C	40 °C	43 °C
Harmony 1.0	32.10±6.33	63.55±3.13	152.79±9.80
YF-L902	137.07±8.61	175.34±23.82	304.19±15.15
TWIST 1.0	228.78±6.33	302.11±31.09	395.52±45.77

The fermentation temperature significantly contributes to EPS concentration because the increased rate of fermentation temperature is attributed to increased metabolic activity of LAB (Figure 1, a, b, c).

Our results are comparable with the findings of several authors (De Vuyst, Degeest, 1999; Laws et al., 2001; De Vuyst et al., 2001) that the higher EPS production of LAB was observed at the higher cultivation temperature, because of this grown-associated EPS production with thermophilic starter cultures and because of the limited number of catabolic pathways that provide energy in LAB (substrate level phosphorylation and secondary metabolic energy generation), cell synthesis is limited, and so is the energy-demanding EPS biosynthesis.

We also observed EPS degradation during the self-life of yoghurt. The reduction of EPS yield seems to be dependent on the starter used and biochemical reactions due to LAB metabolism during the storage of samples. The highest degradation was observed in yoghurt samples where after fermentation and maturation were determined the higher yield of EPS (in samples fermented with starters TWIST 1.0 and YF-L902 at 40 °C and 43 °C).

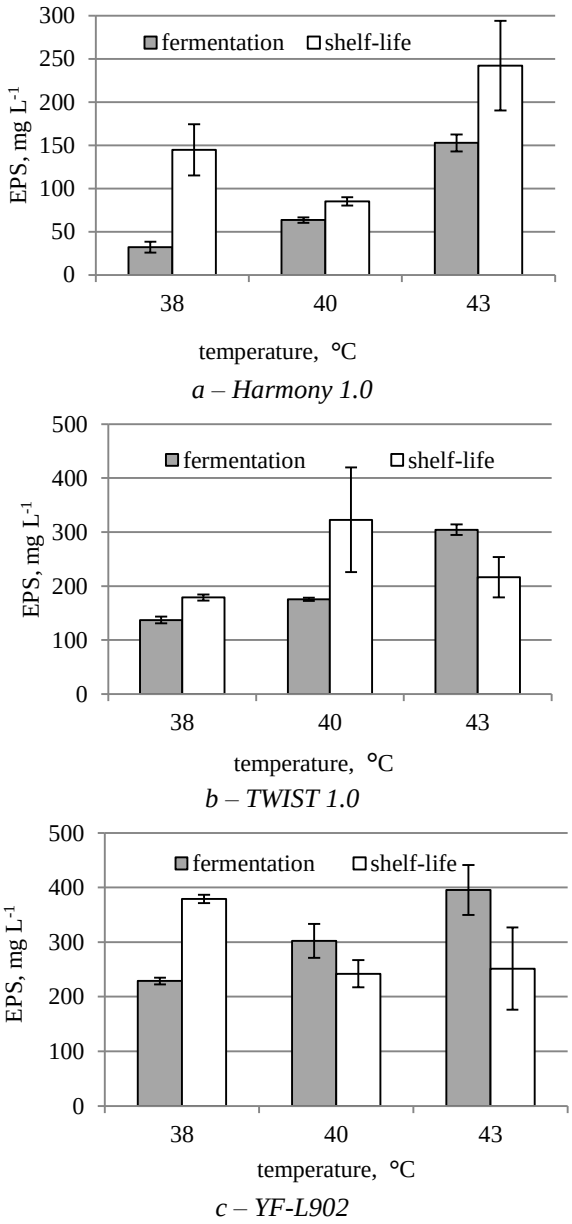


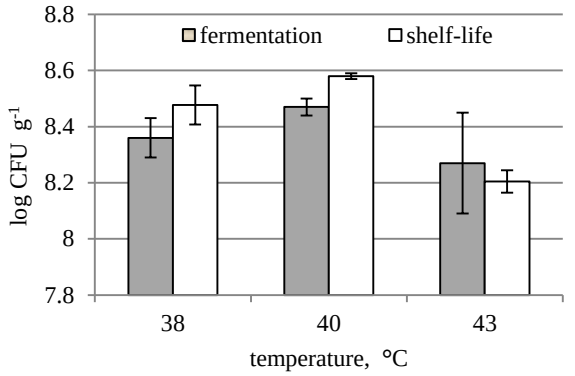
Figure 1. The comparison of EPS concentration in yoghurt samples after fermentation and at the end of shelf-life

We also noticed the tendency to increase EPS concentration during the self-life of product (in samples fermented with starters Harmony 1.0 at 38 °C, 40 °C and 43 °C; TWIST 1.0 at 38 °C and 40 °C; YF-L902 at 38 °C). A possible interpretation of our results in thermophilic starters is that isoprenoid phosphate carriers are primarily needed for cell wall synthesis growth (Petry et al., 2000). This may explain our finding that EPS production is increased in stationary phase cells.

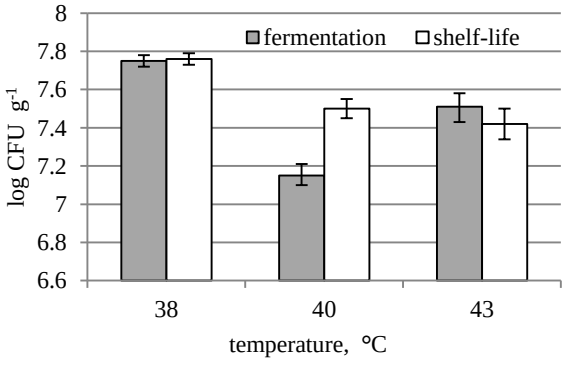
EPS production influences the amount of CFU of LAB in yoghurt samples (Figure 2, a, b, c).

There isn't established the correlation between the amount of EPS and CFU of LAB in samples fermented at different temperatures, but there is found stable CFU concentration in samples with higher EPS

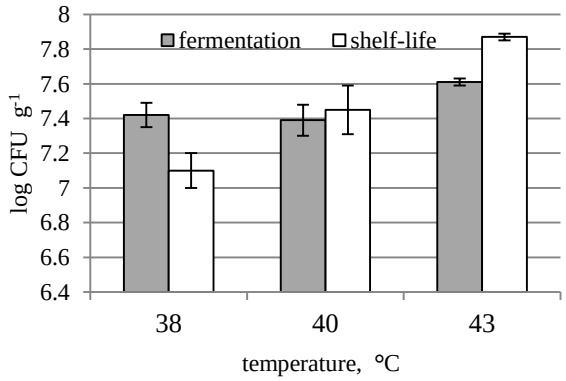
concentration during the shelf-life of yoghurt (Figure 2, b and c). We could explain it with EPS functional properties that EPS in their natural environment are thought to play a role in the protection of the microbial cell.



a – Harmony 1.0



b – TWIST 1.0



c – YF-L902

Figure 2. The changes of CFU of LAB in yoghurt samples after fermentation and during the shelf-life

We also observed that yoghurt samples with the highest initial EPS concentration and the highest degradation level of EPS showed stable CFU of LAB during the shelf-life of samples. We found that the higher reduction of CFU of LAB was noted in yoghurt samples with the increasing yield of EPS during the shelf-life (Harmony 1.0 at 43 °C, YF-L902 at 38 °C). We explain it with the energy-demanding EPS biosynthesis instead of the growing activity of LAB.

A lot of work has been done in the field of isolating and characterising the composition of EPS produced by various strains of lactic acid bacteria (Faber et al., 2002; Grobben et al., 2000; Petry et al., 2000; Van Calsteren et al., 2002; Ruas-Madiedo et al., 2005) but the functionality of EPS in fermented milk is still not completely clear. The fermentation temperature has a crucial role for the development of yoghurt quality and functional properties, too.

Conclusions

Analysing starters' producing EPS showed large variations in concentration from 25.28 to 440.81 mg L^{-1} in samples fermented in different temperatures.

The fermentation temperature significantly contributes to EPS concentration because the increased rate of fermentation temperature is attributed to increased metabolic activity of LAB.

The correlation between the amount of EPS and CFU of LAB in samples fermented at different temperatures is not established, but there is found stable CFU concentration in samples with higher EPS concentration during the shelf-life of yoghurt.

References

1. Behare P. V., Singh R., Kumar M. (2009) exopolysaccharides of lactic acid bacteria: A review. *Journal of Food Science and Technology*, No. 46, p. 1–11.
2. Cerning J. (1990) Exocellular polysaccharides produced by lactic acid bacteria. *FEMS Microbiology Reviews*, No. 87, p. 113–130.
3. Cerning J., Bouillanne C., Landon M., Desmazeaud M. J. (1992) Isolation and characterization of exopolysaccharides from slime-forming mesophilic lactic acid bacteria. *Journal of Dairy Science*, No. 75, p. 692–699.
4. De Vuyst L., De Vin F., Vaningelgem F., Degeest B. (2001) Recent developments in the biosynthesis and applications of heteropolysaccharides from lactic acid bacteria. *International Dairy Journal*, Vol. 11, Issue 9, p. 687–707.
5. De Vuyst L., Degeest B. (1999) Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiology Reviews*, No. 23, p. 153–177.
6. De Vuyst L., Vanderveken F., Van de Ven S., Degeest B. (1998) Production by and isolation of exopolysaccharides from *Streptococcus thermophilus* grown in a milk medium and evidence for their growth-associated biosynthesis. *Journal of Applied Microbiology*, No. 84, p. 1059–1068.
7. Degeest B., Vaningelgem F., De Vuyst L. (2001) Microbial physiology, fermentation kinetics, and process engineering of heteropolysaccharide production by lactic acid bacteria. *International Dairy Journal*, No. 11, p. 747–757.
8. Degeest B., De Vuyst L. (1998) Process characteristics of exopolysaccharide production by *Streptococcus thermophilus*. *Polymer Preprints*, No. 39 (1), p. 697–698.
9. Doleyres Y., Schaub L., Lacroix C. (2005) Comparison of the functionality of exopolysaccharides produced in situ or added as bioingredients of yoghurt properties. *Journal of Dairy Science*, Vol. 88, Issue 12, p. 4146–4156.
10. Faber E. J., van Kuik J. A., Kamerling J. P., Vliegenthart J. F. G. (2002) Modelling of the structure in

aqueous solution of the exopolysaccharide produced by *L.helveticus* 766. *Biopolymers*, No. 63, p. 66–76
11. Folkenberg M. D., Dejmek P., Skriver A., Guldager H. S., Ipsen R. (2006) Sensory and rheological screening of exopolysaccharide producing strains of bacterial yoghurt cultures. *International Dairy Journal*, Vol. 16, p. 111–118.
12. Gancel F., Novel G. (1994). Exopolysaccharide production by *Streptococcus salivarius ssp. thermophilus* cultures. 2. Distinct modes of polymer production and degradation among clonal variants. *Journal of Dairy Science*, No. 77, p. 689–695.
13. Gassem, M. A., Schmidt, K. A. and Frank J. F. (1997) Exopolysaccharide production from whey lactose by fermentation with *Lactobacillus delbrueckii ssp. bulgaricus*. *Journal of Food Sci*ence, No. 62 (207), p. 171–173.
14. Grobben G. J., Chin-Joe I., Kitzen V. A., Boels I. C., Boer F., Sikkema J., Smith M. R., De Bont J. A. M. (1998) Enhancement of exopolysaccharide production by *Lactobacillus delbrueckii subsp. bulgaricus* NCFB 2772 with simplified defined medium. *Applied Environmental Microbiology*, No. 64, p. 1333–1337.
15. Grobben G. J., Boels I. C., Sikkema J., Smith M. R., De Bont J. A. M. (2000) Influence of ions on growth and production of exopolysaccharides by *Lactobacillus delbrueckii subsp. bulgaricus* NCFB 2772. *Journal of Dairy Research*, No. 67, p. 131–135
16. Grobben G. J., Sikkema J., Smith M. R., De Bont J. A. M. (1995) Production of extracellular polysaccharides by *Lactobacillus delbrueckii ssp. bulgaricus* NCFB 2772 grown in a chemically defined medium. *Journal of Applied Bacteriology*, No. 79, p. 103–107.
17. Hidalgo-Cantabrana C., Lopez P., Gueimonde M. (2012) Immune modulation capability of exopolysaccharides synthesised by lactic acid bacteria and bifidobacteria. *Probiotics and Antimicrobial Proteins*, No. 4**,** 227-237.
18. Jolly L. (2002) Exploiting exopolysaccharides from lactic acid bacteria. *Antonie Van Leeuwenhoek*, Vol. 82, p. 367–374.
19. Laws A., Gu Y., Marshall V. (2001) Biosynthesis, characterisation, and design of bacterial exopolysaccharides from lactic acid bacteria. *Biotechnology Advances*, No. 19, p. 1–28.
20. Mozzi F., Savoy de Giori G., Oliver G., Font de Valdez G. (1994) Effect of culture pH on the growth characteristics and polysaccharide production by *Lactobacillus casei*. *Milchwissenshaft*, No. 50, p.80–81.
21. Mozzi F., Savoy De Giori G., Oliver G., Font De Valdez G. (1996) Exopolysaccharide production by *Lactobacillus casei* under controlled pH. *Biotechnology Letters*, No. 18, p. 435–439.
22. O'Connor E. B., Barrett E., Fitzgerald G. (2007) Production of vitamins, exopolysaccharides and bacteriocins by probiotic bacteria. **In**: *Probiotic Dairy Products.* A. Y. Tamime *(eds). Oxford: Blackwell Publishing Ltd. p.* 167–194.
23. Petry S., Furlan S., Crepean M. J., Cerning J., Desmazeaud M. (2000) Factors affecting exocellular polysaccharide production by *Lactobacillus delbrueckii subsp. bulgaricus* grown in a chemically defined medium. *Applied Environmental Microbiology*, No. 66, p. 3427–3431.
24. Ruas-Madiedo P., de los Reyes-Gavilán C. G. (2005) Methods for the screening, isolation, and characterization of exopolysaccharides produced by lactic acid bacteria. *Journal of Dairy Science*, Vol. 88, p. 843–856.
25. Ruas-Madiedo P., Salazar N., de los Reyes-Gavilán C.G. (2010) Exopolysaccharides produced by lactic acid bacteria in food and probiotic applications. **In**: Microbial glycobiology – structures, relevance and applications. A. Moran, O. Holst, P. J. Brennan, M. von Itzstein (eds.). Amsterdam: Elsevier, p. 885–902.
26. Ruas-Madiedo P., Hugenholtz J., Zoon P. (2001) An overview of the functionality of exopolysaccharides produced by lactic acid bacteria. *International Dairy Journal*, Vol. 12, p. 163–171.
27. Van Calsteren M. R., Pan-Roblot C., Begin A., Roy D. (2002) Structure determination of the exopolysaccharide produced by *Lactobacillus rhamnosus* strains RW-9595M and R. *Biochemistry Journal*, No. 363, p. 7–17.
28. Welman A. D., Maddox I. S. (2003) Exopolysaccharides from lactic acid bacteria: perspectives and challenges. *Trends in Biotechnology*, Vol. 21, No. 6, p. 269–274.

EVALUATION OF PRESERVATION CONDITIONS ON NUTS PROPERTIES

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Abstract

Dried fruits like nuts are very much appreciated, but because of their hygroscopicity may be susceptible to undesirable changes during storage. Therefore, this work was undertaken to study the effect of different storage conditions on three types of nuts commonly consumed in Portugal (almond, hazelnut and walnut). The samples were originated from different countries and while most had the internal skin on, one sample had it off. The storage conditions tested were: ambient temperature, high temperatures (30 and 50 °C) and low temperatures (refrigeration at + 2 °C and freezing at – 15 °C). The characteristics evaluated were water activity, moisture content, colour and texture.

The results obtained in the present work allowed concluding that the storage conditions that best preserve the characteristics of nuts are those at low temperatures, because, while the treatments at high temperatures induced in general more changes, the refrigeration and freezing systems had a lower effect on the products characteristics, particularly moisture, water activity, hardness and friability. Also the results indicate that the nuts stored under all conditions tested had values of water activity lower than 0.6, thus guaranteeing stability at the microbial and enzymatic levels. It was further concluded that the internal skin had a great influence on the characteristics of the nuts, particularly texture and colour, for all treatments tested.

Keywords: almond, colour, hazelnut, walnut, texture.

Introduction

Tree types of nuts are used worldwide in confectionary, culinary and bakery food applications due to their desirable flavour attributes and high energy density.

Almonds (*Prunus dulcis*, syn *Prunus amygdalus*, *Amygdalus communis* L. and *Amygdalus dulcis* Mill) are one of the most important commercial tree nut crops worldwide (Xiao et al., 2014). Almonds provide important nutrients such as vitamin E, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), arginine, and magnesium. Almonds also contain considerable amounts of potential prebiotic indigestible carbohydrates (Bolling et al., 2010; Liu et al.).

The common hazel plant (*Corylus avellana* L.) is a shrub native to Europe and Asia that grows in temperate climates. Hazelnut plays a major role in human nutrition and health because of its special composition of fat (around 60%), most of which are highly rich in MUFA (mainly oleic), protein, carbohydrate, dietary fiber, vitamins (vitamin E), minerals, phytosterols (mainly β-sitosterol), squalene and antioxidant phenols (Oliveira et al., 2008; Yada et al., 2013).

Walnut tree (*Juglans regia* L.) was originated from Southeast Asia and east Mediterranean areas and was introduced in Europe by the Romans. Walnuts, despite the pleasant taste, were considered for long, inconvenient for human consumption because they are very rich in fat. However they bring a great benefit to health because they help control bad cholesterol and therefore help to protect the heart. They are particularly rich in minerals like copper and zinc, required for formation of haemoglobin in the blood (Almeida, 2013; Tapia et al., 2013).

The storage conditions are of major importance so as to maintain the integrity and quality of nuts, and thus prevent spoilage (Freitas-Silva, Venâncio, 2011; Ma et al., 2013). Post-harvest handling and storage might deteriorate both nut nutritional value and taste. It is well known that many factors, such as a high drying temperature or prolonged storage under air and / or at a relatively high temperature and humidity have negative effects on various nut quality attributes (Tsantili et al., 2011).

The aim of this work was to study the effect of different storage conditions on nuts (almond, hazelnut and walnut), namely ambient temperature, high temperatures and low temperatures. The characteristics evaluated were water activity, moisture content, colour and texture.

Materials and Methods

Sampling

The fruits evaluated in this study were almond, hazelnut and nut. The fruits were all without the outer shell, but in some cases they had the inner skin (seed coat) and in other cases not.

The almond samples were: from Spain with skin (A-SP-s), Portugal with skin (A-PT-s) and from United States with and without skin (A-US-s and A-US-n). The hazelnut samples were: from Spain with skin (H-SP-s), Portugal with skin (H-PT-s) and from Turkey without skin (H-TR-n). The walnut samples were: from Chile with skin (W-CH-s), Portugal with skin (W-PT-s), Romania with skin (W-RO-s) and USA with skin (W-US-s).

Determination of water activity and moisture content

The water activity was measured at 25 °C by a hygrometer (Rotronic Hygroskop BT-RS1) connected to a thermal bath. In all cases four determinations were made to calculate the medium value and standard deviation. Moisture content was evaluated by drying in

a stove (WTB-Binder) at 105 °C until reaching constant weight. In all cases three determinations were made.

Evaluation of colour

The colour of all samples was measured using a handheld tristimulus colorimeter (Chroma Meter-CR-400). The parameters measured were the lightness L*, which varies between 0 and 100 (from black to white, respectively), and the coordinates of opposed colour: a* and b*, which vary from – 60 to + 60, where the a* assumes negative values for green and positive values for red, while b* assumes negative values for blue and positive for yellow. The total colour change (ΔE), was the parameter considered for the overall colour difference evaluation, between a sample and the reference fruit (without storage, designated with an index 0:

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

Evaluation of texture

The texture profile analysis (TPA) for all samples was made by a texturometer (TA.XT.Plus from Stable Micro Systems). The text performed was measure force in compression and the probe used was a Blade Set HDP/BS (Warner- Bratzler). Twenty measurements were carried for each fruit and each storage condition. The curve force (N) versus distance (mm) allows to calculate the hardness (the force at first peak) and friability (the distance of first peak).

Storage conditions

The fruits were stored without package for 90 days at ambient conditions, in a stove at two temperatures with controlled temperature but no control over relative humidity, under refrigeration and frozen (Table 1). The use of high temperatures (50 °C) aimed at verifying the effect of these storage conditions on the water absorption and therefore the conservation.

Table 1

Storage conditions for the fruits studied

Description	Temperature, °C	Relative humidity, %
B: Just acquired (base)	–	–
AT: Ambient temperature	23.4±2.5	50.5±6.7
S30: Stove (T controlled but RH not)	30.0±0.0	36.0±3.6
S50: Stove (T controlled but RH not)	50.0±0.0	13.2±1.5
R: Refrigerated	2.3±3.7	48.1±23.3
F: Frozen	-15.4±2.6	61.7±6.2

Results and Discussion

Figure 1 shows that the moisture content of the almond samples was always higher before storage, regardless of the conditions. Comparing the different origins, the almond from Spain had the highest moisture content.

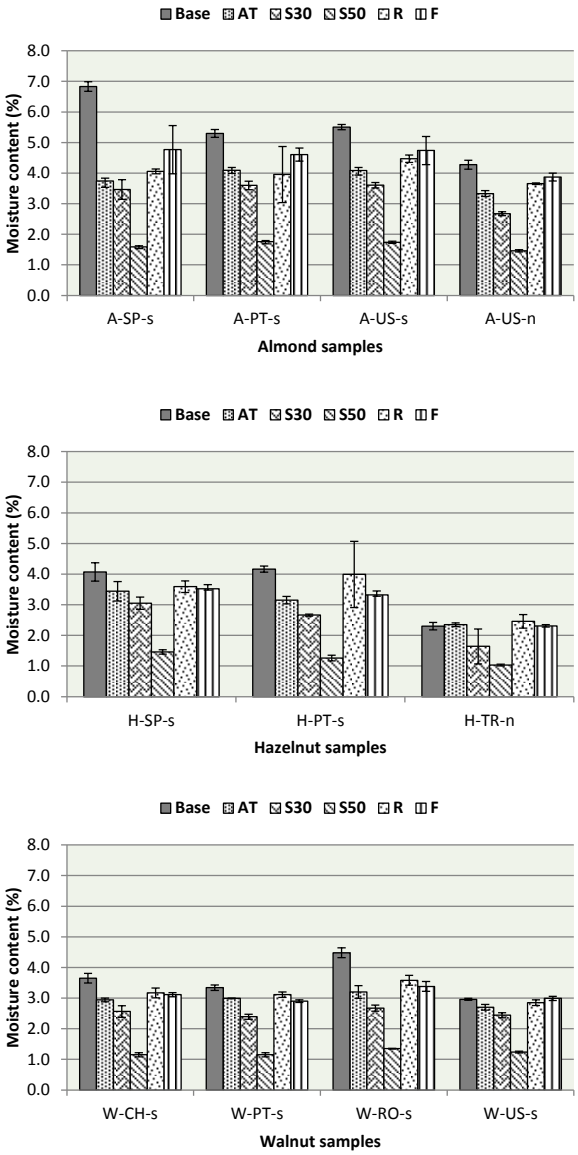

Figure 1. Moisture content of nuts

AT – Ambient temperature; S30, S50 – storage at high temperature (30 and 50 °C); R – refrigerated; F – frozen; Origin: SP – Spain, PT – Portugal, US – United States, RO – Romania, CH – Chile, TR – Turkey

As to the almonds from the United States, the absence of the skin contributed to a further dehydration, because the moisture content of the almond with skin was 1.74% while for the sample without skin was 1.46%.The results also reveal that for all almonds the frozen samples were those that had the moisture content more similar to the base ones. The trend observed for almonds was also observed for hazelnuts and walnuts. However, the relative contents are different, where almonds have higher moisture contents when compared to the other two nuts studied. The values of the water activity for all samples and all storage conditions are presented in Figure 2. The results show that, like what was observed for moisture, the storage of 50 °C produces the almonds with lower

water activity, while the frozen almonds showed the higher water activity among the stored samples. The results for the other two nuts studied, hazelnuts and walnuts, are very similar, but once again the magnitude of the values is different, so that the almonds present much higher water activity. One important issue concerning stability and that may influence is lipid quality oxidation, but that was not studied in this work.

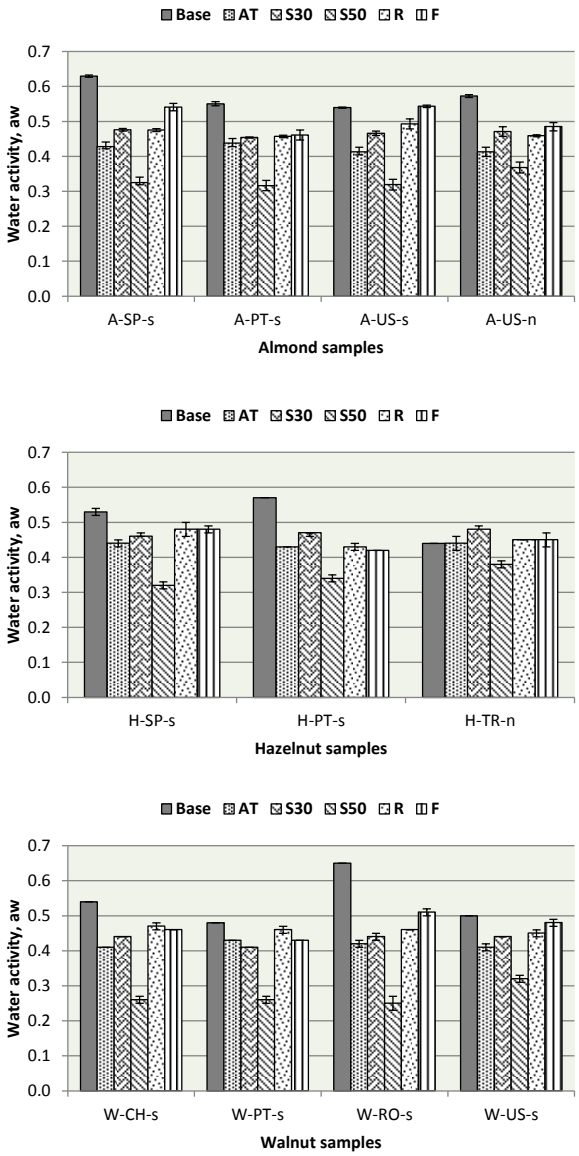

Figure 2. Water activity of nuts

AT – Ambient temperature; S30, S50 – storage at high temperature (30 and 50 °C); R – refrigerated; F – frozen; Origin: SP – Spain, PT – Portugal, US – United States, RO – Romania, CH – Chile, TR – Turkey.

However, and despite the slight changes observed, these findings allow to conclude that in general the samples are stable and not susceptible to spoilage, since for all storage conditions the values of aw are lower than 0.6, thus preventing the development of any bacteria, fungi or yeasts.

Table 2 shows the values of the colour parameters for all the nut the samples before storage.

The values of L*, a* and b*are very similar for the three almond with the skin, but differ much of that of the sample without skin, as naturally expected. The almond samples with the skin present values of L* (brightness) under 50, thus indicating that they are very dark, contrasting with the value of the sample without skin (L*=78.1), which stand in the region approaching white. The values of a* are positive in all cases, thus indicating that the samples have a more or less (depending on the presence of the skin) reddish colouration. In the case of the A-US-n sample, the value is very close to zero, indicating that the predominance of red is very weak. Regarding the values of b*, they are all strongly positive, and so the intensity of the yellow colour is high.

As to the colour parameters for hazelnut, the results are very similar to those of the almonds and the trends observed are the same as discussed above. Also the influence of the internal skin is well evidenced.

Regarding the walnut samples, and because they were in this case all with the internal skin, the results are very close among each other. However, it is worth mentioning that the values of a* and b* are smaller in the skin of the walnuts as compared to the skin of the other two nuts studied.

Table 2

Colour parameters of the nuts

Sample	L^*_0	a^*_0	b^*_0
Almond			
A-SP-s	47.8±3.3	16.5±1.7	32.7±3.3
A-PT-s	43.4±2.9	16.8±1.5	30.5±2.7
A-US-s	47.3±2.6	17.2±1.1	33.9±2.2
A-US-n	78.1±2.2	1.6±1.2	28.1±2.1
Hazelnut			
H-SP-s	42.6±4.1	17.8±1.9	24.5±3.3
H-PT-s	47.2±4.4	18.6±2.0	26.2±2.6
H-TR-n	75.2±3.1	4.8±1.5	31.6±3.1
Walnut			
W-CH-s	40.6±6.4	11.5±1.9	24.2±2.4
W-PT-s	48.2±6.3	9.4±2.4	28.4±3.7
W-RO-s	49.3±6.0	10.3±1.8	31.1±2.8
W-US-s	47.0±6.4	8.9±1.9	26.7±4.1

Figure 3 shows the total colour difference between each sample and the reference, which consisted of the nuts before storage. The values were calculated by equation (1) from the mean values for each case, and therefore no standard deviation is included. The results show that the highest differences were observed for those almonds stored at 50 °C, and that is particularly visible for the almonds with skin. These results indicate that the skin is affected by heat, becoming darker due to the browning processes occurring during storage.

Hence, the results seem to indicate that maybe refrigeration and freezing could be an alternative to preserve the colour of the almonds with skin.

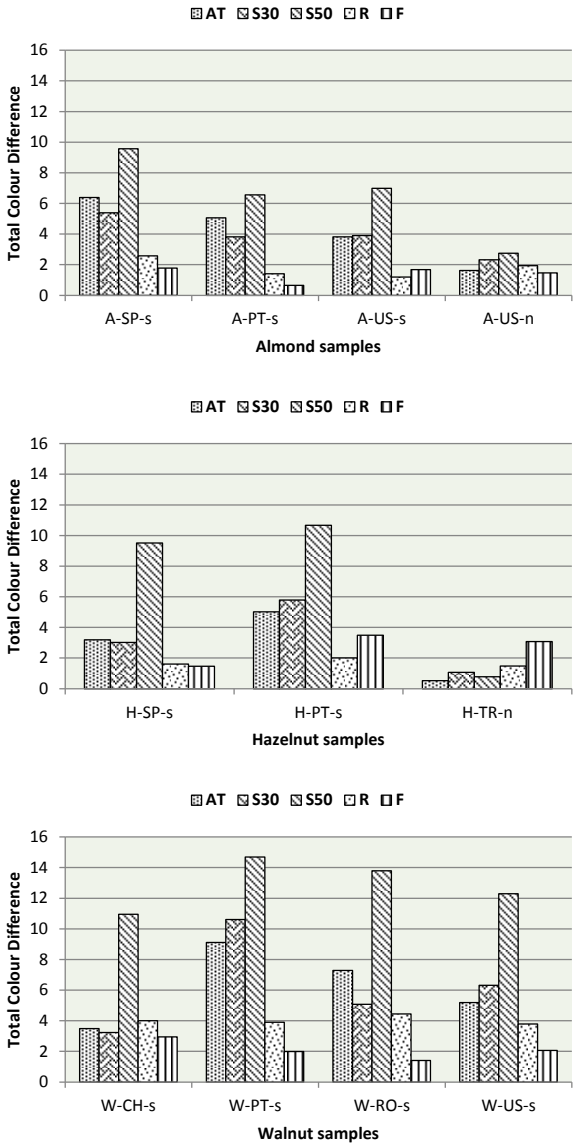

Figure 3. Total colour difference of nuts

AT – Ambient temperature; S30, S50 – storage at high temperature (30 and 50 °C); R – refrigerated; F – frozen; Origin: SP – Spain, PT – Portugal, US – United States, RO – Romania, CH – Chile, TR – Turkey

In relation to the other two nuts, it was also observed that the storage at 50 °C originated the highest colour differences. As to the best method of storage for these two nuts, although in majority of the cases it is freezing like in the case of almonds, there are however some exceptions, in the case of hazelnuts from Portugal and from Turkey.

Hardness is the mechanical strength to crush. It is important as it ensures the physical integrity of the product, allowing it to support the mechanical stress in the process of packing and transportation. The hardness limits are specified according to the diameter and weight of the sample and refers to the minimum resistance to be removed from the container without breaking. The textural attribute hardness was evaluated for almonds and hazelnuts and the results are shown in Figure 4.

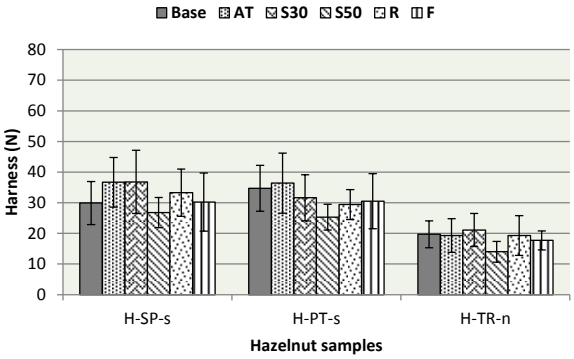

Figure 4. Hardness of almonds and hazelnuts

AT – Ambient temperature; S30, S50 – storage at high temperature (30 and 50 °C); R – refrigerated; F – frozen; Origin: SP – Spain, PT – Portugal, US – United States, TR – Turkey

The almonds from different origins show important differences in terms of this characteristic, being the almonds from Spain the softer among the samples with skin. Naturally that by removing the skin, the hardness decreases, as the results of the two sample from US (with and without skin) indicate. As to the effect of storage conditions, the results are not consistent among the different almonds, because the same treatment produces contradictory effects on different almonds. However, in general, the refrigeration and freezing conditions allow obtaining values closer to the reference samples of almonds.

As to the hazelnuts, it can be observed from Figure 4 that the variability is lower when compared to the almonds, and so are also the values of hardness. In fact, the hardness of the almond samples is almost double of the hazelnut samples. In the case of hazelnuts, all the storage conditions allow obtaining products with a similar hardness to the unstored samples.

The friability respects to the ease with which the fracture occurs in the products, and assumes a particular importance to avoid product loss. Figure 5 presents the results obtained for friability of almonds and hazelnuts, and these are quite consistent with what was previously observed for hardness. Once again the almond without skin shows to be less affected by storage, while the almond from Spain in that which suffers higher changes along storage, regardless of the conditions. Figure 5 also reveals that hazelnuts have lower friability when compared to almonds.

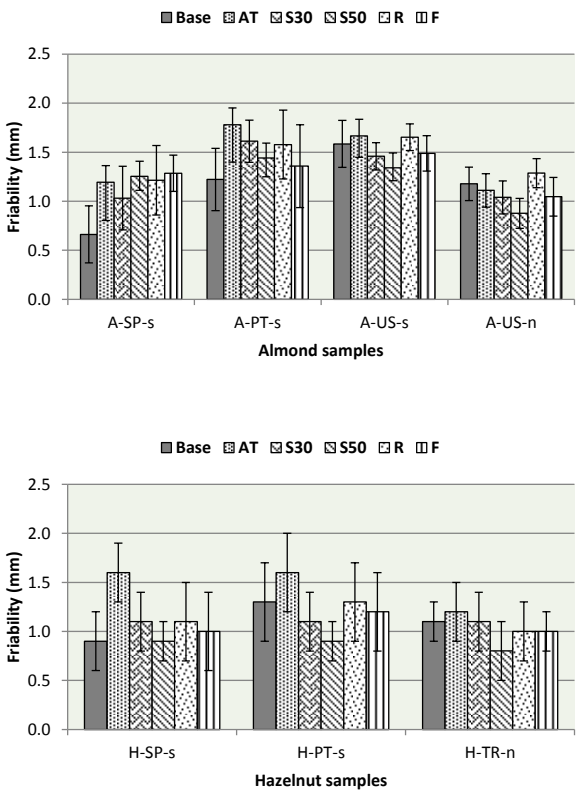

Figure 5. Friability of almonds and hazelnuts

AT – Ambient temperature; S30, S50 – storage at high temperature (30 and 50 °C); R – refrigerated; F – frozen; Origin: SP – Spain, PT – Portugal, US – United States, TR – Turkey

Conclusions

The findings of the present work allowed to draw some general conclusions as to the best storage conditions to preserve the characteristics of nuts. In fact, while the treatments at high temperatures induce in general more changes, the refrigeration and freezing systems are recommended for their lower effect on the products characteristics.

Furthermore, it was also concluded that the nuts stored under all conditions tested were safe from the point of view of the microbiological stability, given the very low water activity observed in all cases. Finally, it is important to note that the presence or absence of the internal skin greatly influences the product properties.

Acknowledgment

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References

1. Almeida C. (2013) Efeitos das condições de conservação nas características de frutos secos. Trabalho de Estágio. (Portugal: Escola Superior Agrária de Viseu).
2. Bolling B.W., McKay D.L., and Blumberg J.B. (2010) The phytochemical composition and antioxidant actions of tree nuts. *Asia Pacific Joural of Clinic Nutrition*, Vol. 19, p. 117–123.
3. Freitas-Silva O., Venâncio A. (2011) Brazil nuts: Benefits and risks associated with contamination by fungi and mycotoxins. *Food Research International*, Vol. 44, p. 1434–1440.
4. Liu Z., Lin X., Huang G., Zhang W., Rao P., Ni L. Prebiotic effects of almonds and almond skins on intestinal microbiota in healthy adult humans. *Anaerobe*, Vol. 26, p. 1–6.
5. Ma Y., Lu X., Liu X., Ma H. (2013) Effect of 60 Coγ-irradiation doses on nutrients and sensory quality of fresh walnuts during storage. *Postharvest Biology and Technology*, Vol. 84, p. 36–42.
6. Oliveira I., Sousa A., Morais J.S., Ferreira I.C.F.R., Bento A., Estevinho L., Pereira J.A. (2008) Chemical composition, and antioxidant and antimicrobial activities of three hazelnut (*Corylus avellana* L.) cultivars. *Food and Chemical Toxicology*, Vol. 46, p. 1801–1807.
7. Tapia M.I., Sánchez-Morgado J.R., García-Parra J., Ramírez R., Hernández T., González-Gómez D. (2013) Comparative study of the nutritional and bioactive compounds content of four walnut (*Juglans regia* L.) cultivars. *Journal of Food Composition and Analysis*, Vol. 31, p. 232–237.
8. Tsantili E., Konstantinidis K., Christopoulos M.V., Roussos P.A. (2011) Total phenolics and flavonoids and total antioxidant capacity in pistachio (*Pistachia vera* L.) nuts in relation to cultivars and storage conditions. *Scientia Horticulture*, Vol. 129, p. 694–701.
9. Xiao L., Lee J., Zhang G., Ebeler, S.E., Wickramasinghe N., Seiber J., Mitchell A.E. (2014) HS-SPME GC/MS characterization of volatiles in raw and dry-roasted almonds (*Prunus dulcis*). *Food Chemistry*, Vol. 151, p. 31–39.
10. Yada S., Huang G., Lapsley K. (2013) Natural variability in the nutrient composition of California-grown almonds. *Journal of Food Composition and Analysis*, Vol. 30, p. 80–85.

THE USE OF FACE READING TECHNOLOGY TO PREDICT CONSUMER ACCEPTANCE OF CONFECTIONERY PRODUCTS

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Abstract

Traditional sensory and consumer tests predict consumer acceptance of new products rather poorly, as evidenced by the high their failure rates in the market. These tests typical reflect conscious processes whereas consumer acceptance may also be based on unconscious processes.

The aims of this work were to examine whether facial expressions measured with the Noldus FaceReader technology can be used for differentiating between differing sugar confectionery products (various types of sweets and chocolates), and to investigate whether facial reactions are able to explain liking ratings on hedonic scales.

Naive consumers (mean age 22 years) were recruited at the Kaunas University of Technology. They were asked to rate the sample with an intentional facial expression, which was recorded and then characterized by FaceReader program (Noldus Information Technology, Wageningen, The Netherlands). The measurements showed significant differences between facial expressions elicited by the different samples of tested sugar confectionery products and reflected the introspective liking ratings well. The positive correlations of facial expression "happy", and negative correlations of "sad" expression intensity against self-reported liking ratings suggest that these may be the most valuable descriptors for explaining the self-reported hedonic quality of sweets and chocolates. It can be concluded that Noldus FaceReader technology is sufficiently accurate for differentiating between sugar confectionery products and can deliver additional information to conventional acceptance tests.

***Keywords*:** sugar confectionery products, consumers, facial expressions, hedonic liking, FaceReader.

Introduction

Up to 80% of all new food products fail in the marketplace, despite the fact that they are typically subjected to a large number of sensory and consumer tests before their market introduction. This suggests that the standard sensory and consumer tests, which typically include sensory analytical profiling and liking tests, have a low predictive validity with respect to general product performance. Possibly, consumer food choice outside the laboratory may be less based on cognitive information processing and rational reasoning, and more on unarticulated / unconscious motives and associations (Dijksterhuis, Smith, 2005; Köster, 2009). Reasons for likes or dislikes of different foods are typically difficult to articulate but may determine much of our food choice. Certain foods are more attractive than other foods because for some reason they make us feel good – i.e. they trigger positive emotions.

For some time, sensory analysts within the commercial sector have looked for better means to connect with marketing. The measurement of emotions might help in the further connection of sensory science and marketing. The measurement of emotions also serves as a further tool to support product development. Measurement of emotions allows us to compare existing products, and measure the emotional response to product prototypes. In these ways, the measurement of emotions can provide a common lexicon for sensory and marketing to communicate and for product development that meet a marketing need. Emotions can be the common language to bring these areas together.

There are many studies showing that tastes and odours elicit different emotions and facial reactions in neonates (Soussignan et al., 1997) children (Soussignan, 1996; Zeinstra et al., 2009) and adults (Greimel et al., 2006; Wendin et al., 2011). In most of these studies quite intense stimuli were used, like for example, concentrated basic taste solutions (Wendin et al., 2011) or odours ranging from fruity to fecal (Soussignan, 1996). The study of emotions in relation to food choice has recently advanced by work of King and Meiselman (2010) and De Smet and Schiffertein (2008). Their results suggest that most emotions related to food are mildly positive, are only partly related to liking, and improve the predictions of food choice.

Facial expressions can be analyzed with the anatomically based Facial Action Coding System (FACS). These FACS analyses are very time-consuming and require trained observers. To overcome these difficulties, different automated facial expression recognition systems like Nviso (Nviso SA, Lausanne, Switzerland), Affdex (Affectiva Inc., Waltham, USA) and FaceReader (Noldus Information Technology, Wageningen, The Netherlands) have been developed. These systems are capable of analyzing facial expression patterns from video data. Currently, these systems are used mainly for research in the fields of psychology, education, market research and consumer behaviour. De Wijk et al. (2012) analyzed the facial expressions elicited by the prospect of tasting or smelling liked or disliked food with FaceReader. The first sight of disliked foods compared to liked foods resulted in increased facial expressions of sadness, disgust, and angriness. However up until now, little work has been published about the measurement of facial expressions elicited directly by the actual tasting of food products using facial expression recognition

technology. Danner et al. (2014) examined consumers' facial reactions elicited by the flavour of orange juice products using FaceReader technology in implicit and an explicit measurement approach. Both, implicit and explicit measurements showed significant differences between facial expressions elicited by the different samples. The explicit measurement reflected the introspective liking ratings well. Especially expressions happy and disgusted showed a high correlation with liking and were good indicators for liked and disliked samples, respectively. To minimize artefacts, caused by eating and drinking, which can be easily misinterpreted by the FaceReader software as emotion, they used liquid samples (juice) which need less processing in the mouth than solid samples.

The aim of this work was to examine whether facial expressions measured with the Noldus FaceReader technology are a sufficiently accurate measure for differentiating between various types of sugar confectionery products (sweets and chocolates), and to investigate whether facial reactions are able to explain such product liking ratings on hedonic scales.

Materials and Methods

Samples and sample preparation

Six different types of sugar confectionery products were tested in the experiment. Each set of tested products was made from 3 to 4 samples with significant differences in flavour or texture (Table 1). All samples were presented in a sequential way, at room temperature (20–22 °C), randomized and coded. Water was provided to rinse the mouth before and between tasting the samples. All tested sugar confectionery products were commercial products delivered by various Lithuanian companies.

Table 1

Samples of sugar confectionery products

Products	Significant difference
Chocolate bars	Different additives: peanuts, hazelnuts, almonds, coffee
Milk chocolate	Different brand names: Milka, Sonata, Pergale
Sweets "Ruta"	Different fillings: almond, hazelnuts, actinidia, granadilla and white chocolate
Sweets "Ruta" with nutty filling	Different coatings: dark (70% or 50% cocoa solids), white, milk chocolate
Sweets "Ruta" with almond filling	Different coatings: dark (70% or 50% cocoa solids), white, milk chocolate
Caramel "Tropic"	-

Participants and measurements

The number of participants in the testing of each type of sugar confectionery products varied from 12 to 20. All participants were students of the Department of Food Technology, Kaunas University of Technology, with an average age of 22 years.

The experiments took place in the Sensory Laboratory at Kaunas University of Technology. At first, participants were introduced to the procedure for tasting. They were asked to taste the whole presented food sample (10 g) at once, take some seconds to think about an impression of it, then give a signal with their right hand and visualize the taste experience of the sample with a facial expression best representing their liking of the sample (explicit measurement). Afterwards, they rated their liking or disliking of the sample on a 7-point hedonic scale, ranging from 1 (dislike extremely) to 7 (like extremely).

The whole procedure was filmed with a Microsoft LifeCam Studio webcam, mounted on the laptop facing the participants, using Media Recorder software (Noldus Information Technology, Wageningen, The Netherlands). The recordings with a resolution of 640×480 at 25 frames per second were saved as AVI files and analyzed frame by frame with FaceReader 5 software (Noldus Information Technology, Wageningen, The Netherlands), scaling the 6 basic emotion patterns (angry, happy, disgusted, sad, scared, surprised) and neutral from 0 (not present at all) to 1 (maximum intensity of the fitted model). For each sample, the section of intentional facial expression (exactly from the point when the subject had finished raising their hand to give the signal until the subject started lowering the hand again) was extracted and used for the statistical analysis. FaceReader contains an image quality bar, which gives a good indication of how well the program is able to model the face depicted in the image. For the best image quality, the main attention was focused on camera position and illumination. For this reason, participants were asked to sit and look frontally into the camera. All of the participants agreed to the use of their data in the context of this experiment.

Statistical analysis

For the statistical analysis, the maximum values of the facial expression patterns (angry, disgusted, happy, sad, scared, surprised, and neutral) of the respective section were used. To examine the correlation between facial expressions and the hedonic liking, a Multiple Linear Regression was performed. All analyses were performed with STATISTICA V10 (StatSoft, Inc., Tulsa, OK, USA). Significance of differences between treated samples was evaluated by using Duncan's multiple range tests at a 5 percent level ($p<0.05$).

Results and Discussion

Results of experiments showed that there were significant differences in facial expressions between tasted types of sugar confectionery product (Fig. 1) as well as between the samples of the same type of products (Fig. 2). Tested types of confectionery products differed significantly concerning the emotion patterns happy, sad and angry. The similar tendencies were found during the analysis of the same type of

confectionery products (sweets). The sweets also were differ in emotion pattern surprised. No significant differences ($p>0.05$) in other facial expressions between tested samples were observed.

It was noticed, that the caramel samples elicited significantly more intense facial reactions of happy than all other tested confectionery products. Sweets "Ruta", especially with dark chocolate (70 % cocoa) elicited the strongest sad facial expressions, significantly differing from all others. The statistical analyses of the self-reported liking ratings identified the caramel "Tropic" as the most liked sample and the chocolate bars as the least liked. The data collected suggest that emotional intensity sometimes tracks with acceptance, and sometimes differs. For example, the acceptances of sweet with different fillings do not track with the emotion profile (Fig. 2). Thus emotions might help to explain acceptance data and why acceptance data might not always predict market success.

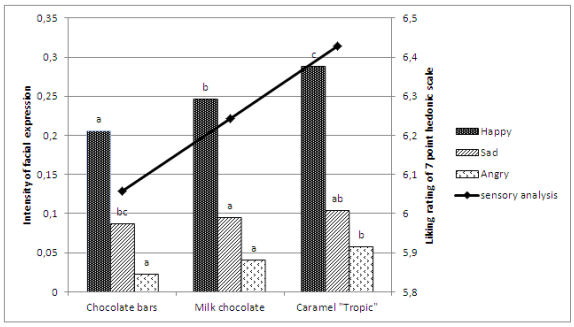

Figure 1. Intensity of facial expressions and self-reported liking of different types of sugar confectionary

While the measurement of emotions gives new information beyond acceptance, it is nevertheless interesting to relate emotions and acceptance. Linear regressions of the mean values of the facial expressions showed positive correlations of happy and negative correlations of sad (Table 2). This indicates that liked samples elicited more intense facial expressions of happy than disliked samples.

This study shows that measuring facial expressions using Noldus FaceReader 5 is a sufficiently accurate method to differentiate between various sugar confectionery samples. Discrimination between liked and disliked samples was possible on the basis of the intensity of elicited happy and sad facial expressions. This supports the findings of Danner et al. (2014) who used the FaceReader technology for study of orange juice and found high correlation of facial reactions happy and disgusted with liking.

It should also be mentioned that some differences in the intensity of facial reactions between participants were observed. The participants could be divided into two groups, the ones who showed clearly visible facial reactions when tasting the samples and those who had a poker face showing only little to almost no facial reactions. This can partially be attributed to the sensory laboratory test setup, where the participants are facing an unfamiliar environment and may feel stressed to a certain degree, or are very concentrated on the task.

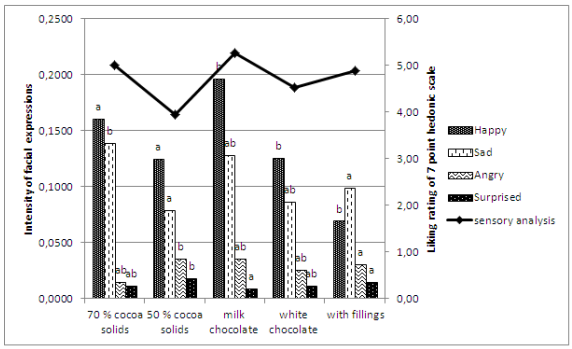

Figure 2. Intensity of facial expressions and self-reported liking of sweets "Ruta" with different coatings

DC1 – dark chocolate coating (70% cocoa solids),
DC2 – dark chocolate coating (50% cocoa solids),
MC – milk chocolate coating, WC – white chocolate coating

Table 2

Correlation of facial expression intensity against self-reported liking of different sugar confectionary products

Products	Emotions				
	N	H	Sa	A	Su
Chocolate bars	-0.39	0.14	-0.70	-0.98	-0.15
Caramel "Tropic"	-0.24	0.68	-0.03	-0.82	-0.54
Milk chocolate	0.72	0.33	-0.49	1.00	0.48
"Ruta"[1]	0.62	0.73	-0.60	0.31	0.50
"Ruta"[2]	-0.85	0.52	-0.36	-0.48	-0.32
"Ruta"[3]	-0.84	0.87	-0.23	-0.96	-0.69

N – neutral; H – happy; Sa – sad; A – angry; Su – surprised; Sc – scared; D –disgusted; [1] – with different fillings; [2] – with nutty filling and different coatings; [3] – with almond filling and different coatings

Further examinations in a more natural environment, also if possible without directly asking the participants to rate the products, could be interesting.

It is also important to point out the limitations and requirements of FaceReader technology. It does not work with children below the age of three. Pose, movement and rotation of the test person are limited. The test person needs to face the camera head on (angle <40°). The face must not be partially obscured by hair or when handling samples.

Motor artefacts, caused by eating and drinking, can be misinterpreted by the FaceReader software as emotion. In more complex tasting situations, like full meals that involve longer and potentially overlapping oral processing actions, motor artefacts can compromise the measurement of facial expressions to a higher degree.

Conclusions

It could be concluded that NoldusFaceReader technology is sufficiently accurate to detect significant differences in facial expressions elicited by different

samples of sugar confectionery products, such as chocolates, sweets, caramel, and can deliver additional information to conventional acceptance tests. However, more research is needed to see how this technology performs in more complex testing procedures, simulated or real life environments.

The positive correlations of happy and negative correlations of sad expression intensity against self-reported liking ratings suggest that these may be the most valuable descriptors for explaining the self-reported hedonic quality of sugar confectionery products.

Acknowledgment

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References

1. Danner L., Sidorkina L., Joechl M., Duerrschmid K. (2014) Make a face! Implicit and explicit measurement of facial expressions elicited by orange juices using face reading technology. *Food Quality and Preference*, Vol. 32, p. 167–172.
2. De Smet P. M. A., Schiffertein H. N. J. (2008) Sources of positive and negative emotions in food experience. *Appetite*, Vol. 50, p. 290–301.
3. De Wijk R. A., Kooijman V., Verhoeven R. H. G., Holthuysen N. T. E., De Graaf C. (2012) Autonomic nervous system responses on and facial expressions to the sight, smell, and taste of liked and disliked foods. *Food Quality and Preference*, Vol. 26, p. 196–203.
4. Dijksterhuis A., Smith P. K. (2005) What do we do unconsciously? And how? *Journal of Consumer Psychology*, Vol. 15, p. 225–229.
5. Greimel E., Macht M., Krumhuber E., Ellgring H. (2006) Facial and affective reactions to tastes and their modulation by sadness and joy. *Physiology and Behavior*, Vol. 89, p. 261–269.
6. King S. C., Meiselman H. L. (2010) Development of a method to measure consumer emotions associated with foods. *Food Quality and Preference*, Vol. 21, p. 168–177.
7. Köster E. P. (2009) Diversity in the determinants of food choice. A psychological perspective. *Food Quality and Preference*, Vol. 20, p. 70–82.
8. Soussignan R. (1996) Children's facial responsiveness to odors: Influences of hedonic valence of odor, gender, age, and social presence. *Developmental Psychology*, Vol. 32, p. 367–379.
9. Soussignan R., Schaal B., Marlier L., Jiang T. (1997) Facial and autonomic responses to biological and artificial olfactory stimuli in human neonates: Reexamining early hedonic discrimination of odors. *Physiology and Behavior*, Vol. 62, p. 745–758.
10. Zeinstra G. G., Koelen M. A., Colindres D., Kok F. J., De Graaf C. (2009) Facial expressions in school-aged children are a good indicator of "dislikes", but not of "likes" *Food Quality and Preference*, Vol. 20, p. 620–624.
11. Wendin K., Allesen-Holm B. H., Bredie W. L. P. (2011) Do facial reactions add new dimensions to measuring sensory responses to basic tastes? *Food Quality and Preference*, Vol. 22, p. 346–354.

THE WETTABILITY AND SURFACE FREE ENERGY OF ACORN STARCH GELS ISOLATED BY ALKALINE AND ENZYMATIC METHODS

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Abstract

Wettability may be a convenient parameter providing information on surface properties of starch gels surface. In food technology, a better understanding of the surface properties of gels can be useful in developing new food products and food components. The gelatinized starch has the property of forming gels, and when it is dehydrated, it forms a hard, transparent, bright, and resistant film. Starch biofilms are produced from gelatinization and after retrogradation of starch. This work aimed to study acorn starch gels wettability and their surface free energy, as well as the colour.

Quercus suber and *Quercus rotundifolia* fruits were dry and flour were produced. Starch was isolated from acorn flours by an alkaline and enzymatic laboratory scale methods. Starch gels films were prepared with starch suspensions of 1.2 and 3% from the isolated acorn starches. Contact angles were measured on dry starch gel films. Colour of films was measured using a colorimeter and the classification by CIELAB system.

The isolation method influenced the wettability of the acorn starch films form heated 3% starch suspensions. The acorn films presented high contact angles, meaning that films are hydrophobic, with less water affinity, for both starch isolation method, between 69.9–74.3° for *Q. rotundifólia* and 68.6–70.2° for *Q. suber*. The surface energy was different for acorn species and isolation methods, and generally it is low, which means that acorn starch presented high intramolecular interactions forces. Films also presented similar transparency and were yellow-brown in colour. Acorn starch films isolated through enzymatic method were browner.

Keywords acorn fruits, starch, films, isolation methods, contact angle.

Introduction

Acorn fruits from *Quercus suber* (QS) and *Quercus rotundifolia* (QR) are important forestry resources in the Central and Southern regions of Portugal, but they can also be important in Greece, Italy and France (Grove, Rackham, 2001). Most of the fruit production goes to animal feeding, mainly of Iberic pigs. These fruits are also consumed in other European countries (Rakic et al., 2006), and there are many different kinds of commercially available processed acorn products, including breads, cakes, soups, snacks, noodles and jelly, which are comprised principally of acorn flours (Kim, Yoo, 2009).

Starch as a natural component, contributes to the characteristic properties of food products such as texture, viscosity, gel formation, adhesion, binding, moisture retention, film formation and product homogeneity in different products like sauces, puddings, confectionary, and a variety of low-fat products. Starch is the main component of acorn flour, 31.4% (Correia, Beirão-da-Costa, 2012), suggesting that these fruits can be a good alternative to conventional starch resources, such as cereals and tubers. However, the properties of starches are not only dependent on the starch source but also highly dependent on the history of the starch itself (Wichmann et al., 2007) as for instance the extraction procedures. It is known that extraction procedures affect both the chemical composition and physical properties of starch, which justify the interest of studying the most suitable one for each individual raw material.

Many uses of starch involve heating it in water, which leads to granule hydration, swelling, and at a high enough temperature, solubilisation of starch molecules. The most important starch properties, with respect to its utilisation, are often related to the properties of gelatinized pastes. The gelatinized starch has the property of forming gels, and when it is dehydrated, it forms a hard, transparent, bright, and resistant film. Starch biofilms are produced from gelatinisation and after retrogradation of starch.

Wettability may be a convenient parameter providing information on surface properties of starch gels`surface. The wettability of a solid surface can be determined by a relatively simple method, measuring the so-called contact angle (Adamson, 1990). The study of starch gels`wettability and their surface free energy may be useful for food science and pharmacology fields. In food technology, a better understanding of the surface properties of gels can be useful in developing new food products and food components. In pharmacology, wettability can help to explain the drug delivery systems and mucosa adhesion (Bialopiotrowicz, 2003). Furthermore, an intense search for new renewable sources to produce edible and biodegradable materials is observed. Edible and biodegradable natural polymer films offer alternative packaging with lower environmental costs. The main renewable and natural biopolymer films are obtained from polysaccharides, lipids and proteins. Furthermore, starch is considered one of the most promising raw materials for developing biodegradable plastic to reduce the environmental impact of plastic wastes, especially from packaging (SinhaRay, Bousmina, 2005).

The objective of this work was to study acorn starch films colour characteristics, their wettability and their surface free energy, important parameters for food and non-food industries.

Materials and Methods

Sampling

Quercus suber Lam. (QS) and *Quercus rotundifolia* Lam. (QR) acorns were collected in "montados", located in Idanha-a-Nova (Centre East of Portugal). The acorns were harvested at full maturity. Three sets of 2 kg were randomly collected for each species. Preparation of acorn fruits was performed as described by Correia and Beirão-da-Costa (2012).

Starch isolation methods

Starch was isolated similarly as from sorghum flours following two methods (Correia, Beirão-da-Costa 2012):

- *Alkaline pH using successively three sieves (A)*

The flours (120 g) were soaked in 2 volumes of 0.25% NaOH at 5ºC for 24 hours. Suspensions were homogenised and screened through a 180 µm sieve. The procedure was then repeated twice. The precipitate was screened successively in 75 and 53 µm sieves. The mixture was centrifuged in a Universal 16 centrifuge (Hettich Zentrifugen Company, Germany) at 800 g 15 minutes, the mucilaginous layer was scraped away and the precipitate was then suspended in water. This last step was repeated twice. Isolated starches were dried for two days at 40 °C in a FD 115 Binder ventilated drying chamber (with an air flow of 300 m^3 per hour).

- *Enzymatic method (E)*

Protease from *Aspergillus oryzae* was purchased from Sigma Chemical Co. One unit of protease was defined as the amount of enzyme that liberated 1.0 µmol of tyrosine per minute from casein at pH 7.5 at 37 °C. The protease was added (900 units) to 120 g individual flours. Water was added (360 mL) and the slurry was adjusted to pH 7.5 (with 0.1 M NaOH or 0.1 M HCl). The slurry was incubated at 37 °C for a period of 2 hours. The slurry was then centrifuged in the same conditions as previously mentioned. The starch fraction was suspended, washed with water (200 mL) and filtered through a 53 µm sieve. The filtrate was centrifuged. The supernatant and tailings were discarded and the starch dried as reported above.

The yields and starch purities are shown in Table 1.

Table 1

Yield and purity of starches isolated from acorn flours through alkaline and enzymatic methods

Sample	Isolation method	Yield, %	Purity, %
QR	Alkaline	88.5 a	98.1 a
	Enzymatic	86.9 b	97.6 a
QS	Alkaline	87.6 a	98.0 a
	Enzymatic	85.4 b	97.8 a

Starch gels and preparation of their films

Starch gels and preparation of their films of 1, 2 and 3% were obtained from the isolated acorn starches, according to Silva et al. (2007). Two hundred milliliters of water suspension of a proper amount (e.g. 1, 2 and 3%) of dry starch powder were poured in a small (250 mL) stainless steel reactor vessel. The vessel was immersed in a thermostated bath at 85 °C and its content was continuously stirred mechanically. After reaching 85 °C, the heating continued during 40 minutes at continuous stirring. The obtained gel was cold, and 15 mL of gelatinised starch was placed in a plastic Petri dish, with 110 mm of diameter and 15 mm of thick. The Petri dish was placed in a dry oven with controlled temperature, at 25 °C for 18 hours (the time necessary to remove the excess of water).

Contact angle measurements

Contact angles were measured on dry starch gel films. The contact angle for MilliQ water (Millipore, Moshlaim, France), formamide pure Fluka AG (Switzerland), ethylene glycol p.a. POCh Gliwice (Poland), and glycerol p.a. POCh Gliwice (Poland) was determined on acorn gel films. Contact angle measurements were carried out by using a contact angle instrument, Contact Angle System OCA20 (Dataphysics, Germany) with multiple portions, and the Sessile and Captive drop method was applied. A 0.1 mL droplet of water (or the other used liquids) was placed on the sheet surface and the image of the drop was captured by a CCD digital video camera. The contact angles were measured on both sides of the drop and averaged. Samples of 30 mm of length and 5 mm of width were applied on a glass surface using double-sided adhesive tape. At least 30 measurements were done for each experiment. The measurements were made at room temperature in a closed box.

Colour parameters of acorn starch films

The colour of all gel films was measured using a handheld tristimulus colorimeter (Chroma Meter-CR-400). The parameters measured were the brightness L*, which varies between 0 and 100 (from black to white, respectively), and the coordinates of opposed colour: a* and b*, which vary from –60 to +60, where the a* assumes negative values for green and positive values for red, while b* assumes negative values for blue and positive for yellow. From L* a* b*, the chroma (c*) and hue angle (h°) were determined. Chroma or saturation, c* (0–60), measures how dull / vivid the object colour is; hue angle, h° (0–360°), expresses the characteristic/dominant colour (0 – red / purple; 90 – yellow; 180 – bluish / green). Films colour measurements were taken by putting films on white paper. The total colour change (ΔE), was the parameter considered for the overall colour difference evaluation, between a sample and the reference fruit (without storage, designated with an index 0):

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

At least 30 measurements were done for each experiment.

Statistical analysis

The obtained results were subject to a one-way analysis of variance (ANOVA) test using the Statistic® vs 8

software. The separation of means or significant difference comparison of all parameters was tested by the Tukey's HSD test. The level of significance used for all the statistical tests was 95%.

Results and Discussion

The isolation methods lead to acorn starches with high purity and yields (Table 1), and the alkaline method was considered to be the better one, mainly because of the higher yields. Considering the experimental conditions for film formation, the 3% gel concentration was the only sample with good results, producing films without breakage. The films showed different colour characteristics depending on the starch source and the isolation method (Figure 1 and Table 1).

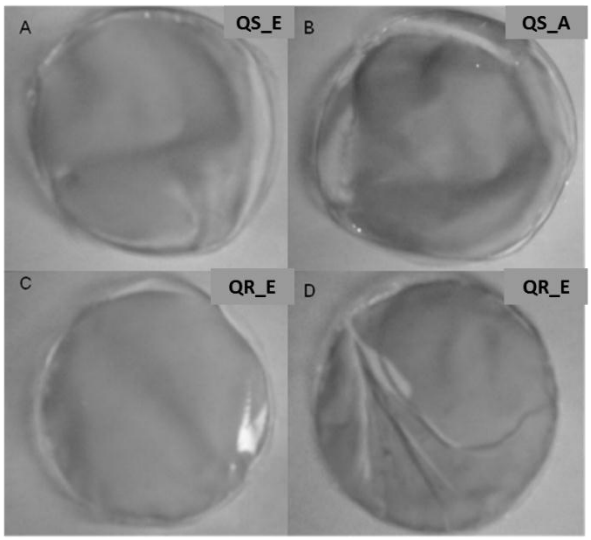

Figure 1. Appearance of acorn starch films(S)
A – Starch alkaline isolation method, E – Starch enzymatic isolation method

The QS_A film seen to be the most distinguished one (Figure 1). The colorimetric results showed that the starch isolation method influences the films colour characteristics, but in different ways. The acorn films presented high transparency and predominant yellow colour (Table 2). QS films presented high absolute values of a* parameter, meaning that they assumed green colour. The QR films formed from starch isolated through enzymatic method were more vivid than the others. The encountered colour difference might be classified according to Drlange (1994) as great (ΔE between 6.0 and 12.0). The results showed that either for the variety QS_A, which corroborate the image showed in Figure 1.

Table 2

Colour parameters of sorghum and corn films

Sample	L*	a*	b*	c*	h°	ΔE
QR_A	89.06 c	-0.35 b	8.25 c	8.25 c	92.42 c	11.51 c
QR_E	90.82 a	0.03 a	3.68 b	3.68 b	89.39 b	6.63 b
QS_A	87.07 b	-0.56 d	12.83 a	12.83 a	92.51 c	16.50 a
QS_E	89.00 c	-0.48 c	8.74 c	8.74 c	93.13 a	12.02 c

Means sharing the same letters in column are not significantly different from each other (Tukey's HSD test, $p<0.05$).

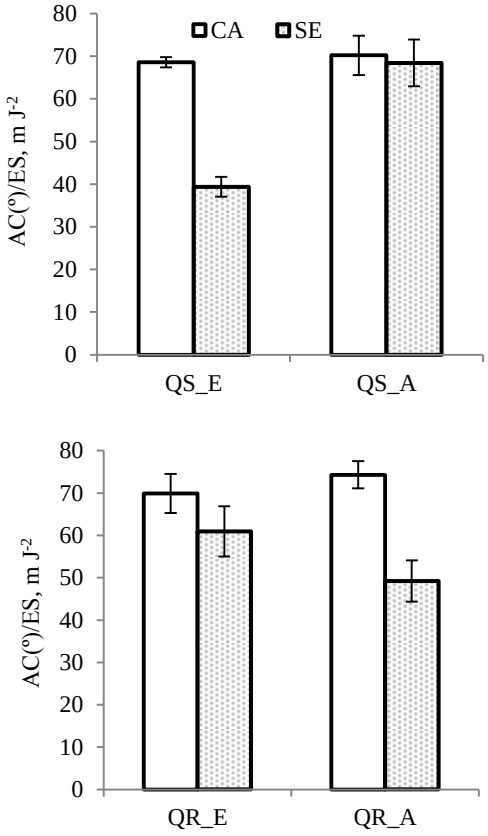

Figure 2. Contact angle (CA) and surface energy (SE) of acorn starch films
E–Enzymatic method, A–Alkaline method

The contact angle of the biofilms formed from sorghum starch presented higher values than commercial corn starch (46°) (Gonçalves et al., 2012), meaning that the acorn films surface is more hydrophobic, with less water affinity (Fig. 2). It could be noticed that the starch isolation method did not affect significantly this characteristic.

The surface energy was greatly influenced by the isolation method but differently and oppositely for QS and QR. Generally all the films presented low (or intermediate values of surface energy, when compared to other tested material, such as sorghum (38.0 and 40.7, respectively for alkaline and enzymatic isolation method) and commercial corn (90.0) starches (Gonçalves et al., 2012) which mean that acorn starches presented lower or intermediate intramolecular interactions forces. This characteristic is lower for QS_E and QR_A.

The results also showed that the interaction forces dispersed and polar part are quite different between species and between isolation methods. For all samples the polar part was high, with the exception of QS films form from starch isolated through enzymatic method (Fig. 3) and this could be related with the lower values for surface free energy (combination of dispersed and

polar interactions forces). It was also noticed that for QS films produced from starch isolated from enzymatic method the dispersed component was higher.

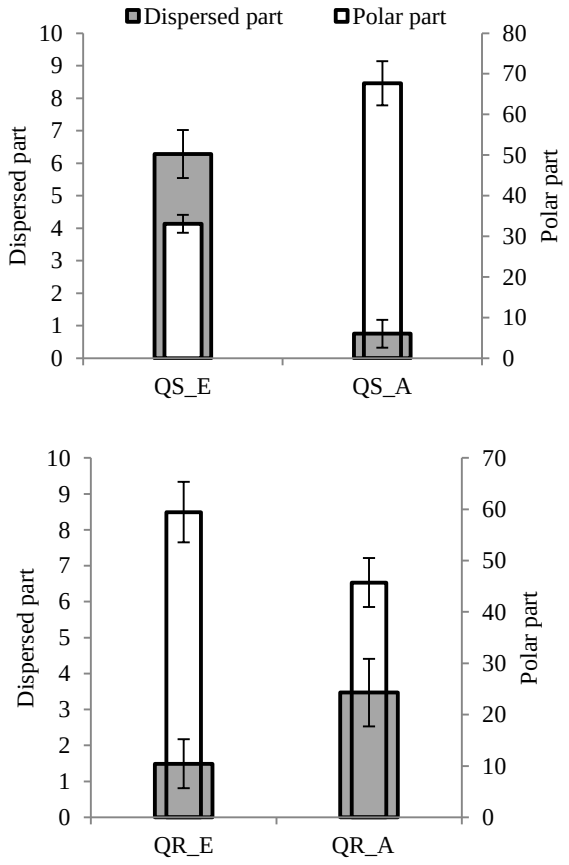

Figure 3. Surface energy of polar and dispersed parts of sorghum and corn starch films
E – Enzymatic method, A –Alkaline method

Conclusions

The results showed that acorn starch could form biodegradable films and appears as a low-cost renewable polymer.

Generally, the isolation method influenced the wettability and colour of the acorn starch films form heated 3% starch suspensions in different ways, and the studied characteristics of acorn films depend also on acorn species.

The acorn films presented high transparency, which is a good characteristic, with a predominant yellow colour, mainly in *Quercus suber* films formed with starch isolated by alkaline method. This yellow colour could influence the surface colour of the product.

Acorn films presented a hydrophobic surface with low water affinity. Moreover, the surface energy was low / intermediate which means that acorn starch presented weak intramolecular interaction forces.

For all samples the interaction force polar part was high, with the exception of QS films from starch isolated through enzymatic method, which could be related with the lower values for surface free energy.

Acknowledgment

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References

1. Adamson A. W. (1990) *Physical Chemistry of Surfaces*. 5th Edition. New York: Wiley-Interscience. 777 p.
2. Bialopiotrowicz T. (2003) Wettability of starch gel films. *Food Hydrocolloids*, No. 17, p. 141–147.
3. Correia P. R., Beirão-da-Costa M. L. (2012) Isolation of starch from chestnut and acorn flours through alkali and enzymatic methods. *Food and Bioproducts Processing*, No. 90, p. 309–316.
4. Drlange (1994) Colour Review. USA: Drlange Application Report No. 8.0 e. Drlange.
5. Gonçalves S., Coelho J., Gil H., Correia P. (2012) The wettability and surface free energy of white sorghum starches gels isolated by alkaline and enzymatic methods. In: International conference of Agriculture Engineering CIGR-AgEng2012, Spain, Valência [e-book]. 5 p.
6. Grove A.T., Rackham O. (2001) The Nature of the Mediterranean Europe: An Ecological Story. New Haven: Yale University Press.
7. Kim, W.-W., Yoo, B. (2009). Rheological behaviour of acorn starch dispersions: effect of concentration and temperature. International *Journal of Food Science and Technology* No. 44, p. 503–509.
8. Rakic, S., Povrenovic, D., Tesevic, V., Simic, M., Maletic, R. (2006). Oak acorn, polyphenols and antioxidant activity in functional food. *Journal of Food Engineering*, No. 74, p. 416–423.
9. Silva W. A., Pereira J., Carvalho C. W., Farrua F. Q. (2007) Determination of color, topographic superficial image and contact angle of the biofilms of diferente starch souces. *Ciência Agrotecnológica, Lavras*, No. 31, p. 154–163.
10. SinhaRay S., Bousmina M. (2005) Biodegradable polymers and their layered silicate nanocomposites: in greening the 21st century materials world. *Progress in Material Science*, No. 50, p. 962–1079.
11. Wichamann B., Ahmt T., Bandsholm O., Blennow A., Young N. (2007) Testing properties of potato starch from different scales of isolation - A ringtest. *Journal of Food Engineering*, No. 79, p. 970–978.

EFFECT OF DIFFERENT TYPES OF HEAT TREATMENT ON INVERTASE ACTIVITY IN HONEY

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Abstract

Honey is important food stuff, which is often consumed fresh or used in other meals. Liquid honey is more demanded in market, so solid honey often is heated in order to melt it. One of the most traditional ways to melt the honey is by heating using thermal treatment – usually in higher temperature than 50 °C. Using microwave ovens are very popular approach to heat food and its popularity is growing, so it is important to understand, how food and its substances are being influenced by microwave heating. One of the most heat-sensitive enzymes is invertase.

The aim of the research is to study and to compare, how invertase activity in honey is affected by heating it in the microwave oven and in the thermostat, while changing microwave oven power, heating duration and heating temperature in thermostat.

p-Nitrophenyl-α-D-glucopyranoside (pNPG) is used as a substrate for the determination of the invertase number in honey. pNPG is split into glucose and p-nitrophenol by α-glucosidase (invertase, sucrase). By adjusting the pH-value to 9.5 the enzymatic reaction is stopped and at the same time nitrophenol is transformed into the nitrophenolate anion, which corresponds to the amount of converted substrate and is determined photometrically at 400 nm.

Analyzing different honey samples, the following results were obtained. When honey samples were heated in 450 W microwave oven, invertase activity significantly decreased after 20 seconds. After 25 seconds invertase activity decreased 5.8 times, after 30 seconds – 12.5 times.

After heating honey samples in thermostat in 60 °C for 120 minutes, invertase activity decreased only twice.

Summarizing research results, we concluded, that invertase activity changes are significantly higher in honey samples, heated in the microwave oven, than honey samples, heated in the thermostat.

Keywords: Honey, invertase activity, thermal treatment, microwave.

Introduction

Food often gets the thermally treated to heat it. Food is heated faster in microwaves than in usual oven. Honey is one of most used foodstuff. Honey in food is used both raw and in confectionery products. In order to better sell honey it's often heated. A small amount of honey can be quickly heated using microwaves.

Microwaves are electromagnetic radiation in the wavelength range between 30 cm to 1 mm (1 GHz–300 GHz), by other data 1 m – 1 mm (300 MHz–300 GHz). Microwaves are essentially short radio waves and in the past they were not separated from radio waves; they are produced by high-frequency power generator, called magnetron (Electromagnetic Energy and Microwaves, 2013).

Microwave radiation intensity depends on the tubes that are used in device. There are separated high power and low power microwave radiation sources. Microwave transmitters and receivers are similar to radio wave transmitters and receivers. Microwave transmitter tubes contain tuned circuit, which is able to perceive or transmit certain frequency bands.

Product heating in microwave oven happens when high-frequency electromagnetic wave energy is transformed in thermal energy; in result products cooking time is two to three times lower than usual. Microwave principle of operation is to broadcast non-ionizing microwave radiation, usually 2.45 GHz, through food. Substances such as water, grease and other receive energy from microwaves in process called dielectric heating.

Many molecules, including water are polar – with a single positive charge on one side and negative charge on the other. Transmitting microwave radiation, water molecules continuously rotates, relative to the prevailing magnetic field that varies 2.45 million times per second. As rotation speed of water molecules increases, product temperature increases as well (Sapunov, 2007; Electromagnetic Energy and Microwaves, 2013).

Affecting food, such as honey, with microwaves denaturates proteins within it, changes vitamin content and also reduces the activity of enzymes (Sapunov, 2007; Hendrickson, 2011).

Honey is losing its biological and antibacterial activity and many of its component volatiles.

Some of the honey quality and biological activity indicators are the enzymes. Enzymes are complex proteins that catalyze reactions within cells. Enzymes have huge impact in human life processes. Honey naturally preserves small amounts of enzymes. Honey mostly contains enzyme amylase, glucose oxidase, catalase and invertase, and their contents can vary depending on the region in which honey is collected (Delaplane, Mayer, 2000).

Enzyme functions and catalyzed reactions determine honey composition and pH level and antibacterial properties.

One of honey quality indicator and thermal treatment indicator is enzyme invertase (Karabournioti, Zervalaki, 2001).

Table 1

Enzyme catalyzed reactions in honey
(Honey enzymes, 2014)

Enzyme in honey	Substratum	Reaction products
Invertase	Sucrose	Glucose and fructose
Glucose oxidase	Glucose	Gluconic acid and hydrogen peroxide
Catalase	Hydrogen peroxide	Water and oxygen
Amylase (Diastase)	Starch	Dextrose, maltose and glucose

Invertase is an enzyme which splits sucrose into glucose and fructose. Invertase activity is expressed as the invertase number or invertase units.

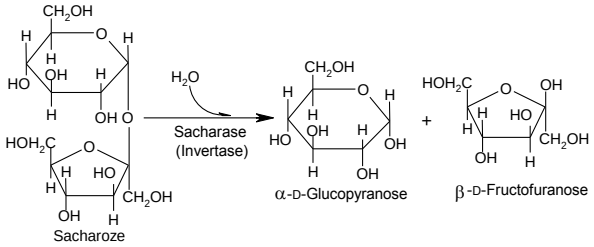

Figure 1. Reaction catalyzed by invertase
(Garret, Grisham, 2005)

Invertase unit is 1 micromole of substrate that is cleaved within 1 minute. Invertase number is the amount in grams of sucrose, which within 1 hour is hydrolyzed by invertase in 100 g honey (1 IN=7.344732 IU kg^{-1}). According to the European Commission's recommendations, invertase number should be higher than 10 (individual honey samples greater than 4) (Bogdanov, 2009; Honey Quality and International Regulatory Standarts, 2014).

The aim was to find out how honey treatment in microwave heating in a thermostat affect invertase activity and hence the quality of honey and its biological properties. To achieve the goal invertase activity were determined in honey samples.

Honey samples were heated in a thermostat, and also treated in microwave oven.

Materials and Methods

In research buckwheat flower honey samples collected in 2013 in Jelgava District Sesavas parish were analyzed. The analysis was performed at the Department of Chemistry of LUA laboratories.

Invertase activity in honey samples were determined spectrophotometrically using Jenway 6300 spectrophotometer.

Research of microwaves influence was analysed, using Samsung microwave oven.

p-Nitrophenyl-α-D-glucopyranoside (pNPG) is used as a substrate for the determination of the invertase number in honey. pNPG is split into glucose and p-nitrophenol by α-glucosidase (invertase, sucrase).

By adjusting the pH-value to 9.5 the enzymatic reaction is stopped and at the same time nitrophenol is transformed into the nitrophenolate anion, which corresponds to the amount of converted substrate and is determined spectrophotometrically at 400 nm (Bogdanov, 2009).

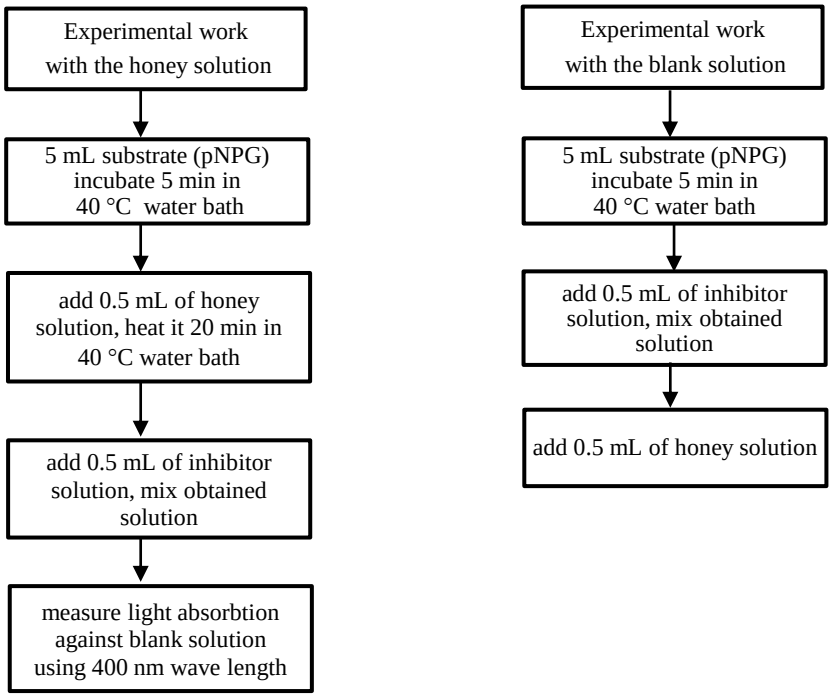

Figure 2. Overall determination scheme of invertase activity

Research on microwave radiation impact were made by treating honey samples in microwave oven 10 s using 180 W, 300 W, 450 W, 600 W in 800 W high power microwave. According from the results, the optimal choice of microwave intensity was chosen.
The thermostat heating temperature was selected by heating the honey samples for 1 h in 50 °C, 60 °C and 70 °C. Then, according from the results the optimal temperature was used in subsequent experiments.
The general methodology for determining invertase activity is shown in Figure 2.

Results and Discussion

Microwave oven power influence on invertase activity in honey is shown in Figure 3.

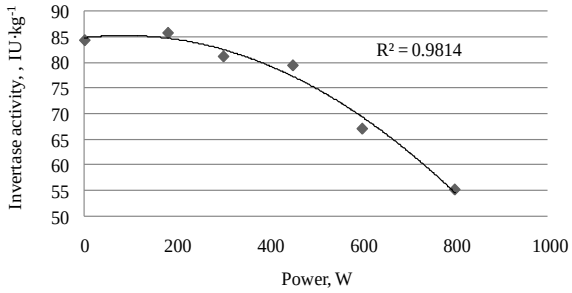

Figure 3. Changes of invertase activity in various microwave oven powers

Honey samples, using 180 W, 300 W, 450 W, 600 W, 800 W microwave oven power, were treated 10 s. As can be seen from the results of Figure 3, increasing microwave power, reduce the invertase activity in honey samples.
Raw honey sample have invertase activity of 85±8 IU, which indicates honey sufficiently high enzyme activity and thus biological - functional properties. This is consistent with the recommendations of the International Honey Commission (IN≥10; IU≥73.45).
To study microwave radiation duration effect on invertase activity, had to opt for microwave power.
It may be more accurate and more appropriate to do this by studying the dynamics of invertase activity decay times by reference to the fresh honey invertase activity.

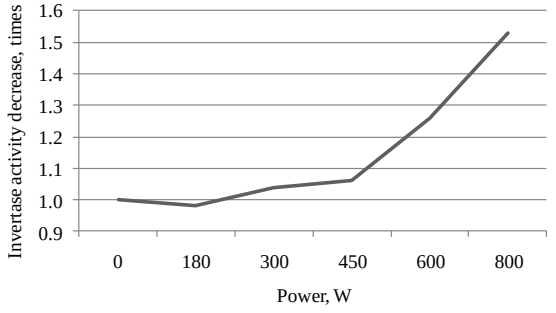

Figure 4. Correlation between decrease of invertase activity and microwave oven powers

As can be seen from the Figure 4 data, a significant decrease in invertase activity occurs from 450 W microwave oven power. Therefore, studies on the effects of microwave radiation on the invertase activity, depending on the microwave radiation exposure time, is carried out using 450 W microwave power.

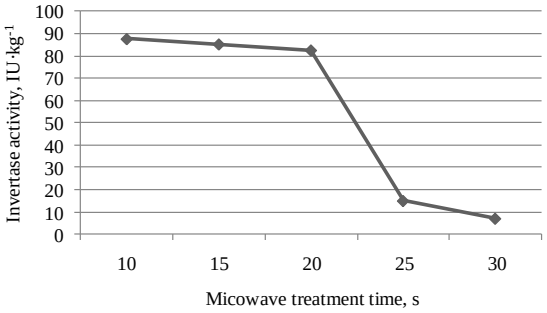

Figure 5. Changes of invertase activity depending on the duration of exposure to microwave radiation

Figure 5 shows that by treating honey samples in a microwave oven power of 450 W, significant changes of invertase activity started to happen after 20 seconds. After 25 seconds of treatment in a given microwave oven power invertase activity decreases 5.8 times, but when treated for 30 seconds – 12.5 times.
It means that significant honey quality changes occur, thus, protein denaturation occurs; it worsens the healing and anti-bacterial properties.
In order to judge the effect of microwave radiation, honey samples were also heated in thermostat using different heating temperatures and heating lengths.
In order to assess the heating effect of temperature on invertase activity in honey were heated for 1 hour at 50 °C, 60 °C and 70 °C.

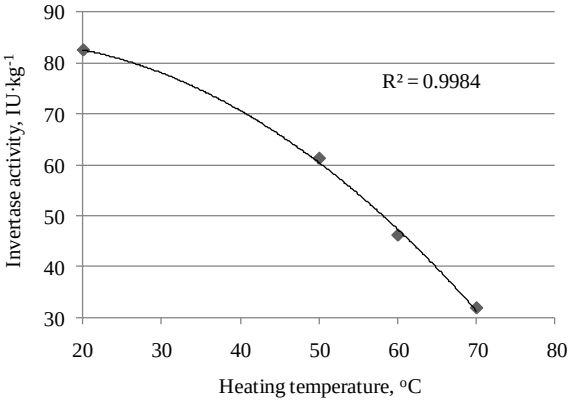

Figure 6. Effect of the heating temperature on invertase activity in honey

As can be seen from Figure 6, increasing the heating temperature, the invertase activity decreases.
In order to characterize the duration of the heating effect of honey on honey invertase activity in honey and correctly select the heating temperature, the

invertase activity change dynamics were characterized by invertase activity decay times relative to the fresh honey (uncooked) invertase activity.

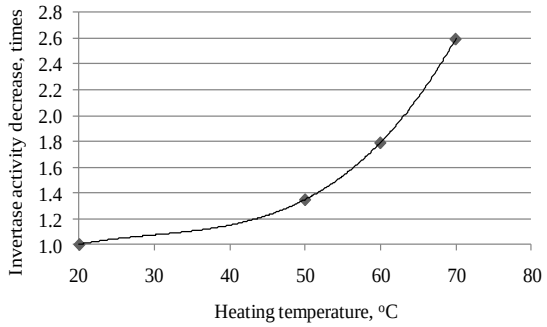

Figure 7. Correlation between decrease of invertase activity and heating temperature

As can be seen from Figure 7, invertase activity starts to decrease significantly from 50 °C temperature. In order to characterize the invertase activity of correlation between the duration of heating, the temperature of 60 °C was chosen because at this temperature for invertase activity has been greatly reduced. Invertase activity of correlation between the duration of the heat treatment is shown in Figure 8.

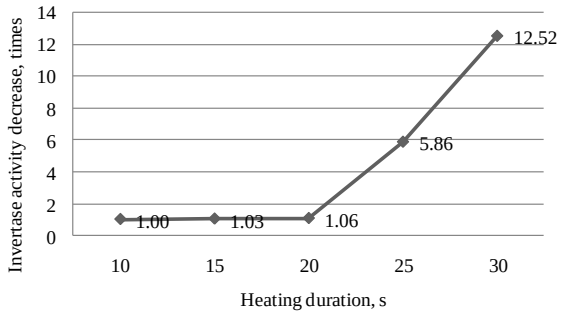

Figure 9. Decrease of invertase activity depending on heating time in microwave oven

As can be seen from the Figure 9 invertase activity changes in the microwave oven heated honey samples are insignificant if honey is heated less than 20 s, on the other hand, from the 20 s invertase activity changes occur very rapidly. On the other hand, if we compare the thermostat heated honey samples, they invertase activity gradually decreased from the beginning of the heating. However the invertase activity decrease is lower than in microwave oven heated honey. For example, at the end of experiment, in thermostat 2 hours heated honey invertase acitivity decreased 2 times (Fig. 10), however in 450 W microwave oven it decreased 5.86 times after 25 s and 12.52 times after 30 s heating.

That means if honey needs to be heated then it should be done using conventional heating. In microwave oven honey can be heated very short time period, and if honey is heated for 30 s or longer, then honey can completely lose its quality.

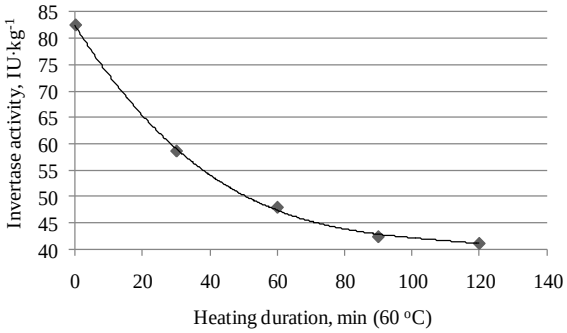

Figure 8. Correlation between invertase activity and the heating time of honey

As you can see from the Figure 8, honey invertase activity changes are not so great when compared to the invertase activity changes in honey samples heated in the microwave.

This is best demonstrated when comparing change in invertase activity decay times in a microwave oven and in a thermostat treated honey samples.

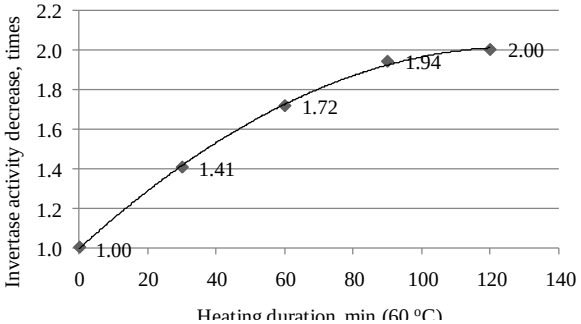

Figure 10. Decrease of invertase activity depending of heat treatment

Conclusions

Treating of the honey samples in a microwave oven, invertase activity changes occur much faster than heating it in an oven.

Treating of the honey samples in Samsung microwave oven significant invertase activity changes occurs if microwave oven power is higher than 450 W.

Treating of the honey samples in a microwave power of 450 W, a significant invertase activity changes occur after 20 s. After 25 s of treatment in a given power invertase activity decreases 5.8 times, while treating the 30 s – 12.5 times.

Heating of the honey samples in a thermostat at 60 °C for 2 h, invertase activity gradually decreases twice.

Treating of the honey samples in microwave oven significantly reduces the activity of biologically active substances.

In thermostat heated honey samples, significant invertase activity changes occurs if heating temperature exceed 50 °C.

Enzymes and other proteins denaturates – lose activity, if they are heated in thermostat or treated in microwave radiation.

The invertase number of analyzed honey samples (11.57) indicates the high quality of honey.

References

1. Bogdanov S. (2009). *Harmonised methods of the international Honey Commission*. [accessed on 20.02.2014]. Available: http://www.beekeeping.com/articles/us/honey_quality.htm
2. Delaplane K., Mayer D.F. (2000) *Crop pillination by bees*. Cambridge: Cabi Pusblishing, 344 p.
3. *Electromagnetic Energy and Microwaves*. [accessed on 27.12.2013.]. Available: http://research.fuseink.com/p75i28jvfap8y/electromagnetic-energy-and-microwaves
4. Garret R.H., Grisham C.M. (2005) *Biochemistry*, 3th Edition. Belmont, Saunders College Publishing Inc.,1100 p.
5. Hendrickson K. (2011) *The Effects of Microwaving on Food*. [accessed on 02.03.2014.]. Available: http://www.livestrong.com/article/371758-the-effects-of-microwaving-on-food/
6. *Honey Enzymes.* [accessed on 02.03.2014.]. Available: http://www.honey.com/images/uploads/general/enzymesofhoney_-final_from_David_Ropa_061010_with_edits_accepted.pdf
7. *Honey Quality and International Regulatory Standarts. Review by the International Honey Commission.* [accessed on 20.02.2014]. Available: http://www.beekeeping.com/articles/us/honey_quality.htm
8. Karabournioti, S., Zervalaki, P. (2001) Die Wirkung der Erwarmung auf HMF und Invertase des Honigs. *Apiacta*, Vol. 36, No 4, S. 178–181.
1. Сапунов Г.С. (2007) *Элементы микроволновой техники. Влияние СВЧ излучения на биологические объекты*. [accessed on 02.03.2014.]. Available: http://www.elremont.ru/small_rbt/bt_rem19.php

COMPARISON OF THREE DIFFERENT TECHNIQUES FOR EXTRACTION OF VOLATILES FROM PISTACHIO NUTS

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Abstract

Pistachio nut (*Pistacia vera* L.) is one of the popular tree nuts of the world and is widely cultivated in saline, dry and hot areas of the Middle East, Mediterranean countries and United States. Iran, USA, Syria and Turkey are the main producer countries of this product. Pistachio nut is widely consumed as a raw or roasted ingredient of many desserts, ice creams, cakes, pastry and in the production of some sausages. The aim of the present study was to determine the appropriate technique for extraction of volatiles from pistachio nuts. Uzun variety which is cultivated in the South-East Region of Turkey was used as the material of the study. Volatiles were extracted with three different extraction techniques – Liquid-Liquid Solvent Extraction (LLSE) at 85±2 °Cfor 6 h by using ethanol and hexane as solvents, Headspace extraction (HS) at 100°C for 1 h, and Solid Phase Micro Extraction (SPME) at 100 °C for 1 h by using DVB/Car/PDMS fiber. Obtained extracts were analyzed by Gas Chromatography/Mass Selective detector (GC/MS). The total number of volatiles extracted by LLSE, HS and SPME techniques were 34, 41, 25, respectively; and the percentage of volatiles identified by these techniques were found to be 67.36%, 72.04% , 92.91%, respectively. Since more than 90% of volatiles could be identified by SPME which also showed high repeatability, it could be recommended as the most appropriate technique for extraction of volatiles from pistachio nuts.

Keywords: pistachio nut, extraction of volatile components, GC/MS analysis.

Introduction

Pistachio nut (*Pistacia vera* L.) is one of the popular tree nuts of the world and is widely cultivated in saline, dry and hot areas of the Middle East, Mediterranean countries and United States (Maskan, Karatas, 1999; Kashani-Nejad et al., 2003). According to the FAOSTAT Iran, USA and Turkey are the main producer countries of pistachio nuts (Anon, 2014a). One of the major cultivar of pistachio nuts grown in Turkey is Uzun and it is mainly preferred for the production of baklava and nut paste because of its special green kernel colour, flavour and texture (Balta, 2002; Gamlı, Hayoglu, 2007).

Different kind of extraction techniques have been studied to determine the volatile compound of different types of nuts such as solvent extraction technique for chestnuts (Morini, Maga, 1995), almonds (Cantalejo, 1997; Vazquez-Araujo et al., 2008), peanuts (El-Kayati et al., 1998; Ku et al., 1998), hazelnuts (Kiefl, Schieberle, 2013) headspace (HS) technique for peanuts (Young, Hovis, 1990; Braddock et al. 1995; Burroni et al., 1997; Ku et al. 1998, Alasalvar et al., 2003); and solid phase micro extraction (SPME) technique for peanuts (Abegaz et al., 2004; Krist et al., 2004. Although the flavour of pistachio nuts has wide appeal, there is not enough study about this topic. Soliman et al (1981) studied the volatiles of roasted pistachio nuts by using vacuum carbon dioxide distillation of acetone extracts and identified the volatiles as pyrazines, pyrrols, aldehydes and some others by using GC/MS. In another study conducted by Kendirci and Altug (2011) the volatile compounds of different varieties of fresh pistachio nuts were extracted by using SPME-GC/MS and it was found that volatiles of fresh pistachio nuts were mainly composed of terpenes like α-pinene, α-terpinolene, limonene, β-myrcene. In another study where volatiles of roasted pistachio nut were analysed, pyrazines, aldehydes, acids and some other compounds were detected (Acena et al. 2011).

In this study, the volatile components of Uzun variety of pistachio nuts grown in Turkey were extracted using three different extraction methods to determine the appropriate technique. For this purpose, Liquid-Liquid Solvent Extraction (LLSE), Headspace extraction (HS) and Solid Phase Micro Extraction (SPME) was applied to the pistachio nuts. Obtained extracts were analyzed by Gas Chromatography equipped with Mass Selective Detectorr (GC/MS).

Materials and Methods

Materials

Uzun variety of pistachio nuts (*Pistacia vera L.*) were obtained from Pistachio Nut Research Institute in Gaziantep, Turkey at the beginning of the harvest season in September 2005.

Liquid Liquid Solvent Extraction (LLSE)

The method described by Heath and Reineccius (1986) was applied to the samples as a LLSE technique. For this purpose, 40 g of ground pistachio nuts were transferred to the sample tube of LLSE apparatus with the help of 100 mL of ethanol; while 125 mL of hexane was in the collector conical flask (Figure 1).

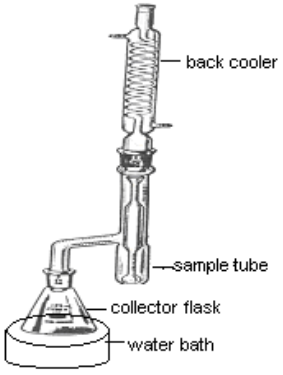

Figure 1. LLSE apparatus

Conical flask was placed in to the water bath at 85±2 °C and extraction was continued for 6 h. Hexane extract was concentrated to 10 mL at room temperature by the help of nitrogen gas. 5 µL of extract was injected in the GC/MS (Hewlett Packard, USA).

Headspace Extraction (HS)

HS extraction was performed by modifying the methods suggested by Young, Hovis (1990), Burroni et al. (1997) and Ku et al. (1998). For this purpose 8 g of ground sample was weighed into a screw top amber vial with black viton septa which was placed into a block heater at 100 °C. After 60 min, 1 mL of headspace was injected to the GC/MS device.

Solid Phase Micro extraction (SPME)

SPME procedure was performed by using a manual SPME device and extractions were carried out by using a 50 / 30 µm DVB/Carboxen/PDMS stable-Flex fiber as suggested in Abegaz et al. (2004) and Krist et al. (2004). 8 grams of dehulled and groundsample was weighed into a screw top amber vial with black viton septa which was placed into a block. SPME fibre was immersed to the headspace of the vial and the volatiles were collected for 60 min at 100 ºC. After sampling, the SPME fiber was removed from the vial and introduced onto the injection port of GC/MS.

GC/MS Analysis

The volatiles were separated using HP-5MS 5% phenylmethylcyloxane column (30.0 m×0.25 mm× 0.25 µm film thickness) that was attached to a Hewlett Packard Model HP-6890 gas chromatograph equipped with a HP 5973 Mass Selective detector. The operating conditions of the GC were as follows: injector temperature of 250 °C, splitless mode, carrier gas of helium (with an inlet flow rate of 1 mL min^{-1}). The temperature gradient used began at 60 °C for 1 min, then was raised to 260 °C at 5 °C min^{-1} and held at this temperature for 45 min. Mass spectra were generated at 70 eV. The mass selective detector was scanned from 30 to 350 at 1 scan s^{-1}. Identification of the volatile compounds was achieved by comparing retention times with those in the MS library (NIST and WILEY).

Interpretation of the Results

The analyses were applied as triplicate for each technique, and the standard deviations from the mean values were calculated and shown on tables.

Results and Discussion

The chromatographs of the volatiles extracted from the pistachio nuts by using LLSE, HS and SPME techniques are shown on Figures 1–3; list of the volatile compounds are given in Tables 1–3.

As it can be seen from Table 1 and Table 4, 34 volatile compounds were extracted from pistachio nuts using LLSE technique. 32.62% of the total peak area of the volatiles could not be identified, while 35.41% them were determined as 9-octadecanoic acid isomers having a fatty character (Anon 2014b).The percentage of α-pinene which is one of the major volatiles of the pistachio nuts (Kendirci, Altug, 2011) was detected to cover only 0.16% of the total peak area. The repeatability of the technique was determined to be low since the standard deviations of the results were high from Table 1.

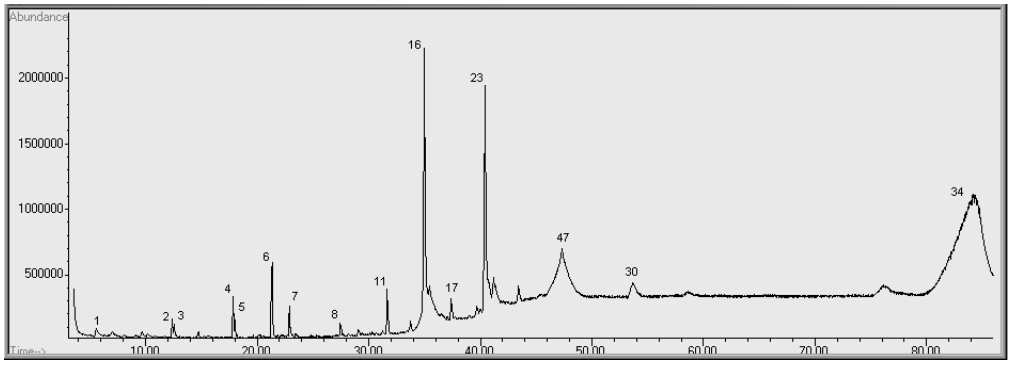

Figure 1. GC/MS chromatograph of the volatiles extracted by using LLSE technique

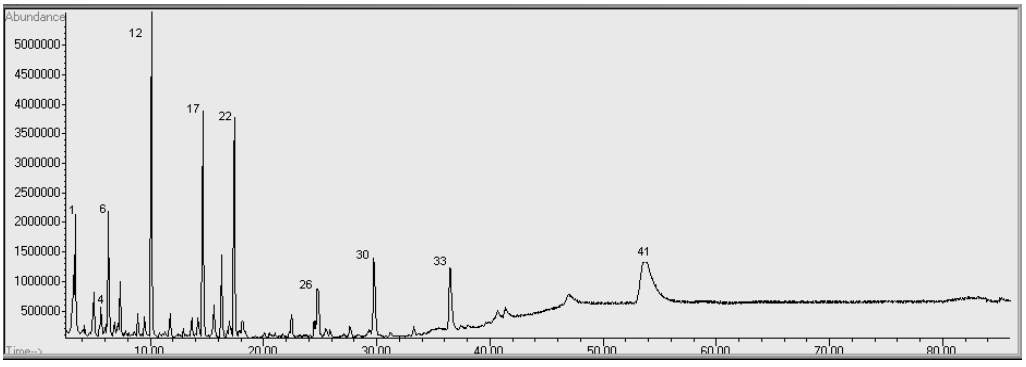

Figure 2. GC/MS chromatograph of the volatiles extracted by using HS technique

Table 1

The volatile compounds extracted by using LLSE technique

Peak	t_R (min)	Volatile compound	Area %
1	5.60	α-pinene	0.16±0.28
2	12.4	1-dodecene	0.57±0.49
3	12.58	dodecane	0.47±0.41
4	17.87	1-tetradecene	1.30±0.97
5	18.02	tetradecane	0.37±0.53
6	21.34	2,6-bis(1,1-dimethylethyl)-4-phenol	1.81±0.97
7	22.89	1-hexadecene	1.13±1.07
8	27.44	5-octadecene (E)	0.59±0.57
9	29.07	hexamethyl pyranonyndane	0.04±0.07
10	31.19	Unidentified	0.04±0.08
11	31.63	Hexadecanoic acid ethylester (ethyl palmitate)	1.10±0.98
12	32.28	n-hexadecanoic acid	0.62±1.07
13	32.85	9-octadecanoic acid (Z), isomer	1.15±1.07
14	33.40	5-eicosene	0.35±0.61
15	33.74	9-octadecanoic acid (Z), isomer	0.63±0.34
16	34.96	ethyl oleate	10.17±7.50
17	35.70	9-octadecanoic acid (Z), isomer	19.46±26.69
18	36.51	9-octadecanoic acid (Z), isomer	1.13±0.78
19	37.40	Unidentified	1.26±0.60
20	39.01	9-octadecanoic acid (Z), isomer	0.07±0.12
21	39.72	9-octadecenal	0.60±0.98
22	40.00	Unidentified	0.14±0.24
23	40.41	9,17-octadecadienal (Z)	13.74±8.86
24	40.8	Unidentified	0.52±0.91
25	41.17	Unidentified	2.91±3.12
26	41.40	1,12-tridecadiene	1.43±2.47
27	43.45	Unidentified	1.38±0.70
28	45.42	9-octadecenoic acid (Z) , 3-hydroxypropyl ester	2.47±4.28
29	47.31	9-octadecanoic acid (Z), isomer	6.55±7.36
30	53.73	9-octadecanoic acid (Z), isomer	3.95±2.08
31	58.77	Unidentified	0.27±4.28
32	61.05	Unidentified	4.15±7.18
33	76.08	Unidentified	2.97±2.38
34	84.28	Unidentified	15.03±26.03

Table 2

The volatile compounds extracted by using HS technique

Peak	t_R (min)	Volatile compound	Area %
1	3.36	hexanal	8.20±0.67
2	4.20	2-ethyl-3-vinyloxirane	0.18±0.25
3	5.04	heptanal	2.32±0.28
4	5.66	α-pinene	0.73±1.04
5	6.05	Unidentified	0.24±0.34
6	6.35	2-heptenal, (E)	4.56±0.31
7	6.85	heptanol	0.32±0.45
8	7.14	1-octen-3-ol	9.29±0.41
9	7.39	octanal	2.54±0.38
10	8.96	octenal	0.89±0.04
11	9.58	penthylcyclopropane	0.92±0.12
12	10.15	nonanal	9.60±0.95
13	11.82	2-nonenal	1.05±0.07
14	12.93	decanal	0.17±0.24
15	13.68	Unidentified	0.31±0.43
16	14.29	Unidentified	0.98±0.04
17	14.71	2-decenal (E)	7.91±0.07
18	15.70	2,4-decadienal (E,E)	1.58±0.16
19	16.39	2,4-decadienal	3.12±0.12
20	16.82	Unidentified	0.12±0.17
21	17.00	Unidentified	0.51±0.72
22	17.50	2-octenal	7.96±0.30
23	18.13	Unidentified	1.00±1.42
24	22.55	8-hexadecane (Z)	1.16±0.08
25	24.47	Unidentified	0.23±0.33
26	24.85	8-heptadecene	2.88±0.43
27	25.51	1-cloro tetradecane	0.27±0.39
28	27.62	7-hexadecenal (Z)	0.30±0.42
29	29.35	Unidentified	0.26±0.37
30	29.82	9-oxabicyclo[6.1.0]nonane	4.83±0.21
31	33.33	Unidentified	0.55±0.15
32	35.58	9-octadecanoic acid (Z)	3.78±5.34
33	36.54	Unidentified	6.87±0.05
34	37.45	E,E-10,12-hexadecadiene-1-ol acetate	0.12±0.17
35	39.83	9-octadecanoic acid (Z), isomer	0.41±0.59
36	40.51	9-octadecanoic acid (Z), isomer	0.97±1.38
37	40.76	9-octadecanoic acid (Z), isomer	2.49±1.31
38	41.46	9-octadecanoic acid (Z), isomer	2.56±1.91
39	46.95	Unidentified	1.28±1.81
40	47.33	Unidentified	2.02±2.85
41	53.94	Unidentified	13.53±4.38

41 volatiles could be extracted from the pistachio nuts and the repeatability of HS technique was higher in comparison with LLSE technique (standard deviations are lower than LLSE) (Table 2). On the other hand, 27.90% of the total extracted volatile compounds could not be identified and the percentage of α-pinene was found to be only 0.16% (Table 4).

Table 3 shows that 25 volatile compounds were extracted from samples by using SPME technique.

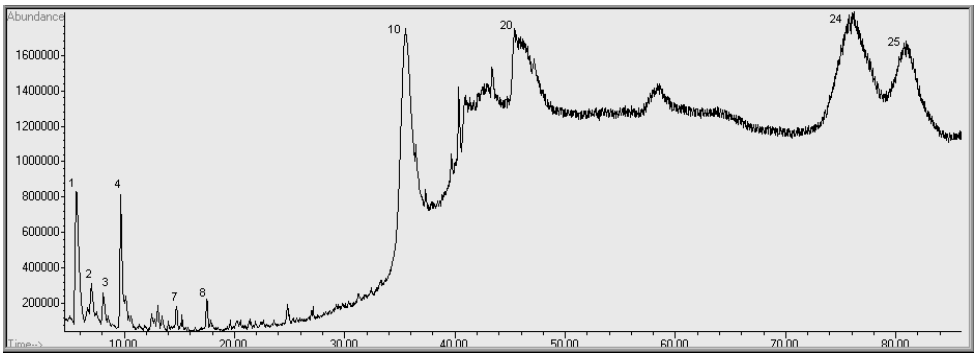

Figure 3. GC/MS chromatograph of the volatiles extracted by using SPME technique

Table 3

The volatile compounds extracted by using SPME technique

Peak	t_R (min)	Volatile compound	Area %
1	5.64	α-pinene	10.23±8.85
2	7.01	β-myrcene	1.80±1.32
3	8.09	limonene	1.41±1.25
4	9.67	α-terpinolene	6.38±5.90
5	10.16	o-isoprophenyltoluene	0.16±0.22
6	13.04	azulene	0.14±0.20
7	14.72	2-decenal	0.61±0.40
8	17.48	2-octenal	0.73±0.56
9	24.78	8-heptadecen	0.15±0.21
10	35.43	9-octadecanoic acid (Z), isomer	31.09±17.36
11	36.44	9-octadecanoic acid (Z), isomer	3.07±0.05
12	37.33	9-octadecanoic acid (Z), isomer	0.24±0.33
13	39.68	9-octadecanoic acid (Z), isomer	0.14±0.20
14	40.33	Unidentified	2.10±1.69
15	40.67	9-octadecanoic acid (Z), isomer	2.85±4.03
16	40.91	Unidentified	0.69±0.98
17	41.29	Unidentified	1.32±0.98
18	41.45	9-octadecanoic acid (Z), isomer	0.56±0.78
19	43.36	Unidentified	1.44±0.87
20	45.33	9-octadecanoic acid (Z), isomer	4.47±1.24
21	46.00	9-octadecanoic acid (Z), isomer	2.43±3.44
22	47.20	9-oxabicyclo[6.1.0]nonane	1.53±2.17
23	58.57	Unidentified	1.54±2.17
24	76.13	9-octadecanoic acid (Z), isomer	15.29±21.63
25	80.97	9-octadecanoic acid (Z), isomer	9.64±13.63

Although the total number of the volatiles extracted by SPME were lower in comparison with the other two techniques, the percentage of unidentified volatiles were much lower (7.09%).

Table 4

The names and the proportion of volatile compound groups extracted by using three different extraction techniques (LLSE, HS and SPME)

	LLSE	HS	SPME
TERPENES	0.16 % - α-pinene	0.73% - α-pinene	19.96% - α-pinene - α-terpinolene - β-myrcene - azulene - limonene
ALDEHYDES	14.34% - 9,17-octadecadienal - 9-octadecenal	50.20% - 2,4-decadienal - 2-decenal - 2-heptenal - 2-octenal - 7-hexadecenal - decanal - heptanal - hexanal - nonanal - 2-nonenal - octanal - 2-octenal - 2,4-decadienal	1.34% - 2-decenal- 2-octenal
ACIDS	47.30% - 9-octadecanoic acid - 9-octadecenoic acid -3-hydroxy-propyl ester - ethyl oleate -hexadecanoiacid ethylester - n-hexadecanoic acid	10.21 % - 9-octadecanoic acid isomers	69.78% - 9-octadecanoic acid isomers
OTHERS	8.06 % - 1-dodecene - dodecane - tetradecane - 1-hexadecene - 1-tetradecene - 5-eikosene - 5-oktadecene - 1,12-tridecadiene - 2,6-bis(1,1-dimethylethyl)-4-phenol -,hexamethyl pyranonyndan	19.97 % - 1-cloro tetradecane - 8-heptadecene - 8-hexadecane - 9-oxabicyclo[6.1.0] nonane - 1-octen-3-ol - 2-ethyl-3-vinyloxirane - penthylcyclopropane - E,E-10,12-hexadecadiene-1-ol asetate - heptanol	1.84% - 8-heptadecen - 9-oxabicyclo [6.1.0] nonane - o-isoprophenyl-toluene

Additionally, it was determined that 69.78% of the total peak area of volatiles was composed of 9-octadecanoic acid isomers (Table 4) which gives fatty character (Anon, 2014b); while 7.79% was limonene and α-terpinolene which gives citrus character (Anon, 2014b), 5.64% was α-pinene which gives pine character (Anon, 2014b), 1.80% was β-myrcene which gives must, balsamic and spice characters (Anon 2014b), and 1.34% was 2-decenal and 2-octenal which give green, nut and fatty characters (Anon, 2014b) of pistachio nuts.

Table 4 shows the proportion of volatile compound groups extracted from pistachio nuts by using three different extraction techniques. As it can be seen from the Table 4, among the extraction techniques examined in this study, SPME was the best technique for extracting the terpene groups of the volatiles which are expected to be one of the main volatile groups of pistachio nuts (Kendirci, Altug, 2011) while aldehydes were the main group of HS technique. Because of the high fat content of the pistachios, mainly fatty acids (47.30 %) were determined by LLSE technique (Table 4).

Conclusions

Among the three extraction technique studied in this study, SPME seems to be the best technique for the extraction of the volatile compounds of pistachio nuts. By this technique the major volatiles which make up pistachio nut flavour could be extracted, and the percentage of unidentified compound were detected to be low in comparison with the other tecniques examined in this study

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References

1. Abegaz E.G., Kerr W.L., Koehler P.E. (2004) The role of moisture in flavor changes of model peanut confections during storage. *LWT-Food Science and Technology.*, Vol. 37(2), p. 215–225.
2. Acena L., Vera L., Guasch J., Busto O., Mestres M. (2011) Determination of roasted pistachio (*Pistacia Vera* L.) key odorants by headspace solid-phase microextraction and gas chromatography-olfactometry. *Journal of Agricultural and Food Chemistry*, Vol. 59, p. 2518–2523.
3. Alasalvar C., Shahidi F., Cadwallader K.R. (2003) Comparison of natural and roasted .turkish tombul hazelnut (*Corylus avellana* L.) volatiles and flavour by DHA/GC/MS and descriptive sensory analysis. *Journal of Agricultural and Food Chemistry*, Vol. 51, p. 5067–5072.
4. Anon. (2014a) FAOSTAT Food and Agricultural Organization of The United Nations database [accessed on 22.03.2014]. Available at: http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID= 567
5. Anon. (2014b) FlavorNet [accessed on 22.03.2014]. Available at: http://www.flavornet.org/flavornet.html
6. Balta F. (2002) Phenotypic differences of nut and yield characteristic in Siirt pistachios (*Pistacia vera* L.) grown in Siirt province. *Journal of American Pomological Society*, Vol. 56, Iss.1, p. 50–56.
7. Braddock J.C., Sims C.A., O'Keefe S.F. (1995) Flavor and oxidative stability of roasted high oleic acid peanuts. *Journal of Agricultural and Food Chemistry*, Vol. 45(8), p. 3190–3192.
8. Burroni V.L., Grosso N.R., Guzman C.A. (1997) Principal volatile components of raw, roasted and fried Argentenian peanut flavors. *Journal of Agricultural and Food Chemistry*, Vol. 45(8), p. 3190–3192.
9. Cantalejo M.J. (1997) Analysis of volatile components derived from raw and roasted earth-almond (*Cyperus esculentus* L.) *Journal of Agricultural and Food Chemistry*, Vol. 45(5), p. 1853–1860.
10. El-Kayati S.E., Fadel H.H.M., Mageed M.A.A., Farghal S.A. (1998) Heat and storage effects on the flavour of peanuts. *Nahrung* Vol. 42(6), p. 416–421.
11. Gamlı O.F., Hayoglu, I. (2007) The effect of the different packaging and storage conditions on the quality of pistachio nut paste. *Journal of Food Engineering*, Vol. 78, p. 443–448.
12. Heath H.B., Reineccius, G. (1986) *Flavor Chemistry and Technology*. New York: AVI Book, Van Nostrand Reinhold Company, Inc.. 442 p.
13. Kashani-Nejad M., Tabil L.G., Mortazavi A., Safe Kordi A. (2003) Effect of drying methods on quality of pistachio nuts. *Drying Technology*, Vol. 21(5), p. 821–838.
14. Kendirci P., Altuğ T. (2011) Investigation of Volatile Compounds and Characterization of Flavor Profiles of Fresh Pistachio Nuts (Pistacia vera L.). *Journal of Food Properties*, Vol. 14(2), p. 319–330.
15. Kiefl J, Schieberle P. (2013) Evaluation of process parameters governing the aroma generation in three hazelnut cultivars (*Corylus avellana* L.) by correlating quantitative key odorant profiling with sensory evaluation. *Journal of Agricultural and Food Chemistry*, Vol. 61, p. 5236–5244.
16. Krist S., Unterweger H., Bandion F., Buchbauer G. (2004) Volatile compound analysis of SPME headspace and extract samples from roasted Italian chestnuts using GC-MS, *European Food Research and Technology*, Vol. 219, p. 470-473.
17. Ku K.L., Lee R.S., Young C.T., Chiou R.Y.Y. (1998) Roasted peanut flavor and related compositional characteristics of peanut kernels of spring and fall crops grown in Taiwan, *Journal of Agricultural and Food Chemistry*, Vol. 46(8), p. 3220–3224.
18. Maskan, M., Karatas, S. (1999) Storage stability of whole-split pistachio nuts (*Pistachia vera* L.) at various conditions. *Food Chemistry*, Vol. 66, p. 227–233.
19. Morini G., Maga J.A. (1995) Volatile compounds in roasted and boiled Chineese chestnuts (*Castanea molissima*). *LWT-Food Science and Technology*, Vol. 28(6), p. 638–640.
20. Soliman M.M.A., Osman F., El Sawy A.A. (1981) Volatile components of roasted pistachio nut. *Agricultural and Biological Chemistry*, Vol. 45(9), p. 2123–2125.
21. Yildiz M., Gurcan Ş.T., Ozdemir M. (1998) Oil composition of pistachio nuts. *Lipid/Fett.* Vol. 3, p. 84–86.
22. Vazquez-Araujo L., Enguix L., Verdu A., Garcia-Garcia E., Carbonell-Barrachina A. (2008). Investigation of aromatic compounds in toasted almonds used for the manufacture of turron. *European Food Research and Technology A.* Vol. 227(12), p. 243–254.
23. Young C.T., Hovis A.R. (1990) A method for the rapid analysis of headspace volatiles of raw and roasted peanuts. *Journal of Food Science*, Vol. 55(1), p. 279–280.

RHEOLOGY, TECHNOLOGICAL AND SENSORY CHARACTERISTICS OF FORTIFIED DRINK PRODUCTS WITH FIBERS

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Abstract

Fruits, vegetables and grain all produce a complex carbohydrate known as fiber that plays an important role in overall health. As fiber passes through gastrointestinal system, it isn't absorbed or digested like protein or fat. Instead, it stays in intestines to produce soft, formed stools that pass easily through body. Fiber also combines with fluid to create a gel-like substance that helps lower the level of cholesterol and glucose in blood. To make yoghurt even more beneficial to health and to replace conventional stabilizers with newer, healthier ones a research has been carried out, during which conventional processing aids used to improve the consistence of yoghurt, were replaced with orange pulp, pectin, bamboo and cane fibers. This study evaluated the effect of the supplementation of the same dietary fiber on the syneresis, stability, pH, acidity, dry matter content, viscosity and sensory evaluation of yogurt, juices and juice drinks. Dry matter content evaluation results showed that all the fibers were able to increase the dry matter content of yoghurt, juices and juice drinks. Viscosity measurements confirmed that fiber improved the consistence of yogurt. Results indicate that the Citri-Fi fiber is an almost applicable ingredient for the design of new high value-added yoghurt, juices and juice drinks.

Keywords: dietary fiber, yoghurt, juice drinks, texture.

Introduction

Fruits, vegetables and grain all produce a complex carbohydrate known as fiber that plays an important role in overall health. As fiber passes through gastrointestinal system, it isn't absorbed or digested like protein or fat. Instead, it stays in intestines to produce soft, formed stools that pass easily through body. Fiber also combines with fluid to create a gel-like substance that helps lower the level of cholesterol and glucose in blood and dietary fiber intake on a daily basis to prevent obesity, atherosclerosis, heart diseases, gut cancer and diabetes (Granato et al., 2010, Ramage et al., 2014).

High quantities of new minimally processed foods have appeared on the market in response to a growing demand for natural products that are perceived by consumers as healthier. Among them are beverages based on a mixture of fruit juices and milk fortified with vitamins, minerals and fiber (Renuka, 2009). These beverages are the most widely consumed functional foods. Today's consumers who being more health conscious are seeking products with greater health benefits and there is a great demand for "health foods".

A major concern of the yoghurt industry is the production and maintenance of a product with optimum consistency and stability. Tamime and Robinson (2007) mentioned that factors known to improve consistency are increasing total solids, manipulation of processing variables and characteristics of starter culture. The addition of stabilizers (such as polysaccharides) improves body and texture, appearance and mouth feel and retards syneresis of yoghurts (Hussein et al., 2011). The addition of polysaccharides as a stabilizer in the manufacture of yoghurt is a common practice. Stabilizers are sometimes referred to as hydrocolloids and have two basic functions in yoghurt: the binding of water and improvements in texture.

For the development of new fermented milks, the influence of modifications in the milk base on texture, rheology and sensorial properties of products has been studied, concerning mainly the lipid content of milk (Santo et al., 2012a, 2012b), the total dietary fiber (DF) contents (Santo et al., 2013), and the addition of proteins to increase total solids (Marafon et al., 2011). Formulation of new food products with ingredients from fruit by-products rich in total DF has increased in recent years. Dietary fiber can be fractioned into two major groups of components, the water-insoluble and the water-soluble fraction. While the insoluble fraction stimulates the intestinal peristalsis, the soluble one promotes the selective growth of the indigenous microbiota, acting as a prebiotic. Therefore it is healthier to consume the total dietetic fiber, instead of just its prebiotic fraction. One of the promising fruit by-products is orange pulp fiber, which, in addition to its functional properties such as the reduction of cholesterol and glucose in blood serum. Moreover, orange pulp fiber enhanced the texture parameters of ice-cream (Crizel et al., 2013). Based on this background, the present study aimed to evaluate some important aspects of the rheology, spontaneous whey separation, acidity of fruit juice beverages (apple juice) and yoghurts enriched with different kinds of fiber.

Materials and Methods

Preparation of apple juice

Fibers were mixed with the apple juice after pasteurization 85 °C, in 200 mlL pasteurized apple juice added 3 g fibers. By analogy, all tests have been carried out with different types of fiber: Potatoes fiber (Campus fiber 110), Orange pulp fiber (Citri-Fi 100). When fibers were placed in the juice, the mixtures were homogenized at 10000 rpm and left in the fridge for 24 hour at 4 °C.

Preparation of yoghurt

Four types of yoghurts were prepared. The fibers used for yoghurt preparation were used: sugar cane fiber (JustFiber SC 200), bamboo fiber (JustFiber BFC 40), Orange pulp fiber (Citri-Fi 100). For the analysis, each yoghurt type was prepared in duplicate in two independent batch fermentations (N=4) 0.5% and 1% (in the final yoghurt) concentrations of fibers (Table 1) were dissolved individually in the 500 mL amount of milk, homogenized, and heated to 85 °C for 5 min. The flasks containing 500 mL of the heat treated skim milk base were inoculated with yoghurt starter cultures (YO-MIX LYO DUC, Danisco, Germany). Afterwards, the flasks with the samples were transferred to a water bath at 42 °C. At pH 4.5, fermentation was stopped and the flasks were cooled to 6 °C in an ice bath and stored in refrigerator at 4 °C.

Table 1

Specification of fiber

Fibre	Appearance	Total dietary fiber, %	Ash, %	pH (10% suspension)
Sugar cane fiber	Fine cream white fiber	99.0	0.5	5.0–7.5
Bamboo fiber	Fibrous white powder	99.0	0.3	4.0–7.0
Orange pulp fiber	Light yellow powder	68.2	2.65	5.0-7.0
Potatoes fiber	White powder	72.0	3.0	5.0–7.0

Methods of analyses

Titratable acidity (using 0.1 N NaOH for titration and phenolphthalein as an indicator) according ISO/TS 11869:2013. Total solids were measured by electronic moisture analyser (KERN MLS 50-3). The viscosity measurements were performed in triplicate at 18 °C in a rotational viscometer (Fungilab, Spain) with spindles R3 (juice) and R2 (yoghurt). Water holding capacity (WHC) was performed as described (Wu et al., 2013). 10 g of samples were centrifuged at 1800 g for 20 min at 25 °C. The sedimentable fraction was calculated by the ratio of the weight of sediment to the weight of the sample. All measurements were performed in triplicate. Arithmetic mean standard deviation was calculated for each sample.

Results and Discussion

Analyses of apple juice beverages

At first step apple juice was fortified with different kinds of fiber, as Sugar cane fiber, Bamboo fiber, Orange pulp fiber and Potatoes fiber. Sensory analysis indicated that only juice with Orange pulp fiber (Citri-Fi) and Potatoes fiber (Campus) was acceptable taste.

Quantitative solids evaluation of juice beverages samples (Table 2) indicated that apple juice with Citri-Fi fiber had highest amount of dry matter. The potato pulp fiber (Campus, 0.5%) reduced the dry matter in samples.

Table 2

Total solids of juice

Samples	Dry matter, %
Citri-Fi M 40 0.5%	11.92
Citri-Fi 200 FG, 0.5%	12.27
Campus, 0.5%	11.02

The apple juice acidity is expressed in the presence of malic acid. It is thought that Citri-Fi 200 FG fiber acids adsorbed more than another fiber used because this kind of fiber is natural mixture of citrus fiber and guar gum (Fig. 1).

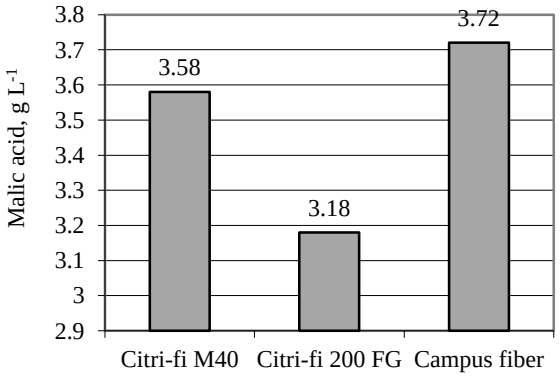

Figure 1. Acidity of apple juice with different fiber

Sedimentation results are presented in Figure 2. The results showed that juice with potato fiber sedimentation was bigger than with citrus fibers.

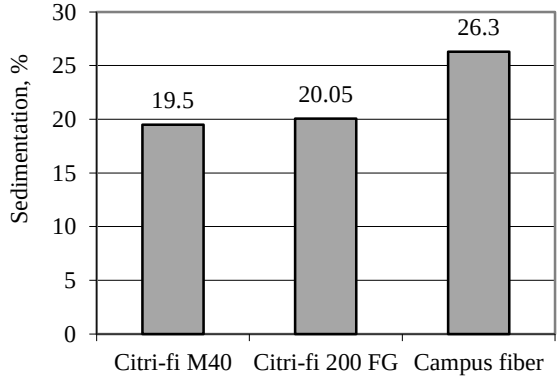

Figure 2. Sedimentation of apple juice with different fiber

Viscosity flow dependency of used kind of fiber is showed on Fig. 3.

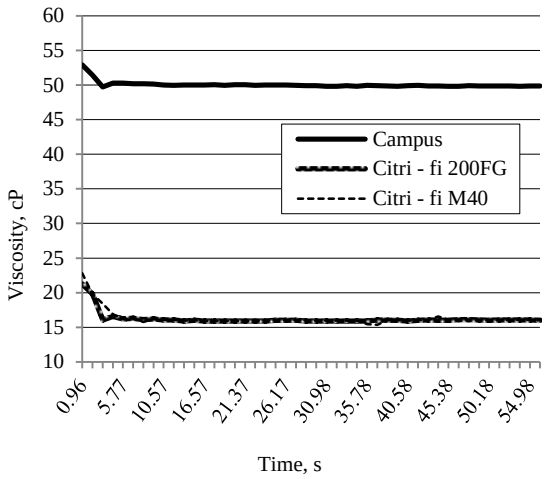

Figure 3. Viscosity flow of apple juice with different fiber

Analyses of yoghurt

Quantitative evaluation of solids of yoghurt samples (Table 3) indicated that yoghurt with sugar cane fiber had highest amount of dry matter. The orange pulp fiber 1% increased significantly the dry matter of samples compare to 0.5%.

Table 3

Total solids of yoghurt

Samples	Dry matter, %
Sugar cane 0,5%	13.47
Sugar cane 1%	13.5
Bamboo 0,5%	12.07
Bamboo 1%	12.75
CitriFi 0.5%	12.31
CitriFi 1%	13.24

The results of titratable acidity at the end of 3 weeks (21 days) exhibited the increasing in Figure 4. The highest influence on acidity was observed in yoghurts supplemented with sugar cane 0.5% and bamboo 0.5% fibers. The addition of fiber to 1% decreased variation of acidity.

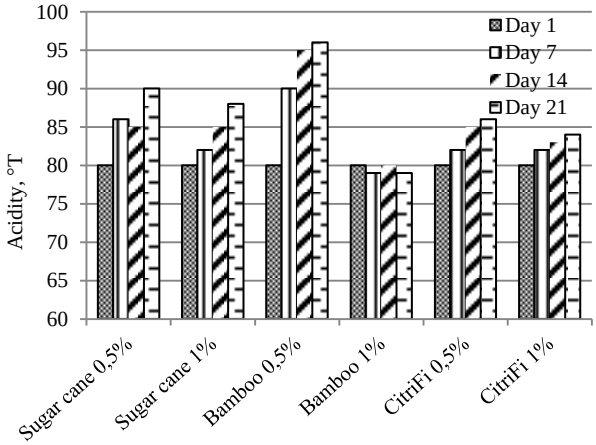

Figure 4. Titratable acidity of yoghurts with different fiber

The stability of yoghurt samples with fiber were characterized by sedimentation measurements as shown in Figure 5. The 1 day results showed that kind of fiber had no influence to sedimentable fraction. Otherwise, after 3 weeks the content of sedimentable fraction decreased. The experimental results were in agreement with increasing acidity when stability of yoghurt decreased. Therefore, the stability of samples with 1% of bamboo and Citri-Fi was constant.

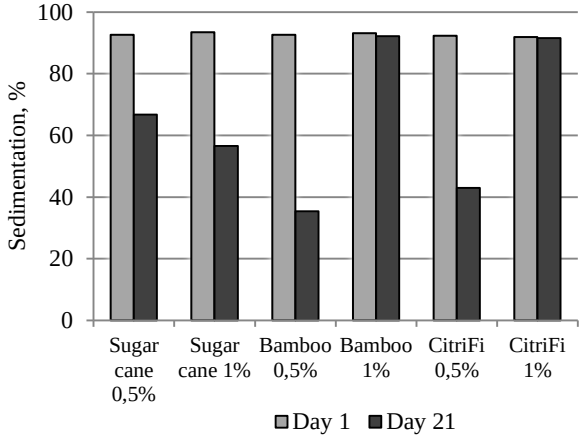

Figure 5. Sedimentation of yoghurts with different fiber

As can be seen from Figures 6, 7, 8, the viscosity of yoghurt with different fiber exhibited shear-thinning flow behaviour. The viscosity of yoghurts with sugar cane (Figure 6) decreased during time. During cold storage the behaviour of viscosity didn`t changed.

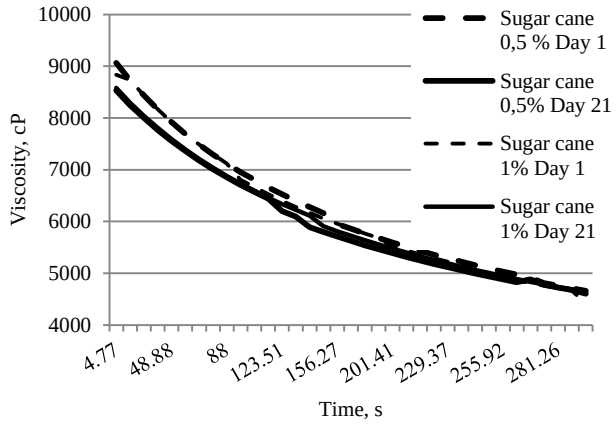

Figure 6. Viscosity flow of yoghurts with sugar cane fiber

The same viscosity decreasing during storage was observed with Citri-Fi fiber (Figure 7). Results indicated that concentration of fiber had no influence.
The viscosity results with bamboo fiber didn`t showed the significant differences (Figure 8). Moreover, yoghurts with sugar cane and bamboo fiber were less viscous at the end of storage. The Citri-Fi fiber helped yoghurt to keep structure more constant.

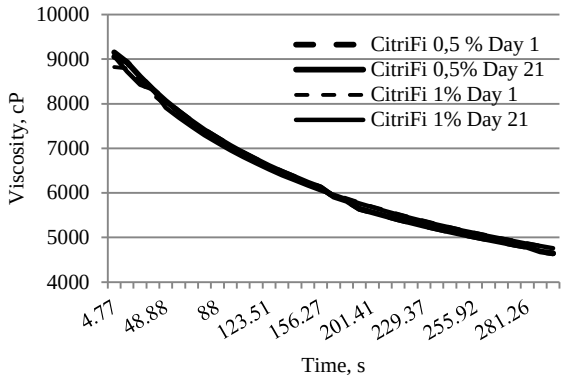

Figure 7. Viscosity flow of yoghurts with orange pulp fiber

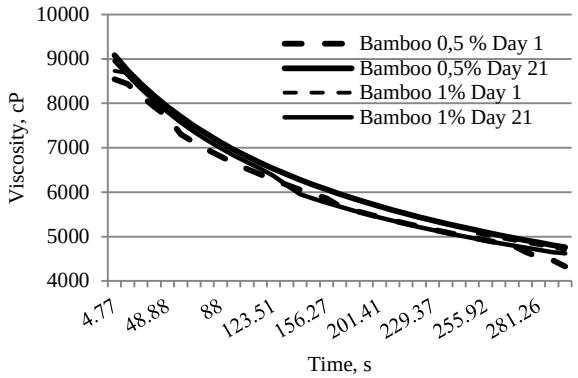

Figure 8. Viscosity flow of yoghurts with bamboo fiber

Conclusions

Results showed that apple juice could be produced by using citrus and potatoes fiber. The essential physicochemical parameters of apple juice depending on fiber kinds remained essentially unchanged, but juice with Citri-Fi 200 FG fiber which guar gum adsorbed more malic acid.

Enriching yoghurt with bamboo and orange pulp fiber offered a scaffold that strengthened the yoghurt's structure, and increased stability during storage. The experimental results have demonstrated that as a potential stabilizer, the orange pulp fiber – Citri-Fi 100 has shown promise for the stabilising yoghurt system.

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References

1. Crizel T.M., Jablonski A., Rios A.O., Rech R., Flôres S.H. (2013) Dietary fiber from orange byproducts as a potential fat replacer *LWT-Food Science and Technology*, Vol. 53 (1), p. 9–14.
2. Espírito-Santo A. P., Perego P., Converti A., Oliveira M. N. (2012b). Influence of milk type and addition of passion fruit peel powder on fermentation kinetics, texture profile and bacterial viability in probiotic yogurts. *LWT-Food Science and Technology*, Vol. 47(2), p. 393–399.
3. Espírito-Santo A.P., Lagazzo A., Sousa A.L.O.P, Perego P., Converti A., Maricê N. Oliveira (2013) Rheology, spontaneous whey separation, microstructure and sensorial characteristics of probiotic yoghurts enriched with passion fruit fiber. *Food Research International*, No. 50, p. 224-231.
4. Granato D., Branco G.F., Nazzaro F., Cruz A.G., Faria J.A.F. (2010) Functional Foods and Nondairy Probiotic Food Development: Trends, Concepts, and Products *Comprehensive Reviews in Food Science and Food Safety*, Vol.9 (3), p. 292–302.
5. Hussein M.M., Fatma A.M. Hassan H.H. Abdel Daym, Salama A., Enab A.K., Asmaa A. El-Galil A. (2011) Utilization of some plant polysaccharides for improving yoghurt consistency. *Annals of Agricultural Science*, Vol. 56, p. 97–103.
6. Marafon A. P., Sumi A., Granato D., Alcântara M. R., Tamime A. Y., Oliveira M. N. (2011) Effects of partially replacing skimmed milk powder with dairy ingredients on rheology, sensory profiling, and microstructure of probiotic stirred-type yogurt during cold storage. *Journal of Dairy Science*, Vol. 94(11), p. 5330–5340.
7. Santo A.P.E., Cartolano N. S., Silva T. F., Soares F. A. S. M., Gioielli L. A., Perego P. (2012a) Fibers from fruit by-products enhance probiotic viability and fatty acids profile and increase CLA content in yogurts. *International Journal of Food Microbiology*, No. 154, p. 135–144.
8. Ramage S., Farmer A., Eccles K.A., McCargar L. (2014) Healthy strategies for successful weight loss and weight maintenance: a systematic review. *Applied Physiology, Nutrition, and Metabolism*, Vol. 39(1), p. 1–20.
9. Renuka B. Fructooligosaccharide fortification of selected fruit juice beverages: Effect on the quality characteristics (2009) *LWT-Food Science and Technology*, Vol. 42(5), p. 1033–1031.
10. Tamime Y. A., Robinson R. K. (2007). *Yoghurt: Science and technology* (3rd ed.). Boca Raton, FL, USA: CRC.
11. Wu J., Liu J., Dai Q., Zhang H. (2013) The stabilisation of acidified whole milk drinks by carboxymethyl cellulose. *International Dairy Journal*, Vol. 28, p. 40–42.

DEVELOPING OF EDIBLE PACKAGING MATERIAL BASED ON PROTEIN FILM

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Abstract

Nowadays due to the accelerated pace of industrial development in particular in food processing the nessesety of the new kinds of packaging materials with desired functional properties (such as bacteriostatic activity for increasing the shelf life for raw materials and ready products) arised. However, the problem of using of non-degradable plastics in packaging that harms the environment remains unsolved. In this study we have attempted to develop an edible and biodegradable packaging material possessing activity against spoilage causing microrganisms of fruit and vegetable products

Keywords: microbial transglutaminase, gelatin film, strength, elasticity, bacteriostatic activity.

Introduction

In world practice different film-forming materials used for food packaging which are produced as synthetic and biogenic composites. The leadingtrend of scientific developments in this direction is the creation of edible coatings.

The edible films can be obtained from protein, polysaccharide and lipid substances. Among them, the most attractive are the edible protein-based films. They have higher barrier properties than films produced from lipids and polysaccharides. However, poor stability of the protein films to water vapor and their low mechanical strength are limited their using in food packaging. Thus, modification of protein-based films must be aimed primarily at improving the mechanical strength and barrier properties of the packaging material with respect to moisture (Bourtoom, 2009).

Chemical modification helps to achieve increasing plasticity (Park et al., 2008). The most commonly used plastisizers include various polyols (glycerine), oligosaccharides and lipids (monoglycerides, phospholipids) that are destroying the hydrogen bonds between polymer chains, make structure more fluid, thereby increasing the elasticity. However, the barrier properties of the film are reducing (Hettiarachchy, Eswaranandam, 2005); besides these agents significantly increase the hydrophilicity of the material and as a result, increase its vapor permeability.

Successful attempts of increasing the elongation at break of the films were obtained when was applied formaldehyde and ethylene glycol (Wu, Zhang, 2001), however, due to the high toxicity of these compound, they can't use in the food industry.

The most promising approach for modifying the structure of the protein components of the films is the use of enzymatic methods, and a special place among them is the use of microbial transglutaminase (mTG). TG (protein-glutamine γ-glutamyl transferase, EC 2.3.2.13) – common in the nature enzyme involved in vital biological functions. mTG catalyzes an acyl- transfer reaction between the γ-carboxyamide group of peptide - bound glutamine residues (acyl donor) and various primary amines (acyl acceptor), including ε-amino group of lysine residues. This crosslinking may be both intra- and intermolecular that in the latter case leads to an increase in molecular weight protein molecules. The reaction proceeds according to the scheme:

$$R_1\text{-Glu-CO-NH}_2 + H_2N\text{-}R_2 \rightarrow R_1\text{-Glu-CO-NH-}R_2 + NH_3$$

Catalysis involving mTG led to various changes of films properties depending on the protein used but there was an increase barrier and mechanical properties in all cases. Thus, in the case of whey protein concentrate, soy isolates (SI) and their mixtures in various ratios were used, reduction in permeability to oxygen and water vapor were observed, but the elongation at break was increased under these conditions (Su, 2007). The vapor permeability decreased when mixture of gelatin and casein were used (Chambi, 2006). The vapor permeability decreased and the polymeric structure compressed when mixture of gelatin and sodium caseinate were used (Bruno, 2008). Films obtained from fish gelatin and treated by microbial TG had shown reduced transparency and reduced elongation at break, compressive structure and good barrier properties (Yi, 2006). In cases of film material comprising various composites by the enzyme treatment also leads to an improvement of some characteristics of the obtained materials. Thus, pectin – SI films prepared using mTG, show an increase in strength characteristics, but the elasticity of the films decreases (Mariniello et al., 2003). Processing transglutaminase caused an increase and decrease in the mechanical strength of the strain. Finally, it has been found that the effectiveness of the barrier to oxygen and carbon dioxide in an enzyme – linked films markedly improved, with decreased permeability to water. These data showed promising application of transglutaminase for the regulation of the mechanical properties and films permeability containing proteins (Di Pierro et al., 2006). The processing enzyme – ovalbumin chitosan films decreases their solubility in water and increases the mechanical strength, barrier properties against gases and water vapour at the same time, and also reduces the

amount of swelling (Di Pierro, 2007).
Along with the development of biodegradable films, there has been actively working on giving them antibacterial properties using different agents: nisin (Rossi-Márquez et al., 2009), propylparaben (Chung et al., 2001), potassium sorbate (Flores et al., 2003), lysozyme (Buonocore et al., 2003).
The protein film can also function as a carrier of antimicrobial agents (Franssen et al., 2002). The ultimate goal of these modifications is to increase the retention and preservation of food raw materials and ready products.
This work proposes to use as a packaging material with preservative properties of gelatin-based film, and its influence on the characteristics of the product is described below.

Materials and Methods

A commercial preparation of mTG Activa EB with an activity of 100 units (Adjinomoto Co), glycerol (Vecton), porcine pig gelatin (LLC Norden), nisin (LLC Norden), potassium sorbate (LLC Norden) were used in the experiments.

Composition of films

In the study the composition of the film material containing gelatin and glycerine was used. There were some variants of films with different compositions at the previous stage. The film with the best organoleptic characteristics like transparency, strength, elasticity was chosen. The enzyme was applied in accordance with the recommendations of technology – supplier (0.01 g g^{-1} protein). Because protein films are very fragile plastisizers are added to the composition for giving them elasticity. Glycerine was used as plastisizer in the study. Composition of analyzed material is shown in a Table 1.

Table 1

Composition of analyzed material

Component	Test sample	Control sample
	%	%
Gelatin	4	4
Glycerine	2	2
mTG, U	4.3 10^{-3}	0
Potassium sorbate	0.1	0.1
Nisin	0.01	0.01
Water	to 100 mL	

Preparation of film

Gelatin was dissolved in water at a temperature of 55–60 °C. After that glycerin was added to the solution of gelatin. The mixture was stirred and then cooled to 25 °C. Then mTG preparation was added, mixed well and placed in an incubator for 30 minutes at 37 °C. Then the predissolved preservative mixture was added to the film-forming solution. After that the vollume of the mixture brought to 100 mL. The obtained solution (40 mL) is uniformly distributed over the surface coated with polyethylene (S=17×17 cm). These films were dried at room temperature and relative humidity of 50–60% within 18 hours.

Mechanical characteristics analisys

Before the test, the film thickness of the total area was measured at 10 points with accuracy of 1 micron. The mechanical characteristics study of the films, namely the tensile strength and elongation at break was performed on a tensile testing machine IR 5071-01S (LLC "Tochpribor Service") under standard conditions:

- distance between the clamps – 50±1 mm;
- sample size: width 15±1 mm, length – 100–150 mm;
- speed in relation to the moving clamp was 250 mm min^{-1};
- measuring range from 0 to 10 kg (100 N).

Tests were conducted in the dry state of the sample, the breaking strength was determined in the longitudinal direction of the sample.
Breaking load (Qmax) and elongation at break (Lmax) was calculated using the following formulas:

$$Q_{max} = \frac{F}{S}, \quad (1)$$

F – the applied load, S – section area of the sample

$$L_{max} = \frac{l-l_0}{l_0}, \quad (2)$$

l – final length of sample, l_0 – initial length of sample.

Bacteriostatical characteristics analisys

The films obtained in the previous paragraph were applied to grapes by immersion method. This kind of berries has been chosen because of its popularity among customers, but it is tend to rapid spoilage during storage. The initial contamination was found by the means of microbiological washout method, after which the control samples without the film and the film-forming composition coated prototypes were left on cold storage during the month (t=0–4 °C, relative humidity 60–65%). At regular intervals (3, 14, 30 days), microbiological washouts were made from them, which were cultivated in the MPA (meat-peptone agar) for 24 hours at 37 °C. Further colonies were counted using the formula:

$$X = \frac{n \times 10^m}{78,5}, \quad (3)$$

n – amount of microorganosms colonies on Petri dish, m – number of 10x dilutions (m = 4 in this study)
All washes were carried out on three samples. After washout, the sample was removed from the cold chamber.

Shrinkage of berries analisys

Berries samples coated with film and control samples without it were stored under described in previous paragraph conditions. At regular times (0, 3, 4, 10, 12, 14 days) they were weighed in triplicate. Further data processing was carried using the formula:

$$\% = \frac{m - m_i}{m} \ , \qquad (4)$$

m – initial weigh, m_i – weight of berry at the day of measurement.

Data processing

All the data were processed in the program Microsoft Office Excel 2010 (Microsoft Corp.).

Results and Discussion

Mechanical characteristics

During the experiments, data were obtained on tensile strength and elongation at break of films of various compositions. The measurement results were compared, the changes in mechanical characteristics percentages shown in Figure 1.

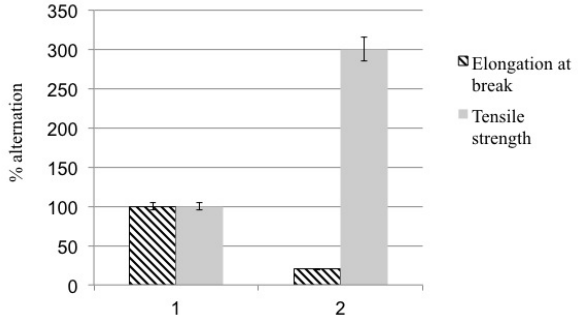

Figure 1. Mechanical characteristics changes of gelatin films causing mTG using
1 – control sample, 2– test sample with mTG

The breaking strength increases along with a decrease in elasticity, which was determined by elongation at break, in all cases of application of the enzyme. In our opinion, these changes occur because of the formation of crosslinks between the polymer chains of the protein, causing their mobility in relation to each other within the polymer network to reduce, by this reason there were decreasing in elasticity and increasing in strength.

Bacteriostatic activity

Manufactures is known to prolong the shelf life of the product by the addition of various preservatives on the surface of the fruit, so before our experiments, all berries were thoroughly washed with warm water for half an hour. Then they were dried for a day at room temperature and constant winding then the experiment was started which results are shown in Table 2 and Figure 2.

Table 2

Microorganisms amount

Day of storage	Test sample	Control sample
	CFU×cm^{-3}	CFU×cm^{-3}
0	(0.6 ±0.03)×10^4	(0.6 ±0.03)×10^4
3	(0.4 ±0.06)×10^4	(2.2 ±0.05)×10^4
14	(3.2±0.04)×10^3	(7.0±0.03)×10^4
30	(0.6 ±0.03)×10^3	(7.5 ±0.02)×10^4

Evaporation is one of the main reasons of weigh losses during storage. Some authors reported that mTG is capable to decrease water vapour permeability (Di Pierro, 2007), so we can suggest that the mTG-treated films can decrease weigh losses because of water evaporation.

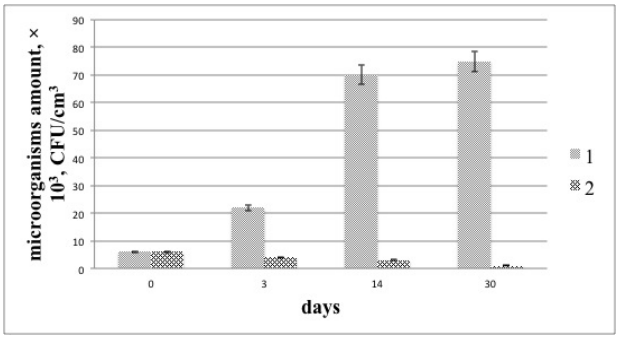

Figure 2. Microbological index changing
1 – control sample, 2– test sample with mTG

As we can see the significant decrease in microorganisms quantity is observed in case of film application. The strong tendency of microphlora reduction is notable during storage for packaged samples, so we can conclude that the coating is capable to decrease and restrain bacteria growth.

Shrinkage during storage

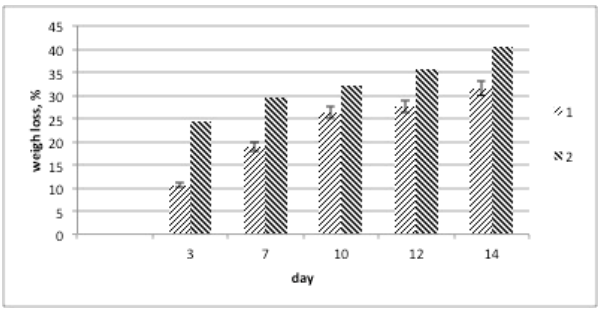

Figure 2. Weight loss during storage
1 – test sample with mTG, 2–control sample

The results obtained showed the decrease in weigh loss during the storage for samples coated in film. It can be concluded that the film could prevent shrinkage.

Conclusions

We have developed and investigated edible packaging material based on porcine gelatin with preservative properties. Because of using cross-linking agent – mirobial transglutaminase, it showed acceptable mechanical properties. At the same time, because of the preservative composition used based on potassium sorbate and nisin, it demonstrated antimicrobial action. Selected packaging material composition had no significant effect on the appearance of the packaged product, and, if desired, can be washed away from the surface of the sample.

References

1. Bourtoom T. (2009) Review Article. Edible protein films: properties enhancement. *International Food Research Journal*, Vol. 16, p. 1–9.
2. Bruno M. (2008) Engineering Properties of Edible Transglutaminase Cross - Linked Caseinate - Based Films. *Food and Biopr. Tech*., Vol. 1, p. 393–404.
3. Buonocore G. G. et al., (2003). Modeling the lysozyme release kinetics from antimicrobial films intended for food packaging applications. *Journal of Food Science*, Vol. 68(4), p. 1365–1370.
4. Chambi H. (2006) Edible films produced with gelatin and casein cross - linked with transglutaminase. *Journal of Food Research* International, Vol. 39, p. 466.
5. Chung D. et al. (2001). Inhibition of Saccharomyces cerevisiae by slow release of propyl paraben from a polymer coating. *Journal of Food Protection*, Vol. 64(9), p. 1420–1424.
6. Di Pierro P. et al. (2006) Chitosan-Whey Protein Edible Films Produced in the Absence or Presence of Transglutaminase: Analysis of Their Mechanical and Barrier Properties. *Biomacromolecules*, Vol.7, p. 744–749.
7. Di Pierro P. (2007) Transglutaminase - catalyzed preparation of chitosan – ovalbumin films. *Enzyme and Microbial Technology*, Vol. 40, p. 437–441.
8. Franssen L. et al. (2002) Modelling of natamycin and potassium sorbate diffusion in whey protein isolate films to application to cheddar cheese. *In Annual meeting and food expo. Anaheim*, California, Session 28.
9. Flores S. et al. (2003) Antimicrobial performance of potassium sorbate supported in tapioca starch edible films. *European Food Research and Technology.*
10. Hettiarachchy N. S., Eswaranandam S. (2005) Edible Films and Coatings from Soybean and Other Protein Sources. *Bailey's Industrial Oil and Fat Products*. p. 374–390
11. Mariniello L et al. (2003) Preparation and mechanical properties of edible pectin - soy flour films obtained in the absence or presence of transglutaminase. *Journal of Biotechnology*, Vol. 102, p. 19.
12. Park H. J. et al. (2008) Water Vapor Permeability And Mechanical Properties Of Grain Protein-Based Films As Effected By Mixtures Of Polyethylene Glycol Amd Glycerin Plasticizers
13. Rossi - Márquez G. et al. (2009) Effect of temperature, pH and film thickness on nisin release from antimicrobial whey protein isolate edible films. *Journal of the Science of Food and Agriculture*, Vol. 89 (14), p. 2492–2497.
14. Su G. (2007) Formation of Edible Soybean Films. *Food Technology*. Vol. 45. pp. 393-404
15. Wu Q. X. and Zhang L. N. (2001) Properties and structure of soy protein isolate-ethylene glycol sheets obtained by compression molding. *Indust. Eng. Chem*. Vol. 40. p. 1879–1883.
16. Yi J.B. (2006) Transglutaminase - Induced Cross - Linking on Properties of Fish Gelatin Films. *Journal of Food Science.* Vol. 71(9), p. 376–38.3

NON-PRICE FACTORS THAT INFLUENCE CONSUMERS' WASTED FOOD AMOUNTS

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Abstract

Each year the price of food products gradually increases, and some economists point out that to reduce the food prices, the food production increase must be managed. But even if some people suffer from hunger, others simply discard their food, so the possible food production increment can also increase wasted food amounts. One of the possible solutions to food product insufficiency is to cut down wasted food amounts. The main aim of this paper is to identify the non-price related factors that influence people to waste their food, look at food wasting matter from seller and buyer point of view, and also to identify the solutions to food wasting reduction. The results are based on research of theoretical guidelines and pilot research conducted in 2013, in which participated 610 respondents. Pilot research results shows that people are less concerned about reducing food wastage when it comes to environmental problems, but are the most motivated if they see a real opportunity to save money. So to motivate people waste less people need to be shown how their food wasting habit affect them economically.

Keywords: packaging, environment, storage, planning, labelling.

Introduction

The global population exceeded seven billion people during 2011 and is predicted to reach 9.3 billion by 2050, with a projected increased food demand of 50–70%. Against this backdrop of rising demand, 868 million people are chronically under-nourished, equating to one in eight people worldwide. At the same time, it is estimated that over one third of all food produced globally for human consumption goes to waste (Bond et al., 2013).

A food-wasting problem can be viewed from very different perspectives, not only as social problem that influence people relationship and values, but also as economical problem that directly or indirectly affects people's incomes and in general can negatively affect the environment.

The aim of this review paper was to summarize the research conducted previously, identify non-price factors that affect food wasting at household level, explore the influence of non-price factors on selected respondents, and provide the evaluation of the problem by identifying the results of the pilot research.

Materials and Methods

Primary sources for theoretical discussion and pilot research survey shape identifying are scientific papers, monographs, fundamental documents that are closely related to the subject, which could be found in the scientific databases and as free sources on the Internet. Papers were selected by the search terms and by the provided references in the studies that were found.

The pilot research was conducted from May till September 2013. The survey was posted on the Internet, and had 48 questions in Latvian regardless peoples' eating habits that influence food wasting. 610 respondents took part in the survey: 345 were women and 265 – men.

Age distribution:

- 18–29 years: 135 respondents or 22%
- 30–49 years: 243 respondents or 40%
- 50–69 years: 196 respondents or 32%
- 70 and more years: 36 respondents or 6%

Results and Discussion

Wasted food is defined as food that is discarded and not fully consumed (Princeton University Dictionary, 2006); it is closely related to attitudes and behaviours. Food gets 'lost' if it is affected by structural causes such as weak infrastructure, technological obsolescence, lack of refrigeration, etc. (Gustavsson et al., 2011). If the food loss problem in poor countries could be solved by investing money in infrastructure, processing and storage technologies and facilities that is mostly by investments then in rich countries to solve a food wasting problem, it is necessary to change people's attitude towards food, their habits and even laws.

The food waste concept, however, is not so strictly defined and it varies from research to research. Moreover, classification itself can be specific to a particular region or culture, and can be affected by the eating habits of the researchers. Thus, the classification not only includes the stage of food that gets discarded, but also it can include the interpretation of what is considered avoidable and unavoidable food waste. While, for example, Langley et al. (2010) consider all preparation by-products and residues of food preparation inedible and therefore unavoidable, researches that cooperate with Waste and Resources Action Programme use an additional subcategory of possibly avoidable food. Possibly avoidable food is considered edible and defined as the food and drink that some people eat and others do not (e. g. bread crusts), or that can be eaten when food is prepared in one way but not in another (e. g. potato skins)' (Household Food …, 2009). As for avoidable food, studies generally agree that wholly unused and partly consumed food would be avoidable, but the classification of the post-preparations and consumption residues differ.

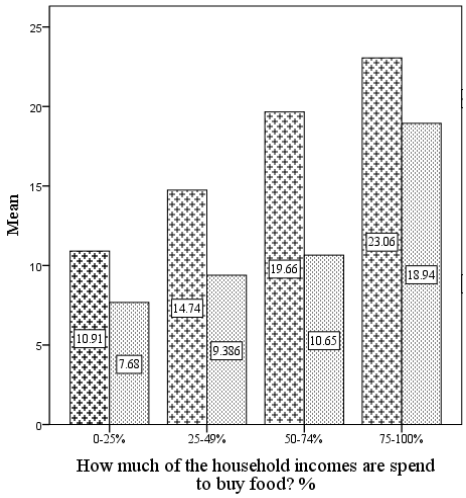

Figure 1. Correlation between incomes and food waste amounts

How much of the discarded waste is so called possibly unavoidable food waste? %

How much of the discarded waste is so called possibly avoidable food waste? %

The pilot research data shows that the bigger part of the average food waste makes a so-called unavoidable food waste, though an avoidable food waste still makes a significant part of the total waste. Research data also shows that with the income growth, the part of the food waste in the total waste also grows, both unavoidable and avoidable. That can also be explained with pickiness this people can afford. For example, when some part of the food seems less suitable for eating (in a case of an unavoidable food waste), and that is why it simply gets discarded. Or also can be explained with the assumption that it is easier for them to throw away suitable food when people just don't want to eat it anymore, or have it cooked in a such a big amount, that it isn't possible to eat it all, before it spoils (in a case of an avoidable food waste).

Economics researchers view a food-wasting problem manly from demand and supply perspective, evaluating what can more affect decrement of wasted food amounts – the increase of food demand or the increase of food supply (Rutten, 2013). Researching how food wasting reduction can affect both sellers / producers and consumers. Assuming that the decrease of wasted food amounts at consumption level can lead to the increase of consumption of the products that previously would be discarded, so basically people would eat more, or consumers would just buy fewer products, so in a way instead of just discarding their food they wouldn't buy it at all, saving some money. The latter possibility would negatively affect sellers and producers of those products. So sellers aren't motivated to decrease food wasting. Though if consumers demand for food products decrease, it does not mean that sellers cannot increase their incomes, they can always try to offer some different products that would be demanded by consumers. Because that, not buying certain food products, consumers would save money, so they have opportunity to buy something else, or even save some money up, so they can buy something more expensive, what previously they couldn't afford.

In general, the food waste decrease can positively or neutral affect consumers, and neutral or negatively – producers / sellers. That is why for producers / sellers it is not beneficial to decrease their own wasted food amounts or that consumers get more cautious about their food waste habits and start to waste less. Because that for producers/sellers their incomes are proportional to the sold products. Thus, it is only possible to decrease wasted food amounts if consumers are informed about food wasting problem in general.

There are several different factors that can affect food thoughtless discarding, and those factors are closely connected to the people's shopping habits, understanding of the labelling, financial and envelopment concern etc.

Table 1

Non-Price Factors that Influence Consumers' Wasted Food Amounts

Factors	Influence positively	Influence negatively
Unawareness of wasted food amounts	No positive influence	Not only food but also money gets wasted
The way of storing	Food can be used more expedient for a longer time	No negative influence
Packaging	Helps food products to stay fresh and suitable for the consumption for a longer time	Non-quality or unsuitable packaging doesn't allow to consume food products fully
Environmental concern	No positive influence	No negative influence
Financial benefits	Helps to save up some money, so people are motivated to waste less	No negative influence
To buy list and meal planning	People make less impulsive purchases. Bigger possibility that all brought food will be consumed	No negative influence
Not understanding / being not able to read labelling of the product	No positive influence	Not knowingly – it is possible to buy food with almost expiring validity Because of the misleading labelling, can buy food products that consumer didn't intend

Source: made by the author

No doubt, that the price can affect how much food can be bought, and therefore discarded in the end, but the price is not the only essential factor that effect food wasting.

One of the factors that affect food wasting is the unawareness of the wasted food amounts**,** and the main reason for that is that discarded food don't stay stored in the house for too long, and if consumers compare bought and discarded food amounts, the discarded part seems insignificant (Jones, 2004). Therefore becoming more aware of the food products that get wasted, would help save up money and maybe also would stimulate people to plan their meals more truthfully.

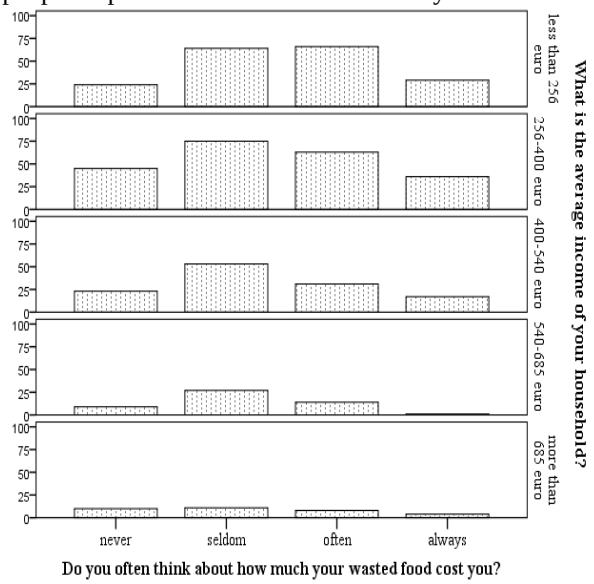

Figure 2. The correlation between the household incomes and concern about wasted food cost (%)

To be able to find out how aware people in Latvia are about food wasting amounts in their household, the pilot research survey contained a question about how often people think about the cost of the food that in the end gets discarded. In addition, to be able to get a more demonstrative answer, this question was analysed by searching the correlations with the question about the average household income.

The results showed that in the households with the lower average incomes people much often think about how much their discarded food cost them, so the lower incomes are the more aware of the wasted food amounts people are. However, as it was stated in the Figure 1, people with higher incomes waste way more, so they are also the ones that think about the cost of the waste way less, but their contribution to the total food waste is the greatest. That is why it is important to make people with higher incomes much more aware of the food waste problems and consequences.

The way people store their food products is closely connected to the fact how long those products can stay fresh and valid for the consumption (Gustavsson et. al., 2011). So right storing helps to use food more expedient for a longer time.

Table 2

Available places of storage and influence on wasted food amounts

		Possibly avoidable food waste of all waste, %			
		0–24	**25–49**	**50–74**	**75–100**
A refrigerator with a freezer	Count	517	35	12	3
	% of Total	85.8	5.7	2.0	0.5
A refrigerator without a freezer	Count	16	0	0	1
	% of Total	2.6	0.0	0.0	0.2
More than one refrigerator with a freezer	Count	35	3	0	0
	% of Total	5.7	0.5	0.0	0.0
A freezer	Count	113	6	0	0
	% of Total	18.5	1.0	0.0	0.0
A storeroom	Count	175	14	6	1
	% of Total	28.7	2.3	1.0	0.2
A cellar	Count	266	16	6	0
	% of Total	43.6	2.6	1.0	0.0
Total	Count	557	37	12	4
	% of Total	91.3	6.1	2.0	0.7

It is hard to explore all possible storing methods of individuals, for that it would be necessary to live together in one household with respondents. But it is possible to search the correlation between existing storing places in the household and the amount of possibly avoidable food waste in total waste. As it is shown in the Table 2, people waste way less 0–24% of all food if they have a proper storage place for it. And the more storing places respondents have, the less food they waste in the end.

Packaging not only prolongs the freshness and validity of the product, but also makes it more safe for a use, so bought food can be used longer, and in the best-case scenario – fully, not wasting much of it (Manalili et al., 2011).

Table 3

Things customers pay attention when buying food

	Responses		Percent of Cases
	Quantity	**Percent**	
Price	549	31.4	90
Ingredient list	294	16.8	48.2
Product's expiration date	471	26.9	77.2
Packaging	106	6.1	17.4
Product's volume or weight	330	18.9	54.1

The pilot research results shows that respondents pay attention to the packaging way less, than to the price and product's expiration date. Even if food validity is closely connected to the packaging.
But when it comes to the environment concerns Baker et al. (2009) came to concussion that it motivates to think about how to reduce food discards way less than the possibility to save some money**.** But people take it positively, that food wasting reduction and money saving can also help to positively impact environment.

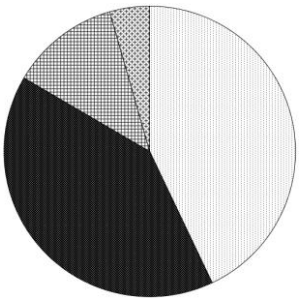

Figure 3. Respondents care about food wasting effect on environment
Do you care about how discarded food affects environment?
□ Never ■ Seldom ▦Often ▩Always

The pilot research results showed similar situation as Baker et al. (2009) described, respondents do not care or seldom care about environment problems that are caused by food wasting.
People admit that planning meals and listing products that need to be bought can help reduce food wasting; because that can help with not buying unneeded products spontaneously. But research also show that no matter that people want to reduce wasted food amounts and eve plan what to buy, for them it is still hard not to make impulsive buys. Sometimes people also just make up their minds and do not want to eat anymore something that they previously planned and have bought food for (Stefan et al., 2012).
So in a way if the planning has a positive impact on food waste reduction, than the shopping routine has an opposite impact. Meaning that no matter how good a person previously thought about that to buy and what to do with bought food, during shopping it still is hard not to buy food, that wasn't planned, and that this action in the end won't be a reason for some food wasting (Stefan et al., 2012).
Gunders (2012) came to conclusion that main part of the consumers does not really plan what they are going to eat. Food gets impulsively bought (sometimes those action influence commercials, or sales, or the wants/needs of the person at that time), but then food gets stored and unused, sometimes food gets bought in such a big amount that it isn't even possible to consume all of it before the expiration day or before it actually spoils. Researcher explains the buy of big amounts of food products with the policy sellers/manufacturers have, when it is more beneficial to the consumers to buy a bigger amount, because calculating per kg, it is more beneficial, because that per kg that product is more cheaper.

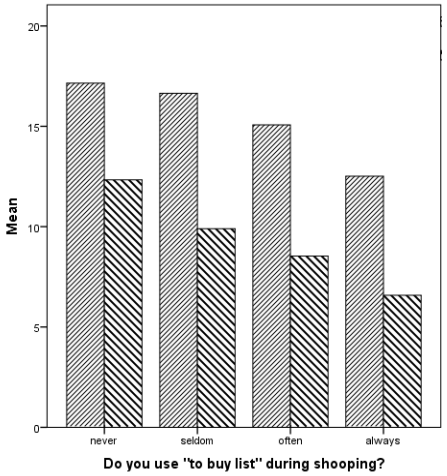

Figure 4. Correlation between meal planning and food waste
▨ How much of the discarded waste is so called possibly unavoidable food waste? %
▧ How much of the discarded waste is so called possibly avoidable food waste? %

The pilot research shows that planning does help with reducing unavoidable and also avoidable food waste. However, there does not exist a significant difference between values. That probably also can be explained by the conclusions Gunders (2012) came up in his research, that even if Latvian respondents plan their meals beforehand, for them it is still hard not to make impulsive buys.
Food labelling can be very misleading, people can buy food that contains ingredients that can cause allergic reactions, so not knowingly a person can buy it, and, if a product consumed fully, a person can have health problems, but if a product wasn't fully consumer, it most likely will be discarded, so in the end – wasted. Food labelling also can mislead people in to thinking that the product is unsuitable for the consumption, assuming that expiration date shows the exact day when product must be discarded obligatory (Manalili et al., 2011).
It is hard to determine if respondents gets mislead by the labelling, because that most of the time (not having a bad allergic reaction, or product having no bad smell or taste) a person does not even understand that wrong/unsuited food was bought.
So it was important to identify answers to the question about if people deliberately buy products, whose validity has almost ended, and correlate those answers with answer to the question how people determine if the food is still valid for the consumption.
Those respondents that buy food whose expiration date is very close, more likely will judge the picked food suitability for the consumption not by date, but by the smell of the product.

Table 4

Food labelling and respondents' food validity determination methods

How do you determine if food is valid for the consumption		Do you buy food whose validity will end soon?			
		never	seldom	often	always
By the expiration date	Count	80	326	50	0
	% within row	17.5	71.5	11	0
	% within column	32.1	29.2	26.7	0
By the look of the product	Count	62	280	45	0
	% within row	16	72.4	11.6	0
	% within column	24.9	25.1	24.1	0
By the smell of the product?	Count	59	306	54	0
	% within row	14.1	73	12.9	0
	% within column	23.7	27.4	28.9	0
By the taste of the product	Count	48	205	38	1
	% within row	16.4	70.2	13	0.3
	% within column	19.3	18.4	20.3	100

Those people, whose food is still valid judged by the date written on the packaging, decide if food is valid for the consumption most often by its' taste.

Conclusions

1. Food thoughtless discarding is affected by different factors, and even if the price of the food plays a very significant role in the future of the bought food, it is not the only factor that affect unavoidable and avoidable food waste.
2. Unawareness, storing, the packaging of the food, the environmental concern, financial benefits, planning and the labelling – all this non-price factors can have both positive and negative effect on food wasting.
3. People in Latvia have similar food wasting tendencies as foreigner researchers have observed during their own research.
4. In order for Latvian people to understand the seriousness of the food wasting problem in the world, people must be more often informed about food wasting problems and consequences. Not only on global scale, but also on personal level – how people's actions affect them financially, because that financial factor is the most effective lever that can make people waste food less.

References

1. Bond M., Meacham T., Bhunnoo R., Benton T.G. (2013) Food waste within global food systems. A Global Food Security report [accessed on 11.03.2014.]. Available http://www.foodsecurity.ac.uk/assets/pdfs/food-waste-report.pdf
2. Food waste: Princeton University Dictionary. (2006) [accessed on 10.03.2014.]. Available http://dictionary.reference.com/browse/food+waste
3. Gunders D. (2012) *Wasted: How America Is Losing Up to 40 Percent of Its Food from Farm to Fork to Landfill*, NRDC: Natural Resources Defense Council, New York City, USA, 26 p.
4. Gustavsson J., Cederberg C., Otterdijk R., Alexandre Meybeck A. (2011) *Global food losses and food waste.* Study conducted for the International Congress. FAO: Food and Agriculture Organization of the United Nations. Rome. Italy. 38 p.
5. *Household Food and Drink Waste in the UK* (2009). Waste and Resources Action Programme. UK: WRAP, Banbury, 95 p.
6. Jones T.W. (2004) Using Contemporary Archaeology and Applied Anthropology to Understand Food Loss in the American Food System. [accessed on 10.03.2014.]. Available Pieejams: http://www.ce.cmu.edu/~gdrg/readings/2006/12/19/Jones_UsingContemporaryArchaeologyAndAppliedAnthropologyToUnderstandFoodLossInAmericanFoodSystem.pdf
7. Langley J., Yoxall A., Heppell G., Rodriguez E.M., Bradbury S., Lewis R., Luxmoore J., Hodzic A., Rowson J. (2010) Food for thought? A UK pilot study testing a methodology for compositional domestic food waste analysis. *Waste Management & Research*, No. 4, p. 220–227.
8. Manalili N.M., Dorado M.A., Otterdijk R. (2011) *Appropriate food packaging solution for developing countries*. Food and agricultural organization of the United Nations. Rome, Italy. p. 37.
9. Rutten M. (2013) *The Economic Impacts of (Reducing) Food Waste and Losses*. Wageningen School of Social Sciences, Wageningen. p. 16.
10. Stefan V., Herpen E., Tudoran A.A., Lähteenmäki L. (2012) Avoiding food waste by Romanian consumers: The importance of planning and shopping routines. *Food Quality and Preference*, Vol. 28, p. 375–381.

CURRENT EATING PATTERNS AND LIFE STYLE ON THE HEALTH OF NAMIK KEMAL UNIVERSITY STUDENTS

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Abstract

The aim of this study to determine the students' current eating patterns and life style on the health. A self-reported questionnaire was administered to 618 students. Students from Namik Kemal University in Turkey participated in this study. Chi-square analyses were conducted for non-parametric variables.

Descriptive statistics of some of the results obtained from the survey study are as follows: 39.8% of the students smokes, 55.3% of them come from the city, which 65.5% stay at a flat with their friends. It was reported that 38.2% of them have breakfast regularly, 45.1% have lunch regularly, and 79.6% have lunch regularly. In order to test whether or not there is any difference between the regular consumption of breakfast, lunch and dinner according to the students' gender, chi-square test was made. In chi-square test made at $p<0.05$ level, it was discovered that there is difference in their consumption between the regular breakfast and lunch according to their gender. It was recognized that male students consume dinner more regularly. The most attractive foods are meat dishes, vegetable dishes, and meat pasty.

***Keywords*:** eating pattern, life style on the health, student.

Introduction

It is obligatory for a living organism to feed itself in order to maintain its life (Çalıştır et al., 2005). Nutrition is to use and take most economically the food which provides sufficiently each of energy and nutrition components that are necessary for growing, development and living healthily and efficiently for a long time without making it lose its nutrition value and destruct health (Tanır et al., 2001). It is put forward scientifically that growing and development are prevented and that health is harmed when any of these components is taken insufficiently or excessively (Baysal, 1993). However, it must not be forgotten that nutrition is a sociological and psychological action as well as a physiological one (Çalıştır et al., 2005). To be healthy in the mind and body and retain health are possible with sufficient and balanced nutrition (Tanır et al., 2001).

Although nutrition is significant for each section of the society, it has a distinct significance for the university youth. Most of the students studying at university have to live away from their family environment for the first time in their lives. Before university, the nutrition habits of student continue as the family life requires, but the differentiating life with university can change the nutrition habits of the student. The changing nutrition attitudes not only concern the mental and physical situation of the student but also are able to affect his school performance. Therefore, determining the nutrition information and habits which the university students have and developing advices suitable for the situation are very important (Erten, 2006).

A considerable number of high education students study away from their families, and this situation causes difficulties in their nutrition, housing, school expenditures and health problems. The studies regarding the nutrition habits of the young at university report that very serious problems are lived in this period, that students usually do not care for meals, that they neglect meals, especially breakfast, that they eat such nutrients as bagel and tea and that satiating hunger and eating are accepted as identical among the young (Yaman, Yabancı, 2006; Karaoğlu et al., 2005). Sufficient and balanced nutrition carries significance on the grounds that it affects people's health and successes and makes the following generations gain a good nutrition habit.

This study planned from that was planned and conducted in order to determine the students' information levels of nutrition and their nutrition habits at Namık Kemal University.

Materials and Methods

This research was conducted to determine the students' current eating patterns and life style on the health of the students surveyed. A self-reported questionnaire was administered to 618 students. Students from Namık Kemal University in Turkey participated in this study. The Questionnaire consisted of five parts and first part is about some personal information about students and their body composition. The second part contains questions that measure students' eating behaviour and its relation to nutrition knowledge. The third part includes some questions about student's attitudes toward nutrition habits. The forth part poses some questions about choosing the foods and the last part collects student's recommendations.The collected data was analysed means, standard deviation and percent were calculated. In addition, this result supported by chi-square test for non-parametric variables.

Results and Discussion

It was determined that 38.3% of the students participating in the research were female, and 61.7% were male; 87.1% were between 18–21 ages, and 20.0% were between 22–25 while 1.9% were over 26; their age average was 20.5. It was found out that 55.3% of them come from the city which 65.5% stay at a flat with their friends which mothers of 48.5% graduated from primary school while fathers of 34.4% graduated from primary school (Table 1).

Table 1

Socio-demographic Ffeatures of the students

Gender	Number	%
Female	237	38.3
Male	381	61.7
Age		
18–21	483	78.1
22–25	123	20.0
26 +	12	1.9
The place left for high education		
Village / Town	84	13.6
County	192	31.1
City	342	55.3
Accommodation		
Flat	405	65.5
Dormitory	141	22.8
With a relative	21	3.4
With family	51	8.3
Where did they nutrition education?		
In school education	138	22.3
Conferences and panels	116	18.8

According to Leigh (1983) smoking is a habit which affects health negatively. While a study conducted on students at Cukurova University reveals that smoking rate of girls was 39.0%, another study conducted on students at Ankara University reports that 27.9% of the students smoke 17–20 cigarettes a day (Yaman, Yabancı, 2006). In this study, 39.8% of the students smoke. The proportion of the students smoking 1–5 cigarettes a day is 12.2% while that of the students smoking 16 and more cigarettes a day is 40.2%.

Table 2

Students' smoking status

Smoking	N	%
Yes	246	39.8
No	372	60.2
Number of cigarettes smoked per day		
1–5	30	12.2
6–10	63	25.6
11–15	54	22.0
16+	99	40.2

As nutrition education can be achieved with an individual's training himself in his own conditions, it can also be provided for individuals by means of formal and common education institutions. In this study, 22.3% of the students stated that they took lessons regarding nutrition whereas 18.8% expressed that they participated in the meetings such as conferences and panel (Table 1). Mazıcıoglu and Öztürk (2003) reported that 47.2% of the students took education regarding nutrition and that 27.7% of those taking education participated in a conference and education on nutrition outside the lesson while Erten (2006) in his study indicated that 27.7% of the students took education related with nutrition and that 98.5% of them took this education at school.

Table 3

The states of the students' consuming meals regularly

	Female	%	Male	%	Total	%	X^2/p values
Breakfast							
I consume	105	44.1	132	34.6	237	38.2	X^2=3.399
Not consume	133	55.9	249	65.3	382	61.8	p<0.05
Lunch							X^2=12.953
I consume	84	35.4	195	51.2	279	45.1	p<0.05
Not consume	153	64.6	186	48.8	339	54.9	
Dinner							X^2=0.018
I consume	189	79.7	303	79.5	492	79.6	p>0.05
Not consume	48	20.3	78	20.5	129	20.4	

In order to test whether or not there is any difference between the regular consumption of breakfast, lunch and dinner according to the students' gender, chi-square test was made. In chi-square testmade at p<0.05 level, it was discovered that there is difference in their consumption between the regular breakfast and lunch according to their gender. It was recognized that male students consume dinner more regularly.

The information about the students' meal consumption is given in Table 3. It was reported that 38.2% of them have breakfast regularly, 45.1% have lunch regularly, and 79.6% have dinner regularly. It was found out the food consumed most at breakfast is pastry, toast, bagel (43%), standard breakfast cheese, onion, jam, etc. (549.8), cornflex+ milk (2.0%), and those consuming only tea or coffee is 4.8%.

It was informed in another study conducted in Ankara that the most preferred food by university students is cheese-onion (67.4%) and bagel (53.7%) (Gülec et al., 2008).

In our study, it was mentioned that breakfast is omitted most while dinner is neglected least. In order to take the energy and food constituents at the suggested level in the youth age, the consumption of the main meal and snack must be approached with care. The number of meals is important in sufficient and balanced nutrition. While neglecting meals, especially breakfast, affects school success negatively in children and young people, it also paves the way for insufficient and unbalanced nutrition (Rampersaud et al., 2005; Dimeglio, 2000). In a study which Tumerdem et al. conducted on the students studying in different faculties of three universities in Istanbul, they discovered that breakfast and lunch are not taken regularly, and hunger is satiated

with hurry while dinners are prepared more carefully than other meals (Tumerdem et al., 1985).

In a study which Arslan et al. (1994) conducted on young people of high education, it was found out that the most neglected meal is breakfast with 31.5%. Saygun (1987) determined the fact that breakfast is most often neglected among other meals. Isıksologlu (1986) and Ozpınar (2002) discovered that the least neglected meal is dinner. In a study which Nnanyelugo and Okeke (1987) performed on the university students in Nigeria, it was determined that the most neglected meal is breakfast by the proportion of 73%. Often, neglecting breakfast can spring from the difficulty of preparing breakfast and worry to be late for school. However, it is reported that breakfast has a very important role in beginning day eagerly, maintaining the activity suitably and preserving the level of sugar blood (Erten, 2006).

Table 4

The average scores and percentage distribution of the students' interest levels regarding food

Food Types	N	1*	2*	3*	4*	5*	Mean Score
Fast food	579	6.8	16.0	9.2	39.8	21.8	3.86
Home cooking	585	1.5	1.5	3.9	33.5	53.9	4.56
Frozen food	570	5.8	20.9	14.6	35.4	15.5	3.37
Canned food	573	11.2	21.8	15.5	31.6	12.6	3.40
Pastry, cookie	579	0.5	5.8	9.2	38.8	38.8	4.33
Milk puddings	570	2.9	5.3	9.2	34.5	39.8	4.34
Pasta types	579	4.4	9.7	10.2	38.8	30.1	4.08
Egg	567	4.9	12.1	13.1	39.3	22.3	3.68
Vegetable dishes	582	6.3	7.6	10.0	42.7	27.5	4.63
Meat dish	575	2.4	5.3	4.9	38.5	41.9	4.71
Potato chips	582	1.9	11.2	8.7	39.8	32.5	4.35
Hamburger	582	5.2	12.6	9.9	38.0	28.5	3.91
Pizza	562	4.4	10.2	8.3	39.3	28.8	3.86
Meat pasty	579	3.6	6.8	7.3	34.0	42.1	4.55
Toast & sandwich	577	2.6	9.2	8.7	42.7	30.1	3.95
Döner	581	1.6	7.4	6.3	43.2	35.4	4.10
Patty types	581	1.1	9.9	7.5	37.7	37.9	4.16
Chocolate, sugars	577	1.6	5.5	7.5	36.9	41.9	4.21

*1It absolutely does not attract my attention, 2 It does not attract my attention, 3 I have no idea, 4 It attracts my attention, 5 It absolutely attracts my attention

The students participating in the survey were asked what kinds of food attract their attention, and Table 3 was made up. The most attractive foods are meat dishes (4.71), vegetable dishes (4.63), and meat pasty (4.55). The least attractive foods are frozen food (3.37), canned food (3.40), eggs (3.68), fast food (3.86), and pizza (3.86) (Table 4).

In recent years, fast-food consumption has been increasing with the increasing importance of time and the effect of the consumption habits imported from developed countries on the university youth. The fact that the young population demands much for this area has increased the fast-food sector in cities especially where universities exist. When the conducted researches are examined, it is seen that most of the students have fast-food consumption habit most (because it is the most practical one). As the young people consume much fast-food, home cooking attracts the students' attention more.

Conclusions

In this study in which the information and habits of university students regarding nutrition were evaluated, it was discovered that the students neglect meals and that the most neglected meal is breakfast (61.8%). 22.3% of the students stated that they took education concerning nutrition, and 18.8% stated that they participated in such meetings as conferences and panels.

The foods mainly attracting the students' attention are meat dishes, vegetable dishes and meat pasty. The foods attracting the students' attention least are frozen food, canned food, eggs, fast-food and pizza, and we can say that the reason for this is that they consume these foods more.

What is important is that necessary precautions should be taken before the students' consumption habits become permanent. Of these precautions, making up healthy nutrition habits and enabling healthy food to be reached come first.

Therefore, individuals should be made to adopt the habit of consuming main meals regularly from their infancy and childhood age. It is though that the fact that the students adopt the right consumption habits is important in terms of protecting their health in their following periods of their lives (Rampersaud et al., 2005). For this reason solving the incompetence in nutrition information and habits with the nutrition education given permanently and effectively, organizing short-term conferences, panels and conversations on nutrition issues at universities at regular intervals will be useful in solving the problems concerning nutrition of the students.

References

1. Arslan P., Karaağaoğlu N., Duyar İ., Güleç E., (1994) Yükseköğrenim gençlerinin beslenme alışkanlıklarının puanlandırma yöntemi ile değerlendirilmesi. *Beslenme ve Diyet Dergisi*; Vol. 22, p. 195–208.
2. Baysal A. (1993) Gençliğin Beslenme Sorunları, *Aile ve Toplum Dergisi*, Vol.3, p.1, accessed on 15/03/2014. Available: [http://www.aile.gov.tr/raporlar/Aile%20ve%20toplum3.pdf].
3. Çalıştır B., Dereli F., Eksen M., Aktaş S., (2005) Muğla Üniversitesi Öğrencilerinin Beslenme Konusunda Bilgi Düzeylerinin Belirlenmesi, *Uluslararası İnsan Bilimleri Dergisi*, Vol. 2, p. 1–8.

4. Dimeglio G., (2000) Nutrition in adolescence. *Pediatratric Rewiev* Vol. 21, p. 32–33.
5. Erten M., (2006) Adıyaman İlinde Eğitim Gören Üniversite Öğrencilerinin Beslenme Bilgilerinin ve Alışkanlıklarının Araştırılması, *Gazi Üniversitesi, Eğitim Bilimleri Enstitüsü, Aile Ekonomisi ve Beslenme Eğitimi Anabilim Dalı, Yüksek Lisans Tezi*, Ankara.
6. Güleç M., Yabancı N., Göçgeldi E., Bakır B., (2008) Ankara'da iki kız öğrenci yurdunda kalan öğrencilerin Beslenme alışkanlıkları, *Gülhane Tıp Dergisi* Vol. 50, p. 102–109
7. Işıksoluğlu M.K., (1986) Yükseköğrenim yapan kız öğrencilerin beslenme durumu ve buna beslenme eğitiminin etkisi. *Beslenme ve Diyet Dergisi* Vol. 15, p. 55–70.
8. Karaoğlu L., Pehlivan E., Genç M.F, Güneş G., Eğri M., (2005) İnönü Üniversitesi ikinci sınıf öğrencileri beslenme alışkanlıkları ve fiziksel aktivite düzeyleri. *Sağlık ve Toplum*; Vol. 15, p. 82–88.
9. Leigh J.P., (1983) Direct and indirect effects of education on health, *Social Science & Medicine*, Vol.17, p. 227–234
10. Mazıcıoğlu M.M., Oztürk A.,(2003) Universite 3 ve 4.sınıf öğrencilerinde beslenme alışkanlıkları ve bunu etkileyen faktörler, *Erciyes Tıp Dergisi* Vol. 25, p. 172–178.
11. Nnanyelugo D.O., Okeke E.C., (1987) Food habits and nutrient intakes of Nigerian University students in traditional halls of residence. *Journal of the American College of Nutrition (JACN)*, Vol. 6, p. 369–374.
12. Ozpınar K. N. (2002) Afyon Kocatepe üniversitesi meslek yüksekokulu öğrencilerinin beslenme bilgi ve alışkanlıkları üzerine bir araştırma. *Gazi Üniversitesi Sağlık Bilimleri Enstitüsü, Yüksek Lisans Tezi* Ankara.
13. Rampersaud G.C., Pereira M.A., Girard B.L., Adams J., Metzl J.D., (2005) Breakfast habits, nutritional status, body weight, and academic performance in children and adolescents. *Journal American Diet Association*, Vol. 105, p. 743–760.
14. Saygun P., (1987) Farklı sosyo-kültürel çevrelerdeki lise son sınıf öğrencilerinin beslenme bilgi ve alışkanlıklarının ölçülmesi. *Hacettepe Üniversitesi Sağlık Bilimleri Enstitüsü. Bilim Uzmanlığı Tezi*. Ankara.
15. Tanır F., Şaşmaz T., Beyhan Y., Bilici S., (2001) Doğankent beldesinde bir tekstil fabrikasında çalışanların beslenme durumu, *Türk Tabipler Birliği Mesleki Sağlık ve Güvenlik Dergisi*, p. 22–25.
16. Tumerdem Y., Güray Ö., Dişçi R., Ayhan B., (1985) Metropolitan bir kentte üniversite gençliğinin beslenme durumu. *Diabet Yıllığı*; Vol. 4, p. 210–218.
17. Yaman M., Yabancı N., (2006) Üniversite öğrencilerinin beslenme alışkanlıklarının değerlendirilmesi. *I. Uluslarası Ev Ekonomisi Kongresi, Ankara. Kongre Özet Kitabı*, p. 214–223.

SHORT COMUNICATION

OPTIMIZATION OF MANUFACTURING TECHNOLOGY OF SOFT CHEESE

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Abstract

The aim of this work was optimization of production processes and determination of the goals settings of the optimal values in production of investigatory soft cheese. Consequently, fat and protein ratio of the prepared pasteurized milk mixture for soft cheese production, and their influence on the fat content in dry matter of final product was determined, moisture of the coagulum and the final product established and the coagulum moisture content and coagulum drying effect on the duration of the final product moisture investigated.

It was established that the largest fat content in dry matter of soft cheese (52.21%) is by the fat/protein ratio of – 1.21, and the lowest (41.69%) – by the ratio of 0.83. Optimal pasteurized mixture of milk fat/protein ratio for soft cheese is from 0.830 to 0.962. The ideal fat/protein ratio for given soft cheese – 0.89. The lowest moisture content of the final product (51.9±0.08%) was obtained by the coagulum moisture at 76.1±0.07%, and the highest – 55.0±0.09%, when coagulum moisture was 71.4±0.13%. The optimal coagulum moisture range, conforming to corporate standards for the final product moisture soft cheese is from 69.01 to 75.55%. The longest drying time of the coagulum (35 min) corresponds to the minimum moisture content of soft cheese – 51.438±0.492%. The maximum moisture content of soft cheese 54.87±0.38% was obtained, when the coagulum drying time was 30 min. This period of time is also optimal drying time for coagulum of investigatory soft cheese.

Keywords: milk, soft cheese, coagulum.

Introduction

Production of cheese has started in the year 8000 BC, according to the various sources. Cheese is ripened or unripened, soft or semi-hard, hard or very hard product (which may have a coating or shell), and the protein (casein) ratio of whey should not exceed the amounts in milk (Sekmokien et al., 2008).

The quality of produced cheese acts the composition, quality and activity of the leaven of the milk used in tanning for the cheese production, decisive. Cultures micro flora catalyzes the enzymatic milk clotting process, stimulates enzymatic curd syneresis and provides unfavorable conditions for the secondary micro flora. Cultures micro flora changes the main milk components (lactose, proteins, lipids) in the compounds, leading to cheese flavor, aroma, nutritional and biological value. Compounds, secreted of cultures micro flora, in particular CO_2, form the porosity and consistency of cheese (Rupsiene, 2003).

Soft cheese is characterized by the fact that there is a considerable amount of moisture, which causes a variety of problems if quantity of humidity is too high. Excessive moisture can cause unwanted micro-organisms appearance and reproduction, therefore, in order to avoid of this, term of realization has to be short what is useless for the company in respect of sales. Also product loses marketable appearance, its organoleptic properties becomes lower (Gudonis, 2012; Masteikiene, 2006).

This investigation was focused on the one kind of dairy product – soft cheese. All the production from entering the establishment of milk to the finished product is analyzed, with the aim of optimization of production processes and determination of the goals settings of the optimal values in production of investigatory soft cheese.

Materials and Methods

This research was carried out in the Department of Biochemistry of LHSU and in the company "Modest".

The cheese-making technology. Milk for the production of cheese is normalized in fat and protein relation. To obtain the corresponding final product oiliness, the appropriate relationship of fat and protein must be used. Normalized mixture is heated to 35–45 °C and cleaned. Finally, in order to obtain the required normalized fat milk and a skim milk of minimum fat content, it must be separated. The following sequence is: filling of production machines, heating of milk, tanning and clotting of milk mixture, curd processing, cheese making, pressing, and the salting. Finally, the cheese is ripened and realized (TI-10-PL-3).

Milk analyzer Lactoscope C4 + (Holland) was used to detection of milk fat, protein, solids and freezing point. This spectrophotometer analyzes the milk and milk products composition using infrared technology. The moisture content was determined using a moisture analyzer KERN MLB-N.

In the statistical analysis of the data arithmetic averages of groups were calculated, the errors were estimated and statistical significance of intergroup was established (ANOVA, Duncan multiple comparisons test). The correlation coefficients were counted and regression analysis (linear and square studied addiction) was conducted for evaluation of relations between the characteristics of subjects. Calculations were made with the computer program Microsoft Excel 2007. The study is considered reliable at $p<0.05$, unreliable – if $p>0.05$.

Results

Soft cheeses are moist, and it causes many problems for the product. Undesirable microorganisms may appear due the high moisture content inside the cheese. In order to avoid this, a short term of product sales is recommended. Product loses marketable appearance, decreases its organoleptic characteristics, at the same. Consistency of the product becomes fragile; the product is crumbling, it is impossible to dice it nicely. This soft cheese is cut by hand and evacuated in the company; these conditions causes large yield losses because of large amounts of scrap and are not suitable for sale.

It was very important to optimize the production technology of soft cheese so as to control the moisture and get the best possible texture of the product, to minimize financial losses of company and to satisfy the buyer with the result. In order to achieve these targets it was necessary to look over the cheese-making technology and perform tests, focusing on normalization of pasteurized milk mixture in respect of fat/protein ratio, estimation of grain moisture and grain drying time impact on the quality of the product and evaluation and determination of the optimum values of these parameters in the production process.

Test of the pasteurized milk mixture fat / protein ratio influence on the final product.

The milk supplied to the company is usually too rich in fat and has too high protein content. Dairy products derived from such non-standardized milk are very creamy and low consistency – soiled, without form and excessive fat content can lead to extraneous taste, also. Therefore, in the milk industry in order to get cheese of standard fat and protein content milk is normalized by normalization instructions with skim milk, buttermilk or sour cream. This is the most important step, which determines the chemical parameters of the forthcoming final product.

Rations of fat and protein in pasteurized milk mixture were presented in Figure 1.

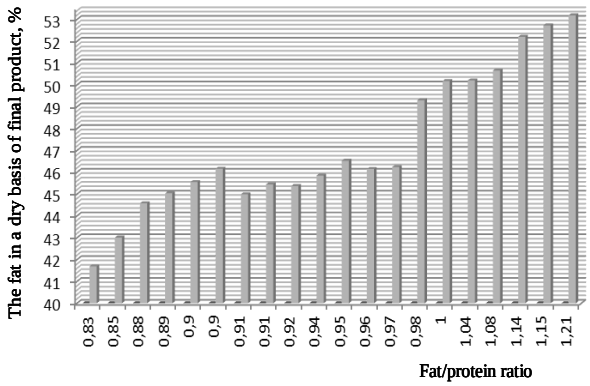


Figure 1. Dependence of the fat in a dry basis of soft cheese on fat/protein ratio of pasteurized milk mixture

The data showed that the increase of fat / protein ratio leads to the growing of the fat in a dry basis of soft cheese, almost evenly. The highest it is in fat / protein ratio of 1.21, and the lowest at 0.83.

The graphical regression analysis of the interdependence of these properties is presented in Figure 2. It was set very strong linear correlation ($r=0.96$, $p<0.001$) among the fat / protein ratio and the fat in a dry basis of soft cheese.

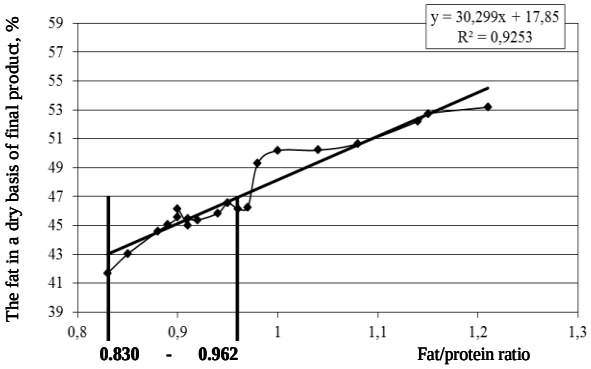


Figure 2. The interdependence regression analysis of fat/protein ratio and fat in the dry basis of soft cheese

The fat content on a dry basis of the final product increases with the increase of the fat / protein ratio. Since the linear equation describes the dependence statistically reliable, we can predict the fat / protein ratio limits, hoping to get optimal fat content on a dry basis of soft cheese. To obtain the final product within the fat content of the dry basis of 45±2%, the fat / protein ratio should range from 0.830 to 0.962.

Influence of grain moisture on the moisture of final product.

Cheese grains obtained during the manufacturing process distinguish the whey and forms a peel. If the peel arises very quickly, the whey fails to stand out from grains, so in grains, and thus the in mass of cheese, could increase the number of micro-organisms activity. Otherwise, when the peel arises very slowly too much whey is eliminated from the grain and the product becomes very dry and crisp. His consistency is crumbling.

During the technological process, when curd cutting, mixing and drying of grains were executed, the samples from the production machines were selected. The moisture contents in the milk samples were determined using a moisture analyzer KERN. In the Table 1 the average values of moisture in grains and in soft cheese are presented.

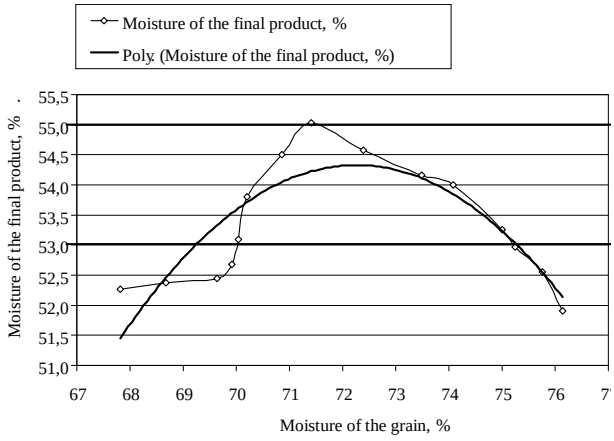
From the data presented in Table 1 it is evident that there is no linear relationship between the moisture of soft cheese and grains: initially, with the increase of grain moisture the moisture content of soft cheese is also growing, but later, after the optimum point is reached, soft cheese moisture begins decline, although grains moisture continues to increase. The maximum value of the final product moisture 55.0±0.09% is reached when the grains moisture is 71.4±0.13%.

Table 1

The average values of moisture in grains and soft cheese, %

Production No.	In grains	In soft cheese
1	67.8±0.11	52.3±0.06
2	68.7±0.11	52.4±0.07
3	69.6±0.22	52.4±0.11
4	69.9±0.09	52.7±0.08
5	70.1±0.07	53.1±0.07
6	70.2±0.09	53.8±0.06
7	70.9±0.07	54.5±0.06
8	71.4±0.13	55.0±0.09
9	72.4±0.09	54.6±0.05
10	73.5±0.09	54.2±0.04
11	74.1±0.05	54.0±0.03
12	75.0±0.06	53.3±0.03
13	75.2±0.06	53.0±0.05
14	75.8±0.04	52.6±0.03
15	76.1±0.07	51.9±0.08

The graphical dependence of the final product moisture on the grain moisture is given in Figure 3.



$y = -0.1455x^2 + 21.034x - 705.63$, $R^2 = 0.7397$

Figure 3. The soft cheese moisture dependence on the grains moisture

The dependence of soft cheese moisture on the grains moisture we described using the quadratic equation. Statistical significance of addiction was evaluated calculating the Fisher criterion ($F=3291.2$, $p<0.001$ ($p=4.01\times10^{-54}$)). Equation can be statistically significant ($p<0.001$) predicted that the moisture of the final product will vary within the permitted limits (53–55%), when grain moisture content will vary in the range from 69.01 to 75.55%, according to the dependence.

The influence of the moisture of soft cheese on grains drying time.

The grains drying time is particularly important process that determines the appearance of the product. It depends on the season of the year and from the composition of the milk changes. The grains drying time is determined by grains moisture, which is important in order to get a good consistency of the final product. The data presented in Table 2 shows influence of soft cheese moisture on the grains drying time.

Table 2

The influence of soft cheese moisture on grains drying time

	Test No.1	Test No.2	Test No.3
Grain drying time	25 min	30 min	35 min
Soft cheese moisture, %	53.44±0.489 a	54.87±0.38 1 bC	51.438±0.492 bD

a, b indicates a statistically significant differences at $p<0.05$. C, D indicates a statistically significant differences at $p<0.001$.

The average moisture content of soft cheese 53.44±0.489% is obtained after drying of grains for 25 min (Table 2). After drying for 30 min the average moisture content of soft cheese is 54.87±0.38%, and drying for 35 min – 51.438±0.492%. In the standard IST 121313693-01:2009 of JC Modest humidity for soft cheese is declared 55±2%, so after drying for 35 min the humidity of soft cheese does not meet the requirements of the standard above, and such a product could not be released to the market.

Discussion

Ratio of fat / protein in milk mixture is very important in milk industry. Too high fat content in the mixture can damage the sensory properties of produced cheese and the product could bee too soft, can not have its inherent consistency, moisture content of product will become too high and it could start multiplying of various bacteria. Too low level of fat content in the milk mixture will make the product lean, dry, it could become free-flowing and crumbly. According to company standard IST 121313693-01:2009, the ideal ratio of fat / protein in a milk mixture was 0.89, during our experiment (Fig.1).

The grains size of the cheese is very important for the moisture content of cheese mass. The whey will excrete much faster from the smaller grains than from the larger. Together with the whey and dissolved substances, releases colloidal and emulsion components of milk.

Because of the distinguishing of whey from the cheese grains, a peel forms on the surface. If the peel arises very quickly, the whey fails to stand out from grains, so in grains, and thus the in mass of cheese, could increase the number of micro-organisms activity. So, it is very important to determine appropriate moisture of grains to obtain standards-compliant product.

Moisture content of final product in SC Modest enterprise standard for soft cheese is defined 53–55% (IST 121313693-01:2009). When the moisture of

grains is 67.818%, the moisture of soft cheese – 52.268% (does not meet to the indicator defined by standard of the company). When grains moisture is 71.418%, moisture of soft cheese – reaches 55.038% (optimal variant of declared parameters), while the moisture of grains was 76.148%, soft cheese moisture was just 51.898% (does not meet to the indicator defined by standard of the company). The optimum range for grains moisture is from 69.01 to 75.55%.

All the studies and analysis of the results were based on our own assumptions and conclusions, as neither Lithuania's nor foreign authors on a similar theme was unable to find and compare.

Conclusions

1. The largest fat content in dry matter of soft cheese (52.21%) is by the fat / protein ratio of – 1.21, and the lowest (41.69%) – by the ratio of 0.83. Optimal pasteurized mixture of milk fat / protein ratio for soft cheese is from 0.830 to 0.962. The ideal fat / protein ratio for given soft cheese – 0.89.
2. The lowest moisture content of producet soft cheese (51.9±0.08%) was obtained by the coagulum moisture at 76.1±0.07%, and the highest – 55.0±0.09%, when coagulum moisture was 71.4±0.13%. The optimal coagulum moisture range, conforming to corporate standards for the final product moisture soft cheese is from 69.01 to 75.55%.
3. The longest drying time of the coagulum (35 min) corresponds to the minimum moisture content of producet soft cheese – 51.438±0.492%. The maximum moisture content of soft cheese 54.87±0.381% was obtained, when the coagulum drying time was 30 min. This period of time is also optimal drying time for coagulum of investigatory soft cheese.

References

1. Gudonis A., (2012) *Milk products technology*. Kaunas: Indra. 256 p.
2. IST 121313693-01:2009. Soft cheeses. Standard of SC Modest.
3. Masteikiene R. R. (2006) *Food Microbiology*, Book No.2, Technologija, Kaunas. 427 p.
4. Rupsienė R. (2003) Influence of leaven on dairy tanning and cheese quality. *Food Chemistry and Technology*, Conference Proceedings, Kaunas, Technologija, p. 83–84.
5. Sekmokiene D., Serniene L., Liutkevicius A. (2008) Advanced processing techniques of skimmed milk, buttermilk and whey. *Food Chemistry and Technology*. 89 p.
6. TI-10-PL-3. *Technological instruction of SC Modest. Production of soft cheeses Brinza and Fetuva*.

SHORT COMUNICATION

PIKE-PERCH FARMING IN RECIRCULATING AQUACULTURE SYSTEMS (RAS) IN THE KALININGRAD REGION

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Abstract

Pike-perch (*Sander Lucioperca* L.) is considered as one of the most valuable food fishes native to Europe. Low fat content (1–2%), highly assimilable protein and a delicate flavour makes pike-perch meat is highly valued by dieticians. Currently, pike-perch available on the market are usually caught by fishers, mainly from Eastern Europe. However, breeding technology is still low, only a few countries (such as Denmark and the Netherlands) have established production. According to predictions of increasing consumption and environmental degradation it'll be necessary to develop reliable technology of pike-perch reproduction as a potential for aquaculture development.

Nowadays, in the EU is actively developing aquaculture in fully "recirculating" systems where water is largely reused. It is known as Recirculating Aquaculture Systems (RAS). In the EU, only the fish which is reared in the RAS may be considered as an eco-friendly product. And food safety concerns are led to the fact that some consumers are willing to pay a premium prices for safe and eco-friendly products, making such cultivation technology more profitable.

The objective of this article is to provide an overview of the current status of pike-perch farming in the Russian Federation with a focus on quality of fish, which is produced in environmentally friendly way by using the RAS technology. We researched the growth features of various pike-perch generations reared experimentally in a local fish hatchery and established the possibility of introducing this species to the market in the Kaliningrad Region.

Keywords: pike-perch, RAS, weight gain.

Introduction

Nowadays, aquaculture is a highly dynamic sector of agriculture. This fact can be illustrated by data of American analytic company "Transparency Market Research" report about aquaculture market development. According to company prediction, aquaculture market production in 2019 will be valued at US$ 195.13 billion. Towards the end of 2015 the volume of aquaculture production presumably to exceed the amount of fish caught, and fishery will cease to be the main source of fish and seafood supplies for human consumption (Transparency Market Research, 2013).The strong environmental restrictions to minimize pollution from hatcheries and aquaculture plants in Northern European countries have sparked a rapid technological development of recirculating aquaculture systems (RAS). The RAS enables to create optimal conditions for farmed fish at all stages of the production process and control the health of farmed fish. The highest possible outcome of fish products per unit area is common to the system in particular. That is a compromise with regard to intensive production and environmental sustainability at the same time (Bregnballe, 2010). Pike-perch (*Sander Lucioperca* L.) is a new species for cultivation in the RAS. This fish is characterized by rapid growth, early maturation and high breeding performance, complemented by excellent taste of meat (Хрусталев, Дельмухаметов, 2010). Thanks to its low fat content (usually 1–2%) and highly assimilable protein, pike-perch meat is highly valued by dieticians and suitable for baby food production (Мукатова, Гайворонская, 2010). The proportion of the unsaturated fatty acids is high and the proportion of the omega-3/omega-6 (n-3/n-6) fatty acids (3:1-4:1) might help to develop the ideal level in the human body (Molnár et al., 2009). Pike-perch is usually sold frozen as gutted whole fish, fillets with skin or skinned fillets. The fillets are usually sold in the following weight categories: 120–170 g, 170–230 g, 230–300 g, 500–800 g, >800 g. This species is less frequently sold fresh, e.g. whole fish, whole gutted fish, fillets with skin and skinned fillets (FAO, 2009). Pike-perch successfully cultivated in the RAS by several European companies (Philipsen, 2008; The David Suzuki Foundation, 2008). However, according to accumulated experience, breeding technology is still poor and only few countries (such as Denmark and the Netherlands) have established production. In Russia, pike-perch farming was conducted during the Soviet times. It was a traditional pond aquaculture, and the fish reared on such fish farm cannot be considered as eco-friendly product nowadays. Recently, in the Kaliningrad region we have made some attempts on pike-perch cultivation in the RAS. During the period from 2007 to 2013 we are managed to form brood stock and develop methods of work with a pike-perch from juvenile to commodity output in a closed system. This is the first Russian experience of pike-perch cultivation in the RAS. The growth of pike-perch in natural reservoirs has been studied appreciably well, but the growth in artificial conditions was not detailed or comprehensive enough. Our goal was to study the growth features of pike-perch at different stages of cultivation process. We researched the growth features of various pike-perch generations reared experimentally in a local fish hatchery. We provided an overview of the current status of pike-perch farming in the Russian Federation with a focus on quality of fish, which is produced in environmentally friendly way by using the RAS technology.

Materials and Methods

The objects of the study were a pike-perch of different generations. The research work was carried out on an experimental RAS at the KMP Aqua Ltd. (Svetly, Kaliningrad region) and industrial RAS at the TPK Baltpticeprom Ltd. (Kaliningrad), during the period from 2007 to 2013. Both plants consisted of biofilters (loaded by pelleted polyethylene), mechanical filters and UV lamps. At different stages of cultivation, the fish were distributed in tanks with volume from 700 L to 7000 L. Oxygen levels in the tanks were maintained by Atlas Capco GX11FF air compressor.

Scheme of the plant is presented on Figure 1.

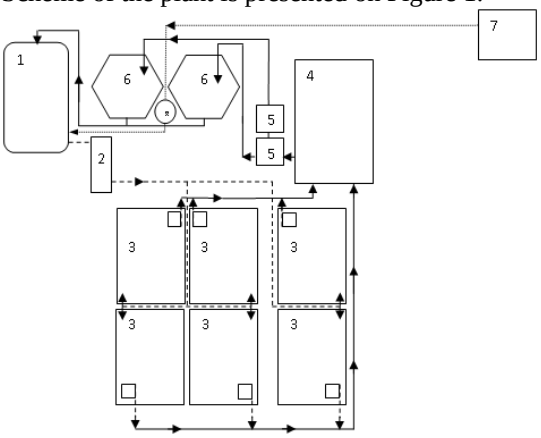

Figure 1. Scheme of the plant of industrial RAS at the TPK Baltpticeprom LLC

1 – degasser, 2 – UV lamps, 3 – tanks, 4 – mechanical filter, 5 – pumps, 6 – biofilters, 7 – air compressor, 8 – oxygenator. —— water output, ------- water input

Such type of plant can be considered as environmentally friendly. There are no any harmful emissions and we had a full control of outputs and effluents.

Oxygen levels in the tanks were maintained above 125% of saturation. The volume of daily replaced water was 10%.

Aller Aqua (Christiansfeld, Denmark) recipes: Aller Futura, Aller Trident, Aller Bronze and Coppens MariCo Focus (Helmond, Netherlands) feeds are used for feeding. All recipes of Aller Aqua are contain raw materials ensure an excellent amino acid profile for good metabolism. The composition includes fish meal, fish oil, wheat, vegetable oils, vitamins and mineral supplements. Aller Futura additionally includes krill meal, while Aller Bronze contains soy flour. Coppens MariCo Focus is a high protein low fat grower with a high proportion of fish meal, additionally includes vitamins and minerals.

Daily ration was changing in accordance with the feed consumption. Estimation of feed conversion ratio (FCR) was performed per months by the equalation:

$$FCR = \frac{Feed\ consumption}{Body\ weight\ gain} \quad (1)$$

Temperature maintenance of water in the tank was maintained by heating panels in the plant placement. To characterize the efficiency of cultivation has been chosen the index of weight gain as the most convenient and commonly used in aquaculture (Купинский, 2007). Sample size during the control analysis was 1% of the total fish amount in a tank.

Results and Discussion

To date, we have received a third generation of pike-perch which has been farmed under the RAS conditions. At cultivation of brood stock we have achieved the weight of fish in 1–1.3 kg in 34 months period. Weight gain velocity of subsequent pike perch generations was higher than that expected for the first generation due to optimization of biotechnological techniques, especially the feeding regime (Fig. 2).

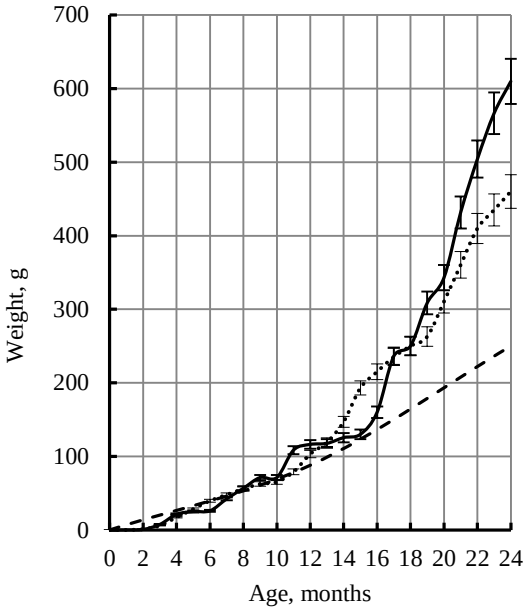

Figure 2. Increase in weight of first and third generations of pike-perch farmed in RAS, and growth of pike-perch in Curonian lagoon during the first two years of life (Голубкова, 2003; Domarkas et al., 2008)

····· First generation, —— Third generation, ------- Curonian lagoon

By the end of the second year of cultivation the pike-perch of third generation reached the weight of 600 g, while the first generation was 460 g. It can be easily seen that the marked differences in the growth rate of pike-perch could be traced from the age of 12–18 months, in particular for its disclosure completeness of growth potency at this age.

During the growing period of third generation, the economical consumption of nutrients to weight gain has been noted. Comparing these data with pike-perch growth from the Curonian Lagoon, it should be emphasized that the growth rate of cultivated pike perch was 2.5 times higher than that of natural pike perch inhabiting the Curonian Lagoon, suggesting the feasibility of cultivation.

The reason why the growth rate of the third generation were higher is optimization of biotechnological techniques, especially the feeding regime as well as a

continual and careful updating of feeding norms in accordance with specific conditions (temperature, oxygen concentration, and configuration and operational features of the RAS, etc.).

The feed conversion ratio was below or close to 1.0, thus made a quite effective feeding of the third generation in the described period. This is also can be attributed to the use of high quality feed (Coppens MariCo Focus) at the last stages of cultivation. A high ratio of protein, energy and low fat contend in the feed is reflected in a higher growth rate of fish, which correspond to lower values of feed conversion ratio. The feed containing 10–12% fat is perfect (Schulz et al., 2007) for the pike-perch.

The survival rate of various pike-perch generations from larvae to market size fish was about 56%, which is comparable and sometimes exceeds the results obtained by European fish farmers (Хрусталев, Дельмухаметов, 2010).

Fishes were produced without hormones, antibiotics, etc., which can be considered as eco-friendly product, especially noting that we had a full control of outputs. Usage of extruded feeds (Aller Trident, Aller Bronze) had no harmful effect on the body composition of fish.

The cost price for product was 3.5 euros per kg. Despite this fact, the first batch of product (3.000 kg) was sold in January–February of 2014 very quickly, which indicates a high consumer demand. It should be noted that the consumer demand for pike-perch can increased and depends on fishing ban periods and freezing period.

Thus, in the third generation of cultivated pike-perch we have achieved better disclosure of growth potency as compared to the first generation, to ensure higher results in the future.

Conclusions

The main feature of this research in the fact that the cultivation of pike-perch in a closed aquaculture system held in the Russian Federation for the first time. As the results showed, the feasibility of cultivation is very high, that allowing the use our biotechnology in the future.

Despite this fact, there is not a fully perfected technique with larvae and fry, especially in survival rate. Terms of pike perch cultivation to market size should be reduced and selected more optimal feed regime for the rearing of marketable fish, and the production must be made cheaper. All these issues will be perspective areas of consideration for the further research.

Acknowledgment

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References

2. Aquaculture (Marine Water, Freshwater and Brackish Water) Market for Carp, Molluscs, Crustaceans, Salmon, Trout and Other Fishes - Global Industry Analysis, Size, Share, Growth, Trends and Forecast, 2013 – 2019 [accessed on 20.11.2013]. Working paper: Transparency Market Research.
3. Available at: http://www.transparencymarketresearch. com/aquaculture-market.html
4. Bregnballe J. (2010) *Guide to Recirculation Aquaculture. An introduction to the new environmentally friendly and highly productive closed fish farming systems.* Copenhagen: Eurofish. 64 p.
5. Cultured Aquatic Species – Pike-Perch [accessed on 1.10.2013.]. Working paper: Food and Agriculture Organization of the United Nations (FAO). Available at: www.fao.org/fishery/culturespecies/Sander_lucioperca/en
6. Domarkas A., Repečka R., Kesminas V., Bukelskis E., Virbickas T., Žiliukas V., Milerienė E., Kaupinis A., Ložys L., Kerosierius L., Poviliūnas J., Radaitytė E. (2008) *Pasienio žuvų išteklių atkūrimas.* Vilnius: Lietuvos hidrobiologų draugija. 144 p.
7. *Global Assessment of Closed System Aquaculture* [accessed on 1.02.2014.]. Working paper: The David Suzuki Foundation. Available at: http://www.davidsuzuki.org/publications/reports/2008/global-assessment-of-closed-system-aquaculture
8. Molnár, T., Szabó, A., Szabó, G., Szabó, C., Hancz Cs. (2006) Effect of different dietary fat content and fat type on the growth and body composition of intensively reared pikeperch Sander lucioperca L. *Aquaculture Nutrition*, Vol. 12, p. 173–182.
9. Philipsen A. (2008) *Excellence fish: production of pikeperch in recirculating system. Percid fish culture, from research to production.* Namur. 67 p.
10. Schulz C., Böhm M., Wirth M., Rennert B. (2007) Effect of dietary protein on growth, feed conversion, body composition and survival of pike perch fingerlings. *Aquaculture Nutrition,* No. 13, p. 373–380.
11. Голубкова Т.А. (2003) *Эколого-биологические характеристика запасов судака Куршского залива Балтийского моря.* Калининград: дисс. канд. биол. наук. 146 р.
12. Купинский С.Б. (2007) *Продукционные возможности объектов аквакультуры*. Астрахань: ДФ АГТУ. 142 р.
13. Мукатова М. Д., Гайворонская М.С. (2010) Консервы для детского питания на основе рыбного сырья. *Вестник АГТУ. Сер. Рыбное хозяйство*, No. 1, p. 156–158.
14. Хрусталев Е. И., Дельмухаметов А. Б. (2010) Рыбоводно-биологические показатели судака при выращивании в искусственных условиях. *Известия КГТУ*, No. 17, p. 15–20.

SHORT COMUNICATION

DETERMITION OF AFLATOXIN CONTAMINATION IN SOME DRIED NUTS AND SPICES BY ELISA

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Abstract

This study aimed to determine the total aflatoxin levels in some dried nuts and spices by ELISA (Enzyme-linked Immunosorband Assay). In this study, 1158 some dried and spices samples (513 hazelnut, 179 pistachio, 244 almond, 52 peanut, 143 walnut, 2 mahlep, 9 sahlep, 8 cinnamon and 8 black pepper) were randomly obtained from markets and spices shops in Istanbul. Total aflatoxin contamination was determined in 156 (30%) of 513 hazelnut, in 93 (51.96%) of 179 pistachio, in 107 (43.85%) of 244 almond, in 23 (55.95%) of 52 walnut, in 23 (44.23%) of 52 peanut, in 2 (100%) of 2 mahlep, in 7 (78.78%) of 9 sahlep, in 6 (75%) of 8 cinnamon and in 6 (75%) of 8 black pepper. Therefore, monitoring of aflatoxins is necessary to ensure that they are not present at levels that may pose health risks to the public and controls should be done strictly and more often by authorities.

***Keywords*:** aflatoxin, nuts, ELISA.

Introduction

Aflatoxins are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic compounds. The main target organ for toxicity and carcinogenicity is the liver (Ayçiçek et al. 2005). Aflatoxins are secondary metabolites produced by species of *Aspergillus*, especially *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins easily occur on feeds and foods during growth, harvest or storage (Çolak et al., 2006).

A review of monitoring studies on the occurrence of aflatoxins in food products has demonstrated that aflatoxins are still being found frequently in food products at levels that are of significant concern for consumer protection (Chun et al., 2007).

The European Commission and Turkish government have set limits for maximum levels of total aflatoxin allowed in groundnuts, nuts, dried fruit and their products. For foods ready for retail sale, these limits are 4 $\mu g kg^{-1}$ (total aflatoxins), and for nuts and dried fruit to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs the limits stand at 10 $\mu g\ kg^{-1}$ (total aflatoxins) (Anonymous, 2002a).

ELISA is widely used for analysis at the field of clinical chemistry, veterinary and food control. ELISA analysis is convenient for simultaneous determination of contaminants in a large number of samples with relatively low cost and short time (Chun et al., 2007).

Mostly, spices are grown in tropical and subtropical regions and harvested in poor sanitary conditions. These improper conditions are convenient for the biosynthesis of aflatoxins. Therefore, growing conditions, harvesting and processing methods, storage conditions and postharvest treatments should be carefully controlled in order to prevent aflatoxin risks due to contaminated spices. In addition, training programs should be presented for producers.

Turkey is the world's second largest nut producer, with about 25% of the total world output. The major nuts that are produced in Turkey are hazelnuts, pistachio nuts, almonds, peanuts and walnuts. Turkey is the world's leading hazelnut producer and exporter followed by Italy and the USA and dominates 80% of world hazelnut trade and export process (Exporters Union, 2006). Most of the supply of approximately 210 000 tons per annum is exported to European Union (EU) countries and elsewhere (Exporters Union, 2006). Turkey is also the third main producer of pistachio nuts with 20% of the world production and takes its position among the first 10 countries in almond production. Nuts may be contaminated and invaded by fungi during their development in the field, during transport or storage, and fungal contamination of nuts may result in the production of mycotoxins (Arrus et al., 2005). Higher contamination risk is expected in those that are being transported for long distances and stored for a long time under unhygienic, unventilated, hot and humid conditions (Basaran, Ozcan, 2009).

The study on the total aflatoxin in some dried nuts and spices is important to ensure the safety of nuts and spices for human consumption. The aim of this study was to determine total aflatoxin concentrations in some dried nuts and spices that some of them were exported to Europe and other countries.

Materials and Methods

A total of 1158 samples of dried nuts and spices (513 hazelnut, 179 pistachio, 244 almond, 52 peanut, 143 walnut, 2 mahlep, 9 sahlep, 8 cinnamon and 8 black pepper) purchased in Istanbul were randomly obtained from markets and spices shops. Samples were stored at 4 °C in plastic bags until the analysis. All samples were analyzed in duplicate and total aflatoxin concentrations were determined by ELISA. According to Ridascreen Aflatoxin Total (Art No.: 4701) test kit manual, 50 µL of the standard solutions or prepared sample in duplicate were added to the wells of micro-titer plate. Then 50 µL of the diluted enzyme conjugate and 50 µL of the diluted antibody solution were added to each well. The solution was mixed gently and incubated for 30 min at room temperature (20–25 °C)

in the dark. The unbound conjugate was removed during washing for three times (ELISA Washer ELX 50, Bio-tek Inst.). Afterwards, 100 µL of substrate/chromogen solution was added to each well, mixed gently and incubated for 30 min at room temperature (20–25 °C) in the dark. Then, 100 µL of the stop solution (1 M H_2SO_4) was added to each well and the absorbance was measured at 450 nm in ELISA plate reader (ELX 800, Bio-tek Inst.). The mean lower detection limit is 0.25 µg kg^{-1} (Çolak et al., 2006; Anonymous 2002b).

Results and Discussion

During the past decades a huge number of scientific papers have demonstrated that the list of raw materials and processed foods actually contaminated by aflatoxins is continuously increasing spanning from peanuts, known to be contaminated by aflatoxins since 60 s, to cereals, coffee, cocoa, dried fruits and spices (Zinedine et al., 2006). Turkey is a leader in the hazelnut production. And hazelnut and its products are among major exported goods of Turkey. Pistachio is important products that have a high commercial value following hazelnuts (Aluç et al., 2005).

In this study, total aflatoxin contamination was determined in 156 (30%) of 513 hazelnut, in 93 (30%) of 179 pistachio, in 107 (30%) of 244 almond, in 23 (30%) of 52 walnut, in 23 (30%) of 52 peanut, in 2 (30%) of 2 mahlep, in 7(30%) of 9 sahlep, in 6 (30%) of 8 cinnamon and in 6 (97.5%) of 8 black pepper (Table 1). Total aflatoxin level in one of the walnut (1 of 52) and one of pistachio (1 of 179) samples were found higher than the Turkish and EU legal limit (10 µg kg^{-1}) (Anonymous, 2002a) (Table 2).

Yildirim et al.(1997) found total aflatoxins in 8 out of 34 red pepper samples (23.5%) in the range of 1.6–15.0 µg kg^{-1}.

Table 1

Total aflatoxin (B1, B2, G1, and G2) contents of some dried nuts

Total Aflatoxin	Hazelnut	Pistachio	Almond	Peanut
Not detected	357 70.00%	86 48.04%	137 56.15%	29 55.77%
>0–0.5 ppb	3 0.30%	7	8	–
>0.5–1 ppb	126 24.50%	48	64	20
>1–2 ppb	24 5.00%	33	26	2
>2–3 ppb	1 0.01%	1	4	1
>3ppb	2 0.01%	4	5	–
Detected samples	156 30.00%	93 51.96%	107 43.85%	23 44.23%
Total	513	179	244	52

In another study performed by Erdogan (2004), it was reported that total aflatoxins was found in 8 red-scaled pepper samples (18.2%) and in 3 red pepper samples (10.7%). Our results were found to be higher than these results. On the other hand, Hazır and Çoksöyler (1998) reported that 46 out of 141 red pepper samples (32.6%) contained aflatoxin. In a similar study in Van, Ağaoğlu (1999) found the highest aflatoxin contamination level of 44.0 µg kg^{-1} in red-scaled pepper.

Aluç, Aluç (2005) checked 367 samples of pistachio produced in 3 year (2002–2004) and it has emerged that 33 samples exceeded the maximum tolerated level in Turkish and European countries (4 ppb). Total aflatoxin was not detected in 75% of pistachio samples. These results are correlated well with our findings.

Abdulkadar et al. (2004) examined total aflatoxin levels in nut from Qatar and found in 23.4% of nut samples with range of 0.53–289 µg kg^{-1}. Aflatoxin contamination was detected in pistachios and peanuts, while other nuts such as almond, cashew nut, walnut and hazel nut were found free from aflatoxins.

In walnut samples, the incidence of AFB1 and AFT was 30%. The contamination levels in walnut samples ranged from 0.56 to 2500 lg kg^{-1} for AFB1 and from 1.24 to 4320 lg kg^{-1} for AFT, respectively. The average contamination levels of walnut with AFB1 and AFT were 360 and 730 lg kg, respectively (Juan et al., 2007). Out of samples, 20% exceeded the maximum limit (2 lg kg^{-1}) fixed for AFB1 by European legislations (European Commission, 2006). Our results are in agreement with the above findings.

Table 2

Total aflatoxin (B1, B2, G1, and G2) contents of some dried nuts and spices

Total Aflatoxin	Walnut	Sahlep	Cinnamon	Black pepper
Not detected	63 44.05%	2 22.20%	2 25.00%	2 25.00%
>0–0.5 ppb	1	–	–	–
>0.5–1 ppb	27	3 33.30%	6 75.00%	6 75.00%
>1–2 ppb	21	4 44.50%	–	–
>2–3 ppb	3	–	–	–
>3ppb	28	–	–	–
Detected samples	80 55.95%	7 78.8%	6 75.00%	6 75.00%
Total	143	9	8	8

In conclusion, aflatoxins continue to pose a health concern via human exposure to contaminated spices and nuts. Aflatoxins cause economic and trade problems at almost every stage of marketing of nuts and spices especially during export. Therefore, monitoring of aflatoxins is necessary to ensure that they are not present at levels that may pose health risks to the public and controls should be done strictly and more often by authorities.

References

1. Abdulkadar A. H. W., Al-Ali A. A., Al-Kildi A. M., Al-Jedah, J. H. (2004) Mycotoxins in food products available in Qatar. *Food Control*, Vol. 15, p. 543–548.
2. Ağaoğlu S. (1999) Van ilinde açıkta satılan kırmızı pul biberlerde aflatoksin B1 varlığının araştırılması. *Van Tıp Dergisi*, Vol. 6(4), p. 28–30.
3. Aluç M. Aluç S. (2005). Antepfıstıklarında Aflatoksin Kirliliği. Türkiye'de Mikotoksin Çalışmaları. *II. Ulusal Mikotoksin Sempozyumu Bildiriler Kitabı* 23-24 Mayıs 2005
4. Aluç M., Yılmaz İ., Arıcı M., Dağlıoğlu O. (2005). Determination Of Aflatoxin in Hazelnuts By HPLC. *1st International Food And Nutrition Congress Food Safety And Quality Through The Food Chain: Farm To Fork-Fork To Farm.* 15-17 June 2005
5. Anonymous (2002a) Turkish Food Codex. Legislation about determination of maximum levels of certain contaminants in foods. *Basbakanlik Basimevi*. Ankara
6. Anonymous (2002b) Enzyme immunoassay for the quantitative analysis of aflatoxins. *Art. no. R 4701. RBiopharm GmbH*, Darmstadt, Germany.
7. Arrus K., Blank G., Clear R., Holley R. A., Abramson D. (2005) Microbiological and aflatoxin evaluation of Brazil nut pods and the effects of unit processing operations. *Journal of Food Protection*, Vol. 68, p. 1060–1065.
8. Ayçiçek H., A. Aksoy Ş. Saygı (2005) Determination of Aflatoxin Levels in Some Dairy and Food Products which Consumed in Ankara, Turkey. *Food Control*, Vol. 16, p. 263–266.
9. Basaran P. Ozcan M. (2009) Occurrence of aflatoxins in various nuts commercialized in Turkey. *Journal of Food Safety*, Vol. 29, p. 95–105.
10. Chun H.S., Hyun Jung Kim A., Hyun E, Jin-Bong Hwang A., Duck-Hwa Chung (2007) Determination of aflatoxin levels in nuts and their products consumed in South Korea. *Food Chemistry*, Vol. 102, p. 385–391.
11. Colak H., Bıngol E. B., Hampıkyan H., Nazlı B. (2006) Determination of Aflatoxin Contamination in Red-Scaled, Red and Black Pepper by ELISA and HPLC. *Journal of Food and Drug Analysis*, Vol. 14(3), p. 292–296.
12. Erdogan A. (2004) The aflatoxin contamination of some pepper types sold in Turkey. *Chemosphere.* 56: 321-325.
13. European Commission (2006) Commission Regulation No. 1881/2006 of December 19th (2006). Setting maximum levels of certain contaminants in foodstuffs. *Official Journal of the European Union No. L364/5 of December 20th*.
14. Exporters Union (2006) Turkish hazelnut export. http://www.ieuhazelnut.org (accessed November 10, 2006)
15. Hazır Z. Çoksöyler NF. (1998) Farklı Bölgelerde ve farklı yöntemlerle elde edilen kırmızı biberlerde aflatoksin düzeyleri. *Gıda Mühendisliği Kongresi*, p. 479–483
16. Juan C., Zinedine A., Molto J.C., Idrisi L., Manes J. (2008) Aflatoxins levels in dried fruits and nuts from Rabat-Salé area, Morocco. *Food Control*, Vol. 9, p. 849–853
17. Yildirim T.,Tanriseven A., Ozkaya S. (1997) Bursa ve Sakarya kırmızıbiberlerinde aflatoksin çalışması. *Gıda Teknolojisi*, Vol. 2, p. 60–63.
18. Zinedine A., Brera C., Elakhdari S., Catano C., Debegnach F., Angelini S. (2006) Natural occurrence of mycotoxins in cereals and spices commercialized in Morocco. *Food Control*, Vol. 17, p. 868–874.

SHORT COMUNICATION

DETERMINATION OF AFLATOXIN LEVELS IN CASHEWS ON TURKISH MARKETS

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Abstract

The problem of food contamination with aflatoxin is one of the current concerns and has received a great deal of attention during the last three decades. Aflatoxins are a group of highly toxic secondary metabolic products as some *Aspergillus* species. Aflatoxins are carcinogenic, mutagenic, teratogenic and immunosuppressive to most animal species and humans. They are considered to be one of the most important food contaminants affecting food safety and public health.

The aim of the study is to determine the level of aflatoxins in cashews on Turkish markets. Samples were analyzed by reverse phase HPLC containing post column derivatization and fluorescence detection after immunoaffinity column clean-up. AOAC (999.07) was used in as the methodology of the study.

In this study, aflatoxin B1 and total aflatoxins (B1+B2+G1+G2) were analyzed in totally 50 samples of cashews. A total of 50 samples of cashew samples were obtained randomly from supermarkets in Istanbul. Aflatoxins were found in 14 out of 50 samples (28%) of cashews (B1: 0.26–0.32 ppb; total: 0.5–0.84 ppb). All positive samples did not exceed the maximum limit of 2 μg kg^{-1} set by EU regulations for aflatoxin B1 and total aflatoxin (4 μg kg^{-1}). Although the aflatoxin contamination in cashew samples is very low, aflatoxin analysis should be done strictly for human health.

***Keywords*:** aflatoxin, cashew, HPLC.

Introduction

The cashew tree *Anacardium occidentale* (French.: acajou, anacarde; Spanish.:marañon, caju, acaju) originates from South and Central America (from Brazil to Mexico). The cashew apple is a pseudocarp, and, in botanical terms, is the thickened stem of a fruit which the actual fruit, the cashew nut, is attached. In its originating countries, the pear-shaped cashew apple is eaten fresh. The fruit is distributed throughout the tropics in this form. It was the development of a roasting method to extract the oil from the shell which turned the nut itself into the main product. Cashew nuts are dried before being sold. After the harvest, cashew nuts need to be immediately placed out in the sun to dry, whilst being continually raked over, until the nuts rattle around in their shells (3% rest moisture). In this form, cashew nuts can be stored for up to 2 years, in ideal storage conditions (dry, dark, cool, well-ventilated). Yet they are usually processed within the same year of harvesting (Anonymous, 2000).

Generally, tropical conditions such as high temperatures and moisture, monsoons, unseasonal rains during harvest, and flash floods lead to fungal proliferation and production of aflatoxins. Poor harvesting practices, improper storage, and less than optimal conditions during transport and marketing can also contribute to fungal growth and increase the risk of aflatoxin contamination (Bhat, Vasanthi, 2003).

Mycotoxins are secondary fungal metabolites that contaminate agricultural commodities and can cause diseases or death in humans and animals have a significant economic impact worldwide. *Aspergillus* is one of the most important large genus genera. More than twenty five genes are involved in pathway synthesized aflatoxins (Pearson et al., 1999).

The most toxic of this group, is the most potent carcinogen known. Because of their high toxicity, the presence of aflatoxins in food commodities is believed to pose a risk to human health (Leszczynska et al., 2000). Aflatoxin B1 (AFB1) is considered as the most dangerous toxic metabolite because of its hepatotoxic, teratogenic, immunosuppressive and mutagenic nature.

Aflatoxins are regulated in more than 75 countries. Currently, the worldwide range of limits for AFB1 and total aflatoxins are 1–20 ng g^{-1} and 0–35 ng g^{-1}, respectively (FAO, 2004). European Commission regulations set limits for AFB1 and total aflatoxins of 2 and 4 μg kg^{-1}, respectively in groundnuts, nuts, dried fruit and cereals since 1998 (Commission Regulation, 2006).

Milhome et al. (2014) reported that 24 samples (34.3%), among 70 samples analyzed, were detected presence of total aflatoxins. However, only two samples (2.8%) showed contamination levels above limit set by EU regulations (4.0 μg kg^{-1}), during 2010–2012 period. Aflatoxin contaminations ranging from 2 to 4 mg kg^{-1} were detected in 22 samples analyzed in 2010–2012, however these samples are in accordance with national and international standards. The incidence of aflatoxins above the allowed limit (EU) has decreased in the last years. In the last year of monitoring (2012) no sample was detected high levels (>4.0 μg kg^{-1}) of total aflatoxins (Milhome et al., 2014).

However, few studies cite the incidence of aflatoxin contamination in cashew nuts. The main objective of this study was to investigate the occurrence and distribution of aflatoxin contamination in cashew nuts imported from India and other tropical countries to assess whether levels of these aflatoxins are affecting food safety and public health.

Materials and Methods

A total of 50 samples of cashews commercialized in Istanbul were randomly obtained from markets and bazaars. Samples were stored at 4 °C in plastic bags until the analysis. All samples were analyzed in duplicate.

Aflatoxins were analyzed in cashew nuts according to the method described by AOAC (2007) first aflatoxin standard solution (containing 1000 ng B1, 200 ng B2, 1000 ng G1 and 200 ng G2 per mL) were prepared in toluene-acetonitrile (98+2). Working standard solutions were prepared daily from these standard solutions acoording to Stroka et al. (2000) For the extraction procedure, 50 g of sample was added with 5.0 g of NaCl, extracted 300 mL methanol: water (80:20 v/v) in a blender at high speed for 3 min and filtered through a Whatman filter paper No.4. 10 mL were diluted with 60 mL of phosphate buffered saline (PBS), and 66 mL of the diluted filtrate was applied to the immunoaffinity column (Aflaprep, R-Biopharm) previously conditioned with 10 mL of PBS (flow rate of about 3 mL min^{-1}). The column was washed with 15 mL of water and air was drawn through the column until dry. Aflatoxins were eluted by applying 1.25 mL of methanol to the column. The eluate was diluted with 1.75 mL of water. A 100 µL aliquot was injected onto the HPLC system (Hewlett Packhard 110 HPLC Chromatograph, equipped with a Hewlett Packard 1100 fluorescence detector). Excitation and emission wavelengths were set at 333 and 460 nm, respectively. The eluate passed through a C18 column (5 µm particle size, 250 mm×4.6 mm). The mobile phase was acetonitrile: water : methanol (17:54:29 v/v/v), and the flow rate was set at 1 mL min^{-1}. The limit of detection of the method was 0.02 µg kg^{-1}. Quantification of each toxin was performed by measuring peak areas at their retention times, and by comparing them with their relevant standard calibration curve. The identity of each toxin was confirmed in all the analyzed samples by injecting sequentially sample extracts and comparing the peak area ratio with their corresponding standard (AOAC, 2007).

Results and Discussion

Due to the contamination of aflatoxins, the nut is considered as a high risk commodity. The problem of aflatoxin contamination is worldwide but in India, the poor harvesting practices, high temperature, high moisture levels and post harvest practices are conducive for fungal growth, proliferation and aflatoxin contamination (Reddy et al. 2011). In Turkey, cashews are imported from India and other tropical countries.

The occurrence of aflatoxins in different types of nuts and nutty products has been reported by several authors from different countries. Chun et al. (2007) found that nut samples were contaminated with aflatoxins (10.6% of incidence) in the range of 0.20–28.2 µg kg^{-1} in South Korea. In China, peanut was found contaminated with aflatoxins, being on the average level of 80.3 µg kg^{-1} and the highest level being 437 µg kg^{-1} (Wang, Liu, 2006). In Turkey, Yentür et al. (2006) reported that AFB1 and total aflatoxins in peanut butter were in the range of 2.06–63.7 ng g^{-1} and 8.16–75.7 ng g^{-1}, respectively. According to Cheraghali et al. (2007), 11.8% and 7.5% of 10,068 Iran pistachio nut samples were above the maximum tolerated level of AFB1 and total aflatoxins, respectively. Abdulkadar et al. (2004) examined total aflatoxin levels in nut from Qatar and found in 23.4% of nut samples with range of 0.53–289 µg kg^{-1}. Aflatoxin contamination was detected in pistachios and peanuts, while other nuts such as almond, cashew nut, walnut and hazel nut were found free from aflatoxins. The incidence and levels of aflatoxins found in this study were relatively low compared to those quoted in the literature.

Table 1

Aflatoxin (B1) and total aflatoxin (B1, B2, G1, and G2) contents of cashew samples

	AFB1	Total Aflatoxin
Not detected	36 (72%)	36 (72%)
>0-0.5 ppb	14 (28%)	–
>0.5–1 ppb	–	14(28%)
>1–2 ppb	–	–
Detected samples	14 (28%)	14 (28%)
Total	50	50

The present study on assessment of aflatoxin contamination in total cashew nuts has been satisfactorily performed by HPLC. In this study, aflatoxin B1 and total aflatoxin (B1+B2+G1+G2) were analyzed in totally 50 samples of cashew. A total of 50 samples of cashew samples were obtained randomly from supermarkets in Istanbul. Aflatoxin was found in 14 out of 50 samples (28%) of cashew (B1: 0.26–0.32 ppb; total: 0.5–0.84 ppb) (Table 1). Any of the positive samples did not exceed the maximum limit of 2 µg kg^{-1} set by EU regulations for AFB1 and AFT (4 µg kg^{-1}). Although the aflatoxin contamination in cashew samples is very low, aflatoxin analysis should be done strictly for human health.

References

1. Abdulkadar A. H. W., Al-Ali A. A., Al-Kildi A. M., Al-Jedah J. H. (2004) Mycotoxins in food products available in Qatar. *Food Control*, Vol. 15, p. 543–548.
2. AOAC (2007) AOAC (999.07). Official methods of analysis, *Association of official analytical chemists*. 18th edition, Washington D C.
3. Bhat R.V.,Vasanthi S. (2003) Mycotoxin food safety risk in developing countries. *Food Safety in Food Securty and Food Trade.* p. 3–17.
4. Cheraghali A.M., Yazdanpanah H. N. Doraki N., Abouhossain G. M., Hassibi M. S., Ali-abadi S. Aliakbarpoor M., Amirahmadi M., Askarian A., Fallah N., Hashemi T., Jalali M., Kalantari N., Khodadadi E., Maddah B., Mohit R., Monseny M., Phaghihy Z., Rahmani A., Setoodeh L., Soleimany E., Zamanian F.

(2007) Incidence of aflatoxins in Iran pistachio nuts. *Food and Chemical Toxicology*, Vol. 45, p. 812–816
5. Chun H.S., Kim H.J., Ok H.E., Hwang J.B., Chung D. H. (2007) Determination of aflatoxin levels in nuts and their products consumed in South Korea. *Food Chemistry*, Vol. 1, p. 385–391.
6. Commission Regulation (2006) (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Communities*, Luxembourg.
7. FAO (2004) Worldwide regulations for mycotoxins in food and feed in 2003. *FAO Food and Nutrition Paper*, No. 81. FAO, Rome
8. Leszczynska J., Kucharska U., Zegota H. (2000) Aflatoxins in nuts assayed by immunological methods. *European Food Research and Technology*. Vol. 210, p. 213–215.
9. Milhome M. A. L., Lima C. G., Delima L. K., Lima F.A.F., Sousa D. O. B., Nascimento R.F. (2014) Occurrence of aflatoxins in cashew nuts produced in northeastern Brazil. *Food Control*, Vol. 42, p. 34–37
10. Naturland (2000) *Cashew Nut.*, Organic Farming in the Tropics and Subtropics Exemplary Description of 20 Crops.
11. Pearson S.M., Candlish A.A.G., Aidoo K.E., Smith J.E. (1999) Determination of aflatoxin levels in pistachio and cashew nuts using immunoaffinity column clean-up with HPLC and fluorescence detection. *Biotechnology Techniques*, Vol. 3, p.97–99.
12. Reddy S.E.C., Sudhakar C., Reddy N.P.E. (2011) Aflatoxin contamination ground nut induced by *Aspergillus flavus* type fungi: A critical review. *International Journal Of Applied Biology and Pharmacuetical Technology*. Vol. 2, p. 180–192.
13. Stroka J., Anklam E., Jörissen U., Gilbert J. (2000) Immino-affinity column clean up with liquid chromotography post column bromination for determination of aflatoxins in peanut butter, pistachio paste, fig paste and paprika powder; collaborative study. *Journal of AOAC international*, Vol. 83, p. 320–340.
14. Wang J., Liu X.M. (2006) Surveillance on contamination of total aflatoxins in corn, peanut, rice, walnut and pine nut in several areas in China. *Chinese Journal of Preventive Veterinary Medicine*, Vol. 4, p. 33–37.
15. Yentür G., Er B., Özkan M.G., Öktem A.B. (2006) Determination of aflatoxins in peanut butter and sesame samples using high-performance liquid chromatography method. *European Food Research Technology*, Vol. 224, p. 167–170.

SHORT COMUNICATION

NATURAL ANTIOXIDANTS IN BLACK CHOKEBERRY MARC EXTRACTS DEPENDING ON THE EXTRACTION METHOD

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Abstract

The aim of this study was to find out the optimal conditions for extraction of natural antioxidants of grinded black chokeberry marc. The marc was extracted using microwave and ultrasound-assisted extraction methods in water/ethanol solutions with different degrees of concentration, using different particle sizes of the marc and different extraction times. Total content of polyphenols and anthocyanins was determined by spectrophotometry. The highest amount of total polyphenols was found in the extracts obtained using the ultrasound method, a 40% ethanol/water solution and a 1 mm diameter particle size extracted for 30 minutes. The microwave method presented the highest amount of anthocyanins in black chokeberry marc extract, using a 70% ethanol/water solution, a 2 mm diameter particles and 20 minute extraction time. It is necessary to find the most effective extraction method in order to develop a production technology for black chokeberry marc extracts rich in natural antioxidants.

Keywords: black chokeberry, natural antioxidants, polyphenols, anthocyanins.

Introduction

Black chokeberry (*Aronia melanocarpa*, Elliott**)** is a natural, rich source of phenolic antioxidants, such as anthocyanins, quercetin derivatives, and, in smaller amounts, vitamin C. Many reports have suggested its anti-proliferative effects against cancer cells, as well as antimutagenic, hepatoprotective, cardioprotective, and antidiabetic activities. Moreover, a series of papers reported the antioxidant properties of black chokeberry extracts or their phenolic constituents, using various, well established in vitro and in vivo models for direct antioxidant capacity, as well as their protective effects against oxidative stress. Recently, the neuroprotective effects of cyanidin-3-O-glycosides, commonly present in black chokeberry, have been tested in mice. (Gironés-Vilaplana, 2012)

The solid waste streams generated during fruit juice production can be alternative and attractive sourcesof valuable bioactive compounds due to their low cost and biorenewable nature. The utilization of wastes represents a sustainable approach to the integral benefit of raw materials minimizing the environmental problems caused by their disposal.

Berries contain high levels of phytochemicals with phenolic structure (phenolic acids, flavonoids, hydrolysable and condensed tannins) that can act as antioxidants and have health-promoting activities. The solid pressing wastes originated during separation of peels, seeds and pulp from the fruit juice are an abundant source of flavonoids, colour pigments and pectins. Berries are rich sources of anthocyanins, which impact the dark red or blue colour to the fruit, and are strong antioxidants (Laroze at al., 2010).

The phenolic content and composition greatly differs with the type of berry, and the extraction yield is greatly affected by the solvent. The recovery of compounds from the solid residue after berry processing into juice has been investigated using both conventional and alternative technologies, such as ultrasound or microwave assisted alcoholic extraction.

It is widely known that the efficiency of solid / liquid extraction processes is affected by critical processing parameters, such as temperature, nature of solvent, structure of solid matrix (mainly particle size) and extraction time. This means that each plant matrix / extraction solvent pair behaves in a unique way, so it should be studied as such. On the other hand, both the particle size of the plant matrix and the temperature on the extraction process are easily manipulated physical conditions. In general, a smaller size and a higher temperature facilitate mass transfer, but quantification of such heuristic rules for each plant source is required before optimisation efforts can be rationally developed (Giao et al., 2009).

The present study was aimed at evaluating the effect of extraction method and particle size on the extraction and recovery of antioxidant compounds remaining in the pressing pomace of black chokeberry.

Materials and Methods

Fruit pomace of black chokeberry, grown in Latvia, was dehydrated for three days at +50 °C and milled. The ground material was passed through a sieve with different meshes. The fractions were separated and packed in polyethylene bags and kept in dark place at room temperature before use. Solid-solvent ratio 1 : 5. All the samples were assayed in triplicate.

The pressing pomaces from berries were ground in a coffee grinder, and four different particle sizes (0.5, 1, 2 and 3 mm) were selected to evaluate the effect on the yield extraction. Different ethanol solutions were used for the pomace extraction using microwave (70% and 40%) and ultrasound-assisted (50% and 40%) extraction methods. Extraction lasted for 30 minutes at 30 °C with ultrasound bath Elmasonic S30H, 50/60 Hz and 1–20 minutes for microwave oven at 90 W (for longer exposure time samples were already boiling).

Total phenolic content of the extracts were measured using Folin-Ciocalteu method as described by Almey et al. (2010). Gallic acid was used as standard. 0.5 mg·mL^{-1} stock standard solution of gallic acid was prepared by dissolving 35 mg of dry gallic acid in 50 mL of 70% ethanol and then diluted to 100 mL with the same solvent. Working standards of between 0.0014 and 0.007 mg mL^{-1} were prepared by diluting the stock solution with distilled water, adding 2.5 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with deionised water) and after 2 minutes adding 2 mL of 7.5% sodium carbonate solution. Samples were prepared likewise from the extract stock solution prepared at concentration of 2–4 mg·mL^{-1} (2 mL of sample solution was taken for 25 mL test tube).

All the samples were incubated at 50 °C in water bath for 15 min, cooled quickly at cold water and filled up with purified water. Then the absorbance was read at 760 nm using Camspec M550 spectrophotometer. The standard calibration curve of gallic acid (0.0014–0.007 mg·mL^{-1}) was plotted.

The pH differential method was conducted for detection of total anthocyanin content as described in detail by Lee et al. (2005) using a spectrophotometer. It is based on the structural change of the anthocyanin chromophore between pH 1.0 and 4.5. The difference in the absorbance 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.

Results and Discussion

Total polyphenols extraction by ultrasound-assisted extraction using different particle sizes lasted for 30 minutes at 30 °C temperature and showed the highest concentration (60±1 GAE g^{-1}) when 40% ethanol solution and 1 mm particle size was used (Figure 1).

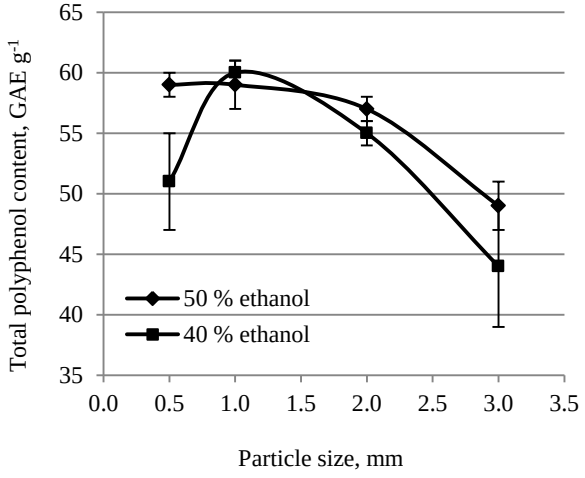

Figure 1. Relevance of particle size and polyphenol content by ultrasound-assisted extraction (30 min)

However almost the same concentration (59±2 GAE g^{-1}) was reached with 50% ethanol solution and 1 mm particle size.

Meanwhile anthocyanins were extracted and the highest amount of total monomeric anthocyanins (4594 ± 412 mg·L^{-1}) was found at 50% ethanol extract when 1 mm particles were used (Figure 2). The extraction solvent concentration was more important in this case comparing to polyphenol extraction. The most effective extraction in both experiments was obtained using 1 mm particle size of black chokeberry marc.

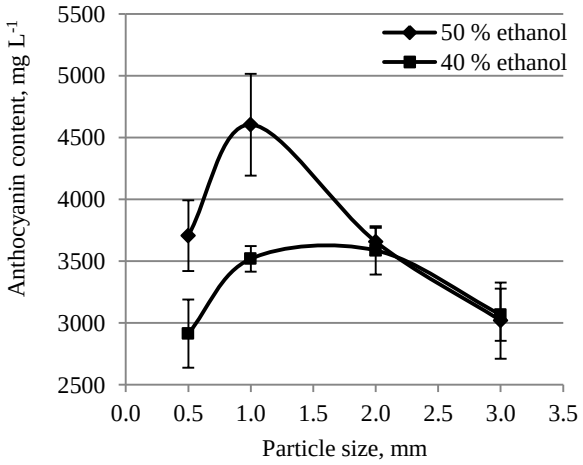

Figure 2. Relevance of particle size and anthocyanin content by ultrasound-assisted extraction (30 min)

The results obtained using microwaves showed at figures 3 and 4. Two particle sizes (2 mm and 3 mm) and two ethanol concentrations (40% and 70%) were investigated.

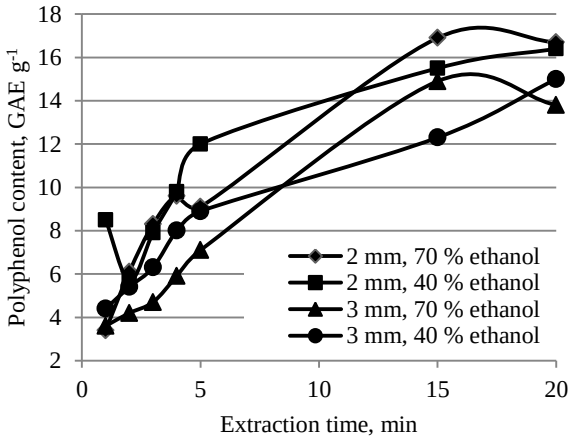

Figure 3. Polyphenol content depending on the extraction time by microwave-assisted extraction

The polyphenol concentration increases by increasing of extraction time, however after 15 minutes it decreases in 70% ethanol extracts. The highest level of polyphenols (16.9±1.2 mg L^{-1}) was achieved with 2 mm particles in 70% ethanol extraction after 15 min. The anthocyanin concentration increases in the same way – by increasing extraction time. The highest total anthocyanin concentration (6517±182 mg·L^{-1}) was obtained with 2 mm particles in 70% ethanol extract

after 20 min. Longer extraction times would not be accurate due to the rise of temperature and solvent evaporation.

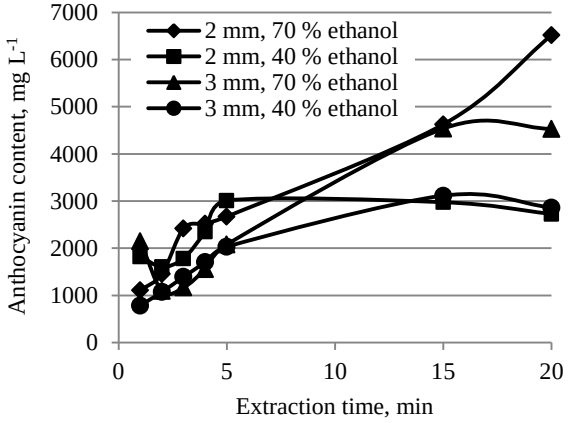

Figure 4. Anthocyanin content depending on the extraction time by microwave-assisted extraction

Microwave technology was more effective for 2 mm than 3 mm particles and 70% ethanol was more effective than 40% ethanol solution for both – polyphenols and anthocyanins

Some authors (Landbo, Mayer, 2001, Wang et al., 2011) investigated extraction efficiency using different solvents, temperature conditions, solvent-solid ratios and particle size and concluded that higher yields in extraction resulted from a decrease in particle size. Other researcher groups working with plant material found out that the most effective extraction is achieved when 0.2 mm (Giao et al., 2009; Goula 2013) and <0.5 mm (Fonseca et al., 2006) particle sizes were used. However smaller particles than 0.5 mm were not investigated in this research due to the available meshes, 1 mm particles were more effective than 0.5 mm. Researcher group investigating black chokeberry found >2 mm particles to be the most effective for ultrasound extraction of polyphenols (d'Alessandro et al, 2012), but it was reached in water extraction at 60 °C temperature and solid-solvent ratio 1:20. Hence each plant material and extraction solvent pair behaves in different way as mentioned above and effect of particle size, time, temperature, solid-solvent ratio, solvent composition and an impact of extraction method need to be evaluated separately. These results are significant for new product developing from black chokeberry juice pressing residues – rich in natural antioxidants.

Conclusions

Polyphenols and anthocyanins were successfully extracted from black chokeberrymarc of different particle size, with different extraction methods, different solvent concentrations and different extraction time. The highest polyphenol yield was obtained from the marc having 1 mm particle size in 40% ethanol extract using ultrasound for 30 minutes. The highest anthocyanin yield was obtained from the marc having 2 mm particle size in 70% ethanol by microwave extraction for 20 minutes. Both extraction methods may be combined to find the most effective extraction method for both – polyphenols and anthocyanins.

References

1. Azlim Almey A.A., Ahmed Jalal Khan C., Syed Zahir I., Mustapha Suleiman K., Aisyah M.R., Kamarul Rahim K. (2010) Total phenolic content and primary antioxidant activity of methanolic and ethanolic extracts of aromatic plants' leaves. *International Food Research Journal*, Vol. 17, p. 1077–1084.
2. d'Alessandro L.G., Kriaa K., Nikov I., Dimitrov K. (2012) Ultrasound sissted extrsction of polyphenol from black chokeberry. *Separation and Purification Technology*, Vol. 93, p. 42–47.
3. Fonseca J.M., Rushing J.W., Thomas R.L., Riley M.B., Rajapakse N.C. (2006) Influence of particle size on extraction yield and quantification of parthenolide in feverfew (Tanacetum Parthenium). **In:** *Proceedings of 4th International Conference on Quality and Safety Issues Related to Botanicals*. Mississippi State University, USA, Oct. 31st, 2006.
4. Giao M.S., Pereira C.I., Fonseca S.C., Pintado M.E., Malcata F.X. (2009) Efect of particle size upon the extent of extraction of antioxidant power from the plants *Agrimonia eupatoria*, *Salvia* sp. and Satureja montana. *Food Chemistry*, Vol. 117, p. 412–416.
5. Gironés-VilaplanaA., Valentão P., Andrade P.B., Ferreres F., Moreno D.A., García-Viguera C. (2012) Phytochemical profile of a blend of black chokeberry and lemon juice with cholinesterase inhibitory effect and antioxidant potential. *Food Chemistry*, Vol. 132, p. 2090–2096.
6. Goula A.M. (2013) Ultrasound-assisted extraction of pomegranate seed oil – Kinetic modeling. *Journal of Food Engineering*, Vol. 117, No. 4, p. 492–498.
7. Landbo A.K., Mayer A.S. (2001) Enzyme-assisted extraction of antioxidative phenols from black currant juice press residues (*Ribes nigrum*). *Journal of Agricultural and Food Chemistry*, Vol. 49, No. 7, p. 3169–3177.
8. Laroze L.E., Díaz-Reinoso B., Moure A., Zúñiga M.E., Domínguez H. (2010) Extraction of antioxidants from several berries pressing wastes using conventional and supercritical solvents. *European Food Research and Technology*, Vol. 231, p. 669–677.
9. Lee J., Durst R.W., Wrolstad R.E. (2005) Determination ofTotal Monomeric Anthocyanin Pigment Contentof Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study. *Journal of AOAC International*, Vol. 88, No. 5, p. 1269 – 1278.
10. Wang Z., Pan Z., Ma H., Atungulu G.G. (2011) Extract of phenolics from pomegranate Peels. *The Open Food Science Journal*, Vol. 5, p. 17–25.

SHORT COMUNICATION

INFLUENCE OF PRE DRYING TREATMENT ON PHYSICAL PROPERTIES OF CARROTS

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Abstract

In the present work, the effect of pre-treatment with ascorbic acid on colour changes and texture was investigated during convective hot air drying at 60ºC. The pre-treatments were done at 0.25% and 1 % of ascorbic acid with treatment times of 60 and 90 minutes.
Changes in colour and total colour difference were evaluated by the CIELab colour system and texture of dried carrots was accessed in terms of hardness, springiness, and cohesiveness.Regarding the total colour difference, soaking carrots in ascorbic acid at both concentrations and treatment times turned out to be an ineffective method for reducing the browning reactions during drying.
With respect to textural attributes non-significant differences were observed in springiness and cohesiveness of untreated and pre-treated dried carrots but the hardness increased at a pre-treatment time of 90 minutes.

Keywords: pre-treatment, ascorbic acid, colour, moisture content, texture.

Introduction

Carrot (*Daucus carota* L.) is one of the most commonly used vegetables for human nutrition due to its pleasant flavour, nutritive value, and great health benefits related to its antioxidant, anticancer, healing, and sedative properties. Carrots are constituted by high vitamin and mineral content and other valuable nutrients presented at lower concentrations (Shivhare et al., 2009).
Although carrots are widely consumed as fresh vegetables, due to their perishable nature, they are also subjected to different processes such as freezing, canning or dehydration to extend their shelf-life for distribution and storage. Drying is one of the oldest methods for the preservation of foods. Deterioration of the chemical and physical properties of dehydrated carrots causes changes in their quality that depend not only on drying conditions but also on the other operations carried out before and after drying (Negi, Roy, 2011). Prior to these processes, some chemical or physical treatments were done to the raw material aimed to inactivate enzymes such as polyphenol oxidases (PPO) and to inhibit some undesirable chemical reactions that cause many adverse changes on the quality of the dried product.
The oxidation of phenolic substrates (natural substances that contribute to the sensorial properties) by polyphenol oxidases (PPO) is one of the major causes of the brown coloration of many fruits and vegetables during ripening, handling, storage, and processing (Queiroz et al., 2008). Other non enzymatic reactions, such as Maillard reaction and caramelization reaction also produce browning pigments (Hiranvarachat et al., 2011).
Ascorbic acid is frequently used for browning control of food products and it has been more effective than its isomer isoascorbic acid. This vitamin acts as an antioxidant because it reduces the o-quinones produced by the catalysis of phenolic compounds by PPO, thus limiting secondary reactions that leads to browning and also contributes to decreasing pH. Since optimum pH to PPO range is 5 to 7.5, lower values inhibit enzymatic activity (Ozoglu, Bayindirli, 2002; Gerrero-Beltrán et al., 2005).
The objective of this work was to study the effects of chemical pre-drying treatment (ascorbic acid) at different concentration/time combinations on some physical properties, namely colour and texture, of dried carrots.

Materials and Methods

Sampling
The carrots used in this study were purchased in a local market, and were hand peeled and cut into slices with thickness of 1cm and diameter of 3 cm.

Pre-treatments before dehydration process
Prior to drying, carrots were pre-treated by the following pre-treatment methods: soaking in aqueous solution of 0.25% ascorbic acid for 60 and 90 min and 1% of ascorbic acid for the same times, at room temperature. Also untreated samples were dried, serving as control. After each pre-treatment, the excess of water on sample surface was superficially removed with paper towel.

Drying experiments
Drying experiments of untreated and treated carrots were carried out in a convection oven at 60 ºC, with an air speed of 0.2 m/s, in order to reduce the average moisture of carrots to about 15 % (w/w). The drying time of the samples was approximately 8 hours.

Evaluation of colour
The colour of all samples was measured using a handheld tristimulus colorimeter (Chroma Meter-CR-400, Konica Minolta).
For each sample, twenty measures were made at different positions of the sample and the obtained values were compared with those of the fresh sample.

The data were reported as average values of these measurements. The colour changes were calculated as:

$$\Delta L = \frac{L - L_0}{L_0}, \quad \Delta a^* = \frac{a^* - a_0^*}{a_0^*} \quad \text{and} \quad \Delta b^* = \frac{b^* - b_0^*}{b_0^*}$$

where L, a*, and b* represent, respectively, the lightness, redness, and yellowness of the samples (the subscript 0 is referred to reference-fresh sample).
The total colour difference (TCD), was the parameter considered for the overall colour difference evaluation between a sample and the reference.

$$TCD = \sqrt{(L - L_0)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

Texture analysis
The texture profile analysis (TPA) for all samples was made by a texturometer (TA.XT.Plus from Stable Micro Systems). The texture profile analysis was carried out by two compression cycles between parallel plates performed using a flat 75 mm diameter plunger, with a 5 s period of time between cycles. TPAs were performed in 20 samples for each state and the measured textural properties were hardness, springiness, and cohesiveness.

Results and Discussion

The moisture content of fresh carrots was 87.02±0.14% (wet basis), that is in the range 80–90% as reported by Prakasha et al. (2004).
Table 1 shows the mean value of moisture content of the dried carrots subjected to pre-treatments. Except for the pre-treatment at 0.25% of ascorbic for 60 minutes, the dryings of the pre-treated samples was similar to those of the untreated samples, all with a drying time 8 hours. Thus, the pre-treatment with ascorbic acid (at both concentrations and time treatment) seems to have no consequence on the effective water diffusivity of carrots during air-drying process as compared with untreated and treated samples.

Table 1

Moisture content of untreated and treated dried carrots

Pre-treatment	Moisture, g 100 g^{-1}
Untreated	16.47±0.63
AA–0.25% for 60 min	21.39±2.76
AA–0.25% for 90 min	15.56±1.23
AA 1% for 60 min	16.57±0.88
AA–1% for 90 min	16.78±2.33

AA – ascorbic acid; Values expressed as means of 3 replicas±standard deviation.

The changes of colour of carrots are illustrated in Table 2. It was found that all pre-treatment methods led to positive ΔL values, which indicates an increase of the brightness of the dried carrots. Except for the treatment at 0.25% of ascorbic acid and treatment time 60 min, the brightness of the pre-treated carrots showed a slight decrease, as compared to the untreated samples. In addition, for both concentrations of ascorbic acid, the increase of treatment time allowed a decrease of approximately 5% in the brightness of pre-dried carrots.

Table 2

Colour parameters of dried carrots

Pre-treatment	ΔL	Δa*	Δb*
Untreated	0.12	0.179	-0.066
AA–0.25% for 60 min.	0.18	-0.001	-0.275
AA–0.25% for 90 min.	0.13	-0.008	-0.249
AA–1% for 60 min.	0.10	0.069	-0.265
AA–1% for 90 min.	0.06	-0.014	-0.252

The drying of untreated carrots led to an increment in a* colour coordinate that implied that the dried samples were redder than the fresh ones. The carrots showed a bright red colour, which is desirable. In all dried untreated and treated samples, Δb* shows negative values, thus meaning that drying decreases yellowness of the carrots. The ascorbic acid soaking leads to similar decrease of Δb* values for both concentration/time combinations.
The total colour change (Figure 1) of untreated dried carrots was 10 and this value increased to around 15 to the samples submitted to the four chemical treatments (values of the chemical-treated samples were statistically similar ($p>0.05$)). This implies that the chemical pre-treatments did not influence positively the colour of carrots during drying.
In fact, carrots soaked in ascorbic acid became less bright and redder than the untreated samples. Therefore, and in order to reduce the total colour difference, it appears that the most appropriate condition of treatment would be at 1% of ascorbic acid and 90 min of treatment time.

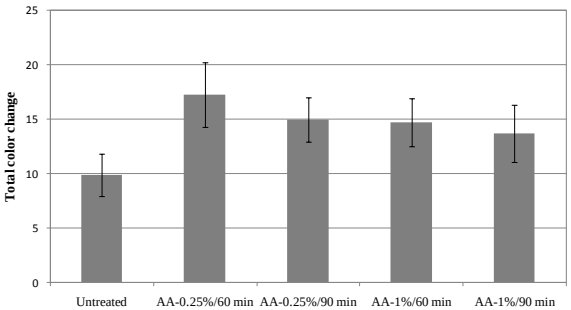

Figure 1. Total colour difference of untreated and pre-drying treated carrots with ascorbic acid

Figure 2 reveals the average values of hardness for carrots untreated and treated with ascorbic acid. The hardness represents the force required to deform the sample in mastication, and it is exerted by compressing the food between the teeth or between the tong and the mouth. The hardness of the untreated dried carrots is 30 N and this value is similar to the ones obtained in the pre-drying treatment for 60 min at 0.25% and 1% of ascorbic acid. Hiranvarachat et al. (2011) observed that the microstructures of untreated hot air carrots

became rigid due to the collapse of cells and pores of the sample with drying and the soaking carrots in water and citric acid did not affect the microstructure after drying. However, when the treatment time was increased to 90 min the hardness of the treated carrots augmented to nearly 45 N, which induced a less intense change of hardness as compared with the fresh carrots.

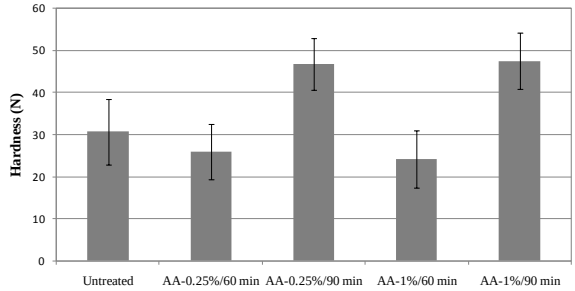

Figure 2. Hardness of untreated and pre-drying treated carrots with ascorbic acid

In Figure 3 the mean values of springiness are presented. The value of springiness of fresh carrots was 85.5%. The springiness measures the degree of recovering shape after removal of the force that deformed the sample and, therefore, is related to the elasticity of the product. The obtained results on untreated and treated dried carrots are not statistically different. Thus, the pre-treatment with ascorbic acid had no visible effect of springiness in the conditions under study.

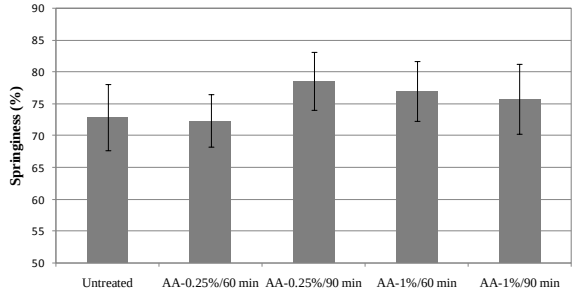

Figure 3. Springiness of untreated and pre-drying treated carrots with ascorbic acid

The cohesiveness accounts for the strength of the internal bonds and measures the degree of deformation before rupture when biting the food with the molars.
The cohesiveness of the fresh carrot was 0.8.

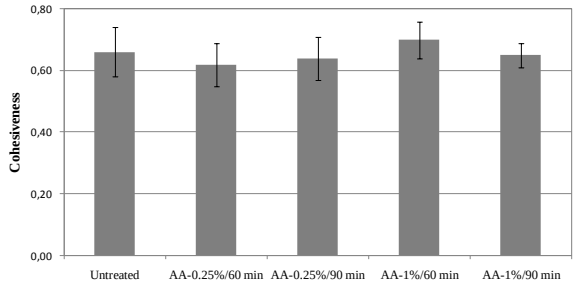

Figure 4. Cohesiveness of untreated and pre-drying treated carrots with ascorbic acid

From Figure 4 it is possible to conclude that the cohesiveness obtained in untreated and treated carrots was very similar, not being observed statistical differences between the different samples. Thus the pre-treatment with ascorbic had no effect on the cohesiveness of dried carrots.

Conclusions

The present work evaluated the effect of pre-treatments with ascorbic acid prior hot convective drying at 60 °C on colour and textural attributes of dried carrots.
The obtained results enabled to conclude that drying allows the samples to be lighter and less reddish than the fresh carrots.
Regarding the total colour difference, the dried carrots soaked with ascorbic acid at both concentrations and pre-treatment times had a higher value when compared with those of the untreated sample. This means that such pre-treatments had not a positive effect on reducing the browning reactions.
With respect to textural attributes, non-significant differences were observed in springiness and cohesiveness of untreated and pre-treated dried carrots.
The hardness of pre-treated dried carrots with ascorbic acid at 0.25% and 1% during 60 min was similar to that obtained for the untreated sample, but this parameter increased if the pre-treatment time raised to 90 min.

Acknowledgment

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References

1. Gerrero-Beltrán J.A., Swanson B.G., Barbosa-Cánovas G.V. (2005) Inhibition of polyphenoloxidase in mango purre with 4-hexylresorcinol, cysteine ans ascorbic acid. *LWT – Food Science and Tecnhology*, Vol. 38, p. 625–630.
2. Hiranvarachat B., Devahastin S., Chiewchan N. (2011) Effects of acid pretreatments on some physicochemical properties of carrot undergoing hot air drying. *Food and Bioproducts Processing*, Vol. 89, p. 116–127.
3. Negi P.S., Roy S.K. (2011) The effect of blanching on quality attributes of dehydrated carrots during long-term storage. *European Food Research and Technology*, Vol. 212, p. 445–448.
4. Ozoglu H., Bayindirli A. (2002) Inhibition of enzymatic browning in cloudy apple juice with antibrowning agents. Food Control, Vol.13, p. 213–221.
5. Prakasha S., Jhab S.K., Datta N. (2004) Performance evaluation of blanched carrots dried by three different driers. *Journal of Food Engineering*, Vol. *62*, p. 305–313.
6. Queiroz C., Lopes M.L.M., Fialho E., Valente-Mesquita V.L. (2008) Polyphenol oxidase: characteristics and mechanisms of browning control. *Food Reviews International*, Vol. 24, p. 361–375.
7. Shivhare U.S., Gupta M., Basu S., Raghavan G.S.V. (2009) Optimization of blanching process for carrots. *Journal Food Process Engineering*, Vol. 32, p. 587–605.

SHORT COMUNICATION

EGG PASTA (*ERIŞTE*) PRODUCED FROM WHOLE GRAIN OAT FLOUR

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Abstract

In this study, egg pastas (*erişte*s) were produced by incorporating refined or whole grain oat flours (ROF, WOF, respectively) at different levels (25, 50 and 100%; w/w) instead of bread wheat flour (BWF) according to the traditional *erişte* production. Some chemical and quality properties of *erişte*s were investigated and compared to the control *erişte*s prepared with BWF or durum semolina. The highest ash, protein and titration acidity contents were obtained with the *erişte* prepared with 100% WOF. The brightness (L*) values of the oat incorporated *erişte*s were lower than the controls. An increasing of WOF level caused gradually an increase in the redness (a*) of *erişte*s. The yellowness (b*) values of WOF incorporated *erişte*s were higher than ROF incorporated *erişte*s and control *erişte* produced with BWF. Cooking properties of *erişte*s prepared with oat flours were generally lower than those of controls. Total dietary fiber contents of *erişte*s prepared with ROF or WOF were in the ranges of 5.29–9.16%, or 8.41–18.25%, respectively. WOF containing uncooked *erişte*s had higher β-glucan content than those of ROF containing and control ones. β-glucan contents of oat included *erişte*s were in the range of 0.67–3.35% in uncooked form. Total phenolic compound contents of all cooked *erişte*s considerably decreased. Antioxidant activities of oat included uncooked *erişte*s were higher than that of controls.

Keywords: oat; whole grain; egg pasta; erişte; β-glucan.

Introduction

Oats have great potential as a health-promoting raw material in several types of foods. Oats have traditionally been used mainly as oatmeal, bran or flakes, which are used to produce porridge, bread and breakfast cereals. Oats are also used in various forms as an ingredient in pasta, biscuits, snack bars and beverages (Kaukovirta-Norja, Lehtinen, 2008). Oat ingredients can be added into a variety of consumer products to provide health-promoting properties, to adjust the flavor and visual appearance or to achieve technological goals. The health effects associated with oats relate essentially to the total dietary fibre and β-glucan contents of oat products (Kaukovirta-Norja, Lehtinen, 2008). The β-glucan of oat and barley has constantly been shown to lower low-density lipoprotein (LDL) and total cholesterol in concern with elevated cholesterol (Jones, 2008).

Pasta products have been consumed in Mediterranean countries for many centuries and it takes the second place after bread in consumption over the world (Torres et al., 2007). Fresh egg pasta (*tagliatalle, fettuccine and tagliolini*) is a typical product in Italy. It is made by hydrating durum wheat with water and eggs. After mixing and kneading, sheeting-rolls are used for shaping. Egg pasta, which is also a traditional product in west Black Sea region of Turkey, is usually called as '*Erişte*'. It is generally produced with a homemade style. The main ingredients of *erişte* are wheat flour, water, salt and egg. Semolina is rarely used in its production. The traditional production method differs in many ways from the industrial production method used in pasta companies. It is well known that production parameters highly affect not only quality characteristics but also sensorial and nutritional properties of pasta products (Petitot et al., 2007; De Zorzi et al., 2007; Manthey, Schorno, 2002; Alamprese et al., 2008). In the present study, *erişte* was produced by incorporating different levels of *whole grain or refined oat flours* (WOF, ROF, respectively) instead of bread wheat flour (BWF) in order to develop high fiber and healthy *erişteş* for consumers and to help producers for developing an alternative product. Then, some chemical and quality properties of *erişte*s were investigated and compared to the control *erişte*s prepared with BWF or durum semolina. Oat incorporated *erişte*s were also evaluated for some of their nutritional properties such as β-glucan, total dietary fiber, total phenolic compound contents and antioxidant activity.

Materials and Methods

Materials

ROF and WOF were obtained from the cereal flour wholesalers located in the city of İstanbul. Other ingredients including BWF, durum semolina, egg and salt were purchased from local markets. β-glucan and total dietary fiber assay kits were provided from Megazyme International Ireland Limited, Wicklow, Ireland. All other chemicals used in this study were analytical grade.

Methods

In the present study, *erişte*s were produced by incorporating ROF or WOF at different levels (25, 50 and 100%; w/w) instead of BWF in the formulation. *Erişte*s were produced according to traditional method that includes mixing of ingredients (2% salt, 70 g egg, 10% durum semolina added on 720 g flour basis) with reasonable amount of water (250–450 mL), sheeting of dough, pre-drying on the *sadj* (hot metal plate) at around 185 °C for 60–90 seconds, cooling to room temperature, cutting into small strands, final drying at room temperature for 1–3 days and storing in plastic bags. Two controls were produced using BWF and durum semolina. Production of *erişte*s was carried out

as replicate. *Erişte* samples were ground and sieved from 500 μm steel sieve (Retsch, Haan, Germany) for analysis. The remaining parts of *erişte* samples were kept for testing cooking properties.

Moisture, protein (N×6.25), ash, crude oil and titration acidity of the samples were determined according to the Approved Methods of American Association of Cereal Chemists International (AACCI, 2000) following the Methods of 44-01, 46-12, 08-01, 30-10, 31-01, respectively.

Pigment contents of the samples were determined according to AACCI Standard Method No. 14-50 (AACCI, 2000).

The colour properties of *erişte* samples was measured using the *L* a* b** colour space (CIELAB space) with Minolta Spectrophotometer CM-3600d (Tokyo, Japan).

Cooking properties, such as optimum cooking time, volume increase, water absorption, cooking loss, were determined according to AACC Standard Method No.66-50 (AACCI, 2000). Protein loss was determined in cooking water as spectrophotometricaly following the method of Lowry et al. (1951). Total organic matter (TOM), representing the amount of surface material released from cooked *erişte* into the washing water after rinsing, was determined according to D'Egidio et al. (1982).

Total dietary fiber (TDF, %) and β-glucan (%) contents of *erişte*s were determined using Megazyme assay kits.

Total phenolic compounds (TPC, %) of *erişte* samples were determined according to Gutfinger (1981). TPC were extracted with dimethyl sulfoxide. For the preparation of cooked *erişte*s in order to analyze β-glucan and TPC contents, *erişte*s were cooked at optimum cooking time, after removing excess water, samples were lyophilized, then ground and kept for analysis at 4 ˚C. Antioxidant activities of *erişte*s were expressed as DPPH (2,2-diphenyl-1 picryhydrazyl) scavenging activity (%) following the method of Yu et al. (2002). The concentration of DPPH in test solution was 25 μM. β-glucan, TDF, TPC and antioxidant activity results were calculated on dry weight basis (dwb).

All analysis was carried out as triplicate. The data was statistically evaluated by using SPSS for Windows (Version 16.0). GLM variance analysis was applied. When significant differences were found, the Tukey's-b multiple comparison test was used to determine the differences among means.

Results and Discussion

The crude oil and protein contents of ROF and WOF were 8.6 and 12.6%, and 7.3 and 14.8% on dwb, respectively. Total dietary fiber and β-glucan contents of ROF and WOF were 8.2 and 1.83%, and 23.2 and 3.77% on dwb, respectively.

The moisture contents of controls and oat incorporated *erişteş* changed in the range of 12.7–13.6%. The ash contents of oat incorporated *erişte*s were higher than control ones. The crude oil contents of oat incorporated *erişte*s were higher than controls and the crude oil contents of both types of *erişte*s increased with an increasing rate of oat incorporation. The protein contents of controls and oat incorporated *erişte*s changed in the range of 12.2–14.8%. The protein contents of WOF incorporated *erişte*s were generally higher than ROF incorporated ones ($p<0.05$). The titration acidity values of oat included *erişte*s were higher than control *erişte*s, and titration acidity value increased with an increasing level of oat flour in both types of *erişte*s. The highest ash, protein and titration acidity contents were obtained with the *erişte* sample prepared with 100% WOF (data not presented).

The pigment contents of *erişte*s prepared with both flour types gradually increased with increasing levels of ROF and WOF ($p<0.05$). The pigment contents of *erişte*s produced with WOF were higher than the *erişte* samples produced with ROF and control ones. The highest pigment content was obtained with the sample prepared with 50% WOF among the *erişte* samples.

The brightness (L*) values of the oat incorporated *erişte*s were lower than the controls. An increasing of WOF level caused gradually an increase in the redness (a*) of *erişte*s. The yellowness (b*) values of WOF incorporated *erişte*s were higher than ROF incorporated *erişte*s and control *erişte* produced with BWF. According to the statistical variance analysis results, the flour type affected the L*, a* and b* values, in addition, L* values were significantly affected by oat incorporation level ($p<0.05$).

In determining pasta quality, cooking properties are important quality constraints. Cooking properties, such as optimum cooking time (min), volume increase (%), water absorption (%), cooking loss (%), protein loss (%) and total organic matter (TOM, %) values of *erişte*s were studied in this research. It was found that the cooking properties of ROF or WOF including *erişte*s were generally lower than those of the controls. The optimum cooking times of the controls and oat incorporated *erişte*s changed in the range of 9.0–12.5 min. The optimum cooking times of oat included *erişte*s were lower than control ones. Lower optimum cooking time is a desirable for quick preparation of egg pastas. The optimum cooking times of WOF incorporated *erişte*s decreased while increasing an incorporation level. The highest cooking loss value was obtained with 100% WOF included *erişte* (data not presented). The protein loss values of WOF incorporated *erişte*s increased while increasing of WOF incorporation level in the formula ($p<0.05$). According to the statistical variance analysis results, the flour type had not any significant effect on cooking loss, protein loss and TOM values ($p>0.05$). Besides, an incorporation level had significant effect on cooking loss ($p<0.05$), but it had not any significant effect on protein loss and TOM values ($p>0.05$).

TDF contents of *erişte*s were determined only in uncooked form. TDF contents of *erişte*s produced with ROF or WOF were mostly higher than controls ($p<0.05$). TDF contents of *erişte*s produced with WOF were higher than that of the *erişte*s produced with

ROF. TDF contents of uncooked *erişte*s prepared with ROF or WOF were in the ranges of 5.29–9.16%, or 8.41–18.25%, respectively. The highest TDF content was determined in 100% WOF incorporated *erişte* (data not presented).

β-glucan contents of *erişte*s were determined in both uncooked and cooked forms. β-glucan contents of oat included *erişte*s were generally higher than control ones in both forms. β-glucan contents of oat included uncooked *erişte*s increased as increasing of oat flour incorporation level. WOF containing *erişte*s had higher β-glucan content than those of ROF containing and control *erişte*s in uncooked form. β-glucan contents of oat flour included *erişte*s were in the range of 0.67–3.35% in uncooked form. The highest β-glucan content was determined in 100% WOF incorporated *erişte* in its both uncooked and cooked forms.

TPC analysis was carried out in both uncooked and cooked *erişte*s. TPC contents of all cooked *erişte*s considerably decreased, it could be concluded that cooking affected TPC contents harmfully.

Antioxidant activity analysis of *erişte* samples was carried out in uncooked form only. It was found that antioxidant activities of oat flour included *erişte*s were higher than that of control ones. In addition, WOF incorporated *erişte*s had higher antioxidant activity than that of ROF included *erişte* samples (data not presented).

Conclusions

Erişte is a traditional product and it is usually made of BWF in west Black Sea region of Turkey. In this study, it was shown that *erişte* could be produced from oat flours by replacing with BWF in different ratios. Besides, it could be produced from 100% ROF or WOF as an alternative dietary fiber rich product. It is expected that oat flour incorporated *erişte*s will provide important health benefits for consumers and will be substitute product for homemade manufacturers. The results also showed that all oat flour included *erişte*s can be regarded as good sources of dietary fiber.

Acknowledgment

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References

1. Alamprese C., Casiraghi E., Rossi M. (2008) Structural and cooking properties of fresh egg pasta as a function of pasteurization treatment intensity. *Journal of Food Engineering*, Vol. 89, p. 1–7.
2. Approved Methods of the American Association of Cereal Chemists International (AACCI, 2000) 10th ed., St. Paul, MN, USA.
3. D'Egidio M.G., De Stefanis E., Fortini S., Galterio G., Nardi S., Sgrulletta D., Bozzini A. (1982) Standardization of cooking quality analysis in macaroni and pasta products. *Cereal Foods World*, Vol. 27, No. 8, p. 367–368.
4. De Zorzi M., Curioni A., Simonato B., Giannattasio M., Pasini G. (2007) Effect of pasta drying temperature on gastrointestinal digestibility and allergenicity of durum wheat proteins. *Food Chemistry*, Vol. 104, p. 353–363.
5. Gutfinger T. (1981) Polyphenols in olive oils. Journal of American Oil Chemical Society, Vol. 58, p. 966–968.
6. Jones J.M. (2008) Fiber, whole grains, and disease prevention. **In:** *Technology of Functional Cereal Products.* B. R. Hamaker (ed.). Boca Raton, FL, USA: Woodhead Publishing Limited, CRC Press LLC, p. 46–62.
7. Kaukovirta-Norja A., Lehtinen P. (2008) Traditional and modern oat-based foods. **In:** *Technology of Functional Cereal Products*. B. R. Hamaker (ed.). Boca Raton, FL, USA: Woodhead Publishing Limited, CRC Press LLC, p. 215–232.
8. Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. (1951) Protein measurement with folin phenol reagent. *Journal of Biological Chemistry*, Vol. 193, p. 265–275.
9. Manthey F., Schorno AL. (2002) Physical and cooking quality of spaghetti made from whole wheat durum. *Cereal Chemistry*, Vol. 79 (4), p. 504–510.
10. Petitot M., Brossard C., Barron C., Larre C., Morel M.H., Micard V. (2009) Modification of pasta structure induced by high drying temperatures. Effects on the in vitro digestibility of protein and starch fractions and the potential allergenicity of protein hydrolysates. *Food Chemistry*, Vol. 116, p. 401–412.
11. Torres A., Frias J., Granito M., Guerra M., Vidal-Valverde C. (2007) Chemical, biological and sensory evaluation of pasta products supplemented with α-galactoside-free lupin flours. *Journal of the Science of Food and Agriculture*, Vol. 87, p. 74–81.
12. Yu L., Haley S., Perret J., Harris M., Wilson J., Qian M. (2002) Free radical scavenging properties of wheat extracts. *Journal of Agricultural and Food Chemistry*, Vol. 50, p. 1619–1624.

SHORT COMUNICATION

IMPACT OF LOW TEMPERATURE, PROLONGED TIME TREATMENT AND VACUUM DEPTH ON THE PORCINE MUSCLE QUALITY AND SAFETY

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Abstract

The effect of low temperature (53 °C), long time heat treatment (Tc +5 h and Tc + 17 h – holding time, Tc – core temperature equal to the water bath temperature), and vacuum depth (0.3 and 0.6 -10^5 Pa) on texture and microbiological safety parameters in *M.longissimus dorsi* from slaughter pigs were studied. The study involved analysis of meat moisture, toughness, cooking loss and microflora parameters. Decreasing shear force and increasing cooking loss during low temperature and long time treatment was observed at 53 °C temperature along with the treatment duration (Tc +5 h and Tc +17 h). Positive shear force and vacuum depth correlation was oberved in the samples. No correlation between samples moisture content and shear force was found. Microbiological analysis revealed safety of the produced product within two weeks period storage at 4 °C temperature.

***Keywords*:** LTLT, vaccum depth, porcine quality and safety.

Introduction

Gentle treatment technologies have been improved during the last decade by cook-in-bag / container technology, which has been extensively adopted by catering services and food processing plants due to the possibility of increased consistency along the muscle as well as appealing texture and colour of the meat. Studies have shown that meat is more tender and has less cooking loss when heating temperatures are low. A range of heat-induced changes occurs at temperatures between 48 °C and 63 °C, and these events, alone or due to the presence of other proteins can be of significance to toughness, cooking loss and colour of LTLT treated meat (Christensen et al., 2011, Christensen et al., 2013). Clearly temperature, but also duration of cooking has a large effect on the physical properties of meat and the eating quality. But the effect on these physical properties when meat is heated at lower temperatures for a long period of time (LTLT) and different vacuum levels is not clearly understood. The aim of the study was to investigate the combined effect of heating temperature, time and vacuum depth on the physical properties and microbiological stability of LTLT treated *Longissimus dorsi* muscle from slaughter pigs.

Materials and Methods

Samples: Longissimus dorsi (LD) muscles were obtained from both sides of 10 slaughter pigs. Whole muscles were vacuum packed and stored at 4 °C for 2 days. After storage muscles were cut into samples, vacuum packed at different depth of vacuum (0.3×10^5 Pa and 0.6×10^5 Pa) and heat treated at 53 °C for Tc +5 h and Tc + 17 h – holding time (Tc – core temperature equal to the water bath temperature). After thermal treatment, samples were taken from the water bath and immersed in an ice-water bath until the temperature reached 26 °C. The samples were then stored in a storage room at 0–4.0 °C for 24 h up to 14 days before analysis. After storage, sample weights were recorded in order to calculate cooking weight losses, samples moisture analysis conducted (105 °C).

Warner–Bratzler shear force: blocks of 1×1×6 cm were cut from the heat treated samples. Each block was sheared 3 times by Lloyd TA1 Texture Analyzer equipped with a triangular Warner–Bratzler test cell. Mean maximum force required to shear through the samples, Warner–Bratzler Shear Force, was determined from each LTLT treatment.

Microbiological analysis: A surface area of 14.7 cm^2 and 0.3 cm depth was sampled in an aseptic manner from the surface of each muscle (raw, LTLT processed, stored for 7 and 14 days). Homogenates and suitable dilutions were prepared. Total plate count, mezophilic, thermophilic bacteria, *Enterobacteriaceae*, *Escherichia coli* counts, yeast and were performed according to standard procedures: LST EN ISO 4833:2003, LST ISO 21527-1:2008, LST ISO 21528-2:2009, LST ISO 16649-2:2002.

Statistical analysis was performed using the Statistical Analysis System (SAS, 1989). Variance was analyzed by using the general linear model procedure of SAS.

Results and Discussion

Effect of LTLT treatments in different vacuum depth on cooking weight loss, Warner–Bratzler shear force and water content. This study investigated the combined effect of heating duration of Tc + 5 h and Tc + 17 h and vacuum depth of 0.3 and 0.6×10^5 Pa of samples treated at 53 °C temperature evaluating in raw and LTLT treated samples moisture, cooking loss, shear force parameters.

Table 1 shows the results obtained for cooking weight loss (CL), Warner–Bratzler shear force (WB) and moisture.

Table 1

Physical properties analysis of LTLT *Longissimus dorsi* muscle (mean values)

Treatment / Properties	Raw meat sample	T_c+5h, 0.3 10^5 Pa	T_c+5h, 0.6 10^5 Pa	TT_c+17h, 0.3 10^5 Pa	TT_c+17h, 0.6 10^5 Pa
Moisture, %	664.24	667.76	667.88	667.99	557.76
Share force, N	552.43	449.90	884.24	559.38	772.34
Cooking loss, %	NA*	88.72	99.02	116.66	115.12

*NA-not applicable

Among alterations in the meat occurring during cooking are toughness and juiciness changes, both of them being important when assessing eating quality of meat.

As expected, cooking loss increased when thermal treatment time was prolonged from Tc+5 h to Tc+17 h in both samples groups: by 91% for LTLT samples treated in 0.3×10^5 Pa vacuum depth, and 68% for samples treated in 0.6×10^5 Pa vacuum depth. However no significant differences were found ($p>0.05$) within the LTLT samples treated the same time but different vacuum depth. The results are in line with those reported for *Semitendinosus* (ST) muscle from young bulls (Christensen et al., 2013), although controvercial result of the same authors was found for *Longissimus dorsi* muscle from slaughter pigs where increasing time did not affect the cooking loss at any temperature (Christensen et al., 2011).

Moisture content did not differ significantly, except for LTLT samples treated for Tc + 17 h at 0.6×10^5 Pa vacuum depth, where moisture was 11% lower than the avarge of LTLT treated samples. Cristensen et al. (2012) found that juiciness decreased with increasing heating temperature and time in all species, and cooking loss increased with increasing temperature.

Shear force (WB) mean values increased as the vacuum depth increased, significant differences ($p<0.05$) being observed between the mean values obtained in samples treated for 5 h in 0.3 and $0{,}6\times10^5$ Pa vacuum depth where shear force increased by 69 % and by 23 % in samples treated of 0.3 and $0{,}6\times10^5$ Pa for 17 h. Both heating time and vacuum depth significantly affected shear force for LTLT porcine samples treated for 5 h at 53 °C temperature, the linear relationship was found for the experiment, although for 17 hours treated LTLT samples there was inverse effect registered between vacuum depth and shear force parameters (table 1). Mortensen et al. (2012) performed sensory analysis of eating quality and found increased tenderness of ST from young bulls with increasing heating times from 3 to 12 h at 56 °C, 58 °C and 60 °C, although in contrast to the results of the current decreased shear force with increasing heating time was found in cows, where WB-PF of ST decreased with increasing temperature and only minor decreases of WB-PF were observed with increased heating time, except at 55 °C where a significant decrease in WB-PF was observed with increasing temperature from 2.5 h to 19.5 h (Christensen et al., 2013).

Effect of LTLT treatments on product microbiological quality

The temperature and treatment duration primarily defines the sensory quality, microbial safety and shelf-life of LTLT products. A number of national and international recommendations exist, such as good manufacturing practice (GMP). Significant process parameter differences are evident among the various GMP recommendations; nevertheless, all of them, invariably, focus upon microbiological safety (Vaudagna et. al., 2002). The results from the current microbiological investigation of the raw meat (LD) and LTLT treated LD from pigs are presented and the effect of 53 °C treatment for 5 and 17 h regime on samples safety discussed below.

Microbiological analysis of raw *M.Longissimus dorsi* revealed the pollution status of the raw material ready to use for LTLT treatment. Total plate count varied within the limits of 2.6×10^6 to 1.1×10^7 CFU g^{-1}, mesophilic bacterial count – 2.0×10^6 to 0.8×10^7 CFU g^{-1}, thermophilic bacteria count – 1.0×10^4 to 5.1×10^4 CFU g^{-1}, yeast and mold count within the limits of 3.8×10^2 to 6.0×10^2 CFU g^{-1}, spores of thermophilic microorganisms, *Enterobacteriaceae* and *E. coli* have not been identified in the samples. Microbiological data suggested that the initial contamination of the raw material was high enough, but no defects and deterioration of the samples were observed. Bacterial spores that can survive heat treatment have not been found.

Treatment of 53 °C temperature for 8 h (Tc +5 h) and vacuum depth of 0.3×10^5 Pa and 0.6×10^5 Pa significantly diminished microbiological contamination in the LTLT treated samples within the 1st shelf life day compared to those of raw meat samples. Samples treated with different vacuum depth of 0.3×10^5 Pa and 0.6×10^5 Pa revealed that total plate count varied within the limits of 1.6×10^2 and 2.0×10^1 CFU g^{-1}, mesophilic bacteria count – 1.1×10^2 and 1.4×10^1 CFU g^{-1} thermophilic bacteria count within the limits of 7.5×10^1 and 4.3×10^1 CFU g^{-1} respectively. Yeast and molds, mesophilic and thermophilic bacteria spores, *Enterobacteriaceae* and *E. coli* have been identified in the LTLT samples.

LTLT samples (treatment of 53 °C temperature for 8 h (Tc +5 h) and vacuum depth of 0.3×10^5 Pa and 0.6×10^5 Pa) were kept for 14 days and analysed for microbiological contamination on the 7th and 14th day of the storage. A minor growth of micro-organisms in the samples stored for 7 days was registered; however

significant microorganisms' increase (with no threat to human safety) in the samples kept for 14 days was identified. Samples treated with different vacuum depth of 0.3×10^5 Pa and 0.6×10^5 Pa revealed that total plate count varied within the limits of 3.2×10^4 and 3.0×10^4 CFU g^{-1}, mesophilic bacterial count – 2.7×10^4 and 1.4×10^4 CFU g^{-1}, thermophilic bacteria count - $1.1x10^4$ and 0.8×10^4 CFU/g respectively. Yeast and molds, mesophilic and thermophilic bacteria spores, Enterobacteriaceae and E. coli have not been identified in the LTLT samples within the 14 days of storage.

Treatment of 53 °C temperature for 20 h (Tc +17 h) and vacuum depth of 0.3×10^5 Pa and 0.6×10^5 Pa significantly diminished (by 10 times) microbiological pollution in LTLT samples compared those of raw meat samples within 7 days of storage. The results revealed that microbiological indicators level was similar to those of 8 h (Tc + 5 h) treatment within the whole storage period (14 days).

Both LTLT treatments for 8 and 20 h duration with different vacuum depth enabled to reduce the marginal pollution of raw meat samples from 2.6×10^6–1.1×10^7 CFU g^{-1} to 2.0×10^1–3.5×10^2 CFU g^{-1} in LTLT samples already at the 1st day of storage, with inactivation of yeast and molds.

Conclusions

Application of low temperature and long time treatment (53 °C, Tc +5 h and Tc + 17 h – holding time, Tc – core temperature equal to the water bath temperature, and vacuum depth of 0.3 and 0.6 -10^5 Pa) revealed that:

- cooking loss increased due to low temperature treatment prolongation from Tc +5 h to Tc + 17 h in LTLT *M.Longissimus dorsi* samples;
- shear force (WB) mean values increased as the vacuum depth increased, significant differences ($p<0.05$) being observed between the mean values obtained in samples treated for 5 h in 0.3 and $0{,}6\times10^5$ Pa vacuum depth where shear force increased by 69% and in samples treated of 0.3 and 0.6×10^5 Pa for 17 h by 23%.

Analyzing the obtained microbiological analysis results it can be concluded that employing LTLT treatment and different depth of the vacuum (0.3×10^5 Pa and 0.6×10^5 Pa) the microbiological safety of the LTLT product was ensured for a maximum period of 14 days, as longer storage times would increase the risk of mesophilic and thermophilic bacteria development.

References

1. Christensen L., Ertbjerg P., Aaslyng M.D., Christensen M. (2011) Effect of prolonged heat treatment 48 °C to 63^0C on toughness, cooking loss and colour of pork. *Meat Science,* Vol. 88, p. 280–285.
2. Christensen L., Gunvig A., Tørngren M.A., Aaslyng M.D., Knøchel S., Christensen M. (2012) Sensory characteristics of meat cooked for prolonged times at low temperature. *Meat Science,* Vol. 90, p. 485–486.
3. Christensen L., Ertbjerg P., Loje H. Van den Berg F.W.J, Christensen M. (2013) Relationship between meat toughness and properties of connective tissue from cows and young bulls heat treated at low temperatures for prolonged times. *Meat Science,* Vol. 93, p. 787–795.
4. Mortensen L. M., Frøst M. B., Skibsted L. H., Risbo J. (2012) Effect of time and temperature on sensory properties in low-temperature long-time sous-vide cooking of beef. *Journal of Culinary Science and Technology,* Vol. 10, p. 75–109.
5. Vaudagna S.R, Sanchez G., Neira M.S., Insani E.M., Picallo A.B., Gallinger1 M.M., Lasta J.A. (2002) Sous vide cooked beef muscles: effects of low temperature–long time (LT–LT) treatments on their quality characteristics and storage stability. *International Journal of Food Science and Technology,* Vol. 37 p. 425–441.

SHORT COMUNICATION

STANDARD METHODS AND CRITERIA TO PREDICT BREAD CEREAL QUALITY – DO THEY STILL MEET THE DEMANDS OF MODERN RAW MATERIALS AND THEIR PROCESSING?

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Abstract

Reliable prediction of the baking behaviour of bread cereals (wheat, rye) is of utmost importance in the respective production, trade and processing. Consequently, there is a great interest in easy to handle, rapid and precise methods. A broad variety of such methods has been established by international standardisation organisations like ICC, AACCI, ISO and CEN, respectively, to assess a wide range of criteria of grain raw material and products thereof. In wheat because of its gluten functionality in baking performance protein quantity and quality assessment criteria play a decisive role. In addition, starch characteristics may be of significance.
In this respect, it has become tradition in practical wheat trade and buying decision making to measure raw protein content (by the Kjeldahl or Dumas method, preferably by NIR), protein quality (as sedimentation value), and Falling number, only. Data received are then put into relation to the bread volume tentatively to be expected. In rye starch and pentosan quantity and quality play the crucial role in baking performance. Thus, the amylogram data gelatinisation maximum and gelatinisation temperature serve as criteria of quality prediction. The presentation will show that progress in wheat and rye breeding on the one hand and the increased diversity in baked goods on the market have made the prediction reliability of traditional methods questionable, at least in specific cases. Furthermore, the technical procedure of performing individual methods will have to be evaluated to avoid misleading results.

Keywords: Wheat, rye, baking quality, standard methods.

Introduction

On different levels of the bread cereal (wheat, rye) production and utilization chain it is of utmost interest to sufficiently predict the tentative processing, i.e. baking quality of the raw material. In early stages of their selection process breeders would like to know if the wheat or rye material they work with will finally fulfil the demands of the processing industry after a ten to 14 years breeding and variety releasing process. Cereal traders and/or millers are permanently facing the challenge to select the right quality material they are aiming at for their specific interest and bakers, finally, need to know the specific processing quality of the milling products they ordered. Therefore, since many decades cereal scientists in close cooperation with practice have elaborated and established a system of methods suitable for mostly rapidly predicting the processing, especially baking performance of a raw material lot of interest and/or a milling product prepared thereof. Basically a baking test would mirror best and most comprehensively the real processing (baking) quality. However, baking tests afford specific and expensive equipment and infrastructure and they are rather time consuming and thus not suitable to be practised under time constriction during harvest and/or primary cereal uptake in mills or trading points.
As a consequence a broad range of so-called indirect methods has been established the results of which are put into relation to a specific dough making and subsequent baking performance thus enabling prediction of the baking result. To facilitate international cereal trade a wide range of methods has been standardised by international standardisation organisations such as the International Association of Cereal Science and Technology (ICC), the American Association of Cereal Chemists International (AACCI), the International Standardisation Organisation (ISO) and the Comité Européen de Normalisation (CEN).
As in wheat baking performance is to a high extent caused by its protein, specifically by its gluten fraction and by the starch fraction, specific (rapid)methods are designed to measure protein (or gluten) quantity and quality, respectively, and starch quality. In rye, the baking quality of which is characterized predominantly by the carbohydrate fraction, i.e. starch and pentosans. Consequently, methods aim at describing this fraction in its dough and gel forming capacity.
For a long period of time it had become practical use in wheat evaluation – at least in the phase of primary uptake of cereal material at harvest – to measure total protein content and sedimentation value as protein (gluten) quality criterion and in addition Hagberg-Perten Falling Number to get information about the starch. This had turned out to be sufficient as the wheat variety material so far on the market showed a good linear relationship between protein characteristics and baking performance mostly taken as volume of the baked good. In specific years with pre-harvest sprouting Falling number was of additional significance.
In rye, more susceptible to pre-harvest sprouting, Falling Number and starch gelatinisation patterns as measured by means of the Brabender amylograph equipment, i.e. starch gelatinisation maximum and temperature at gelatinisation maximum were sufficient to handle rye milling products for bread making.
In most recent years, however, there is – at least in Germany – more and more complaining among wheat and rye breeders, farmers, traders and millers that these traditional methods are no longer sufficient to predict

wheat and rye baking quality. This is specifically conferred to modern German wheat varieties and to a certain extent to rye cultivars it is the intention of the following to describe the given situation in Germany and to consider the question of still existing significance of the established quality assessment system.

Material and Methods

Data for bread wheat *(Triticum aestivum)* and rye *(Secale cereale)* are derived from the official yearly German quality assessments of bread cereals performed by law by Max Rubner-Institute (MRI) - Department of Safety and Quality of Cereals during recent years as well as from processing quality assessments performed in the framework of the releasing process for new varieties.

Analytical methods applied are according to the ICC standardization protocol as follows (ICC 2001): Total protein (ICC Standard No. 105/2), sedimentation value (ICC Standard No. 116/1), falling number (ICC Standard No. 107/1). As baking test the RMT (Rapid Mix Test) with rolls has been chosen being the respective standard procedure in Germany (Arbeitsgemeinschaft Getreideforschung e.V., 1994).

For quality assessment of rye the Brabender Amylograph procedure (ICC Standard No. 126/1) was applied.

Results and Discussion

With respect to baking quality much progress in breeding has been made during the last decades with wheat rather than with rye. Therefore, emphasis is laid in the following on wheat. In Germany – and to a certain extent in other countries too – it has been well established in the wheat business to look at raw protein content as measure of protein quantity. And as there is sufficiently narrow relation protein content enabled anticipating the gluten content of a wheat sample. As a certain measure of protein quality sedimentation value added further information. Starch characteristics, i.e. it's gelatinization capacity and indirectly the sample's status of amylase activity could (and of course, can) be derived from falling number measurements, the significance of these data being of utmost relevance in harvest years with pre-harvest sprouting as a problem.

Taking this into account the "3 pillars" - protein content, sedimentation value, and falling number – were mostly sufficient to evaluate the baking quality of a wheat sample (Fig. 1). This was especially due if the variety or a blend of varieties in a sample were known.

It has not been earlier than 1969 that Prof. Bolling of the former German Federal Research Institute of Cereal Processing established a system of equations (see Fig. 2) enabling the prediction of the baking volume of a roll in the Rapid-Mix-Test (RMT) to be expected knowing total protein content and the sedimentation volume of a wheat sample.

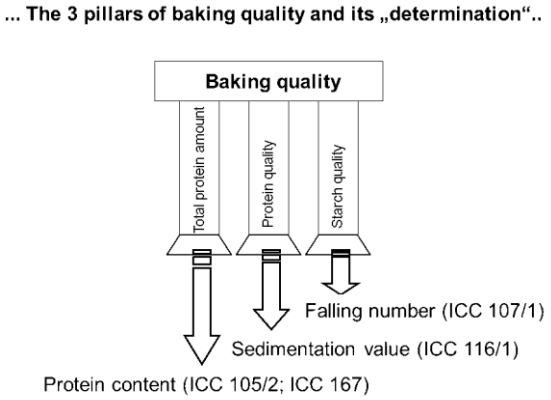

Fig. 1: The "3 pillars" of wheat baking quality measurement

As for a long time until the late nineties of the last century and the early years of the 21st century there mostly was a positive linear relationship between raw protein content and baking quality in the sense of volume forming in yeast leavened baked goods. Therefore it had become use to measure total protein content of a wheat sample, only, and to evaluate from this the baking quality to be expected. This procedure had been supported by the fact that it more and more easy to measure protein content rapidly by near-infrared spectroscopy (NIR). Consequently, it had become tradition internationally to pay wheat quality for its protein content.

RMT-volume estimation by the Bolling (1969) regression equation

Basis : Grain protein content : Sedimentation value

Volume yield = 10 protein + 3 sedimentation value + 420
(valid for E and A wheats)

Volume yield = 17 protein + 3 sedimentation value + 306
(valid for B wheat)

Fig. 2: Regression equations to estimate Rapid-Mix-Test (RMT) volume of wheat sample

More recently, however, more and more wheat cultivars have been released where obviously the narrow relationship between increasing protein content and increasing baking volume does no longer exist. This is to be demonstrated in Figure 3, showing the cultivars Pamier and Kranich representing the "old" relationships. Toras and Tarso, on the contrary, do only have a very weak relationship between protein content and baking performance. Reliable prediction of the baking quality of a wheat sample of these varieties or containing these varieties has become a challenge.

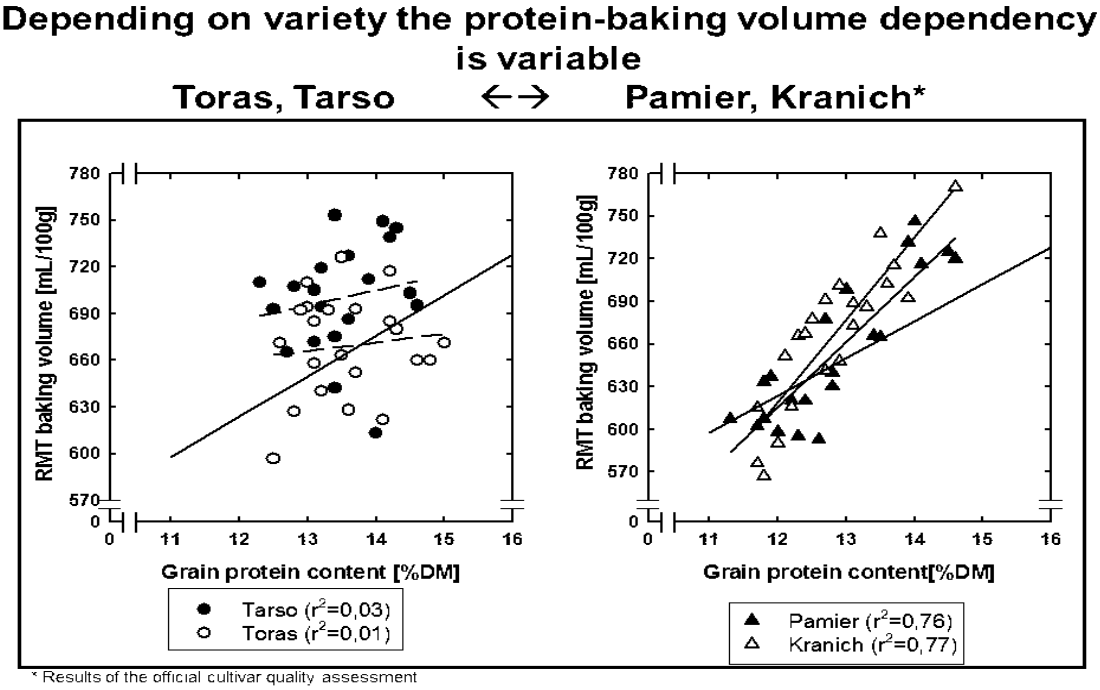

Fig. 3: Protein content/RMT baking volume interactions in selected German wheat varieties

Baking quality evaluation of wheat has become additionally more complicated as there are modern varieties on the market that sore relatively low total protein amounts (and gluten quantities, respectively) but because of the high functionality of the gluten proteins the baking performance of such wheat is much better than expected from classical measurement results.

Also it may be necessary to review the sedimentation value method as it can be shown that results alter in dependence on the milling equipment used to prepare the flours resulting into different protein content of the test sample flours (Seling, unpublished).

Low falling number values are traditionally believed to result in bad baking performance of wheat flour. Samples with low falling number values, however, have been identified during recent harvest years that showed nearly no restriction in baking performance. Own starch related studies with respective samples make assume that starch kernel integrity coinciding with strong gluten functionality are more important than falling number values acquires under conditions of a surplus of boiling water in the FN vessels (Muenzing and Lindhauer, unpublished).

Rye baking quality, finally, is also sometimes questioned as it is believed that flours from modern (German) rye varieties cause problems in baking performance. Specifically, bakers complain about so-called dry baking, i.e. reduced crumb humidity and limited shelf - life of breads. Additionally, crumb cracks are said to occur more often using modern rye varieties then it happened with older varieties.

It must remain open, so far, if the traditional criteria gelatinization and temperature at gelatinization maximum derived from the Brabender amylogram are still sufficient for describing the baking quality of modern varieties. Fact is that modern rye varieties tend to be more pre-harvest sprouting resistant and, thus, they tend to have higher falling numbers.

References

1. Arbeitsgemeinschaft Getreideforschung e.V. (Hrsg.) (1994) Standard-Methoden für Getreide, Mehl und Brot. *Verlag Moritz Schäfer*, 327 p.
2. Bolling H. (1969) Wissenschaftliche Grundlagen für eine Gradierung von Weizen. *Die Mühle + Mischfuttertechnik*, Vol. 106 (46), p. 799–801.
3. ICC - International Association for Cereal Science and Technology (2001) ICC Standards, Vienna.
4. Seling S., Lindhauer M.G. (2011) Impact of granulation and protein content on the sedimentation value of wheat. **In:** *Wheat Science Dynamics: Challenges and Opportunities*. R.N. Chibbar, J. Dexter (eds). Proceedings of International Wheat Quality Conference IV, Saskatoon, Canada, June 2-6, 2009, p. 221-22.

ANALYTIC STUDY OF UNIVERSITY STUDENTS' NUTRITIONAL HABITS AND ATTITUDES AS A PART OF SOCIETY IN TURKEY

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Abstract

The importance of proper nutrition as one of the enhancing nutrition attitudes, knowledge and important aspects of lifestyle were emphasized in the practices of students which has high importance, because recent years and the trend towards healthier diets subsequently will lead to more food-conscious increased.

The aim of this research is to understanding the nutrition knowledge, attitude and food habits of students at higher education programmes. This study has been carried out using subjects of 618 students consisting of 237 female and 381 male students attend from the educational programs present at the Namik Kemal University .A Likert type scale was used to evaluate each of the questions. The collected data was analysed by t-test, one-way ANOVA and Pearson correlation coefficient ($P<0.05$). In addition, this result supported by principal component analysis (PCA), descriptive statistical analysis which students' attitudes on nutritional habits. In research there was significant differences in the knowledge level between students of different departments ($F=3.06$; $p<0.001$). Principal component analysis was used to identify four main dietary patterns, and analysis of variance employed to examine the characteristics associated with them. Factor analysis reduced the 15 independent variables into four factor groups. This paper provides a unique insight into a wide range of nutritional habits among young's' in Turkey (e.g. vegetables, fast foods, milk products etc.-related) and reflects on the responses obtained from policy makers' towards food habits in Turkey.

***Keywords*:** students' nutritional habits, proper nutrition, eating attitudes, factor analysis.

Introduction

Food and nutrition have an essential role in children and young people's achievement at all stages of education. There is evidence that young people's food choices can affect their attendance and behaviour as well as their health. There have been considerable changes in human lifestyle all over the world in recent decades. The importance of proper nutrition as one of the enhancing nutrition attitudes, knowledge and important aspects of lifestyle were emphasized in the practices of students has high importance, because recent years and the trend toward healthier diets have subsequently lead to more food-conscious individuals. The main goal of healthy nutrition plans is to obtain the appropriate and necessary nutrition to remain healthy, to be physically prepared and to lead a healthy life. For this reason to promote the health level of a society, and the attitudes of its people, must be taken into account (Azizi et al., 2011). Given that one of the main goals of universities is to broaden the knowledge of the people in a society, the enhancement of the nutrition attitudes, knowledge and practices of its students is of high importance, as this will subsequently lead to a more food conscious society and more healthy people. Some studies have shown that most students are not familiar with the healthy foods needed for their body in different conditions (Cotugna et al., 2005; O'dea, 2004).

Elhassan et al., (2013) was to assess nutrition knowledge, attitude and practices among Ahfad University students. The other research showed that the majority of students (83.6%) eat three meals during the day regularly and no difference was found between men and women (Ruka et al., 2005). O'dea also exposed that 85% of men and 87% of women, who are overweight, decide to go on a diet to lose weight; also13% of men and 20% of women refuse to eat breakfast. He also reported that students do not have the necessary information and training regarding weight control, nutrition needs and diets (O'dea, Abraham, 2001). According to Gates students with normal weight have a more healthy diet and better points in terms of nutrition knowledge and attitudes compared the others (Gates, De Lucia, 1998).

There is no significant difference between knowledge and attitude between overweight and normal weight persons. In normal weight persons, the body mass index and body fat percentage were related to their attitude to nutrition (Lowry et al., 2000; Mitchell et al., 1999).

The aim of this research was to assess the nutrition knowledge, attitude and food habits of students at higher education programmes.

Materials and Methods

The study is a descriptive cross-sectional, community based study. The study included 618 students consisting of 237 female and 381 male students attend from the educational programs present at the Namik Kemal University are faculties of engineering, arts design and architecture sciences, economics and administrative sciences, theology, medicine, veterinary medicine, agriculture, foreign languages, health sciences, and five vocational schools.

The Questionnaire consisted of five parts and first part is about some personal information about students and their body composition. The second part contains questions that measure students' eating behaviour and its relation to nutrition knowledge. The third part includes some questions about student's attitudes toward nutrition habits. The forth part poses some questions about choosing the foods and the last part collects student's recommendations. In two and third parts the Comprehensive Assessment of Nutrition

Knowledge, Attitudes, and Practices CANKAP (Cunningham, Skinner, et al., 1981) test was used. Likert type scales (Triola, 1992) were used for those responses to the items testing nutritional behaviour. The university students identified their eating behaviour using a five-point scale, ranging from "Never", "Seldom", "Sometimes, "Usually, and "Always".

The collected data was analysed means, standard deviation and percents were calculated for the scores from the nutrition knowledge, attitude and food habits sections. Pearson's correlation coefficient were used to assess the correlation between nutrition knowledge, the attitude and analysis of variations (ANOVA) was used to evaluate nutrition knowledge and the attitude between majors, and an independent t-test was used to compare the nutrition knowledge and attitude between males and females. Statistical results were considered to be significant at $p \leq 0.05$. In addition, this result supported by principal component analysis (PCA), descriptive statistical analysis which students' attitudes on nutritional habits.

Results and Discussion

Table 1 shows the basic demographic characteristics of the students.

Table 1

The basic demographic characteristics

Variable	n	%
Gender		
Male	381	61.7
Female	237	38.3
Age		
18 -21	483	78.1
22-25	123	20.0
Above 26+	12	1.9
Current place of residence		
urban area	534	86.4
rural area	84	13.6
Permanent residence		
dormitory	141	22.5
at home with their friends	405	65.5
with family	51	8.3
with a relative	21	3.4
Family income		
less than 1000 TL	237	38.4
1000-2000 TL	273	44.17
more than 2500TL	108	17.48

Most (61.7%) of the participants were male. The mean age of the students was 20.5 years; nearly 78.1% of the total participants were aged between 18 and 21 years.

The family income of the majority of students was more than 2500 TL (17.48%), then between 1000 and 2000 TL (44.17%), then less than 1000 (38.4%).The mean values and standard deviation for age, height, weight and the body mass index (BMI) of the students are shown in Tables 2and 3.

It is known that age has a central role in the potential to improve especially the dietary habits and lifestyle behaviours of children and young adolescents and improving nutrition knowledge in young people may translate into educating them in good dietary habits. In order to promote healthy nutritional behaviours and prevent overweight and obesity, it is important to target this population with interventions concerning their eating habits and lifestyles (Grosso et al., 2012).

Table 2

Anthropometric characteristics of the students

Gender	Height(cm) (mean±sd)	Weight(kg) (mean±sd)	Age(yr) (mean±sd)
Male	177.9±5.9	70.8±11.5	20.8±2.2
Female	164.46±7.8	57.5±12.3	20.3±1.6
Total	171.15±8,9	66.78±13.2	20.5±2.4

Table 3

Body mass index of the students

Groups	Male		Female	
	Number	(%)	Number	(%)
Thinness (less than 20 kg/m^2)	86	22.6	60	25.3
Normal weight (20-25 kg/m^2)	169	44.3	67	28.3
Overweight (25-30 kg/m^2)	52	13.6	39	16.4
Obese (More than 30 kg/m^2)	25	6.6	19	8.1
No reply	49	12.9	52	21.9
Total	381	100.0	237	100.0

The results concerning nutrition practices also showed that 27.2% of the participants eat fish once or twice a month. In addition, 36.8% of the participants eat breakfast every day and 26.6% of the participants eat fruit every day, while 10% eat fruit only once or twice a week. Also only 10.8% of the participants drink milk every day. Only 20.8% of the participants said yes to the question of "Do you have any nutrition lessons?" Also there was a positive and significant correlation between diet and attitude of both female and male students. 22.8% of students were stayed at dormitory and 65.5% were stayed at home with their friends. No significant relationship was detected between the family income, ($p=0.334$), current place of resident ($p=0.574$). 90.3% of the students leave the meal and 61.6% they retard lunch. About the reason why they ate incomplete 48.3% of student told that they didn't have time. 70.1 % of the students have been educated in nutrition and 53.7% of the students didn't believe that they were healthily nourished. 20.6% of the students

prefer eating their meals at university refectory/dormitory-lodgings.

In this study was identified that the top five ranking foods consumed at high frequencies on daily basis included bread (93.4%), milk (44.9%), fresh vegetables (38%), cheese (35.4%) and egg (17.7%). While the top five foods indicated as never consumed by the students are fish (26.0%), lamb (25.4%) and beef (23.6%). The results from eating behaviour questions indicated that students in this study do not use a food guide to help them choose the food they eat. Consumption of fresh vegetables has been widely believed to promote good health; and protect human body from various diseases particularly those associated with deficiency of vitamins and minerals (Dietary Guidelines For Americans, 2014).

The frequency of consumption of milk and the frequency of consumption of nutriments in the fruit and vegetable group among students was under the necessary level. The most frequently consumed drinks were, successively water, tea and coffee. Results from eating practices showed that a high percentage of the students have unhealthy eating practices with less than or more than recommended dietary guidelines for most food groups therefore major changes in eating habits of this sample are required.

Table 4

Distribution of nutrition knowledge scores according to gender and BMI of university students

Variable	Nutrition knowledge score			
	n	**mean**	**sd**	**p**
Gender				0.001
Female	237	4.6	2.4	
Male	381	4.9	3.1	
BMI Classification				0.002
Thinness	86	4.8	2.3	
Normal weight	169	5.0	2.4	
Overweight	52	4.4	2.0	
Obese	25	4.2	2.1	
No reply	49	-	-	

Findings regarding the association between gender and BMI and nutrition knowledge are reported in Table 4. Means and standard deviations of nutrition knowledge scores are shown to highlight gender and BMI classifications. Higher nutrition knowledge scores were significantly associated with being thinness or normal weight ($p<0.002$).

Regarding the percentage of correct answers according to different constructs, among the group of questions concerning food nutrients the best basic knowledge was found on the item regarding the definition of vitamins (57.8% correct answers) and the worst on the item about balanced diet (37.7% correct answers). Knowledge about food contents was found to be generally acceptable, with about half of the students responding correctly to all items with the exception of the one regarding pasta and bread content (52.7% correct answers).

In this research was found that there was a positive and significant correlation between the knowledge and attitude level of both genders. Also knowledge level of male subjects was higher than that of female subjects. There was also a positive and significant correlation between the nutrition attitude and practice ($r=0.48$; $p<0.00$). It was obtained knowledge level of food engineering, medicine and health science are highest and arts design and architecture sciences and vocational schools are lowest. Using the one-way ANOVA, there was a significant differences in the knowledge level between students of different educational programs ($F=3.06$; $p<0.001$). Findings showed that students' knowledge of nutritional has a statistically significant influence on their nutritional behaviour ($t=2.885$, $p=0.004<0.01$).

Principial Component Analysis (PCA)

Principial Component Analysis (PCA) is recognised as being a powerful tool for pattern recognition, classification, modelling, and other aspects of data evaluation (Csomos et al., 2002; Škrbić, Onjia, 2002; Slavković et al., 2004). It eliminates the redundancy from the data, reducing their dimensionality by revealing several underlying components.

Initially, the aptness of the data for the PCA has been analysed with the KMO (Kaiser-Mayer-Olkin) test. The KMO value was 0.624, and the fact that the KMO value is higher than 0.50 shows that the variants are suitable for PCA and the number is sufficient. In addition to that, a global test has been made, according to the result; it has been shown that the samples drawn are at a level that can represent the population.

The principal components account for the total variance of the original variables (Table 5). The first principal component (PC1) accounts for the maximum of the total variance, the second (PC2) is uncorrelated with the first one and accounts for the maximum of the residual variance, and so on, until the total variance is accounted for. For a practical problem, it is sometimes possible to retain only a few components, accounting for a large percentage of the total variance.

Using the results of PCA, the four most important factors were identified, these being those that explained a high proportion of original variance and had Eigenvalue higher than one. These four factors combined to explain 68.25% of the total variance. The contribution of the variables to the main factors obtained in the PCA of components of nutritional habits and attitudes and variance explained are shown in (Table 5).

The data were analysed using factor analysis (Principal Components with Varimax Rotation). Factor Analysis reduced the 15 independent variables into four factor groups. Each factor group contains independent variables that are highly correlated with each other, but

no correlations exist among the factor groups. These factors can be defined as follows.

Table 5

Principal components loadings for nutritional habits and attitudes

		Factor Score	Percent of Total Variance Explained	Eigen-value
F1 (Dietary quality)	I'm carrying on a family tradition, my eating habits	0.828	27.21	3.077
	I think I have a healthy diet	0.712		
	I see my friends around me that the healthy diet	0.677		
	It is important that the taste of food is very nutritious	0.528		
F2 (Diet-related attitudes)	if you do enough exercise, you can eat whatever you like	0.701	18.64	1.911
	I have enough knowledge about balanced and healthy diet	0.627		
	I eat three meals every day	0.596		
	Food sold in the canteen affecting my eating habits	0.437		
	Sold in the canteen, toast, sandwiches, fast food more than I would prefer to eat school	0.431		
F3 (Nutritional knowledge)	I think, I'm a balanced diet	-0.662	11.28	1.514
	Generally, I prefer to eat the school cafeteria	-0.616		
	Schools are required to sell only healthy foods	0.590		
	In terms of nutritional value of the meals in the cafeteria does not meet my needs	0.504		
F4 (Healthy lifestyle)	I eat enough vegetables for my health	0.888	11.12	1.220
	I do 30 minutes of physical activity at least five days of the week	0.870		

The first factor summarizes four variables related to the *dietary quality* and explains 27.21% of the variance after varimax rotation.

The second factor contains five items that describe students' *diet-related attitudes* and explains 28.64% of the variance.

The third factor contains four statements and describes the *nutritional knowledge*. This factor explains 11.28% of the original variance.

The fourth factor summarizes two variables related to the healthy lifestyle and explains 11.12% of the variance after varimax rotation.

Conclusion

From this study it could be concluded that students should pay more attention to nutrition. Since university student will form the main body of families and professionals in every region and every society and they will represent the future parents (Bano et al., 2013). The time they spend at college is a golden period for learning and can promote nutrition knowledge, the attitude and practices of students. Therefore, an improvement in the learning environment related to nutrition, need to be emphasized on college campuses. Besides, media was the major source of information and not all students were aware of the health hazards of soft drinks and low intake of fruits and vegetables. The significant association between the students' study field and their nutritional knowledge magnifies the role of education. The students' attitude and practices needed improvement, emphasizing the need for further studies and a practical nutrition education programmes.

The main goal of nutrition plans is to obtain the appropriate and necessary nutrition to remain healthy, to be physically prepared and to lead a healthy life. For this reason to promote the health level of a society, the attitudes of its people must be taken into account. Given that one of the main goals of universities is to broaden the knowledge of the people in a society, the enhancement of the nutrition attitudes, knowledge and practices of its students is of high importance, as this will subsequently lead to a more food conscious society and more healthy people.

In order to remain healthy, physically active and enjoy a healthier life style it is necessary to obtain good nutritional knowledge and implement it. Mitchell et al (1999) identified that people with normal weight have a more healthy diet and better points in terms of nutrition knowledge and attitudes compared the others. The knowledge, attitude and practice must be considers in people in order to promote society health. According to Elhassan et al. (2013) one of the main goals of universities is to broaden knowledge of people of the society, so enhancing the nutrition attitudes, knowledge and practice of students have high importance because this subsequently will lead to more food-conscious society and more healthy people especially young adults.

In this research, the concept of healthy nutrition has been planned and conducted in order to determine the manners and the behaviours of the students that receive education at Namik Kemal University, towards nutritional habits. A significant association was found between the study field of students and their nutritional knowledge magnifying the role of education. The factors that affect students' nutrition selection, the evaluation of the concepts of healthy nutrition, personal opinions concerning nutrition levels, reasons of inclining towards healthy nutrition and research of the information obtained in line with the information sources about nutrition constitute the objective of this study. Some researchers have shown that nutrition knowledge was highly and positively related to the behavior toward nutrition (Mahe, 2000; Saegert, Young, 1983; Read et al., 1988).

The study indicated that, university students often miss meals, the most leaving out meal was lunch, the reason therefore was ‘don't have enough time’ and they had an unhealthy nutritional pattern. Activities like conferences with participation on a voluntary basis appeared to be more effective than previous obligatory lectures on the eating habits of the students. Reflection on these findings has led to one possible conclusion that young population should be educated and encouraged to promote healthier diets and lifestyles.

References

1. Azizi M., Aghaee N., Ebrahimi M., Ranjbar K. (2011) Nutrition Knowledge. The Attitude and Practices of College Students, UDC 351.778.2:796.071, Facta Universitatis, Series: *Physical Education and Sport*, Vol. 9, No.3, p. 349–357.
2. Bano R., AlShammari E., Fatima S.B., Al-Shammar N.A. (2013) A comparative study of Knowledge, Attitude, Practice of nutrition and non-nutrition student towards a balanced diet in Hail University, *Journal of Nursing and Health Science (*IOSR-JNHS), Vol. 2, Iss. 3, p. 29–36.
3. Cotugna N. Connie E. Vickery R.D. Sheldon M. (2005) Sports Nutrition for Young Athletes. *Journal Scandinavia Nutrition*, Vol. 21(6), p. 323–328.
4. Csomos E., Heberger K., Simon-Sarkadi L. (2002) Principal component analysis of biogenic amines and polyphenols in Hungarian wines, *Journal of Agricultural and Food Chemistry*, Vol. 50, p. 3768–3774.
5. Cunningham J.L., Skinner J.D., Cagle L.C., Miller S.W., Teets S.T. (1981) Development of CANKAP-a multidime nsional measure of nutritional beliefs. *Journal of Nutrition Education, 13, 109-114.*
6. Dietary Guidelines For Americans, (accessed on 09.04.20 14). Available: *http://www.health.gov/dietaryguidelines/ dga95/9dietgui.htm.*
7. Elhassan M. R., Gamal H. E., Mohammed G. S. S. (2013) Nutrition Knowledge Attitude And Practices Among Students Of Ahfad Unıversıty For Women, *Indian Journal Of Science Results*, Vol. 4(1), p. 25–34.
8. Gates G.E., De Lucıa B.A. (1998) Influences of lifestyle patterns on diet. *Journal American Association of nutrition*, Vol. 989(9), p. 82–87.
9. Grosso G., Mistretta A., Turconi G., Cena H. Roggi C., Galvano F. (2012) Nutrition knowledge and other determinants of food intake and lifestyle habits in children and young adolescents living in a rural area of Sicily, South Italy. *Public Health Nutrition*, p. 1–10.
10. Lowry R., Galuska D.A., Fulton J.E., Wechsler H., Kann L. (2000) Physical activity, food choice, and weight management goals and practices among US college students. *American Journal of Preventive Medicine*, Vol. 18(6), p.18–27.
11. Mahe M. P. (2000) The Correlation between Nutrition Knowledge and Eating Behaviour in an American School: The Role of Ethnicity, *Nutrition and Health*, Vol. 14, p. 89–107.
12. Mıtchell R.D., Ebel A.P., Nathanson H. (1999) Dietary practices and knowledge of and attitudes toward nutrition in normal weight and overweight women. *Journal American Association of Nutrition*, Vol. 99(9), p. 67–72.
13. O'dea A.J. (2004) School-based health education strategies for the improvement of body image and prevention of eating problems. An overview of safe and successful interventions. *Journal of Health Education*, Vol. 105 (1), p. 11–33.
14. O'dea A.J., Abraham S. (2001) Knowledge, beliefs, attitudes, and behaviors related to weight control, eating disorders, and body image in Australian trainee home economics and physical education teachers. *Journal Nutrition Education*, Vol. 33(6), p. 332–340.
15. Read M.H., Harveywebster M., Usinger-Lesquereux J. (1988) Adolescent compliance with dietary guidelines: Health and education implications. *Adolescence*, XXIII, 91.
16. Ruka S., Toyama K.., Amamoto R.L., Shınfuku N. (2005) Nutritional knowledge, food habits and health attitude of Chinese university students–a cross sectional study. *Journal of Nutrition*, Vol. 4(4), p. 1475–1480.
17. Saegert J., Young E.A. (1983) Nutrition knowledge and health food consumption. *Nutrition and Behavior*, Vol. 1, p. 103–113.
18. Škrbić B., Onjia A. (2002) Multivariate analyses of microelement contents in wheat cultivated in Serbia, *Food Control*, Vol. 18, Iss. 4 , May 2007, p. 338–345
19. Slavković L., Škrbić B., Miljević N., Onjia A.(2004) Principal component analysis of trace elements in industrial soils, *Environmental Chemistry Letters* 2, p. 105–108.
20. Triola M.F. (1992) *Elementary Statistics, 5th edn.* Addison Wesley Publishing Company; United States.

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