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VIII FOOD SCIENCE and TECHNOLOGY
Comparative study of three drying methods: freeze, hot air-assisted freeze and infrared-assisted freeze modes

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Abstract. The dehydration tests were conducted at three drying methods to evaluate the drying curves and the energy uptake. Apple (Malus domestica L.) cubes were dried under different processing conditions applying freeze drying (FD), freeze drying assisted by hot air and freeze drying assisted by infrared radiation. Control samples were produced using regular freeze drying without the pre-drying. Hot air combined with freeze drying (HAD-FD) at 60 and 80°C air temperatures was investigated. The infrared-freeze drying (IR-FD) is a relatively new processing method. The Idared apple cubes were dried with 5 kW m⁻² IR power intensity. It was observed that the infrared power level and hot air temperature affected the drying rate and time of freeze drying. The infrared radiation heating had a higher drying rate than hot air during the pre-dehydration. The water activity, colour, firmness and rehydration ratio (RR) of finished products were measured. The dried material produced with IR-FD had desirable colour, higher rehydration rate and lower firmness than dried by HAD-FD ones. The quality of single-stage FD samples was close to IR-FD materials. It was observed that the IR-FD method drastically decreased the energy consumption, compared to FD and HAD-FD drying treatments. The mathematical models such as Henderson-Pabis and third-degree polynomial are used to describe the drying kinetics of food material. It was found that those mathematical models performed adequately in predicting the changes of moisture ratio.

Key words: Combination or hybrid drying, quality assessment, energy uptake, modelling.

INTRODUCTION

Apple is an important material for many food products and apple plantations are cultivated all over the world in many countries. Apple is a high moisture food with moisture content of 80–85% (in w.b.). Unsuitable preservation and storage methods cause losses of fruits which range from 10% to 30% (Togrul, 2005).

The technique of drying is probably the oldest method of food preservation practiced by mankind. Drying of foods is mainly aimed at reducing the moisture to extend the shelf life. The major challenge during dehydration of food is to reduce the energy consumption and the water content of the material to the desired level without substantial loss of colour, appearance, flavour, taste and chemical components. In prepare of functional foods and ready-to-eat foods, freeze-drying or lyophilisation (FD) method is used generally. Freeze drying is a dehydration operation with the sublimation of ice from frozen material. Because of the absence of liquid water and the low temperature (approx. 20°C) used in the operation process, most of deterioration and microbiological reaction are stopped (Lin et al., 2007). Three main steps are involved in
FD process: freezing, sublimation and desorption. In this method shrinkage is eliminated, minimum loss of flavour, aroma, vitamins, and near-perfect preservation results are obtained. Although freeze drying can be applied to manufacture products with complete structural retention, it is an expensive process, due to its long drying time (approx. 20–30h) (Antal et al., 2014). In recent years, freeze drying is combined with various other dehydration methods such as hot air, infrared radiation, microwave and microwave-vacuum.

Foodstuffs with high moisture content can be effectively dried using combination methods, as it provides the synergistic effect. Hybrid drying, like hot air-freeze drying (Xu et al., 2005) and infrared-freeze drying (Wang et al., 2014) have been successfully employed in order to improve the effectiveness of dehydration. Pan et al. (2008) used sequential infrared and freeze drying to produce high-quality dried fruits at reduced cost. The products dried using infrared-freeze drying had better colour, higher shrinkage, higher crispiness but poor rehydration capacity compared to those produced by using single stage freeze drying. According to earlier reports, hot air (HAD) pre-drying can reduce by about half the drying time needed for traditional freeze drying (Kumar et al., 2001). The optimization of IR and HAD application for FD operation is an innovative development that can amplify drying efficiency, shorten drying time and enhance product quality (Chakraborty et al., 2011).

Hot air drying (HAD) is the most commonly employed commercial technique for drying vegetables and fruits, in which heat is transferred from the hot air to the product by convection, and evaporated water is transported to the air also by convection (Lewicki, 1998). However, the major disadvantage associated with hot air dehydrating is the long drying time even at temperatures near 60°C, resulting in the degradation of material quality (Kumar et al., 2005).

Infrared (IR) drying is based on the action of IR wavelength radiation from heat source, which interacts with the internal structure of the sample and thus increases its temperature and favours the evaporation of its moisture (Celma et al., 2008). During drying, the infrared rays penetrate into the wet sample to a certain depth and increase their temperature without heating the surrounding air. Then, the diffusion rate of the water through the material increases and consequently the radiation properties of the samples are changed due to decreasing moisture content, which diffused out of the materials into the air (Laohavanich & Wongpichet, 2008). IR drying is gaining popularity in food processing because of its inherent advantages over hot air drying. IR drying has many advantages including uniform heating, high heat transfer rate, reduced processing time and energy uptake and improved product quality (Sandu, 1986; Vishwanathan et al., 2013).

The objectives of this research were to compare the freeze drying with the combination drying (IR-FD and HAD-FD), for apple, considering drying time, energy consumption, change of physical properties (colour, water activity, rehydration rate and hardness), and additionally to find a model to describe the FD, HAD-FD and IR-FD drying characteristics.

To our knowledge, no work or little detailed information is available the effect of hybrid drying (HAD-FD and IR-FD) on the quality and drying characteristic of apple.
MATERIALS AND METHODS

Raw material
Ripe *Idared* apples (*Malus domestica* L.) were picked from the orchard near Nyíregyháza and stored in a refrigerator (5°C) until use. The apples were cored with a household tool, washed with tap water and cut into cubes with 5 mm thickness. The cause of relatively thin sample: Increases in thickness reduce the penetration of the IR radiation and thereby decreases the drying rate (Kumar et al., 2005).

The samples were divided into nine groups, each group of samples weighed 100 g. The initial mass of apple cubes was measured using a balance (model JKH-500, Jadever Co., Taiwan) with 0.1 g precision. The sliced apple samples were subjected to dryers immediately after cutting to avoid surface enzymatic browning.

Determination of moisture content
Moisture content of the raw and dried apple dices was determined by the gravimetric method (model LP306, LaborMIM, Hungary). At regular time intervals during the drying process, samples were taken out and dried for 8 h at 105°C until constant weight. Weighing was performed on a digital balance (JKH-500, Jadever Co., Taiwan) and then moisture content was calculated. Moisture content was expressed in wet matter (g 100 g⁻¹ fresh matter, %) and in dry matter (kg moisture kg dry matter⁻¹). The initial moisture content of the apple was found to be 84.8% (wet basis: w.b.), 5.578 kg H₂O kg dry matter⁻¹ (dry basis: d.b.). The tests were performed in triplicate.

Drying procedure
The apple slices were dried by different drying methods with the optimal drying technology until final moisture content (5–6%, wet basis: w.b.). The applied drying methods are described under-mentioned. The drying process was continued until no moisture content was recorded (The samples was dried until it reached the equilibrium moisture content, otherwise no change in moisture content). The moisture loss was recorded at 1 min intervals during the drying process in order to determine the drying curves. The experimental data sets from the different drying runs were expressed as moisture ratio (MR) versus drying time (t). All the experiments were repeated thrice and the average of three results for each treatment was used in this paper. The dried products – before quality assessment – was cooled and packed in low-density polyethylene (LDPE) bags that were heat-sealed.

**Convective drying** (HAD) was carried out in a hot-air dryer (model LP306, LaborMIM, Hungary) at 60 and 80°C with an air flow rate of 1 m/s. Air humidity was regulated at ≈20%. The samples (100 g) were spread uniformly, in single layer on the trays of dryer. After 1h, the trays were taken out of the equipment, weighed, and then put back in the dryer. During the drying process, the weight of the apple cubes was recorded to construct a drying curve, and the temperature (material and air), air velocity, air humidity was measured using a Testo 4510 type meter (Testo GmbH, Germany). The mass was measured on an analytical balance (model JKH-500, Jadever Co., Taiwan) with a precision of ± 0.1 g. The apples were dehydrated until they reached the final moisture content (6%, w.b.).
Infrared drying (IR) was conducted by a quartz infrared heater, with nearly 80% efficiency in converting electrical energy to infrared energy was used for effective drying. The chamber wall was formed from aluminized steel, with a length of 15 cm, a breadth of 15 cm, and a height of 25 cm, equipped with a single door opening at the top, which allowed insertion and removal of the sample. In the drying chamber, a pair of quartz glass emitters (220 V, maximum power of per lamp 300 W) was positioned above the sample support. Infrared radiation, with wavelengths expressed in microns, can be accurately measured, controlled, and applied to the product. The wavelength of radiation between 2.4–3.0 µm and the heating intensity was maintained at 5 kW m\(^{-2}\) (Infrared intensity is usually expressed as radiation power per unit area). The quartz glass emitter is located at a distance of 15 cm from the apple surface. The sample tray was supported on a balance (a precision of ± 0.1 g, model Precisa, Precisa Instruments AG, Swiss) to monitor the sample weight change during drying. The samples were spread uniformly in a monolayer on the aluminium tray. A vent was provided at the top of the chamber for the exit of moist air. The experiment was carried out for 60°C drying temperature. The emitter temperature and relative humidity was measured by Testo 4510 type meter (Testo GmbH, Germany) at the top of chamber. However, the relative humidity was not controlled during the laboratory test.

Freeze drying (FD) was performed in a laboratory-scale Armfield FT-33 freeze-dryer (Armfield Ltd., England). In the FD process, the apple dices were spread uniformly in a single layer on a stainless steel tray. The apple samples (100 g) were frozen at -21°C in a freezing/heating chamber and freeze dried to a moisture content of 5–6% (w.b.) at an absolute pressure of 85–90 Pa with a chamber temperature of 20°C and a condenser temperature of -48°C. Thermocouples (four pieces) of freeze drier were inserted into the apple cubes. The weight loss of the samples was followed by a data logger and a RS-232 attached to a PC computer, acquired the data readings from platform cell, which is placed within the sample chamber.

For hybrid or combination drying the apple samples were dried by FD drier by coupling with the HAD and IR devices before the freeze drying step until the final moisture content was between 4.88–6.03% (w/w). The samples after pre-drying procedure (HAD, IR) immediately placed into the FD. The experimental samples dried by HAD at 60°C for 3 h (HAD-FD1), HAD at 80°C for 3 h (HAD-FD2), IR at 60°C for 3 min (IR-FD3), IR at 60°C for 4 min (IR-FD4) and IR at 60°C for 5 min (IR-FD5) then dried by FD were chosen for further quality evaluation. The drying parameters are in agreement with above-mentioned ones (points of 1–3). The Table 1 demonstrates the main details of different drying process.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition of the process</th>
<th>Drying temp., IR intensity</th>
<th>Pre-drying time</th>
</tr>
</thead>
<tbody>
<tr>
<td>FD</td>
<td>single-stage of freeze drying</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HAD-FD1</td>
<td>hot air pre- and freeze finish-drying</td>
<td>60°C</td>
<td>3 h</td>
</tr>
<tr>
<td>HAD-FD2</td>
<td>hot air pre- and freeze finish-drying</td>
<td>80°C</td>
<td>3 h</td>
</tr>
<tr>
<td>IR-FD3</td>
<td>infrared pre- and freeze finish-drying</td>
<td>60°C, 5 kW m(^{-2})</td>
<td>3 min</td>
</tr>
<tr>
<td>IR-FD4</td>
<td>infrared pre- and freeze finish-drying</td>
<td>60°C, 5 kW m(^{-2})</td>
<td>4 min</td>
</tr>
<tr>
<td>IR-FD5</td>
<td>infrared pre- and freeze finish-drying</td>
<td>60°C, 5 kW m(^{-2})</td>
<td>5 min</td>
</tr>
</tbody>
</table>
Mathematical modelling of drying curve

Mathematical modelling of drying is important for optimum management of operating parameters and prediction of performance of the drying system (Jain & Pathare, 2004). There are several empirical approaches for modelling the drying kinetics. Henderson and Pabis (exponential) and third-degree polynomial models were used to fit the drying curves (MR versus drying time) in this study (Table 2). The moisture content of samples is defined by (1):

\[ M_t = \frac{m_t - m_f}{m_f}, \]

where: \( M_t \)– the moisture content at time \( t \) on dry basis, kg H\(_2\)O kg dm\(^{-1}\); \( m_f \)– the weight of material at specific \( t \), kg; \( m_f \)– the dry matter weight of the material, kg.

The dimensionless moisture ratio (MR) was calculated as (2):

\[ MR = \frac{M_t - M_e}{M_0 - M_e}, \]

where: \( M_t \) – the moisture content at time \( t \) on dry basis; \( M_e \) – the equilibrium moisture content, kg H\(_2\)O kg dm\(^{-1}\); \( M_0 \) – the initial moisture content, kg H\(_2\)O kg dm\(^{-1}\).

For infrared drying, Fasina et al. (1998) explained that the \( M_e \) has been numerically set zero, since prolonged exposure of food to IR radiation eventually causes the burning of the samples, which happens only at nearly zero moisture content. Therefore the moisture ratio (MR) was simplified to \( M_t/M_0 \) instead of \((M_t-M_e)/(M_0-M_e)\)–not only at IR. The selected mathematical models are identified in Table 2.

**Table 2.** Mathematical models for modelling drying of apple

<table>
<thead>
<tr>
<th>Model designation</th>
<th>Model equation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Henderson and Pabis</td>
<td>( MR = a \cdot e^{-k_t} )</td>
<td>Doymaz, 2012</td>
</tr>
<tr>
<td>Third-degree polynomial</td>
<td>( MR = a \cdot t^3 + b \cdot t^2 + c \cdot t + d )</td>
<td>Antal et al., 2014</td>
</tr>
</tbody>
</table>

\( MR \)– the dimensionless moisture ratio; \( a, b, c, d \)–the drying coefficients; \( k \)–the drying constant; \( t \)– the drying time (min, h).

The coefficient of determination \( (R^2) \) and root mean square error \( (RMSE) \) were calculated to evaluate the fitting of two models to experimental data. The higher values of the \( R^2 \) and the lower values of the RMSE were chosen for goodness of fit. These statistical parameters can be calculated as (3, 4):

\[ R^2 = \frac{\text{residual sum of squares}}{\text{corrected total sum of squares}}, \]

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\[
RMSE = \sqrt{\frac{1}{N} \cdot \sum_{i=1}^{N} (MR_{\text{exp},i} - MR_{\text{pre},i})^2},
\]

where: \(N\) – the number of observations; \(exp\) – the experimental data; \(pre\) – the predicted data; \(MR\) – moisture ratio.

**Measuring of energy uptake of driers**

Energy used in the drying and heating process is important for production processes in the industrial and household sectors. However, the price of energy is extremely expensive; therefore, there are a strong incentive to invent processes that will use energy efficiently. Currently, widely used drying processes are complicated and inefficient; moreover, it is generally damaging to the environment. What is needed is a simplified, lower-cost approach to this process one that will be replicable in a range of situations (Jindarat et al., 2011). The total energy consumption (\(E\), kWh) during FD, HAD-FD and IR-FD was measured by an energy-cost-checker (model EKM 265, Conrad Electronic GmbH, Germany). Analysis was performed in triplicate.

**Colour measurement**

The colour of apple cubes was measured just before and immediately after drying treatment using a ColorLite sph900 colorimeter (ColorLite GmbH, Germany). The colorimeter (illuminant D65, 10° observer angle) was calibrated against a standard ceramic white tile. For each drying experiment the colour measurement was performed on ten dried samples and the colour values were compared with those of fresh samples (control). The powder obtained by grinding the dried material in a domestic mixer was used for colour estimation. The spectrophotometer supplied with special adapter. MA38 adapter converts the scanning spot from 3.5 to 38 mm. This device can be used to measure apple powders (the samples were examined from different points). All experiments were performed in triplicate and the average values were reported.

An important factor characterizing the variation of colour in the test sample is total colour difference. The total colour change (\(\Delta E\)) was evaluated as (5):

\[
\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2},
\]

where: \(L^*\) – the degree of lightness (100) and darkness (0); \(a^*\) – the degree of redness (+) and greenness (–); \(b^*\) – the degree of yellowness (+) and blueness (–). Subscript ‘zero’ refers to the colour reading of fresh apple cubes as the control. A larger \(\Delta E\) value denotes greater colour change from the control (fresh) sample.

Both chroma (\(C\)) and hue (\(h\)) are derived from \(a^*\) and \(b^*\) using the following equations (6, 7):

\[
C = \sqrt{a^2 + b^2}
\]

\[
h = \arctan\left(\frac{b}{a}\right)
\]
The chroma describes the vividness or dullness of a colour. The hue angle (h) an indicator of browning colour, expresses the colour nuance and values are defined as follows: red-purple: 0°, yellow: 90°, bluish-green: 180°, and blue: 270°.

**Determination of water activity (a\textsubscript{w})**

The water activity (a\textsubscript{w}) shows how tightly the water is bound in the food material. Low water activity foods are those with water activity levels lower than 0.8. Therefore, the targeted a\textsubscript{w} of dried products was 0.6, the general level limits for the growth of yeast, molds and bacteria (Shi et al., 2008). Approximately 3 g of chopped dried apple samples were placed in the sample holder of a Novasina Labmaster (model CH-8853, Novasina AG, Switzerland) a\textsubscript{w}-meter. The temperature and duration for testing were at 25°C and 30 min. The tests were replicated three times.

**Texture (hardness) test**

Texture is considered one of the most important criteria concerning eating quality of dried apple cubes (Deng & Zhao, 2008). The texture characteristics of the fresh and dehydrated apple were measured using a CT3-4500 (Brookfield Engineering Laboratories, Middleboro, USA) texture analyser fitted with a spherical probe. Compression test was carried out to generate a plot of force (N) vs. time (s). This plot was used to determine the value of hardness. The parameters that have been used were the following: 4.5 kg force load cell, 2 mm s\textsuperscript{-1} test speed, 20 mm travel distance and 4 mm diameter of cylindrical probe. The maximum depth of penetration was 3 mm and trigger force was 10 g. A 115 mm diameter plate (rotary base table) was used as a base while compressing the apple samples. The samples were kept in a room temperature at 23°C until analysis. The penetrometer measurements are reported in Newtons (N). Ten samples were tested and the average values were reported.

**Rehydration capacity process**

The rehydration characteristics of the dried material are always used as an index of structural quality, and it largely depends on the dehydration conditions employed (Vishwanathan et al., 2010). The measurement of the water rehydration ratio was based on the following procedure. 100 ml of distilled water was brought to a temperature of 22°C in a constant temperature water bath. Then a precisely weighed 0.5 g sample of the dried material was placed in a plastic vessel and immersed for 30 and 60 min. Afterwards, the samples were taken out (when the time reached 30 and 60 min) and blotted with tissue paper to eliminate excess water on the surface. The weights of dried and rehydrated specimens were measured with an electronic digital balance (model JKH-500, Jadever, Taiwan) having a sensitivity of 0.1 g. The RR values were determined in triplicate. Rehydration ratio (RR) of dehydrated samples was estimated using the equation given below (8):

\[
RR = \frac{W_r}{W_d},
\]

where: \(W_r\) – the drained weight of the rehydrated sample, g; \(W_d\) – the weight of the dry sample used for rehydration, g.
Statistical analysis
Data analyses were determined using the PASW Statistics 18 software (IBM Corp.,
USA), and analyses of variance were conducted by ANOVA procedure, Duncan test.
Mean values were considered significantly different when $P < 0.05$. The parameters of
model were calculated using Table Curve 2D Windows v. 2.03 (Jandel Scientific, San
Rafael, CA)

RESULTS AND DISCUSSION

Drying kinetics of FD, HAD-FD and IR-FD drying
The time required for drying the apple and the final moisture content of sample
under different dehydration modes is presented in Table 3. The reduction in drying time
was between 45.5% and 27.3% with hybrid drying (IR-FD and HAD-FD), respectively
as compared to freeze drying (FD).

The Fig. 1 shows that the traditional FD process required the longest drying time
(22 h). This is because FD, under vacuum conditions, supplies the sublimation heat by
conduction. The rate of heat transfer is slow and thus drying takes a long time (Duan et
al., 2012).

The required HAD-FD1-2 and IR-FD3-5 drying times for obtaining the final
products were 16, 14, 14, 12 hours, respectively. The infrared-assisted freeze drying (IR-
FD4-5) reduced the drying time considerably over hot air-assisted freeze drying (HAD-
FD1-2) in both the cases. The results showed the synergistic effect of IR-FD drying. The
reduction in drying time could lead to consuming lesser energy for processing.

Table 3. Effect of drying methods on moisture content, drying time of dehydrated apple

<table>
<thead>
<tr>
<th>Drying (Symbol)</th>
<th>Final moisture cont.</th>
<th>Total drying time</th>
<th>Reduction in FD time</th>
</tr>
</thead>
<tbody>
<tr>
<td>FD</td>
<td>5.78</td>
<td>0.151</td>
<td>22$^{d}$</td>
</tr>
<tr>
<td>HAD-FD1</td>
<td>6.03</td>
<td>0.180</td>
<td>16$^{c}$</td>
</tr>
<tr>
<td>HAD-FD2</td>
<td>5.98</td>
<td>0.167</td>
<td>14$^{b}$</td>
</tr>
<tr>
<td>IR-FD3</td>
<td>4.88</td>
<td>0.109</td>
<td>14$^{b}$</td>
</tr>
<tr>
<td>IR-FD4</td>
<td>5.11</td>
<td>0.130</td>
<td>12$^{a}$</td>
</tr>
<tr>
<td>IR-FD5</td>
<td>5.07</td>
<td>0.126</td>
<td>12$^{a}$</td>
</tr>
</tbody>
</table>

Means with different letters in the same column were significantly different at the level $P < 0.05$.

The apple cubes of initial moisture content of 84.8% (w.b.) was dried to the final
moisture content of 4.88–6.03% (w.b.) until no further changes in their mass were
observed. The final moisture content of the IR-FD3 treated apple was lower than the
apple dried with other modes.

Two drying model have been used to describe drying kinetics. The model constants
and coefficients of this models used for moisture ratio change with time are presented in
Table 4. An increase in drying temperature resulted in higher values of $k$ in cases of
HAD, as it largely depends on the hot air temperature.

The acceptability of the model is based on a value for the coefficient of
determination ($R^2$) which should be close to one, and low values for the root mean square
error (RMSE). The values of $R^2$ and RMSE of the third-degree polynomial model are
between 0.9923–0.9996 and 0.017204–0.005328, respectively. Similarly, the values $R^2$
and RMSE of the Henderson-Pabis model are between 0.9550–0.9864 and 0.096721–0.026763, respectively.

Consequently, it can be stated that the Henderson-Pabis and polynomial models give an adequate description of the drying characteristic. The high values of $R^2 (> 0.95)$ and low parameters of RMSE (< 0.1) indicated that the calculated results were in good agreement with the experimental data. Therefore, these models can be proposed for predicting changes in moisture ratio with time.

The changes in the moisture ratio (MR) with time during HAD, IR, HAD-FD and IR-FD of apple cubes are given in Figs 1–2. In addition, Figs 1–2 shows the variation of the MR calculated with the selected models, with change points (This point shows where joined the various drying methods in succession). The change points were placed in drying curve before reach to inflexion point of curve and the falling rate period. The dimensionless moisture contents of the products at change points for HAD-FD1-2 and IR-FD3-5 were 1.952, 1.394 and 3.904, 2.510, 1.952, respectively. In Figs. 1–2, it was observed that when the change point is higher, the drying time of HAD-FD and IR-FD increased significantly.

### Table 4. Curve fitting criteria for drying models

<table>
<thead>
<tr>
<th>Drying method</th>
<th>Model parameters</th>
<th>Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>FD</td>
<td>$k$ 0.000186, $a$ -0.00607, $b$ -0.00115, $c$ 1.015071</td>
<td>$R^2$ 0.9996, RMSE 0.005328</td>
</tr>
<tr>
<td>HAD1</td>
<td>$k$ 0.483, $a$ 2.0407, $b$ –, $c$ –, $d$ –</td>
<td>$R^2$ 0.9864, RMSE 0.026763</td>
</tr>
<tr>
<td>HAD2</td>
<td>$k$ 0.634, $a$ 2.4694, $b$ –, $c$ –, $d$ –</td>
<td>$R^2$ 0.9762, RMSE 0.064325</td>
</tr>
<tr>
<td>HAD-FD1</td>
<td>$k$ 0.000104, $a$ -0.00293, $b$ -0.00133, $d$ 0.383225</td>
<td>$R^2$ 0.9991, RMSE 0.007651</td>
</tr>
<tr>
<td>HAD-FD2</td>
<td>$k$ 0.000041, $a$ 0.000745, $b$ -0.04705, $d$ 0.427263</td>
<td>$R^2$ 0.9923, RMSE 0.017204</td>
</tr>
<tr>
<td>IR</td>
<td>$k$ 0.559, $a$ 2.3757, $b$ –, $c$ –, $d$ –</td>
<td>$R^2$ 0.9550, RMSE 0.096721</td>
</tr>
<tr>
<td>IR-FD3</td>
<td>$k$ 0.000073, $a$ -0.00286, $b$ -0.00077, $d$ 0.704602</td>
<td>$R^2$ 0.9995, RMSE 0.005991</td>
</tr>
<tr>
<td>IR-FD4</td>
<td>$k$ 0.0000938, $a$ -0.00355, $b$ 0.014072, $d$ 0.455318</td>
<td>$R^2$ 0.9988, RMSE 0.009663</td>
</tr>
<tr>
<td>IR-FD5</td>
<td>$k$ 0.000020, $a$ -0.00045, $b$ -0.01713, $d$ 0.417317</td>
<td>$R^2$ 0.9980, RMSE 0.010974</td>
</tr>
</tbody>
</table>

The drying curve begins with a warm-up period (at IR and HAD), where the material is heated. In freezing period (at FD) the material is cooled. As the sample warm up (freezing – 0.5 h after warm up at FD), the drying rate increases to a peak drying rate that is maintained for a period of time known as the constant drying rate period. Eventually, the moisture content of the material drops to a level known as critical moisture content, where the high rate of evaporation cannot be maintained. This is the beginning of the falling drying rate period (Haghi, 2001). Constant drying rate period was not detected or very brief stage in IR drying curve. This could be because of the quick drying on the surface of sample at high temperature (Pan et al., 2008).

It can be observed that the moisture ratio decreases with drying time. The effect of temperature on drying is significant in case of hot air drying (HAD). By increasing the temperature from 60°C to 80°C, drying time is decreased 2 hours. However, very high air temperature (higher than 80°C) could lead to steady of the product resulting in its weak quality (Kerekes & Antal, 2006). As seen from Fig. 1, MR of HAD decreased exponentially with time, which shows a typical drying trend.
Hybrid drying had a higher average rate of mass transfer, which is resulted in a shorter drying time over the FD drying. The free water (large amount of moisture) is removed quickly during beginning of IR and HAD process, therefore accelerated the drying rate of FD.

IR drying had much higher drying rate compared with the HAD drying under same drying temperature (60°C). The HAD is a slow process relying on heat conduction from outer surface towards the interior. The rapid diffusion of moisture and direct heat transfer to the material due to infrared drying (IR) resulted in a faster drying process. Since quartz glass emitter heating provides mid-infrared radiation which means high penetration depth, radiation was accumulated in the material (inner layer). According to Nowak & Lewicki (2004), the drying kinetics of apple with infrared energy was dependent on distance between emitters and surface of sample. A decrease in IR-FD processing time by nearly 14.3% was observed when IR drying time was increased from 3 to 4–5 min. The MR of IR decreased exponentially with drying time (Tirawanichakul et al., 2008). Wang et al. (2014) reported that mid-infrared-assisted freeze dried mushroom had lower energy uptake compared to FD product. It is observed that electricity consumption of IR-FD4 and IR-FD5 are almost equal. This is due to same drying time at FD finishing-drying (12 h), which is increased additionally by 4 and 5 min treatment time (at IR pre-drying).

Figure 1. Variation of experimental and predicted moisture ratio with drying time at HAD-FD.

Lin et al. (2007) stated that application of far infrared (wavelength range up to 4 µm) in freeze drying of yam slices could reduce drying time by 25%. Similarly, it can be seen that IR heating was positive effect on moisture loss in the infrared-assisted freeze drying (Fig. 2).
Electricity energy consumption of drying processes

The energy uptake for drying was estimated based on the power input. The results are given in Fig. 3. The energy consumption values for IR-FD4-5 drying mode were slightly lower (6.52 and 6.53 kWh) as compared to HAD-FD2 drying (6.7 kWh) in apple. The IR-FD3-5 and HAD-FD1-2 hybrid drying also gave significantly lower energy uptake values (7.78, 6.7 and 7.58, 6.52, 6.53 kWh) than FD drying (11.88 kWh). This might be due primarily to the higher drying rate and lower energy uptake of IR. This is because infrared waves can penetrate into the interior of the apple, where it is converted to thermal energy, providing a rapid heating mechanism.

The energy consumption obtained in the drying process using FD was almost two fold higher than IR-FD4-5 and HAD-FD2. This trend was also observed by other researchers (Xu et al., 2005). In addition, the change points in drying curve decreases, as well as the consume energy decreases significantly, except of IR-FD5.

Figure 3. Energy consumption during FD, HAD-FD and IR-FD of Idared apple. Means with different letters indicate a significant difference \( (P < 0.05) \) in a column.
Evaluation of quality

Table 5 illustrates the colour changes of Idared apple samples undergoing various drying methods. The colour values measured using colour measurement system (Hunter Lab, USA) as total colour change ($\Delta E$) indicated less variation with infrared-assisted freeze dried (IR-FD) samples compared to FD. Zhu & Pan (2009) stated that the surface colour did not change very significantly during short processing time. In addition, in case of relatively high radiation intensity treatment (5 kW m$^{-2}$) occurred unacceptable colour change after 6 min drying time.

The HAD pre-dried product had a greater colour change ($\Delta E$) than IR pre-dried apple. Compared to fresh apple cubes, the $\Delta E$ in the FD samples were increased by 5.01. For the examined fresh apple, the parameter $a^*$ is negative indicating the green colour of the apple samples. It was found that lightness (L) of HAD-FD apple decreased and $\Delta E$ of HAD-FD apple increased significantly with increasing hot air temperature (from 60°C to 80°C), while redness (a) increased with increasing hot air temperature due to browning reaction occurring during dehydration process. The low L parameter indicated that HAD pre-dried product colour shift towards the darker region.

As shown in Table 5, the FD and IR-FD dried apple gives slightly higher values of lightness (L), redness (a) and yellowness (b). The values of $L^*$ parameter of the FD and IR-FD dried apple cubes increases if compared with those measured on fresh sample, thus the luminance of the treated apple is improved by FD and IR-FD drying. The freezing rate has a marked effect in the lightness of the freeze dried samples: frozen apple slices maintained a whiter colour (Ceballos et al., 2012). Similarly to our results, Boudhrioua et al. (2009) established that value of $L^*$ parameter of the IR dried olive leaves increases compared to lightness of fresh olive leaves samples. According to Pan et al. (2008), the IR pre-drying resulted in significantly higher values of lightness (L) and yellowness (b) of banana slices than the fresh and FD samples. The hybrid drying induces deterioration of the greenness parameters (a). In fact, $a^*$ colour parameter become positive. It was found that lightness (L) of IR-FD apple decreased and redness (a) and yellowness (b) of IR-FD apple increased with decreasing the change point in the drying curve.

<table>
<thead>
<tr>
<th>Drying method (Symbol)</th>
<th>Colour parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
</tr>
<tr>
<td>Raw mat.</td>
<td>75.92</td>
</tr>
<tr>
<td>FD</td>
<td>79.63</td>
</tr>
<tr>
<td>HAD-FD1</td>
<td>71.56</td>
</tr>
<tr>
<td>HAD-FD2</td>
<td>69.88</td>
</tr>
<tr>
<td>IR-FD3</td>
<td>80.75</td>
</tr>
<tr>
<td>IR-FD4</td>
<td>79.22</td>
</tr>
<tr>
<td>IR-FD5</td>
<td>78.94</td>
</tr>
</tbody>
</table>

Means with different letters in the same column were significantly different at the level $P < 0.05$.

The FD samples had higher hue angle (h) values than HAD-FD samples, but lower than IR-FD products, except of IR-FD5. The hue angle of fresh apple is yellow-green colour (hue of 95.24°). The hue angle value of FD and IR-FD product remained range of 87.73°–83.4°, which is yellow colour. The elapsed time increased at IR-FD (change point), the hue angles were decreased from 87.73° to 83.4°. This meant that there was
decreased in yellow colour when the change point was varied from MR = 0.7 to 0.35. Due to heat damage of HAD pre-dried sample, the hue angle value changed drastically from $95.24^\circ$ to $72.6^\circ$–$68.7^\circ$ (orange colour). The hue values of HAD-FD decreased when the air temperature changed from 60°C to 80°C. The chroma (C) of all dried samples was found in range of dullness (11.08–22.37). The HAD-FD1-2 products had significantly lower chroma (C) values than the others.

The water activity of dried apple cubes in all cases is below 0.6, hence the samples can be deemed to be safe from common microbial damage. The dried apple cubes retained low water activity value, range from 0.180 to 0.249 (Table 6). Our results reveal that IR-FD drying process could give steady $a_w$ values for long term storage.

The hardness values for apple dices dried by combination drying and FD methods are shown in Table 7. From Table 7, the FD process was not significant effect to hardness of fresh apple cubes. The textural superiority of the apple samples dried with FD was observed when compared to the textures of the apple dried by combination dried.

### Table 6. Water activity ($a_w$) of Idared apple cubes

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Fresh</th>
<th>FD</th>
<th>HAD-FD1</th>
<th>HAD-FD2</th>
<th>IR-FD3</th>
<th>IR-FD4</th>
<th>IR-FD5</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_w$ (-)</td>
<td>0.961&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.186&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.249&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.220&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.180&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.186&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.181&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values in the same line not sharing the same superscript are significantly different ($P < 0.05$).

This phenomenon due to fine-pored structure and smooth cell walls of FD dried samples (Rother et al., 2011). As a result, the increase of air temperature at HAD-FD resulted significant increasing of firmness value (from 19.67 to 22.33). In the case of HAD pre-drying the relatively high air temperature leads to solid surface, collapsed cellular tissues, changes in cell size and cell size distribution of sample (Lewicki & Jukubczyk, 2004; Shih et al., 2008). It is observed that firmness value in samples dried by IR-FD increased significantly as compared to FD method. On the whole, for the surface hardness values in dried samples, FD and IR-FD methods are significantly better than the HAD-FD method, except of IR-FD4.

### Table 7. Texture of Idared dried apples associated with different drying method

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Fresh</th>
<th>FD</th>
<th>HAD-FD1</th>
<th>HAD-FD2</th>
<th>IR-FD3</th>
<th>IR-FD4</th>
<th>IR-FD5</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F$ (N)</td>
<td>4.70&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.67&lt;sup&gt;e&lt;/sup&gt;</td>
<td>22.33&lt;sup&gt;g&lt;/sup&gt;</td>
<td>14.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.81&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>17.51&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values in the same line not sharing the same superscript are significantly different ($P < 0.05$).

The rehydration ratio of dried Idared apple dices dehydrated by two combinations and FD is presented in Fig. 4. The water uptake of dried apple cubes is dependent on the extent of the structural failure to the apple samples during drying. The higher rehydration ratios (RR) with IR-FD3-5 (30 min: 24.5%, 28.6%, 28.6%; 60 min: 18.1%, 21.3%, 30.4%) dried apple indicated an improved product structure as compared to FD dried samples.

The rapid heating with IR and quicker diffusion of water vapour within the sample might be facilitating the material to retain its porous structure, thereby increasing its ability to absorb higher amount of water during rehydration (Vishwanathan et al., 2010).
As revealed in the graph, the rehydration ratio of HAD pre-dried sample was a significantly lower than FD and IR pre-dried ones. The long period of HAD drying and the high temperature may contribute to a decrease in water uptake. Similarly to our result, Shih et al. (2008) stated that rehydration ratio of HAD-FD product was significantly lower than samples dried by IR-FD. According to Sharma et al. (2005), the onion slices dried under IR-HAD conditions had better rehydration ratio as compared to conventionally dried sample.

In addition, when the soaking time was increased from 30 min to 60 min, the RR of samples was increased slightly. It was observed, when change point of IR and FD drying modes is decreased (from MR = 0.7 to 0.35), the values of rehydration ratio increased slightly.

It can be seen from above results that under IR-assisted drying have a pronounced influence on RR of apple cubes. This conclusion is in accord with that of other authors (Lin et al., 2007).

CONCLUSIONS

Apple cubes drying using freeze drying (FD), hot air-assisted freeze drying (HAD-FD) and infrared-assisted freeze drying (IR-FD) were studied. The effect of drying processes on the energy consumption, colour, water activity, firmness and rehydration ratio was examined.

When processed the samples under hybrid drying (HAD-FD and IR-FD) resulted a higher drying rate. A decrease in processing time for all changes points in comparison to single-stage FD drying was observed. The HAD-FD and IR-FD drying reduced the processing time dramatically (27.3–45.5%) and consumed significantly less electricity energy (34.5–45.1%) compared to FD. Drying with application of IR is much faster than HAD and IR pre-treated product had significantly better quality (colour, water activity, hardness and rehydration ratio).

Based our experimental study, the IR-FD3-5 methods are better when drying time, energy consumption, rehydration ratio were compared to FD. The IR pre-drying produced significantly firmer texture product compared to FD drying. The FD drying is demonstrated to preserve the colour of Idared apple samples. However, the total colour changes are significantly affected by hybrid drying methods compared to FD. Between
the total colour difference of FD and IR pre-dried products was little difference. Increasing the temperature from 60°C to 80°C, showed reduction in drying time caused significant negative effect on the quality of HAD-FD products.

The applied mathematical models – Henderson-Pabis and third-degree polynomial – performed well for describing the drying behaviour of the process on the basis of statistical parameters such as coefficient of determination and root mean square error.

The drying process combining infrared (treatment time: 5 min) and lyophilisation provides a potential alternative to the freeze drying of apple. Further research is required to determine the adequate change point in drying curve and chemical component of final product.

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Effects of dairy cow diet supplementation with carrots on milk composition, concentration of cow blood serum carotenes, and butter oil fat-soluble antioxidative substances

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Abstract. Fat-soluble constituents of milk – \(\beta\)-carotene and \(\alpha\)-tocopherol – are essential for quality and nutritional value of milk and dairy products. Provision of fat-soluble antioxidants and vitamins such as carotenoids and vitamin E necessary for cow organism and milk synthesis depends on their concentration in fodder. The aim of this study was to estimate the effect of cow feed supplementation by carrots on the total carotene concentration in cow blood serum, on fat, protein, lactose concentration in milk, and milk yield, as well as to investigate the effects on \(\beta\)-carotene and \(\alpha\)-tocopherol concentration in butter oil and intensity of its yellow colour. A total 20 cows of Latvian brown (\(n = 8\)) and Danish red (\(n = 12\)) breed were divided into control (CG) and experimental group (EG). In the EG, cow feed was supplemented with seven kg of carrots per cow per day for six weeks at the end of the indoor period (March–May). Milk samples from indoor period (\(n = 100\)) and grazing (\(n = 20\)) were used for butter oil extraction.

The carotene concentration observed in blood of animals before the experiment was insufficient taking into account that the recommended \(\beta\)-carotene concentration in serum is above 3.0 mg l\(^{-1}\) level. During indoor period the increase in carotene concentration in blood of cows was significant in both groups (\(P < 0.05\)) but in EG it was more explicit showing the positive effect of carrot supplementation. Carrot supplementation did not change milk fat, protein, lactose concentration, and yield (\(P > 0.05\)). At the same time it contributed in more stable \(\beta\)-carotene, as well as 30% higher \(\alpha\)-tocopherol concentration and more intense yellow colour of butter oil samples during the indoor period of the experiment (\(P < 0.05\)).

Key words: dairy cow, milk, butter oil, carrot, fat-soluble antioxidants.
INTRODUCTION

The fat fraction of milk is an effective delivery system for fat-soluble vitamins and antioxidative substances in human food. Their content influences not only nutritional value, but also the complex interplay of pro- and antioxidants in milk and dairy products that is important for quality maintenance (Baldi & Pinotti, 2008). However, it has been noted that sensorial properties and oxidative stability during indoor period, especially winter and spring, are declining (Jensen, 2002; Schreiner & Windisch, 2006). Provision of fat-soluble antioxidants and vitamins such as carotenoids and vitamin E necessary for cow organism and milk synthesis depends on their concentration in fodder. Carotenoid concentrations in feeds are highly variable and decrease during the feed storage (National Research Council, 2001; Nozière et al., 2006a; Calderón et al., 2007). Also higher amounts of certain types of feed, especially maize silage or feed based on grains and concentrates increase the risk of insufficient provision of carotenoids (Nozière et al., 2006a; Georges, 2009). The concentration of antioxidative substances in milk and butter oil is also affected by cow health condition and welfare. Because of the current intensification of milk production, problems caused by the oxidative stress of animals are increasing. Mastitis is a major source of economic loss on dairy farms (Meglia, 2004; Beecher et al., 2013). It is known that fat-soluble vitamins, E, A, and carotene are crucial to increase the resistance of the cow to mastitis (Chew, 1995; Meglia, 2004). Various studies indicate that carotenoids may have their own independent role in fulfilling specific animal health functions in order to maintain udder and reproductive health (Arechiga et al., 1998; de Ondarza et al., 2009; Kaewlamun et al., 2011). Many bioactives belonging to carotenoid class neutralise singlet oxygen, other reactive oxygen species, and inhibit free radical and light-initiated oxidative reactions (Pokorny & Parkanyiova, 2005). β-carotene, independent of its provitamin A function, as an antioxidant hinders superoxide formation within the phagocyte (Sordillo et al., 1997) and can enhance the bactericidal ability of neutrophils as reviewed by Chew, 1995. Yet there are not many studies in the world about usage of natural sources of carotene and even more – in practice emphasis usually is put only on the necessity to avoid from the deficit of vitamins A and E. Administration of the natural vs. synthetic form of carotene and other bioactives has potential of better bioavailability and enhanced transfer to milk (Meglia, 2004; Baldi et al., 2008; Politis, 2012). One of the richest sources of carotene is carrot roots historically used as indoor season feed for dairy cattle to enhance yellow colouring in butter and other dairy products. However, because of their varying quality during the spring months and labour-consuming preparation process, carrots are usually replaced by silage. Nevertheless, the economic benefits of carrot feeding may be indirect. Carrot roots are used to provide energy for livestock; although low in protein they enhance forage intake levels. Also owing to soluble sugars and other components contained in carrots, digestive processes, as well as milk secretion can be improved (Fuller et al., 2004; de Ondarza et al., 2009). The aim of the study was to estimate the effect of cow feed supplementation by carrot roots on the total carotene concentration in cow blood serum, on fat, protein, lactose concentration in milk, and milk yield. As well our aim was to investigate the effects on β-carotene and α-tocopherol concentration in butter oil and intensity of its yellow colour.
MATERIAL AND METHODS

Animals and experimental design

The trial was performed in a low-input conventional dairy farm in Latvia specializing on the red cattle breeds – Latvian Brown and Danish Red – that represent the typical herd of Latvia. A total of twenty dairy cows were used in the study. Two cow groups – one experimental and one control group – with 10 cows in each of them were formed taking into account following factors: 1) cow breed, 2) parity (1–6 times), 3) stage of lactation (3–6 months), 4) milk fat and protein concentration, as well as 5) productivity. Cow distribution by breeds – Latvian Brown (n = 4) and Danish Red (n = 6) – in each group was similar. During indoor period cows were housed in a tie-stall barn and fed individually. The feeding and milking of cows was held twice a day. The basic feed of both groups was equal (see Table 1); also cow’s mineral salt licks were freely available. EG feed was supplemented with carrots – 7 kg per cow per day.

Table 1. The composition of cows feed during experiment (per cow per day)

<table>
<thead>
<tr>
<th>Cow groups</th>
<th>Indoor period</th>
<th>Outdoor period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>basic feed composition</td>
<td>supplement feed</td>
</tr>
<tr>
<td>CG</td>
<td>haylage*, hay–ad libitum; mixed feed concentrate** – 2 kg; beet molasses – 0.5</td>
<td>217</td>
</tr>
<tr>
<td>EG</td>
<td>carrots 7 kg</td>
<td>299</td>
</tr>
</tbody>
</table>

CG = control group, EG = experimental group; * mainly consisting of perennial ryegrass; ** feed additive for dairy cattle ‘LUX’ (‘Tukuma Straume’, Latvia), contained following: wheat, barley, sunflower meal, wheat bran, rapeseed cake, fodder yeast, calcium carbonate, sugar beet molasses, sodium chloride, etc. vitamin and mineral supplements.

Feed supplementation was conducted at the end of the indoor period from the end of March and during April and May. The total period of the feed supplementation was 42 days. During outdoor period cows were grazed between both milkings, as well as received hay in the barn. Both groups received equal feed; the total concentration of carotene and α-tocopherol in forage of the respective sampling day is shown in Table 1.

Sample collection and preparation for analyses

A total of 120 milk samples were obtained from the morning milking both on indoor period and when the animals were on the pasture. Milk samples were obtained from healthy cows with somatic cell count less than 400,000 in ml. During the indoor period, milk samples were taken one day before the feed supplementation (n = 20) and several times repeatedly – 1, 3, 5, and 6 weeks from the beginning of the feed supplementation (n = 80). Milk samples from the pasture period were taken 7 weeks after the end of the feed supplementation experiment and the beginning of the grazing period or 13 weeks from the launch of the whole experiment (n = 20).
**Milk fat, protein, lactose concentration and yield**

Concentration of milk fat, protein, and lactose was determined in line by infrared spectroscopy with the ISO 9622:1999 standard method (International Organization for Standardization, 1999) at the Laboratory of Milk Quality Control of the joint-stock company 'Stock Breeding and Artificial Insemination Station of Sigulda'.

**Extraction of butter oil**

Each group’s pooled milk samples for butter oil extraction were obtained by pouring together equal quantities (1 l) of fresh milk from individual cows. For butter oil extraction, milk was warmed up to 42 ± 3°C. Subsequently milk was creamed with a separator obtaining cream having approximately 30% fat content. Than cream was ripened at 4–6°C, for 20 ± 1 hour, and churned till the formation of butter. Buttermilk was removed, and butter was rinsed with cold distilled water. Afterwards butter was warmed up to 45 ± 5°C and centrifuged 14,360 × g, for 10 minutes at 40°C to separate pure butter oil that was used for β-carotene, α-tocopherol, and colour analyses. Till further analyses butter oil samples were frozen at -20°C temperature.

**β-carotene and α-tocopherol concentration in butter oil**

β-carotene and α-tocopherol concentration in butter oil was found out based on the standard methods ISO 9936:2006 (International Organization for Standardization, 2006), EN 12823-2:2000 (European Committee for Standardization, 2000), and method of Granelli and Helmersson (1996) with slight modifications. In a glass baker 0.4 grams of oil were weighted adding 5 ml of ethanol with butylated hydroxytoluene (0.2%) (Supelco) and 10 ml of 2 M ethanolic KOH solution, then mixed. Saponification was performed in 54 ± 1°C temperature for 1 h. Then 20 ml of deionized water and 4 ml of hexane (Sigma Aldrich, Chromasolv) were added and tubes were centrifugated for 5 min 9,400 × g, at 4 ± 1°C temperature. The upper lipid layer was transferred in a test tube, and the extraction was repeated two more times. The extract was evaporated in vacuum at 40 ± 1°C temperature. In the test tube containing the residue, 0.2 ml of methanol (Sigma Aldrich, Chromasolv) was added. The solution was centrifugated for 5 min 34,000 × g at 4 ± 1°C temperature. The samples prepared were analysed by high-performance liquid chromatograph (Waters Alliance 2695) with a photodiode detector (Waters) using YMC Carotenoid column (5μm, 4.6 × 250 mm) at a temperature of 40 ± 1°C. Sample injection volume was 50 μl. The mobile liquid phase was prepared from methanol, methyl tert-butyl ether (Sigma Aldrich, CHROMASOLV), and deionized water in various proportions with the flow rate 1 ml min⁻¹. Analyses of β-carotene and α-tocopherol concentration in butter oil were made in the Agency of the Latvia University of Agriculture ‘Research Institute of Biotechnology and Veterinary Medicine ‘Sigra’.

**Blood sample collection and storage**

Cows blood samples were taken from the v. jugularis externa after morning milking and collected into 5 ml vacutainers without stabilizer. Then samples were stored at room temperature until the blood coagulates and serum separates (1h). Thereafter the tubes were centrifugated (3,000 rpm, 4°C, 10 min) and serum was stored at -18 °C until analyses.
Total carotene concentration in cow blood serum

Experiment covered a comparison of the total carotene concentration in blood serum of animals one day before and at the end of the feed supplementation experiment – after 6-week feed supplementation with carrots – that was determined by the spectrophotometric method. Carotene determination with spectrophotometric method was performed in the Institute of Food Safety, Animal Health and Environment ‘BIOR’.

Yellow colour intensity of butter oil

Samples were heated to 40 ± 1°C before carrying out analyses. Then oil was poured into a transparent plastic Petri plates so as to avoid the formation of air bubbles and held in 25 ± 1°C for the temperature stabilization. The colour of butter oil samples was measured using a ColorTec – PCM colour meter, USA (CIE 1976 L*a*b* colour model), which has been calibrated according to a standard. The color was determined in 7 different surface points. Colour measurements were made at the Faculty of Food Technology of the Latvia University of Agriculture in the Research Laboratory of Packaging Materials’ Attributes. The data were processed with ColorSOF QCW data program. Colors range was read in three coordinate system: L*, which characterizes the degree of brightness, where L = 100 - white, but L = 0 - black, a* is the measure of the – a (green) to + a (red) and b* factor – from -b (blue) to + b (yellow). The factor b* values were used to compare yellow colour intensity of samples.

All analytical reagents used in the analysis were of analytical or higher purity.

Statistical data analyses

Samples were analysed at least in duplicate. Statistical data processing of the acquired results was carried out using MS Excel and SPSS 17 application. Data are presented as the mean ± standard error of means (s.e.m.). The hypotheses suggested were tested by Student’s t test; factors were considered significant if \( P < 0.05 \).

RESULTS AND DISCUSSION

Total carotene concentration in cow blood serum

In order to better assess the impact of feed supplementation on quality and composition of the milk, it was necessary to find out whether the additionally fed carrots increase the carotene concentration in cow organism. Results of the total carotene concentration in blood serum of animals before and after the 6-week feed supplementation with carrots are shown in Fig. 1. The concentration of β-carotene in cow blood can serve as an indicator showing whether the amount of carotenoids in feed is sufficient. A serum β-carotene level of 3.0 mg l\(^{-1}\) has been suggested as the level below which the supplementation is beneficial for udder health (Frye et al., 1991; Jukola et al., 1996). Before the experiment, the average carotene concentration in blood serum of animals of both groups was similar (\( P > 0.05 \), 2.5 and 2.6 mg l\(^{-1}\) in the CG and EG, respectively, and it was below the recommended level.
After the 6-week feed supplementation period, the carotene concentration in CG and EG cow blood serum was 4.0 mg l\(^{-1}\) and 6.2 mg l\(^{-1}\), respectively. A significant increase \((P < 0.05)\) may be associated with a composition differences in separate lots of the basic feed. Therefore the concentration increase in the EG accounted for 2.6 times, while in the CG – 1.7 times. This demonstrates that diet supplementation with 7 kg of carrots may improve dairy cow carotene status. Insufficient carotene concentration (below 3.0 mg l\(^{-1}\) level) has been observed also in other studies giving a cause to a warning. A total of 35% of cows in a study on herds from various regions of France had less than 1.5 mg l\(^{-1}\) \(\beta\)-carotene in the blood plasma showing a strong \(\beta\)-carotene deficiency, while 71% had less than 3.5 mg l\(^{-1}\) \(\beta\)-carotene (Georges, 2009). The status was improved by the contribution of grass silage or alfalfa and the best amelioration was attained by cow grazing. Similar observations were made in Canada, where mean serum \(\beta\)-carotene concentration from 20 Holstein cow herds was lower than preferable – 1.12 mg l\(^{-1}\) (LeBlanc et al., 2004). Previous studies showed that also in organic farm system, \(\beta\)-carotene and vitamin E concentration in feed may be insufficient to provide the preferable \(\beta\)-carotene and \(\alpha\)-tocopherol level in cow blood serum during the calving period, as well as in blood of calves (Johansson et al., 2012). Therefore farms working under this system need to supplement animal food with \(\beta\)-carotene, vitamin A, and E that could reduce the incidence of mastitis. The observation was made in several studies about the effect of cow feed enrichment with \(\beta\)-carotene additives. Supplementation during the dry period showed positive effect on \(\beta\)-carotene level in cow blood and colostrum that is important also for the health of calves (Kaewlamun et al., 2011). However, there is a lack of relevant information in the scientific literature about effects of carrot supplementation on cow blood carotene level.

**Milk fat, protein, lactose concentration and yield**

Milk quality parameters of samples obtained before and during the feed supplementation experiment, as well as milk yield between the groups were compared. The summary of indicators characterising concentration of milk fat, protein, lactose, and yield is given in the Table 2. The average bulk milk quality parameters and yield before
the feed supplementation experiment as well as during it did not indicate significant differences between groups except lactose concentration that was significantly lower in EG ($P < 0.05$) milk. The significant difference in lactose concentration, as well as insignificant differences in milk yield and other parameters between groups before the experiment may be related to the individual characteristics of the animals, as feed composition was the same before the experiment.

Table 2. Comparison of average milk quality parameters and yield before and during feed supplementation experiment

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Before experiment</th>
<th>During supplementation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CG</td>
<td>EG</td>
</tr>
<tr>
<td>Fat, $\text{g kg}^{-1}$</td>
<td>41.6</td>
<td>44.8</td>
</tr>
<tr>
<td>Protein, $\text{g kg}^{-1}$</td>
<td>32.9</td>
<td>33.7</td>
</tr>
<tr>
<td>Lactose, $\text{g kg}^{-1}$</td>
<td>47.8</td>
<td>46.5</td>
</tr>
<tr>
<td>Milk yield, $\text{kg day}^{-1}$</td>
<td>19.65</td>
<td>16.51</td>
</tr>
</tbody>
</table>

CG = control group, EG = experimental group, Pooled s.e. = pooled standard error, * = significant difference, ns = non-significant difference between dietary treatments (Student’s $t$ test, $P > 0.05$).

Comparison of the average bulk milk quality parameters and yield before the feed supplementation experiment and during it did not indicate significant changes in any of the groups ($P > 0.05$). The possible explanation of the results could be that the effect of supplemental feeding may occur only after a longer period of time and is affected by individual health condition of each animal. The similar to our results were obtained in the study of Schreiner and Windisch (2006). Feeding additionally 10 kg carrots with and without 1 kg rapeseed oil per cow per day in iso-caloric diets showed no effects on the total milk yield, fat and protein concentration. At the same time the contradictory results were acquired by other authors. Increase in milk yield by 11% was observed in a study of Arechiga et al. (1998) in which heat-stressed cows feed was supplemented with 400 mg of β-carotene per cow per day. Oldham et al. (1991) observed that after supplementing the feed with 300 mg of β-carotene per cow per day, milk production increased by 6.4% and fat concentration reduced by 4.6%. In the study of de Ondarza et al. (2009), supplementation of 425 mg β-carotene per day did not affect the milk production, but significantly ($P < 0.05$) higher fat concentration (+0.1%) was observed, especially for early lactation cows and cows in their third or greater lactation. Dissimilar basic feed uptake of β-carotene, duration of supplementation or stage of lactation can be some of the explanations of such diverse effects of feed supplementation with carotenoid additives in different studies.

**β-carotene and α-tocopherol concentration in butter oil**

Changes of the β-carotene and α-tocopherol concentration in butter oil samples during the experiment are presented in Fig. 2. Average β-carotene concentration before the experiment was higher in the CG butter oil – 5.2 vs. 4.3 mg kg$^{-1}$ fat in the EG oil samples ($P < 0.05$). The average concentration of α-tocopherol did not differ significantly ($P > 0.05$) and was 9.6 and 10.0 mg kg$^{-1}$ fat in the CG and EG, respectively. During the supplementation with carrots, average concentration of β-carotene in butter oil samples acquired from the EG did not change, compared to β-carotene concentration before the experiment ($P > 0.05$), but in samples
of the CG it decreased ($P < 0.05$). It comprised 3.7 and 4.2 mg kg$^{-1}$ fat in the CG and EG. The difference between the groups was not significant ($P > 0.05$); however, a tendency of marginally higher $\beta$-carotene concentration in samples of the EG was observed. The negative tendency regarding changes in $\beta$-carotene concentration in the CG butter oil may be related to carotene deficit in diet as described above considering that fat yield – the average quantity of the milk fat daily produced by a cow – remained unchanged (0.86 kg day$^{-1}$ during supplementation vs. 0.85 kg day$^{-1}$ before the experiment). At the same time, $\beta$-carotene concentration in butter oil samples of the EG was more stable with respect to the similar fat yield before and during supplementation (0.80 and 0.79 kg day$^{-1}$). Within the supplementation experiment (indoor period), average concentration of $\alpha$-tocopherol in butter oil samples acquired from the EG, increased ($P > 0.05$) reaching average 12.4 mg kg$^{-1}$ fat. The tendency of changes in average $\alpha$-tocopherol concentration in the CG was opposite – it decreased ($P > 0.05$) to average 8.6 mg kg$^{-1}$ fat and thus was lower by 31% if compared to the EG ($P < 0.05$).

![Figure 2](image_url)

**Figure 2.** $\beta$-carotene and $\alpha$-tocopherol concentration (means ± s.e.m.) in butter oil. CG = control group, EG = experimental group. *Significant difference between dietary treatments (Student’s $t$ test, $P < 0.05$).

The positive effect towards more stable $\beta$-carotene concentration in butter oil samples of the EG, as well as its higher $\alpha$-tocopherol concentration during the experiment ($P < 0.05$) may be related to higher total amount of fat-soluble antioxidative substances ingested by feed. The antioxidant system is an integrated system and deficiencies of one component can affect antioxidative efficiency of others (Baldi et al., 2008). It is likely that greater quantities of $\alpha$-tocopherol remain and can be utilised in milk lipid synthesis because body’s necessity for antioxidative substances may be met much better. Similar effect towards increase in tocopherol concentration in milk was observed in another earlier experiment that covered supplementation of Holstein and Latvian brown cow feed with carrots and red palm oil feed additives (Antone et al., 2011). Also the study of Nałęcz-Tarwacka et al. (2003) conducted with Black & White
cows showed higher levels of vitamins A, D, E, and carotene in cow milk achieved by carrot supplementation, yet the statistically significant differences \((P < 0.05)\) were confirmed only for vitamin A. Experiment of Schreiner and Windisch (2006) also showed that carrot supplementation increases carotene content in butter. However, it is known that there is a limitation of daily secretion of \(\alpha\)-tocopherol and \(\beta\)-carotene being independent of milk yield and fat content. Thus, diet supplementation can change the vitamin content of the milk only within certain limits (Baldi & Pinotti, 2008).

Values of fat-soluble antioxidant concentration in milk fat reported by other scientists during the indoor period are similar to or higher than our data. \(\beta\)-carotene concentration in milk fat found by Butler et al. (2008) was higher (5.5–6.3 mg kg\(^{-1}\) fat) but that of Calderón et al. (2007) – similar to our results: by feeding grass silage, \(\beta\)-carotene comprised 4.2 mg kg\(^{-1}\) fat. Regarding the milk fat \(\alpha\)-tocopherol concentration during the indoor period, butter oil in the CG in our experiment was noticeably lower than in the studies mentioned above. In the study of Butler et al. (2008), \(\alpha\)-tocopherol concentration was 23.1–23.9 mg kg\(^{-1}\) fat, and in the study of Calderón et al. (2007) – 11.31 mg kg\(^{-1}\) fat. The differences of results between different studies may be explained by variations in feed quality, composition (the usage of hay and haylage in our experiment), cow breeds, or other factors.

In comparison with the indoor period, concentration of fat-soluble antioxidants in butter oil samples obtained in grazing season grew considerably in both groups \((P < 0.05)\). Average concentration of \(\beta\)-carotene increased to 6.5 and 7.7 mg kg\(^{-1}\) fat, whereas concentration of \(\alpha\)-tocopherol – to 16.5 and 21.7 mg kg\(^{-1}\) fat, in the CG and EG, respectively. Observations of animal feeding and housing impact have been reported very widely. Results of the study on changes in milk fat-soluble antioxidant concentration during grazing period conducted by other scientists are similar to our results. It has been ascertained that milk fat-soluble antioxidant concentration largely depends upon the animal nutrition, and most notably it increases in summer during the grazing season (Coulon et al., 2003). For example, Butler’s et al. (2008) findings show that \(\beta\)-carotene concentration in milk fat increased from 5.5–6.3 mg kg\(^{-1}\) fat during the indoor season to 6.0–9.3 mg kg\(^{-1}\) fat during the grazing season, but \(\alpha\)-tocopherol concentration in milk fat during the grazing season reached 21.4–32.0 mg kg\(^{-1}\) fat compared to 23.1–23.9 mg kg\(^{-1}\) fat during the indoor season. Although pasture grass is the richest source of natural antioxidants used in cattle diets since the ancient times, nowadays smaller use of grazing and higher milk production intensity, as well as other reasons can adversely affect quantity of antioxidants in butter oil.

**Yellow colour intensity of butter oil**

As carotenoids also are yellow-, orange- and red-coloured pigments, changes in yellow colour intensity of butter oil may indicate carotene richness of the feed. Feeding animals with carrots may contribute to the colour of milk and products thereof as one of the most important sensory properties. Regression analysis indicated a positive relationship between the total carotene amount ingested by feed and yellow colour intensity of butter oil \((P < 0.05, r^2 = 0.55)\) (Fig. 3.). The medium close relationship shows that other factors could affect the colour as well.
Figure 3. Correlation between yellow colour intensity (b) of butter oil and total amount of carotene (mg per cow per day) ingested by feed.

Changes in yellow colour intensity of butter oil during the experiment are presented in the Fig. 4. During the feed supplementation, yellow colour of butter oil samples acquired from the EG was stronger ($P < 0.05$) than the one of butter oil yielded from the CG. After 1-, 3- and 5-week supplementation periods, the intensity of yellow colour in the EG butter oil gradually increased ($P < 0.05$), while in samples of the CG it remained constant also showing a tendency to decrease insignificantly ($P > 0.05$) when compared to the colour intensity before the experiment. Measurements showed the decrement in colour intensity of butter oil at the end of the indoor period. It may be related to the deficit of yellow pigments – β-carotene and retinol – due to reduced body reserves or to increased milk yield resulting in dilution effect (Nozière et al., 2006a and 2006b; Calderón et al., 2007).

Figure 4. Changes in yellow colour intensity (mean ± s.e.m.; b) of butter oil. CG = control group, EG = experimental group. *Significant difference between dietary treatments (Student’s $t$ test, $P < 0.05$).

During the pasture period, yellow colour intensity of butter oil produced from milk of both groups increased ($P < 0.05$). Such pronounced colour changes coincide with the increase of β-carotene concentration in butter oil samples obtained from the pasture period. Intensity of yellow colour sometimes may also serve as an indicator of the basic feed type consumed by herd. It is believed that colour measurements should be taken 4–5 weeks after changing composition of basic feed because effect left by carotenoid intake
may be dilatory (Nozière et al., 2006b). Our study shows that butter oil colour differences, compared to the control samples, were apparent earlier – after 1–3 weeks from the cow feed supplementation with carrots. This can be explained by the fact that studies were conducted with the red varieties of cows that are either more efficient at absorbing β-carotene or less efficient at converting it to retinol (National Research Council, 2001; Nozière et al., 2006a). Subsequently their carotene transition in milk should be more noticeable.

CONCLUSIONS

Carrots as a natural source of carotene are relatively inexpensive and can be grown in many parts of Europe and other regions. Milk producers can be suggested to add this vegetable to the feed of dairy cows in order to improve cow carotene status and ensure increased quality and nutritional value of milk and high-fat dairy products. Cow feed supplementation with carrot roots can help to maintain optimal β-carotene level in cow blood, as well as to provide a more stable or higher level of fat-soluble antioxidants (β-carotene and α-tocopherol) in butter oil and its yellow colour intensity during the indoor period. Increased concentration of biologically active compounds such as β-carotene and α-tocopherol can give higher nutritional value to butter and other dairy products. Yellowish colour may improve consumption of dairy products, especially among consumers preferring goods produced within the environmentally friendly organic farming system since such products are associated with natural or pasture feeding. Nevertheless, further research should be necessary regarding effects thereof on milk composition and dairy product shelf-life.

REFERENCES


Effects of degradation preventive agents on storage stability of anthocyanins in sour cherry concentrate

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Abstract. In this study the effects of sugar (sucrose, 25%), gallic acid (700 mg kg\(^{-1}\)) and ascorbic acid (700 mg kg\(^{-1}\)) were used in sour cherry concentrate in order to prevent the degradation of main anthocyanin compounds (cyanidin-3-glucosylrutinoside (Cy-3GR), cyanidin-3-rutinoside (Cy-3R) and cyanidin-3-glucoside (Cy-3G)) which are natural bioactive pigments responsible for red, blue and purple color of many fruits and vegetables. Thermal degradation of anthocyanins was evaluated by determination of anthocyanin content and calculation of the reaction rate constant, half-life of degradation, activation energy. Anthocyanin content decreased at all of the storage temperatures, as an example; there were 75, 51 and 55% reductions in Cy-3G contents of control samples (with no preventive agent) stored at 45, 24 and 4°C, respectively. The values of half-life time were above 200 days in most cases at all storage temperatures for sugar treated samples. Cy-3-GR (activation energy values 35.6-84.4 kJ mol\(^{-1}\)) was found to be the most unstable among the other anthocyanins. The most contributing agent on anthocyanin stability was sugar, whereas ascorbic acid exhibited the lowest effect in terms of preventing anthocyanin degradation.

Key words: sour cherry, anthocyanins, degradation kinetics, storage.

INTRODUCTION

Anthocyanins, the biggest group of water-soluble natural pigments, are a sub-class of flavonoids. They give attractive colours of flowers, fruits (especially berries) and vegetables, as well as their products (Mazza & Brouillard, 1990). Except as colorants, anthocyanins have multiple biological roles, e.g. antioxidant activity, anti-inflammatory action, inhibition of blood platelet aggregation and antimicrobial activity, treatment of diabetic retinopathy and prevention of cholesterol-induced atherosclerosis (Mazza & Miniati, 1993; Espin et al., 2000). Anthocyanins are stable under acidic conditions, but under normal processing and storage conditions they transform to colourless compounds and subsequently to insoluble brown pigments. Thermal treatments and storage temperature have the most important influence on anthocyanin stability (Zorić et al., 2014).

Number of factors influences the stability of anthocyanins, like temperature, pH, light, oxygen, enzymes, structure and concentration of the anthocyanins, presence of ascorbic acid, sugars, sulphite salts, metal ions and copigments (Gradinaru et al., 2003; Tsai & Huang, 2004). Garcia-Viguera et al. (1999) and Iacobucci & Sweeny (1983) proposed a free radical mechanism where cleavage of the pyrillium ring resulted as a consequence of oxidation initiated by activation of molecular oxygen induced by
ascorbic acid. Anthocyanin copigmentation reactions are common in nature and result from the association of metal ions or colorless polyphenolics (cofactors) to anthocyanins under acidic conditions. Copigment complexes also serve to enhance color and stability characteristics of anthocyanins in low acid conditions where anthocyanins are normally colorless. Several studies have suggested an increase in anthocyanin stability in the presence of cofactors (Boulton, 2001; Brenes et al., 2005).

Many studies were conducted with the aim of improving stability of anthocyanins through addition of different additives, like acids, sugars, salts, hydrocolloids and different phenolic compounds (Rein & Heinonen, 2004; Brenes et al., 2005). Fortification of fruit and berry juices with ascorbic acid is a common method to protect against oxidation and to increase the nutritional value of a food product. Ascorbic acid is thought to have several different roles in anthocyanin color stability. In fruit and berry products, by copigmentation the color of juices (Wilska-Jeszka & Korzuchowska, 1996; Dufour & Sauvaitre, 2000), purees, jams and syrups could be enhanced and stabilized, improving consumer acceptance and prolonging product shelf-life. Brenes et al. (2005) reported the total anthocyanins in grape juice ranging between 600-800 mg L⁻¹. The anthocyanin content of twenty different pomegranate varieties from Iran was reported between 5.56–30.11 mg 100g⁻¹ (Tehranifer et al., 2010). The values of total anthocyanins in red currant juices were reported between 34.3–47.9 mg 100g⁻¹ by Kopjar et al. (2009). Filiman et al. (2011) determined anthocyanins between 107–176 mg 100g⁻¹ in four sour cherry varieties grown in Romania.

The chemical stability of anthocyanins in the presence of ascorbic acid, gallic acid and sucrose and storage temperature effects has not been studied upon sour cherry concentrate until now. The aim of this research was to study the kinetics of individual anthocyanins in sour cherry concentrate, in order to advance the knowledge of the thermal stability of the main anthocyanins, and to apply previously reported mathematical models enabling the prediction of the degradation of these compounds during storage.

**MATERIALS METHODS**

**Laboratory-scale preparation of sour cherry concentrate**

Sour cherries were purchased from a local market in June 2010. The samples were immediately transported to laboratory and subsequently pitted, washed and squeezed in a fruit juicer (Philips fruit juicer, HR1861). A juice of 14–16 °Brix was obtained. The obtained juice was pasteurized in an autoclave at 85°C for 30 min. The juice was filtrated through a filter paper. Before concentration it was partitioned into four batches for application of treatments: gallic and ascorbic acids were added seperately to contain final concentration in the concentrate models of 700 mg kg⁻¹. Sucrose was incorporated in order to have a final level of 25%. The initial sugar concentration of sour cherry juice is approximately 8%. For a 100 mL of juice, 2 g of sugar was added and the solution was concentrated with a volumetrically ratio of 2 : 5. The final sugar concentration was about 25%. A non-fortified batch was prepared as control. The samples were concentrated by using a vacuum evaporator at 40°C until a 6°Brix of 55 was obtained. Treatments were sealed in 100 mL screw-cap glass bottles. The samples were stored at three different temperatures, 4°C (in refrigerator), 24°C (room temperature) and at 45°C (in oven) for 6 months. The anthocyanin contents of samples were determined at 0, 20, 50, 90, 120 and 150th days of storage.
Extraction of anthocyanins

The extraction of anthocyanins from sour cherry concentrate was performed according to a previously described procedure (Elez Garofulić et al., 2013). 4 g concentrate was mixed with 8 mL of 80% aqueous methanol solution containing 0.1% HCl (by volume), in a water bath at 60°C for 20 min. Afterwards, the extracts were filtered through Whatman No. 40 filter paper (Whatman, GE Healthcare Bio-Sciences, Pittsburgh, PA, USA), transferred into 10 mL volumetric flasks, and made up to volume with extraction solvent. Extracts were stored at -20°C in an inert nitrogen gas atmosphere before the analysis.

HPLC analysis

The anthocyanins were simultaneously analysed by a direct injection of the extracts, previously filtered through a 0.45 mm pore size membrane filter (Macherey-Nagel GmbH & Co. KG, Düren, Germany). Chromatographic separation was performed using HPLC analysis with Agilent 1260 Infinity quaternary LC system (Agilent Technologies, Santa Clara, CA, USA) equipped with diode array detector (DAD), an automatic injector and ChemStation software. The separation of phenolic compounds was performed on a Nucleosil 100-5C18, 5 mm (250 mm × 4.6 mm i.d.) column (Macherey-Nagel). The solvent composition and the gradient conditions used were as described previously by Mitrić et al. (2012) and Zorić et al. (2014). Mobile phase A contained 3% of formic acid in water, while solution B contained 3% of formic acid in 80% acetonitrile. The used elution program was as follows: from 0 to 28 min 0% B, from 28 to 35 min 25% B, from 35 to 40 min 50% B, from 40 to 45 min 80% B, and finally for the last 10 min again 0% B. The flow rate was 0.8 mL min⁻¹ and the injection volume was 50 µL. Detection was performed with UV/VIS–photo diode array detector by scanning from 220 to 570 nm. Identification of phenols was carried out by comparing retention times and spectral data with those of the authentic standards (anthocyanins were identified at 520 nm).

The quantifications of anthocyanins were made by the external standard method. All anthocyanin standards, cyanidin-3-glucoside (Cy-3-G) and cyanidin-3-rutinoside (Cy-3-R) were prepared as stock solutions in acidified methanol (1% of formic acid in methanol, by volume) at a concentration of 100 mg L⁻¹. Cyanidin-3-glucosylrutinoside (Cy-3-GR) identification was done in comparison with Cy-3-G. Identification was made by matching the retention time of the separated peaks and the retention time of the authentic standards. Additionally, identification was confirmed using characteristic UV/VIS spectra, polarity, and previous literature reports (Del Bo’ et al., 2010; Fracassetti et al., 2013). Under the current chromatographic conditions, the limit of detection (LOD) and limit of quantification (LOQ) were determined to be 100 ng mg⁻¹ (S : N (signal-to-noise ratio) > 5) and 200 ng mg⁻¹ (S : N > 10), respectively.
Degradation Kinetic Studies

The thermal degradation of anthocyanins was performed according to the method reported by Kechinski et al. (2010). Degradation is a temperature-dependent process, as described by the Arrhenius equation: 

\[ k = k_0 \times e^{-\frac{E_a}{RT}} \]

where: 
- \( k_0 \) is the frequency factor (per min), 
- \( E_a \) the activation energy (J mol\(^{-1}\)), 
- \( R \) the universal gas constant (8.314 J mol\(^{-1}\) K\(^{-1}\)), and 
- \( T \) the absolute temperature (K).

Statistical Analysis

Data were reported as mean values of at least four experiments. Statistical analysis was performed by means of Statistica software (Statsoft Inc., Tulsa, OK, USA). One-way ANOVA was performed to determine the variation among the samples stored at different temperatures. Differences between means were evaluated by the Duncan test. Differences were considered to be significant at \( P \leq 0.05 \).

RESULTS AND DISCUSSION

In the presented research, the major anthocyanins determined in freeze-dried sour cherries were Cy-3-GR, Cy-3-R and Cy-3-G with the initial values in control group 245.6 ± 22.1, 187.4 ± 18.4 and 41.5 ± 4.0 mg kg\(^{-1}\), respectively. The initial and final contents of anthocyanins in sour cherry concentrate after storage at different temperatures of 4, 24 and 45°C was plotted and presented in Fig. 1. Mass fractions at all temperatures followed the first order reaction kinetics with a coefficient of determination \( R^2 \), ranging from 0.84 to 0.99 (Table 1). These results are in accordance with previous reports (Zorić et al., 2014; Harbourne et al., 2008).

Anthocyanins displayed decay, directly related to the storage temperature. For all of the three compounds, the correlation indices (\( R^2 \)) were > 0.85, demonstrating a direct correlation between anthocyanin concentration decrease and storage time. The increases in degradation rate of anthocyanins during processing and storage as the temperature rises were also previously reported (Maccarone et al., 1985; Fracassetti et al., 2013).

Cy-3-GR was the most unstable anthocyanin in all three storage temperatures (\( E_a = 35.57-84.28 \) kJ mol\(^{-1}\)). Cy-3-G followed it (\( E_a = 124.04-266.62 \) kJ mol\(^{-1}\)), whereas this compound was the most unstable with the addition of gallic acid, ascorbic acid and sucrose at 45°C.

The most stable anthocyanin compound Cy-3-R (\( E_a = 95.44-825.33 \) kJ mol\(^{-1}\)) showed an exception for ascorbic acid added samples at 45°C storage with the lowest half-life time (\( t_{1/2} \)). Ascorbic acid has been reported to show different roles in anthocyanin stability. Ascorbic acid accelerated decomposition of anthocyanins and enhances polymer pigment formation and bleaches anthocyanin pigments (Marti et al., 2002). Direct condensation between anthocyanins and ascorbic acid has been postulated as a mechanism for anthocyanin degradation (Poei-Langston & Wrolstad, 1981). Also the formation of hydrogen peroxide from ascorbic acid oxidation can influence anthocyanin stability (Talcott et al., 2003). However, the stability of acylated anthocyanins has been observed to increase in the presence of ascorbic acid (Del Pozo-Insfran et al., 2004).
Figure 1. The initial and final contents (mg L$^{-1}$) of anthocyanins in sour cherry concentrate after storage at 4, 24 and 45°C for 5 months.
The $t_{1/2}$ values of anthocyanins were presented in Table 1. The half-life at 45°C of Cy-3-G and Cy-3-R was 53.3 and 6.5 days, respectively, which were lower than the values obtained for the control group samples. This indicates a negative effect of ascorbic acid on these compounds (a more dramatic reduction of half-life of Cy-3-R) at high storage temperatures. Cy-3-R was more susceptible to high temperatures than Cy-3-GR, while Cy-3-G was unstable.

**Table 1.** Activation energy ($E_a$) and half-life ($t_{1/2}$) of individual anthocyanins of the sour cherry concentrate stored at 4, 24, and 45°C.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Treatments</th>
<th>$t_{1/2}$ (days)</th>
<th>$E_a$ (kJ mol$^{-1}$)</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cy-3-GR$^a$</td>
<td>4</td>
<td>80.0</td>
<td>35.57</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>77.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>63.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cy-3-G</td>
<td>4</td>
<td>138.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>173.3</td>
<td>143.16</td>
<td>0.84</td>
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<tr>
<td></td>
<td>45</td>
<td>73.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cy-3-R</td>
<td>4</td>
<td>346.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>231.0</td>
<td>95.44</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>224.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cy-3-GR</td>
<td>4</td>
<td>99.0</td>
<td></td>
<td>0.99</td>
</tr>
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<td></td>
<td>24</td>
<td>86.6</td>
<td>44.89</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>77.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>(700 mg kg$^{-1}$)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cy-3-G</td>
<td>4</td>
<td>231.0</td>
<td>259.31</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>173.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>53.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cy-3-R</td>
<td>4</td>
<td>599.0</td>
<td></td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>24</td>
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<td>825.33</td>
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<tr>
<td></td>
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<td>6.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cy-3-GR</td>
<td>4</td>
<td>138.6</td>
<td></td>
<td>0.95</td>
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<td>24</td>
<td>99.0</td>
<td>84.38</td>
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<td></td>
<td>45</td>
<td>86.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>(700 mg kg$^{-1}$)</td>
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<td></td>
<td></td>
</tr>
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<td>Cy-3-G</td>
<td>4</td>
<td>346.5</td>
<td>266.62</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>231.0</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>45</td>
<td>77.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cy-3-R</td>
<td>4</td>
<td>693.0</td>
<td>155.72</td>
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</tr>
<tr>
<td></td>
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<td>693.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>346.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cy-3-GR</td>
<td>4</td>
<td>346.0</td>
<td>71.79</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>297.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>231.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar</td>
<td>(25%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cy-3-G</td>
<td>4</td>
<td>348.0</td>
<td>124.04</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>230.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>173.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cy-3-R</td>
<td>4</td>
<td>758.0</td>
<td>538.99</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>690.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>346.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^{a}$Cy-3GR: cyanidin-3-glucosylrutinoside, Cy-3R: cyanidin-3-rutinoside, Cy-3G: cyanidin-3-glucoside.

Among the degradation preventive agents, sucrose was the most effective, gallic and ascorbic acid followed. This effect was more evident for Cy-3-GR at 45°C. The protective effect of sucrose on degradation of anthocyanins was attributed to the inhibition
of enzymatic reactions or the hindering of different condensation reactions (Wrolstad et al., 1990) and also its lowering of water activity (De Ancos et al., 1999b).

CONCLUSION

The results supported that the duration and temperature of storage have a strong influence on the anthocyanin stability. Rapid and high anthocyanin degradation indicates that it is very important to identify suitable storage conditions, for which further studies are still needed. The enhancement of sour cherry concentrate with sucrose and gallic acid can be regarded as a promising mean of improving stability of anthocyanins. The use of ascorbic acid should be avoided at elevated temperatures of storage, as ascorbic acid led to a dramatic reduction of half-life of Cy-3-R during storage at 45°C.

REFERENCES


**Pork quality of autochthonous genotype Casertana, crossbred Casertana x Duroc and hybrid Pen ar Lan in relation to farming systems**

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**Abstract.** In the last decades, the development of livestock has coincided with improvements of the animals performance. The swine has been strongly selected for several traits that determined a significant spread of some genetic types, more productive than the old autochthonous genetic types (AGT). Therefore, the AGT suffered a growing demographic contraction. The AGT are able to reduce the loss of genetic variability, potentially useful for the new generation and they play an important economic role for their productive capacity in harsh environments; furthermore, they can be used to obtain natural and ‘traditional’ products. In the current research the black AGT Casertana (CT) was compared with the crossbred CT×Duroc in relation to gender (castrated males and entire females) and farming systems: Open Air and Outdoor (plus access to the bush) for some qualitative properties of meat. In addition ‘Fiocco’ ham, a traditional product, from CT, CT×DU and Pen ar Lan pigs was analyzed. The results showed that the CT pigs had a significantly higher percentage of fat, a thicker adipose tissue, and their meat had lower values of hardness, chewiness, shear force, and appeared significantly redder than other genotypes meat. The farming systems and gender did not affect the carcass composition and physical traits of meat.

**Key words:** pork, meat quality, autochthonous genetic type, Casertana pig.

**INTRODUCTION**

In the last decades, the development of livestock coincided with a socio-economic progress that in many countries has led to an increase in the demand of livestock products. The strong selective activity of pig breeds resulted in a greater spread of certain genetic types, more productive than others and, therefore, a growing demographic contraction of the ancient autochthonous genetic types (AGT). In the ‘70 FAO called for the attention of the political, scientific, and operational communities on the danger of extinction of AGT, the only ones able to play an important role in some harsh areas; each AGT fits in harmoniously with its breeding nutritional and extranutritional specific features (Casabianca & Matassino, 2006). The Casertana pig (CT) is a black AGT reared in South of Italy and, as other native breeds of southern European countries, provides meat with an additional value due to the quality of the raw meat and cured products (Zullo et al., 2003; Barone et al., 2006, 2008a, 2008b; Castellano et al., 2008; Pugliese & Sirtori, 2012). CT pigs are prone to adipogenesis, have a strong aptitude for fat
deposition and have a high percentage of body fat, producing more than double backfat thickness as LW pigs (Murgiano et al., 2010). Another characteristic of AGT is the slow growth that allows the pigs to reach a commercial slaughter weight (about 150 kg) at a considerably older age than improved pigs and, obviously, the rearing of them is very expensive (Maiorano, 2009).

To improve the productivity of AGT without modifying the quality of meat, or reducing the amount of intramuscular fat, the cross with the Duroc is often being employed (Edwards, 2005; Pugliese & Sirtori, 2012). The Duroc pig is also suitable for outdoor systems and appears to have a genetic predisposition to deposit intramuscular fat (Edwards, 2005).

The autochthonous genetic types are better suited to outdoor system than improved breeds. The products (raw or typical cured products) obtained from pigs reared with this system transfer to the consumers the so-called argument of ‘ethical quality’, because of conditions of animal wellbeing, of physical activity, and of the capacity of the animals to express the natural behaviour of the species.

The aim of this research was to evaluate the effects of genotype, rearing systems and gender on pigs carcass traits and on qualitative characteristics of raw meat and ‘traditional’ product: 'Fiocco' (culatello) ham.

**MATERIALS AND METHODS**

A total of 64 pigs [29 entire females (EF) and 35 castrated males (CM)] belonging to three genotypes: 23 Casertana (CT), 32 CT crossed with Duroc boar (CT×DU) and 9 Pen ar Lan (PAL) were analyzed (Table 1). The PAL pigs were fed 100% concentrate without outdoor area (Intensive system), while the others two genotypes were reared in adjacent outdoor areas called Open Air and Outdoor, providing about 210 m² per pig. The Outdoor group, when the environmental conditions allowed, was moved to the bush, about 2400 m², where pigs were left free until late evening, integrating their diet with indigenous resources such as acorns, berries, tubers, brooms, and wild plants and their fruits.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Rearing system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Open Air</td>
</tr>
<tr>
<td>CT</td>
<td>CM</td>
</tr>
<tr>
<td>CT×DU</td>
<td>10</td>
</tr>
<tr>
<td>PAL</td>
<td></td>
</tr>
</tbody>
</table>

Both genetic types CT and CT×DU had received the feed (the same concentrate of PAL) twice a day (morning and evening) on the basis of live weight and had benefited of open air pasture with Alfalfa, Couch Grass (*Cynodon dactylon*), Clover (*Trifolium*) and wild grasses.

The concentrate, the same for all genotypes, had the following chemical composition: 85.71% dry matter (DM) and on a dry matter basis: 19.4% crude protein (CP), 7.41% acid detergent fiber (ADF), 19.39% neutral detergent fiber (NDF), 4.5% ether extract (EE) and 7.4% ash.
Animals were conventionally slaughtered and, after 72 hours of ageing, the left side of each carcass was dissected into primary fatty cuts (backfat, belly, jowl, kidney fat) and lean cuts (ham, shoulder, loin, neck). For each cut the dissection into meat, fat (subcutaneous fat with skin), and bone was made and their weight were registered in order to calculate the percentage of meat and fat on carcass. The backfat thickness was measured with a ruler at three different locations: shoulder, loin and leg.

The instrumental evaluation of colour (CIEL*a*b*) and rheological traits (texture profile analysis - TPA and Warner Bratzler Shear Force–WBS) of meat was carried out on Longissimus muscle of the two genotypes CT and CT×DU. The colour was measured using a U-3000 spectrophotometer equipped with integrating sphere. The operating conditions of the instrument were: observer at 2°, source D65. Two, 1.3 cm thick samples were allowed to bloom for 1 h at 4°C, before recording the data.

The TPA variables, determined with Texturometer (Zenken, Tokio), were hardness (the total energy required for the first deformation per chew, kg), cohesiveness (A2 per A1, where A1 is the area under the first deformation per chew and A2 is the area under the second deformation per chew, texturometer unit), springiness (sample capacity to recover its original shape after the deforming force has been removed, mm) and chewiness (hardness×cohesiveness×springiness). WBS values were determined with Instron universal testing machine (Mod 5565) and represents the force (kg) required to cut a meat sample of 2.54 cm of diameter.

Colour and TPA parameters were also detected on ‘Fiocco’ (culatello) ham, at the end of the seasoning period (12 months), for all 3 genotypes.

The analysis of variance (ANOVA) was performed by the GLM procedure of SAS using a factorial model were genetic type, gender and rearing system were considered main factors. The statistical significance of comparisons between mean values was tested with Student t-test.

RESULTS AND DISCUSSION

The comparison between genotypes about slaughtering and dissection parameters showed a higher fat thickness in Casertana, especially in shoulder and buttock region ($P < 0.01$); Duroc sired genotypes had no determined differences at the loin level (Table 2). Fatty cuts accounted for 34% of the chilled carcass in CT, 31% in CT×DU and only 27% in PAL ($P < 0.05$), while the percentage of lean cuts was not statistically different among the three genotypes (Table 2). This suggests a different development and/or growth of tissues in the considered genetic types. Recently, Maiorano (2009) noted that the slower bone ossification in local breeds than that in improved pigs, conditions the muscle growth and fat deposition. As it is well known, the use of the Duroc breed is usually planned in order to increase the quantity and quality of the meat (Sellier, 1998) and to improve other characteristics such as prolificity, growth rate, feed efficiency, lean content (López-Bote, 1998). Blanchard et al. (1999) reported that slaughtered pigs possessing 50% Duroc genes in comparison to 0% Duroc genes produced more tender meat, had improved pork flavour, and a higher overall acceptability. The results of the present experiment, however, showed that the WBS, hardness, and chewiness of the meat in CT were significantly better compared to Duroc sired genotypes ($P < 0.01$) (Table 3). The meat of CT was also redder, with an $a^*$ value approximately two times that of CT×DU (6.66 vs 3.42; $P < 0.01$), and also with higher values of $b^*$ ($P < 0.01$) and Chroma ($P < 0.001$).
Table 2. Effects of genotype, rearing system and gender on carcass traits* (mean value ± SE)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Genotype</th>
<th>Rearing system</th>
<th>Gender</th>
<th>FC, kg</th>
<th>FT Shoulder, mm</th>
<th>FT Loin, mm</th>
<th>FT Leg, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC, kg</td>
<td>CT</td>
<td>CT×DU</td>
<td>PAL</td>
<td>Open Air</td>
<td>Outdoor (+bush)</td>
<td>CM</td>
<td>EF</td>
</tr>
<tr>
<td></td>
<td>144.35 ± 3.12</td>
<td>163.14 ± 2.85</td>
<td>160.00 ± 5.21</td>
<td>155.02 ± 3.01</td>
<td>153.97 ± 2.88</td>
<td>157.70 ± 2.84</td>
<td>152.30 ± 3.04</td>
</tr>
<tr>
<td>LC, kg</td>
<td>94.02 ± 2.12</td>
<td>104.63 ± 1.89</td>
<td>106.17 ± 3.52</td>
<td>100.55 ± 2.06</td>
<td>98.95 ± 1.97</td>
<td>101.23 ± 1.94</td>
<td>98.28 ± 2.08</td>
</tr>
<tr>
<td>LC CC⁻¹, %</td>
<td>65.27 ± 0.51</td>
<td>64.04 ± 0.46</td>
<td>66.37 ± 0.85</td>
<td>64.85 ± 0.53</td>
<td>64.44 ± 0.51</td>
<td>64.64 ± 0.50</td>
<td>64.65 ± 0.54</td>
</tr>
<tr>
<td>Shoulder, kg</td>
<td>19.90 ± 0.45</td>
<td>24.60 ± 1.26</td>
<td>24.24 ± 0.75</td>
<td>22.43 ± 0.44</td>
<td>22.23 ± 0.42</td>
<td>22.66 ± 0.41</td>
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</tr>
<tr>
<td>Loin, kg</td>
<td>24.97 ± 1.41</td>
<td>22.09 ± 1.26</td>
<td>19.97 ± 1.46</td>
<td>24.49 ± 1.40</td>
<td>22.85 ± 1.38</td>
<td>23.59 ± 1.38</td>
<td>23.75 ± 1.48</td>
</tr>
<tr>
<td>Ham, kg</td>
<td>16.81 ± 0.76</td>
<td>20.28 ± 1.27</td>
<td>22.81 ± 0.79</td>
<td>18.04 ± 0.79</td>
<td>19.09 ± 0.76</td>
<td>19.23 ± 0.74</td>
<td>17.90 ± 0.80</td>
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<td>Fiocco, kg</td>
<td>18.83 ± 0.37</td>
<td>22.81 ± 0.62</td>
<td>23.46 ± 0.36</td>
<td>21.02 ± 0.34</td>
<td>20.78 ± 0.33</td>
<td>21.00 ± 0.33</td>
<td>20.80 ± 0.36</td>
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<td>Neck, kg</td>
<td>13.09 ± 0.37</td>
<td>14.86 ± 0.33</td>
<td>15.67 ± 0.36</td>
<td>14.05 ± 0.34</td>
<td>14.04 ± 0.34</td>
<td>14.27 ± 0.34</td>
<td>13.82 ± 0.36</td>
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<td>FC, kg</td>
<td>48.02 ± 1.29</td>
<td>50.85 ± 1.15</td>
<td>3.12 ± 2.15</td>
<td>49.06 ± 1.31</td>
<td>50.04 ± 1.26</td>
<td>50.39 ± 1.24</td>
<td>48.71 ± 1.33</td>
</tr>
<tr>
<td>FC CC⁻¹, %</td>
<td>33.62 ± 0.84</td>
<td>31.19 ± 1.40</td>
<td>26.93 ± 1.40</td>
<td>31.75 ± 1.40</td>
<td>32.90 ± 1.40</td>
<td>32.22 ± 1.40</td>
<td>32.44 ± 0.88</td>
</tr>
<tr>
<td>Backfat, kg</td>
<td>12.93 ± 0.58</td>
<td>12.89 ± 0.53</td>
<td>7.13 ± 0.97</td>
<td>12.97 ± 0.58</td>
<td>13.06 ± 0.55</td>
<td>13.04 ± 0.55</td>
<td>12.99 ± 0.59</td>
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<tr>
<td>Belly, kg</td>
<td>22.02 ± 0.44</td>
<td>25.70 ± 0.39</td>
<td>26.75 ± 0.73</td>
<td>23.87 ± 0.40</td>
<td>24.00 ± 0.39</td>
<td>23.98 ± 0.39</td>
<td>23.90 ± 0.41</td>
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<td>Jowl, kg</td>
<td>5.40 ± 0.18</td>
<td>5.82 ± 0.16</td>
<td>5.54 ± 0.18</td>
<td>5.63 ± 0.18</td>
<td>5.62 ± 0.17</td>
<td>5.70 ± 0.17</td>
<td>5.55 ± 0.18</td>
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<tr>
<td>Kidney fat, kg</td>
<td>7.67 ± 1.06</td>
<td>6.44 ± 0.95</td>
<td>3.70 ± 1.76</td>
<td>6.60 ± 1.11</td>
<td>7.34 ± 1.06</td>
<td>7.67 ± 1.04</td>
<td>6.27 ± 1.12</td>
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<tr>
<td>FT Shoulder, mm</td>
<td>67.38 ± 2.75</td>
<td>58.97 ± 2.46</td>
<td>46.70 ± 4.59</td>
<td>59.46 ± 2.67</td>
<td>67.20 ± 2.56</td>
<td>63.75 ± 2.52</td>
<td>62.91 ± 2.70</td>
</tr>
<tr>
<td>FT Loin, mm</td>
<td>44.78 ± 2.49</td>
<td>38.79 ± 2.23</td>
<td>28.55 ± 4.15</td>
<td>39.14 ± 2.48</td>
<td>44.65 ± 2.37</td>
<td>41.73 ± 2.34</td>
<td>42.07 ± 2.50</td>
</tr>
<tr>
<td>FT Leg, mm</td>
<td>49.02 ± 2.41</td>
<td>39.44 ± 2.15</td>
<td>24.57 ± 4.01</td>
<td>42.87 ± 2.41</td>
<td>46.1 ± 2.31</td>
<td>47.31 ± 2.27</td>
<td>41.66 ± 2.43</td>
</tr>
</tbody>
</table>

* CC—Cold carcass, LC—Lean cuts; FC—Fatty cuts; FT—Fat thickness; Means with the same superscripts letters, within a factor, are not different (lower cases P < 0.05, and upper cases P < 0.01).

The hue angle showed a different behaviour and was significantly higher in CT×DU (P < 0.01) (Table 3), suggesting a shift within the red hue towards the yellow part of the spectrum (Dugan et al., 1997). According to Pugliese et al. (2005), the redder meat of AGT CT could be due to slower growth and higher slaughter age compared to crossbreed, with the older age groups reared under Open Air system (19–20 vs 15–16 months).
### Table 3. Effects of genotype, rearing system and gender on meat quality* (mean value ± SE)

<table>
<thead>
<tr>
<th>Trait</th>
<th>Genotype</th>
<th>Rearing system</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CT</td>
<td>CT×DU</td>
<td>Open Air</td>
</tr>
<tr>
<td>Hardness, kg</td>
<td>1.52^B ± 0.29^A ±</td>
<td>1.92 ± 0.14 ±</td>
<td>1.89 ± 0.22 ±</td>
</tr>
<tr>
<td>Cohesiveness, TU</td>
<td>0.579 ± 0.590 ±</td>
<td>0.564 ± 0.02 ±</td>
<td>0.605 ± 0.02 ±</td>
</tr>
<tr>
<td>Springiness, mm</td>
<td>11.83 ± 12.54 ±</td>
<td>12.07 ± 0.21 ±</td>
<td>12.29 ± 0.33 ±</td>
</tr>
<tr>
<td>Chewiness, TU</td>
<td>1025^B ± 1743^A ±</td>
<td>1334 ± 234 ±</td>
<td>1433 ± 133 ±</td>
</tr>
<tr>
<td>WBS, kg</td>
<td>6.87^b ± 9.23^a ±</td>
<td>6.77^b ± 0.55 ±</td>
<td>9.35^a ± 0.73 ±</td>
</tr>
<tr>
<td>L*</td>
<td>53.16 ± 55.20 ±</td>
<td>53.09 ± 1.31 ±</td>
<td>55.26 ± 1.26 ±</td>
</tr>
<tr>
<td>a*</td>
<td>6.66^a ± 3.42^b ±</td>
<td>4.76 ± 0.50 ±</td>
<td>5.31 ± 0.37 ±</td>
</tr>
<tr>
<td>b*</td>
<td>17.19^A ± 15.82^B ±</td>
<td>16.51 ± 0.31 ±</td>
<td>16.50 ± 0.23 ±</td>
</tr>
<tr>
<td>Chroma</td>
<td>18.51^A ± 16.25^B ±</td>
<td>17.29 ± 0.42 ±</td>
<td>17.47 ± 0.32 ±</td>
</tr>
<tr>
<td>Hue</td>
<td>1.21^b ± 1.36^a ±</td>
<td>1.30 ± 0.02 ±</td>
<td>1.27 ± 0.02 ±</td>
</tr>
</tbody>
</table>

*Means with the same superscripts letters, within a factor, are not different (lower cases P < 0.05, and upper cases P < 0.01); TU=Texturometer unit.

Regarding ‘Fiocco’ ham, the product obtained from CT (18.83 kg at dissection) at the end of seasoning showed greater hardness (4.65 vs 3.27 and 3.15 kg; P < 0.01) and chewiness values (P < 0.05), a lower springiness value (P < 0.05), a higher redness index (a* value) (P < 0.05) and a lower b* value (P < 0.01) while the product obtained from the CT×DU was significantly less red than PAL (P < 0.05) (Table 4). Carcass composition and meat quality (longissimus muscle) were not influenced by the rearing system, although the pigs that had access to bush showed a higher fat thickness at the shoulder region (67.20 mm vs 59.46 mm; P < 0.05) (Table 2).

### Table 4. Effects of genotype on qualitative traits of cured ‘Fiocco’ ham* (mean value ± SE)

<table>
<thead>
<tr>
<th>Traits</th>
<th>Genotype</th>
<th>PAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CT</td>
<td>CT×DU</td>
</tr>
<tr>
<td>Hardness, kg</td>
<td>4.65^A ± 0.265</td>
<td>3.15^B ± 0.28</td>
</tr>
<tr>
<td>Cohesiveness, TU</td>
<td>0.592 ± 0.016</td>
<td>0.624 ± 0.016</td>
</tr>
<tr>
<td>Springiness, mm</td>
<td>13.06^a ± 0.24</td>
<td>14.11^a ± 0.25</td>
</tr>
<tr>
<td>Chewiness, TU</td>
<td>3653^B ± 267</td>
<td>2913^a ± 278</td>
</tr>
<tr>
<td>L*</td>
<td>44.22^b ± 0.92</td>
<td>45.03^b ± 0.65</td>
</tr>
<tr>
<td>a*</td>
<td>3.44^a ± 0.62</td>
<td>1.96^b ± 0.43</td>
</tr>
<tr>
<td>b*</td>
<td>11.95^A ± 0.55</td>
<td>13.87^B ± 0.38</td>
</tr>
<tr>
<td>Chroma</td>
<td>13.10^B ± 0.57</td>
<td>14.26^AB ± 0.40</td>
</tr>
<tr>
<td>Hue</td>
<td>0.44 ± 0.23</td>
<td>0.73 ± 0.16</td>
</tr>
</tbody>
</table>

*Means with the same superscripts letters, within a factor, are not different (lower cases P < 0.05, and upper cases P < 0.01); TU = Texturometer unit.
According to Pugliese & Sirtori (2012), the effects of rearing systems on the physical traits of pork, both autochthonous and improved breeds, are often contradictory. Cinta senese breed showed higher percentage of intermuscular fat and bones in outdoor system than indoor reared pigs, also higher shear force and a* and lower L* value (Pugliese et al., 2005). Outdoor reared Nero Siciliano pigs produced lighter and yellower meat, probably due to their higher intramuscular fat content (Pugliese et al., 2004). Enfält et al. (1997) showed on outdoor crossbred pigs (Yorkshire, or Yorkshire × Landrace sows, and Duroc or Yorkshire as terminal sire) that this system produces leaner carcasses and meat with lower ultimate pH, higher drip loss, shear force, and higher internal reflectance values.

Gender did not significantly influence meat quality (Table 3) as well as the carcass cuts composition (Table 2). Castellano et al. (2007) on AGT CT, showed that gender did not affect significantly rheological characteristics of meat, but only colour, with a lower value of a* (P < 0.001) and a higher value of b* (P < 0.001) in entire females in comparison with castrated males.

CONCLUSIONS

The ancient autochthonous genetic type Casertana pigs confirm the aptitude to produce carcasses with significantly higher percentage of fat and better texture parameters of meat. These traits make the Casertana suitable for the production of high value-added processed products, like 'fiocco' ham.

The primary attributes of meat quality were not influenced by rearing system and gender, but the quality is a combination of real (objective) and perceived attributes of the product. Indeed the positive characteristics related to consumer perceptions, such as the environmental impact, cultural and socio-economic aspects of the production system, are high successful to guide the consumers choices.

ACKNOWLEDGEMENTS. The research was supported by Ministry of Agricultural Food and Forestry Policies (DM 2606-16.04.2008, reg 6401/1-19.07.2008).

REFERENCES


Detection of bacteriocin-producing lactic acid bacteria from milk in various farms in north-east Algeria by a new procedure

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¹University of Setif 1, Nature and Live Sciences Faculty, Department of Microbiology, 19000, Setif, Algeria; *Correspondence: dabhoc@yahoo.fr

Abstract. Twelve samples of bacteriocin-producing lactic acid bacteria were isolated from raw milk. The screening procedure has the advantage of differentiating directly on agar plates active colonies among the natural microbial population without subsequent culture. Five of milk isolates had effective inhibitory activity against Staphylococcus, Bacillus and all Listeria monocytogenes strains tested. In addition, two bacteriocinogenic isolates were effective against Gram-negative bacteria including Pseudomonas aeruginosa and Escherichia coli. The action of the bacteriocins was eliminated by a proteolytic enzyme. Simulation tests in liquid medium showed a 3 log reduction of Listeria growth in presence of bacteriocin during a period stockage of 14 days at 4°C.

Key words: bacteriocin, lactic acid bacteria, screening procedure, Listeria monocytogenes.

INTRODUCTION

Lactic acid bacteria (LAB) have long been used in the processes of fermentation (milk, meat, vegetables) by participating in organoleptic properties and by ensuring a better conservation of the products. This conservation is mainly due to the production of organic acids by these bacteria (lactic acid, acetic acid) with concomitant decrease of pH that inhibits undesirable contamination flora. Other LAB metabolites such as: hydrogen peroxide, diacetyl, some enzymes, antibiotics and reuterin can also contribute towards the overall preservative potential of these products. In addition, the LABs can synthesize and excrete antimicrobial compounds of a proteinaceous nature known as bacteriocins (Gibbs, 1987; Axelsson et al., 1993, O’Sullivan et al., 2002).

Research on bacteriocins has been the object of particular attention because of their potential advantages for applying them as natural food biopreservatives (Jeppesen & Huss, 1993; Deegan et al., 2006). Combination of these bacterial metabolites and traditional methods of conservation (heat and chemical treatments) led a higher inhibitory action than that of the hurdles applied separately, with the inherent improvement of nutritional and organoleptic quality of foods sanitized by combined treatments (Aymerich et al, 1998).

Some bacteriocins are inhibitory towards a broad spectrum of bacteria which include spoilage microorganisms responsible for modifications of food texture and food-borne pathogens such as L. monocytogenes and Clostridium botulinum (Blom et al., 1999). Since the bacteriocins may be hydrolysed in the human digestive tract and are active at low pH, these properties render them useful as substitutes of some antibiotics.
used for pharmaceutical purposes (Piard & Desmazeaud, 1992). Also, the emergence of bacterial resistance has lead researchers to combine efforts towards developing novel antimicrobial alternatives (Parisien et al., 2007)

The present report describes the development of an original and appropriate methodology for the detection of antimicrobial producing LAB in local fresh and fermented milks, for the purpose of selecting those with inhibitory effects against L. monocytogenes and/or other pathogenic/food borne microorganisms. This work also describes the simulation of the bacteriocin extracts activity in liquid medium and identification of producer LAB.

**MATERIALS AND METHODS**

**Strains and culture conditions**

Lactic acid bacteria (LAB) strains tested for antimicrobial activity were isolated from fresh and fermented milks (curdled milk and buttermilk). These milks came from various farms situated in north-east Algeria (Bazer, Mezloug, Bousselam). A total of 90 samples were collected in 3 farms at a rate of 30 samples per farm corresponding to 10 samples for each type of milk. Samples were collected aseptically in sterile flasks and then rapidly forwarded to the laboratory. The indicator strains used for screening bacteriocin detection are shown in Table 1. Listeria strains were cultured at 30°C in tryptone soya yeast (TSY), MRS or M17 for LAB at 30°C and in nutrient medium for the other types of organisms at an incubation temperature of 37°C.

**Screening for bacteriocinogenic lactic acid bacteria (LAB) by the mixture method**

The technique of detecting cells active against on indicator organisms was based on a novel approach which consisted of adding 0.5 ml of appropriate serial decimal dilutions of the milk to 2 ml of an overnight culture of L. monocytogenes CLIP74910 diluted first one hundred times in tryptone soya yeast (TSY) broth. Milk flora was appropriately diluted in order to obtain well isolated colonies. The surface of Petri dishes containing MRS or M17 agar was inoculated by spreading 0.1 ml of this mixture from every dilution. Milk and indicator organism were cultured at the same time contrary to the traditional procedure where the indicator organism is inoculated into soft agar that is then poured over the plate onto which the milk flora has been grown. Incubation was carried out at 30°C over periods of few days to one week in the search for inhibition zones.

Each whitish colony presenting an inhibition zone was isolated and inoculated into MRS or M17 broth. After incubation from 1 to 2 days at 30°C, a fraction of the culture was streaked onto MRS agar to verify the purity of the isolated cultures. After a second subculture in MRS broth, cultures were preserved at -15°C in cryotubes containing MRS or M17 broth supplemented with 15% of glycerin until use.
Detection of antagonistic activity of isolated cultures

Supernatant fluids were obtained by growing thenhibitory producer strains overnight in MRS or M17 broth. After incubation at 30°C over 18 to 20 h, the cultures were centrifuged and the cell-free supernatant recovered and divided into aliquots that were untreated (crude extract), lyophilized, precipitated with ammonium sulfate subjected to adsorption-desorption method as described by Yang, R. et al. (1992).

Table 1. Indicator strains and origin

<table>
<thead>
<tr>
<th>Strains</th>
<th>References</th>
<th>Origin</th>
</tr>
</thead>
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<tr>
<td><em>Listeria monocytogenes</em></td>
<td>CLIP 74910</td>
<td>Pasteur Institute, Paris, France</td>
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<tr>
<td><em>Listeria monocytogenes</em></td>
<td>CLIP 74904</td>
<td></td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>CLIP 74903</td>
<td></td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>CLIP 74902</td>
<td></td>
</tr>
<tr>
<td><em>Listeria ivanovii</em></td>
<td>CLIP 12229</td>
<td></td>
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<tr>
<td><em>Lactobacillus casei</em></td>
<td>subsp.B445</td>
<td>Process Engineering Laboratory, Nancy University, France</td>
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<tr>
<td><em>Bacillus rhamnosus</em></td>
<td></td>
<td></td>
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<td><em>Escherichia coli</em></td>
<td>CIP 7424</td>
<td>Department of Biology, Setif University, Algeria</td>
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<tr>
<td><em>Staphylococcus aureus</em></td>
<td>CIP 7625</td>
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<td><em>Pseudomonas aeruginosa</em></td>
<td>76110</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
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<tr>
<td><em>Escherichia coli</em></td>
<td>K12</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas syringae</em></td>
<td>11</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus megaterium</em></td>
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<tr>
<td><em>Escherichia coli</em></td>
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<td><em>Escherichia coli</em></td>
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<td><em>Escherichia coli</em></td>
<td>931</td>
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<tr>
<td><em>Escherichia coli</em></td>
<td>ATCC 122</td>
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<td><em>Escherichia coli</em></td>
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<tr>
<td><em>Pseudomonas aeruginosa</em></td>
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<tr>
<td><em>Pseudomonas aeruginosa</em></td>
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<td></td>
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<tr>
<td><em>Staphylococcus aureus</em></td>
<td>290</td>
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<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>766</td>
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<td><em>Staphylococcus aureus</em></td>
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<tr>
<td><em>Bacillus sp.</em></td>
<td>19</td>
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<tr>
<td><em>Proteus mirabilis</em></td>
<td>198</td>
<td>Parasitology Laboratory, El Eulma Hospital, Algeria</td>
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<tr>
<td><em>Staphylococcus aureus</em></td>
<td>71</td>
<td></td>
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<tr>
<td><em>Staphylococcus aureus</em></td>
<td>76</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus sp.</em></td>
<td>105</td>
<td></td>
</tr>
</tbody>
</table>

For the first method, 50 ml aliquots of cell-free cultures were lyophilized (freezing step at -15°C during 24 h; sublimation step for 24 h) and suspended in 5 ml of distilled water (LS supernatant). The ammonium sulfate precipitation of cell-free supernatants was performed as follow: a volume of 50 ml of culture supernatant were made up to 60% saturation by addition of ammonium sulfate and kept overnight at 4°C with gentle stirring. After centrifugation (10,000 × g, 20 min, 4°C), the sedimented pellet was recovered and suspended in 3 ml of 0.1 M potassium phosphate buffer at pH6 (ASPS supernatant). For adsorption-desorption method, a 100 ml of supernatant culture was

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used and its pH adjusted at 6.5 to allow adsorption of the bacteriocin to the wall of the producer cell. Then, a temperature of 70°C for 30 min was applied to the culture to kill cells and to inactivate proteolytic enzymes. Cells were then removed by centrifugation at 10,000 × g (20 min, 4°C), and washed twice with 5 mM sodium phosphate buffer at pH 6.5. Cell precipitates were suspended in 5 ml of 100 mM NaCl solution adjusted to pH 2 for allowing desorption of the bacteriocin. Stirring was applied for 2 hour at 4°C and the supernatant (ADS) was recovered after centrifugation at 18,000 × g (30 min, 4°C).

To exclude inhibitory effects of hydrogen peroxide or organic acids, the cell-free extract solutions were dialyzed overnight at 4°C by a dialysis membrane with a 3.5 kDal cutoff against 1.0 liter of distilled water with two changes of distilled water. After dialysis, the solution in the dialysis bag was filter-sterilized (0.2 µm pore-size filter) or heated (70°C, 20 min). Samples were stored at -15°C until use.

The cell-free extracts were tested for bacteriocin activity against indicator bacteria by using agar diffusion methods (agar spot test or agar well test). The agar spot technique was performed as follows: a fraction of 0.1 ml of an overnight culture of indicator bacteria was poured onto an appropriate medium agar plate. Then, one drop of each supernatant fluid with antibacterial activity was spotted on the plate. After incubation for 24h at temperatures optimal for the indicator bacteria, inhibition was indicated by a clear zone around spots (Yang et al., 1992; Cintas et al., 1998). Concerning the agar well test, a quantity of 20 ml of TSY agar was poured onto a sterile Petri dish. Then, the plate was recovered with a 0.3 ml of molten agar (0.7% agar) inoculated with indicator organisms. Wells of uniform diameter (6 mm) were bored in the agar. Aliquots (150 µl) of the tested cell-free supernatants or of positive and negative controls were dispensed in wells, and plates were incubated overnight at optimal temperature during 24 h. Inhibition of growth was determined by an area of inhibition surrounding each agar well (Herranz et al., 2001).

**Sensitivity to heat and pronase**

Cell-free extracts previously concentrated by the adsorption-desorption method were subjected to heating (63 and 70°C for 30 min; 80; 90 and 100°C for 10 min) and protease treatment (100 µl of pronase E at 2 mg ml⁻¹ added to 100 µl of bacteriocin solution and incubated at 37°C for 1 h). The residual activity was measured by the agar spot test against a sensitive indicator lawn. An untreated preparation of bacteriocin served as the control.

**Identification of bacteriocinogenic cultures**

Cultural, morphological and physiological characteristics of selected isolates and their behavior in certain physico-chemical conditions were determined. Identification tests enclosed Gram coloration, mannitol mobility, catalase, cytochrome-oxydase, peroxydase, sugar fermentation profiles and fermentative type. The fermentation of sugars included glucose, lactose, galactose, fructose, saccharose and glycerin. Development of strains was verified under a wide range of temperature, pH and NaCl concentrations.
Proteins quantification of bacteriocins extracts

Considering that bacteriocins are of protein nature, their quantity was estimated according to Macart et al. (1986) method for quantification of protein by using the following reagent: 0.004% (P per V) of blue of Coomassie G 250; 4% (vol vol\(^{-1}\)) of ethanol (96%); 0.003% (wt vol\(^{-1}\)) SDS and 10% (vol vol\(^{-1}\)) of phosphoric acid (85%). This product was found to be stable for over 3 months. Serum bovine albumin (BSA) dissolved in distilled water at 2 mg ml\(^{-1}\) served as a standard. Fractions of 100 µl of standard solutions and bacteriocin samples at appropriate dilutions were added to 2 ml of reagent and mixed. After 10 minutes, the optical density (OD) of the mixture was measured at 595 nm by using a spectrophotometer (Genesys).

Bacteriocin sensitivity measurement

The growth rate of _L. monocytogenes_ CLIP74910 on TSY in presence or absence of bacteriocin were estimated according to the method of Huang et al. (1994). The tubes containing 10 ml of TSY broth were inoculated with 100 µl of overnight _Listeria_ cultures corresponding approximatively to a final population of 10\(^6\) UFC ml\(^{-1}\) and, to which we added 0.2 ml of bacteriocin preparation. A tube without addition bacteriocin extract served as a negative control. Because _Listeria_ is a psychrophilic bacterium, tubes were placed in refrigeration at 4°C, and the OD was measured at 600 nm at time 0 and at 2 days intervals until 14 incubation days. Cultures at 37°C were also performed and samples were removed at different time intervals for OD measurements at 600 nm and for viable counts. Measurements of plating counts and OD were carried out at 37°C for 0, 4 h, 8 h, 24 h, 30 h, and 48 h on _Listeria_ cultures in order to establish a standard curve.

RESULTS AND DISCUSSION

The detection of antibacterial-producing strains in milk and fermented milks was performed directly on MRS and M17 agar by inoculating these media with a combination of an appropriate dilution of the product under study and an overnight indicator culture of _L. monocytogenes_. The colonies showing halos after incubation of plates at 30°C for 2–7 days were selected and, their visualization is reported in Fig. 1. A total of 40 and 10 strains grown on MRS and M17 agar respectively, exhibited inhibitory activity against _L. monocytogenes_ strain (results not shown). Only 30 isolates were found by the agar spot assay to secrete antibacterial substances into the growth medium that could be detected without concentration of the supernatant fluids. Results also shows that twelve of these isolates secreted antibacterial compounds that preserved their inhibitory effect after dialysis. This fact and their sensitivity to pronase and the positive correlation between inhibitory activity and protein concentration suggest that the 12 supernatants are bacteriocins.

Their activity was stable under heating (60°C for 30 min or 100°C for 10 min). Such temperature stability is very convenient to use the bacteriocin extracts as a food preservative since many processing procedures involve a heating treatment. Many authors have demonstrated bacteriocin resistance to pasteurization and high temperature (121°C for 30 min) (Deraz et al. 2005). Thermoresistance is a characteristic of lactic acid bacteria bacteriocins having low-molecular-weight and a simple structure (Desmazeaud, 1994).
A wide range of Gram-positive and Gram-negative bacteria were used to check the inhibition spectrum of these 12 supernatants. Results are shown in table 2. Inhibition activity was demonstrated by test strains against practically all tested and 40Z had the advantage of being effective against Gram-negative bacteria so inhibiting some strains of *Pseudomonas*, *Klebsiella* and *E.coli*. Most of the bacteriocins produced by LAB are active only against LAB and other gram-positive bacteria (Line et al., 2008; Ray, 1996; Bhunia et al., 1991). None of the tested strains displayed inhibitory activity against *Lactobacillus casei* subsp. *rhamnosus* and *Proteus mirabilis*. *Listeria*, *Staphylococcus* and *Bacillus* strains. In addition, two bacteriocin extracts from isolates 28Z.

![Screening of bacteriocinogenic strains from milk by mixture method.](image)

The resistance of Gram-negative bacteria to bacteriocins seems to be due to the complexity of their cellular wall in comparison to Gram-positive bacteria, containing lipopolysaccharides (LPS) which are absent in Gram-positive bacteria. The results of the present work suggest that the 28Z and 40Z antibacterial extracts could destabilize the LPS layer of Gram-negative bacteria (Kalchayanand et al., 1992; Motta et al., 2008) and, correspond qualitatively to those described by several authors. Rodriguez et al. (2005) described a slight inhibitory effect of bacteriocins of LAB in cheeses on the survival of *E. coli*, which cannot be related to differences in pH values. The authors attributed the effect to a higher sensitivity of injured cells of Gram-negative bacteria to bacteriocins, an injury due to a prolonged acid exposition at the low pH values of cheese (Kalchayanand et al., 1992). Tan et al. (2000) described inhibition of both an ampicillin resistant *E. coli* and a *Salmonella typhii* strains by a bacteriocin produced by *Enterococcus faecalis VRE* 1492. Ponce et al. (2008) described similar inhibition activity on Gram-negative foodborne pathogens for some LAB strains from organic vegetables. In recent study, LAB isolated from olives had developed antimicrobial activity against *Listeria, E. coli* and *Enterococcus* strains (Gaamouche et al., 2014).
Concerning antimicrobial substances lost after dialysis for 38 remainder supernatants, it is probable that are formed by organic acids, hydrogen peroxide (Muriana & Luchansky, 1993) or non-proteinaceous low molecular mass compounds (LMMC) (Niku-Paavola et al., 1999) capable to cross the 3.5 kDa cutoff dialysis membrane used.

Table 2. Inhibitory spectrum of the antibacterial substances produced by selected isolates.

<table>
<thead>
<tr>
<th>Indicator strains</th>
<th>1Z</th>
<th>7Z</th>
<th>9Z</th>
<th>12Z</th>
<th>15Z</th>
<th>23Z</th>
<th>28Z</th>
<th>32Z</th>
<th>36Z</th>
<th>40Z</th>
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<td>Pseud. aeruginosa 254</td>
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<td>Pseud. aeruginosa 152</td>
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+ inhibition of indicator strain;
- absence of inhibition of indicator strain;
± weak inhibition.

Fig. 2 shows the inhibitory activity of 5 isolates exhibiting the strongest activity against Listeria when the agar well diffusion test was used. According to physiological assays, morphological characteristics and carbohydrate fermentation of these isolates (data not shown), all of them produce whitish, bulging and mucous colonies similar to those of lactic acid bacteria on MRS or M17 agar plate, are Gram-positive, catalase-
negative, oxydase-negative, benzidine-negative and non motile. On the basis of their biochemical and morphological characteristics, strains 1Z and 28Z seem to be, respectively, identical to *Pediococcus acidilactici* and *P. pentosaceus*, the strain 7Z to *Streptococcus thermophilus*, the strain 15Z to *Lactococcus plantarum* and the strain 40Z to *Lactobacillus* sp.

![Figure 2](image.png)

**Figure 2.** Activity of bacteriocin extracts against *Listeria monocyogens* CLIP 74910 using agar well diffusion assay. Bacteriocin extracts are adsorbed/desorbed, dialyzed and filter-sterilized before testing against indicator lawn. Cn – negative control (sterile MRS broth); Cp – positive control (filter-sterilized Nisaplin solution at 10%).

The growth patterns of four strains of *L. monocytogenes* and one strain of *L. ivanovii* in TSY broth subjected to antibacterial compounds derived from supernatant broths of the active strains B1Z, B7Z, B15Z, B28Z, B40Z were also determined. The results are shown in Fig. 3. All *Listeria* strains grew in TSY with/without the antibacterial substances produced by either isolates. However, The OD measured in TSY supplemented with antibacterial substances produced by either isolates were significantly lower than O.D. detected in the control TSY at the initiation of the experiment and at the end of incubation period. Analysis was done within 24 h old-cultures indicating that the anti-*Listeria* effects of either supernatant manifested themselves rapidly. After this initial reduction, the population of *Listeria* remained essentially constant throughout the first 3 days. Thereafter, OD values increased progressively to 14 days with values fluctuating approximately from 0.02 to 0.15. By way of contrast, in the absence of bacteriocin, values of OD are much higher and vary from 0.02 to 0.55 with an acceleration of growth rate after 5 at 7 days incubation depending on *Listeria* strain tested. This acceleration is seen from an inflection on the growth curve. OD values of controls are about 3.5 to 5 times higher than OD of strains in presence of bacteriocins at the same incubation period.
Figure 3. Populations of *Listeria monocytogenes* strains 74903 (a); 74910 (b); 743904 (c); 74902 (d) and *Listeria ivanovi* 12229 (last graph) incubated at 4°C for up to 14 days in TSY supplemented with bacteriocin preparations derived from 5 selected isolates.
In this study, bacteriocin extracts inhibited markedly the growth of *L. monocytogenes* strains tested in liquid media. The reduction of *Listeria* populations in presence of bacteriocins is about of 3 log as according to the standard curve obtained (data not shown). Similar results were obtained by Huang and al. (1994) having used milk which was experimentally contaminated with *Listeria* cultures in presence and in absence of pediocin 5. In another study, tests were made on raw milk contaminated artificially by *L. monocytogenes* in both the presence and in the absence of a bacteriocin produced by *Carnobacterium piscicola JG 126*. Under these conditions, piscicolin 126 reduced the number of *Listeria* from 4 to 5 log during the first day of storage (Wan et al., 1997). However, the *Listeria* growth appeared again after an incubation period of 24 h. This phenomenon was also observed for the nisin with apparition of mutants resistant to this bioactive component (Wan et al., 1995). As reported by Altuntas et al. (2012), all *L. monocytogenes* isolated from foods were sensitive to the cell-free supernatant of a bacteriocin-producing strain *P. acidilactici* while some *Listeria* strains were resistant to antibiotics. The results suggest the possibility to use the bacteriocinogenic strains or their bacteriocins as supplements to food in order to reduce unwanted contamination.

**CONCLUSION**

The used method allowed direct detection of isolates with antagonistic activity onto agar plate by facilitating the distinction of active colonies among the microbial population. This screening procedure is easy to execute and could be used as an alternative method to traditional antagonism tests. Further research is needed to identify compounds produced by the selected LABs, their purification and sequencing. This type of work is in progress in our laboratory. Bacteriocins active against pathogens and food spoiling microorganisms are presented as an interesting alternative to chemical preservatives in a variety of industrial applications.

**REFERENCES**


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Functional properties of tarhana enriched with whey concentrate

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Abstract. Whey concentrate is often sold as a nutritional supplement and include proteins, minerals, vitamins and other components (low levels of fat and low levels of lactose). Whey concentrates is well known for their high nutritional value and versatile functional properties in food products. The aim of this study was to enrich tarhana by using whey concentrate (WC) instead of yoghurt. Foaming capacity and foam stability, water and oil absorption capacity, emulsifying activity as a functional properties, colour properties and sweetness, body-texture, colour-appearance, mouthfeel, acerbity, homogeneity, consistency and overall acceptability as a sensorial characteristics of tarhana samples were determined. The highest foaming capacity values were obtained with 50% WC substitution, but 50% WC addition gave the lowest foam stability values. Oil absorption capacity and emulsifying activity values of tarhana samples were decreased by increasing WC levels. Tarhana samples containing WC were lighter according to colour values than the control tarhana samples made with yoghurt. Tarhana soup prepared with 12.5% WC addition was similar to the control in homogeneity and overall acceptability.

Key words: tarhana, whey concentrate, color, foaming capacity, water absorption capacity, sensorial characteristics.

INTRODUCTION

Tarhana, which is prepared by fermenting drain derivates combined with yoghurt and other flavoring ingredients, and drying and grinding into granulated form. Tarhana powder, a semi ready traditional food product in Turkey, is used in soup making (İbanoğlu et al., 1995; Dağlıoğlu, 2000). It is a good source of proteins, vitamins and minerals, and therefore is used largely for feeding people of all age in the form of soup (Tarakçı et al., 2004; Bilgiçli et al., 2006). Tarhana powder has an acidic sour taste with a yeasty flavor due to the its low pH (3.4–4.4) and it also has low moisture content (6–10%) which is a poor medium for pathogens and spoilage organisms (İbanoğlu & İbanoğlu, 1997; Dağlıoğlu, 2000; Erkan et al., 2006).

Whey is a major by-product of dairy industries in manufacturing cheese, casein etc. Whey solids contain half of the precious milk solids, and are not yet fully utilized in various food formulations. Thus, large quantities of whey are drained off and disposed as waste (Khamrui & Rajorhia, 1998). Whey products contain proteins, a wide variety of minerals, vitamins and other constituents such as lactose and lipids. Whey proteins are the best quality proteins available. They have high protein efficiency ratio (PER =
3.6) and possess all essential amino acids. Whey proteins are used as food ingredients because of their unique nutritional and functional characteristics like emulsifying, gelling, thickening, foaming and water binding capacity (Kinsella & Whitehead, 1989). An important functional property of whey proteins is the ability, under appropriate conditions, to form heat induced gel structures capable of immobilize large quantities of water and other food components (Hermansson & Akesson, 1975). They can modify some or all of the organoleptic, visual, hydration, surfactant, structural, textural and rheological properties of food, resulting in improved consumer acceptance of the food product. Whey proteins in their native state are highly soluble in food and beverage systems and are used for applications such as whipping/foaming. They act at oil/water interfaces to form and stabilize emulsions. In their undenatured form, whey proteins have the ability to form rigid, heat-induced irreversible gels that hold water, fat and provide structural support. In addition, they play an important role in controlling the texture of many food products, and are used to modify the rheological properties of foods (Harper, 2000; Hudson et al., 2000).

Quality tarhana soup has to be good in homogeneity and mouth feeling in sensory properties, with phase separation as long as possible. Yoghurt used in tarhana products is very well in sensory properties in consumer acceptance and is a healthy and nutritious food (Tamime & Robinson, 1999; McKinley, 2005). In literature soy yoghurt (Koca et al., 2002) corn flour and whey (Tarakçı et al., 2004), rye, maize, soy bean (Hafez & Hamada, 1984, Öner et al., 1993, Köse & Çağındı, 2002), oat and barley (Tamime et al., 1997, Erkan et al., 2006) wheat germ and bran (Bilgiçli et al., 2006) buckwheat (Bilgiçli, 2009) whole wheat meal and bulgur (Toufeili et al., 1998) were used instead of cow’s milk yoghurt and wheat flour.’ And also in our another study, Ertaş et al. (2009) reported that it is possible to use of whey concentrate (WC) instead of yoghurt as an ingredient in tarhana making, with its high mineral and lactose contents and low fat and acidity. The purpose of this study was to enrich tarhana by using WC instead of yoghurt and, to determine the effect of WC addition on the functional and sensory properties of tarhana samples and to use waste material in tarhana production instead of yoghurt as a dairy by product.

MATERIALS AND METHODS

Materials

The ingredients used in tarhana preparation were purchased from local markets in Konya, Turkey. To prepare tarhana, commercial wheat flour (Selva Food Industry Inc.) with a crude protein content of 11.54% (Nx5.7, w w⁻¹, dry basis), pepper (Capsicum annuum) paste had 22% total dry solids (TDS) and medium sized dry onions (Allium cepa) were used. The spices used were in powder form (i.e., salt, paprika (Capsicum annuum)). The yoghurt was full-fat strained yoghurt (concentrated, ‘Süzme’ yoghurt) made of cow’s milk. WC and yoghurt were obtained from ENKA Dairy Plant, in Konya, Turkey. WC is a byproduct of ‘white cheese’ production as rennet coagulated and non-cooked curd type cheese, like ‘feta’. WC is produced with the integration of nano-filtration (15–20% TDS) for pre-concentration, and falling film evaporators (60–65% TDS).
**Preparation of tarhana samples**

To prepare control tarhana sample at laboratory conditions, wheat flour (200 g), yoghurt (80.0 g, having approximately 25 g of total solid content corresponding 12.5% (w w\(^{-1}\)) based on the 200 g flour), pepper paste (20 g), finely chopped onions (30 g), paprika (4 g), and salt (2 g) were mixed using a Hobart mixer for 5 min at the highest speed with distilled water (100 ml) added.

The other tarhana samples supplemented with WC were prepared as described above, with the additions of WC of 12.5% (40.84 g), 25.0% (81.68 g) and 50.0% (163.36 g) (w w\(^{-1}\)), instead of yoghurt. The resultant mixtures were placed in sealed plastic container and fermented at 30 °C for 7 days.

During the fermentation, the mixture was mixed manually at every 12 hr intervals. Each fermented mixture was divided into 2 cm diameter pieces by hand, placed on aluminum trays and dried at 55 ºC for 48 hr to 6% moisture (w w\(^{-1}\), dry basis) in an air convection oven (Özköseoğlu PFS-9, Turkey). The dried samples were ground into granulated form in a hammer mill (FN-3100 Laboratory Mill; Perten Instruments AB) equipped with 1 mm opening screen. Tarhana samples were kept in closed glass containers at room temperature until used for analysis.

**Chemical properties**

The AOAC methods were used for the determination of total solid content (%), crude protein (N x 6.38), crude fat (%), ash content, titratable acidity (as % lactic acid), pH and salt (%) for yoghurt and WC (AOAC, 2000).

**Color measurement**

Color measurement was performed on tarhana powder using a Hunter Lab Color Quest II Minolta CR 400 (Konica Minolta Sensing, Inc., Osaka, Japan). The \( L^* \), \( a^* \) and \( b^* \) were determined according to the CIELab color space system, where \( L^* \) corresponds to light/dark chromaticity, \( a^* \) to green/red chromaticity and \( b^* \) to blue/yellow chromaticity with illuminate D63 as reference. From \( a^* \) and \( b^* \) values, the hue angle \((\tan^{-1} b^*/a^*))\) and chroma (SI) \((\sqrt{a^{*2}+b^{*2}}))\) were calculated.

**Foaming capacity and foam stability**

The method of Hayta et al., (2002) was used for foaming capacity and foam stability. 10 g tarhana powder was dispersed in 25 ml distilled water and stirred for 20 min by means of a magnetic stirrer. The mixture was centrifuged (Nüve, NF 800 R) at 4000 \( \times \) g for 20 min. Supernatant obtained was filtered (Whatman No. 1) and transferred to a Waring blender and whipped for 2 min at high-speed setting. The solution was slowly poured into a cylinder, and the volume of the foam was recorded after 10 s. Foaming capacity was expressed as the volume (mL) of gas incorporated per mL of solution. Foam stability was recorded as the time passed until the half of the original foam volume had disappeared.

**Water and oil absorption capacity**

The method of Hayta et al., (2002) was used for determination of water and oil absorption capacity of tarhana powder. Tarhana powder (5.0 g) with 25 ml distilled water or with 25 ml sunflower oil was mixed at 25°C in 50 mL centrifuge tubes. Dispersions
were stirred and then centrifuged. Water and oil absorption capacity values were expressed as grams of water or oil absorbed per gram of tarhana.

**Emulsifying activity**
Tarhana powder (10 g) was dispersed in 25 ml distilled water at 25°C and stirred for 20 min and then centrifuged at 4000 × g for 20 min. Supernatant obtained were mixed with equal volumes of sunflower oil and homogenized for 5 min at low-speed setting in a Waring blender. The homogenized mixture was transferred into a measuring cylinder. Emulsifying activity was expressed as percent volume of the emulsified layer in total volume of the mixture (Hayta et al., 2002).

**Sensorial characteristics**
To determine sensory properties of tarhana soup, 25 g tarhana powder (dry basis) was mixed with 250 ml distilled water (10°C) and simmered for 10 min over medium heat with constant stirring. Seven panelists from Food Engineering Department who were familiar with tarhana soup were asked to score the tarhana soups in terms of sweetness, body-texture, colour-appearance, mouth feel, acerbity, homogeneity, consistency and overall acceptability using 10 point hedonic scale with 1–2 dislike, 5–6 acceptable, 9–10 like extremely. The samples were coded with numbers and served to the panelists at random to guard against a bias.

**Statistical Analysis**
The data obtained was summarized by one way ANOVA according to factorial design application. Costat software was used to perform the statistical analysis. Duncan’s multiple range tests were used to differentiate among the mean values. Standard deviations were calculated using the same software (Costat, 1990).

**RESULTS AND DISCUSSION**

**Analytical Results**
The chemical compositions of the yoghurt and WC used in tarhana formulation were presented in Table 1. The solid amount of WC was twofold of the yoghurt’s. The protein amount and acidity values of WC were lower than those of yoghurt. The fat

<table>
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<th>Components</th>
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<th>WC</th>
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<tr>
<td>Total dry solids %</td>
<td>30.50 ± 0.07</td>
<td>61.19 ± 0.08</td>
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<td>Crude protein (N x 6.38) %</td>
<td>7.50 ± 0.24</td>
<td>5.83 ± 0.31</td>
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<tr>
<td>Crude fat %</td>
<td>3.80 ± 0.06</td>
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<td>Ash content %</td>
<td>2.20 ± 0.04</td>
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<td>Titratable acidity (as lactic acid) %</td>
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<td>0.13 ± 0.02</td>
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<td>pH</td>
<td>4.10 ± 0.04</td>
<td>5.48 ± 0.02</td>
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<tr>
<td>Salt %</td>
<td>nd 3</td>
<td>3.48 ± 0.07</td>
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1Values represent the mean of triplicate determinations for each sample, 2based on dry matter, 3nd: not determined.
content of WC is about 1/3 of that of yoghurt. In ash contents, WC was superior due to mineral content. In mineral content, WC has more Ca, K, Mg, Na and P and Zn contents than yoghurt (data not shown), and WC is superior for crude ash and salt (NaCl) involvement. Also chemical and nutritional advantages of this study are mentioned in our previous study (Ertaş et al., 2009).

**Color of tarhana powder**
The attractiveness and brightness of tarhana powder and its soup in the color coming from the natural ingredients, is one of the main quality factors. Color values of the tarhana samples were shown in Fig.1. The color parameters, $L^*$, $a^*$ and $b^*$ values ranged between 71.45–78.11; 7.19–9.13; and 20.49–24.28 respectively. The results agree with those of previous studies conducted by Bilgiçli & Elgün (2005), and Erkan et al. (2006). WC addition showed the higher $L^*$ and lower $a^*$ values than yoghurt addition. The color parameters $a^*$ and $b^*$ tended to decrease, whereas parameter $L^*$ tends to increase with increasing WC levels. WC addition led to more bright and attractive color (reddish orange tarhana color depending on the tarhana formulation) to tarhana powder. The chroma value (colour intensity or saturation) of control was 25.94, which was higher than those of 12.5% WC (23.09), 25.0% WC (22.16) and 50% WC (22.07). The highest hue angle value was obtained with 50% WC addition.

![Figure 1. The effects of WC (%) on the color of tarhana solid ($L^*$ = brightness, $a^*$ = redness, $b^*$ = yellowness, Chroma = $((a^*^2 + b^*^2)^{1/2})$, Hue angle = ($\tan^{-1} b^*/a^*$).](image)
Foaming capacity and stability

The all functional properties of the tarhana samples were presented in Table 2. The foaming capacity and stability of tarhana samples as a measure of viscose structure stability ranged between 0.55–0.77 (ml ml⁻¹). Significant differences (P < 0.01) were observed in the foaming capacity of tarhana samples with different WC content. The WC addition in tarhana samples increased the foaming capacity while decreasing foam stability of the samples. Some food proteins are competent of forming good foams and their capacity to form and save stable foams depends on the type and concentration of proteins, the degree of denaturation, pH, ions, and processing methods (Işık and Gökalp, 1996; Dağlıoğlu, 2000). 50% WC addition into tarhana recipe formed the least foam stability. Sosulski & McCurdy, (1987), reported that the loss of soluble low-molecular weight proteins is responsible to less foam stability. In this study, lower foam stability values were determined WC added tarhana samples compared to control. It may be due to the low-molecular weight proteins of WC than casein proteins of yoghurt.

Water absorption capacity

In viscous foods, the water absorption capacity is an important functional property (Sosulski et al., 1976). The water absorption capacity ranged from 0.41–0.73 ml g⁻¹. (Table 2). Bilgiçli (2009) reported that water absorption capacity values of tarhana samples enriched with buckwheat flour ranged from 0.50 to 0.63ml g⁻¹. WC addition decreased the water absorption capacity of the tarhana samples. This functional property depends on the protein content but mainly on the physical interactions between water and protein (Cheftel et al., 1989).

Table 2. The effect of WC (%) on the functional properties of tarhana solid¹ ²

<table>
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<tr>
<th>Samples</th>
<th>FC (ml ml⁻¹)</th>
<th>FS (min)</th>
<th>WAC (ml g⁻¹)</th>
<th>OAC (ml g⁻¹)</th>
<th>EA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.55 ± 0.01c**</td>
<td>7.09 ± 0.05a**</td>
<td>0.73 ± 0.01a**</td>
<td>0.76 ± 0.01b*</td>
<td>94.05 ± 0.81ab*</td>
</tr>
<tr>
<td>WC 12.5</td>
<td>0.62 ± 0.02bc</td>
<td>2.21 ± 0.03b</td>
<td>0.56 ± 0.03b</td>
<td>0.93 ± 0.04a</td>
<td>95.82 ± 0.05a</td>
</tr>
<tr>
<td>WC 25.0</td>
<td>0.69 ± 0.01ab</td>
<td>0.42 ± 0.05c</td>
<td>0.44 ± 0.01c</td>
<td>0.82 ± 0.04ab</td>
<td>93.79 ± 0.05b</td>
</tr>
<tr>
<td>WC 50.0</td>
<td>0.77 ± 0.02a</td>
<td>0.29 ± 0.02c</td>
<td>0.41 ± 0.02c</td>
<td>0.77 ± 0.02b</td>
<td>93.52 ± 0.06b</td>
</tr>
</tbody>
</table>

¹Means with different superscripts in the same column are significantly different (P < 0.01).
²Values are the average of triplicate measurements on duplicate sample ± standard deviation.
³FC – Foaming Capacity, FS – Foam Stability, WAC – Water Absorption Capacity, OAC – Oil Absorption Capacity, EA – Emulsifying Activity, * P < 0.05, ** P < 0.01.

The low water absorption capacity values can be explained by structural changes in starch and proteins present in tarhana ingredients (Pyler, 1982). The control tarhana sample with yoghurt gave the highest water absorption capacity values (0.73 ml g⁻¹). This can be attributed to the high casein content and acidity of yoghurt used in tarhana production (Table 1). Casein with gel structure absorbs and binds much more water than the serum proteins, and proteolytic activity can cause more swelling capacity of the dough components in acidic medium of the yoghurt (Muir et al., 2000). Similar results were also reported by some researchers (Çelik et al., 2005). Hayta et al. (2002), reported the water absorption capacity of yoghurt between 0.45–2.28 ml g⁻¹ in tarhana made with different drying methods.
**Oil absorption capacity**

This property is important in the prevention of phase separation and in sensory properties. Moreover, oil absorption capacity shows the degree of hydrophobicity of the food system. It is provide that fat distribution as homogeneous due to the increase of fat globules stuck tarhana granules in tarhana. Oil absorption capacity of tarhana samples varied between 0.76 to 0.93 ml g\(^{-1}\) (Table 2). 12.5% WC added tarhana samples gave the highest oil absorption capacity (0.93 ml g\(^{-1}\)). Also 25% of WC addition gave higher oil absorption capacity than that of yoghurt added control samples.

**Emulsifying activity**

The emulsifying activity related to the area of stabilized oil droplets at interface. Therefore it is a function of the oil content and protein concentration (Hayta et al., 2002). In this research, the mean value of emulsifying activity of tarhana samples was 94.29%.

Hayta et al. (2002) and Çelik et al. (2005) reported that emulsifying activity of tarhana samples were 82.6 to 90.0% and 89.0 to 91.5% respectively. As shown in Table 2, 12.5% WC addition gave the highest emulsifying activity values (95.82%), which were also higher than the values of control samples. More than 12.5% WC supplementation into tarhana formulation decreased the emulsifying activity sharply. WC supplementation up to 12.5% levels was more satisfying than yoghurt addition. The probable factors in these results are the increase in the foaming and oil absorption capacities of tarhana samples with 12.5% WC level (Table 2). Decreasing the foam stability at over the 12.5% WC addition level in tarhana formulation, can cause to release sticking the fat globules stuck foam and a phase separation problem. The correlation coefficients given in Table 3 show that the oil absorption capacity of tarhana solid is the most effective factor on emulsifying activity.

**Sensory properties of tarhana**

The sensory properties of tarhana soups were illustrated in Fig. 2. The results of the sensory evaluations showed that utilization of WC in tarhana preparation resulted acceptable soup properties compared to traditional yoghurt used tarhana. The samples containing WC was lighter according to color values than the control tarhana samples with yoghurt. Tarhana soup prepared with 12.5% WC addition was similar to the control in homogeneity and overall acceptability. High levels of WC increased sweetness and decreased the overall acceptability, but not contributed to the other parameters. Tarhana soups with 12.5% WC were more liked by panelists than control and further WC added

**Table 3. Correlation coefficients among the functional properties of tarhana solids**

<table>
<thead>
<tr>
<th></th>
<th>FC</th>
<th>FS</th>
<th>WAC</th>
<th>OAC</th>
<th>EA</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FS</td>
<td>-0.876**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WAC</td>
<td>-0.939**</td>
<td>0.974**</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OAC</td>
<td>-0.122</td>
<td>-0.256</td>
<td>-0.068</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>EA</td>
<td>-0.472</td>
<td>0.119</td>
<td>0.300</td>
<td>0.835**</td>
<td>1</td>
</tr>
</tbody>
</table>

**Sensory properties of tarhana**

The sensory properties of tarhana soups were illustrated in Fig. 2. The results of the sensory evaluations showed that utilization of WC in tarhana preparation resulted acceptable soup properties compared to traditional yoghurt used tarhana. The samples containing WC was lighter according to color values than the control tarhana samples with yoghurt. Tarhana soup prepared with 12.5% WC addition was similar to the control in homogeneity and overall acceptability. High levels of WC increased sweetness and decreased the overall acceptability, but not contributed to the other parameters. Tarhana soups with 12.5% WC were more liked by panelists than control and further WC added
Tarhana samples. Also tarhana soups with 25% WC have acceptable scores in terms of all sensory properties.

**Figure 2.** The effects of WC (%) on the sensory properties of tarhana soup.

Tarakçı et al. (2004) reported that higher amounts of whey added tarhana samples gave higher body, smell, flavour and overall acceptability values than the other samples. The control samples gave higher acerbity values than WC added samples. Panelists expressed an enhancing sweet taste for the soups with increased amounts of WC.

**CONCLUSIONS**

Tarhana samples were successfully produced by WC supplementation. The results of this research showed that 25% level WC can be used instead of yoghurt in tarhana production. WC addition decreased the color a* and b* values of tarhana samples while the parameter L* tends to increase with increasing WC levels. WC addition gave more bright and attractive color to tarhana solids than that of the control samples. WC addition increased the foam capacity and gave higher oil absorption capacity values than that of control sample. Utilization of WC in tarhana preparation resulted in acceptable soup properties up to 25% level in dry basis. In further studies, lactic acid bacteria can be added to improve the natural microbial flora of WC in tarhana formulation.
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Costat, 1990. Costata reference manual (Version 2.1) Copyright Coltort Software. P.O. Box. 1149, Berkery, CA, 94701. USA.


The effect of blanching temperature on the quality of microwave-vacuum dried mushroom *Cantharellus cibarius*

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**Abstract.** The objective of this study was to evaluate the effect of blanching temperature on structure, colour, chemical composition, and rehydration capacity of microwave-vacuum dried chanterelle (\textit{Cantharellus cibarius}). Fruiting bodies of chanterelle were collected from the forests in Jelgava region of Latvia. Prior to drying, fresh mushrooms were blanched in water at various temperatures of 70, 80, 90 and 100ºC for 3 min, then cooled in water (20°C). After blanching mushrooms were dried in a microwave-vacuum drier according to the specially designed program. The content of dry matter of chanterelle was 9.5 ± 0.5%. The results revealed that weight loss at 70–90ºC was significantly smaller compared to blanching at 100ºC temperature. The results indicated the tendency of smaller changes in microstructure, weight loss and colour for samples blanched at 70–80°C temperature comparing to the samples unblanched or blanched at higher temperatures. Electrical conductivity in water extract of microwave-vacuum dried chanterelle decreased with increased blanching temperature. Titratable acidity of chanterelle significantly reduced after blanching due to leakage of soluble acids into blanching water.

**Key words:** protein content, total phenols, structure, aroma profile, rehydration capacity.

**INTRODUCTION**

Wild mushrooms have long been appreciated for their content of proteins and fiber (Kalač, 2009; Pereira et al., 2012), aroma and flavour (Tsai et al., 2009; Dermiki et al., 2013), minerals, vitamins and other biologically active substances (Mattila et al., 2000; Chye et al., 2008), as well as their therapeutic potential (Hong et al., 2012).

Kumari et al. (2011) in their study concluded that the chemical composition and energy values of the wild edible mushrooms of \textit{Cantharellus} species clearly indicate that they provide key nutrients such as protein and carbohydrates. These varieties of mushrooms can also be used in low-caloric diets for their low contents of fat and energy. Besides, they are also good sources of useful amino acids and contain bioactive compounds. \textit{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 \textit{Cantharellus cibarius} is 118 kJ 100 g\(^{-1}\) of fresh mushrooms (Barros et al., 2008). 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). However, edible mushrooms are characterized by a short shelf life due to post-harvest changes resulting from the activity of enzymes such as polyphenol oxidase (PPO) that is responsible for browning reactions during storage (Keyhani & Keyhani, 2011).

Dehydration is among the most popular methods for shelf-life extension of highly perishable foods. Convective drying is widely used; however, several disadvantages of this method have been reported: degradation of important nutritional substances due to relatively long drying times and high temperatures (Marfil et al., 2008; Vega-Gálvez et al., 2012), changes in product colour and texture (Kotwaliwale et al., 2007), decrease in rehydration ability due to shrinkage (Giri & Prasad, 2007). Microwave-vacuum drying have been successfully applied to overcome the mentioned limitations in drying of apple slices (Schulze et al., 2014), green peas (Zielinska et al., 2013), rosemary (Calín-Sánchez et al., 2011), potatoes (Wang et al., 2010), button mushrooms (Giri & Prasad, 2007).

During microwave-vacuum drying the energy of microwaves is absorbed by water located in the whole volume of the material being dried. This creates a large vapour pressure in the centre of the material, allowing rapid transfer of moisture to the surrounding vacuum and preventing structural collapse (Lin et al., 1998; Figiel, 2010). As a consequence, the rate of drying is considerably higher than in traditional methods of dehydration (Sharma & Prasad, 2004). Giri and Prasad (2007) reported a reduction of 70–90% in the drying time of mushrooms, when hot air drying was replaced with microwave vacuum drying. Decreasing the pressure during microwave heating reduced the boiling point of water and thereby the drying temperature. (Sham et al., 2001) observed that the puffing phenomenon, that accompanies the rapid process of dehydration, creates a porous texture of the food, thus facilitating rehydration.

Pre-treatment is common in most processing operations to improve final product quality or to accelerate its drying kinetics (Lewicki, 1998). Among pre-treatments, blanching is one of the most extensive with the aim of denaturing or inactivating enzymes that adversely affect product quality (Sanjuan et al., 2000). The effective moisture diffusivity of broccoli increased from $1.987 \times 10^{-8}$ m$^2$s$^{-1}$ as blanching temperature increased from 20°C to 80°C. Increase in blanching temperature and rehydration temperature can increase the rehydration ratio of dried broccoli pieces (Doymaz, 2014). However, since blanching is a heat treatment, changes associated with thermal processing can be expected. These include degrading and leaching of nutritive components, for example, sugars, minerals and vitamins, colour change, loss of turgor in cells, due to thermal destruction of membrane integrity and partial degradation of cell wall polymers (Bahçeci et al., 2005). Significant reductions in the texture, colour, polyphenols and antioxidant capacity were observed due to blanching of York cabbage (Jaiswal et al., 2012).

The objective of this study was to evaluate the effect of blanching temperature on structure, colour, chemical composition, and rehydration capacity of microwave-vacuum dried mushroom *Cantharellus cibarius*.
MATERIALS AND METHODS

Raw materials
Fruiting bodies of chanterelle (Cantharellus cibarius) were collected from the forests in Jelgava region of Latvia. Fresh mushrooms were cleaned from forest debris, washed thoroughly in running tap water, drained on perforated containers, caps exceeding 40 mm in diameter were cut into halves. Samples were processed within the first 24 h after collection.

Pre-treatment and drying
Prior to drying, fresh mushrooms were blanched in water at various temperatures of 70, 80, 90 and 100°C for 3 min, and then cooled with water (20°C). Blanching was done immersing 0.5 kg of chanterelle in 5 l of water at appropriate temperature and then draining the excess water over a strainer. After blanching, mushrooms were dried in a microwave-vacuum drier ‘Musson-1’ (OOO ‘Ingredient’, St. Petersburg, Russia). Characteristic parameters of the drying program are presented in Table 1. All treatments were carried out in triplicate; obtained samples were combined for the further analyses.

Table 1. Characterization of microwave vacuum drying program for chanterelle

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of magnetrons</td>
<td>4–3–2*</td>
</tr>
<tr>
<td>Pressure, kPa</td>
<td>7.47–9.33</td>
</tr>
<tr>
<td>Drum rotation speed, rpm</td>
<td>6</td>
</tr>
<tr>
<td>Drying time, min</td>
<td>12–18</td>
</tr>
<tr>
<td>Product mass, kg</td>
<td>0.7–1.0**</td>
</tr>
</tbody>
</table>

* drier was programmed gradually decreasing number of used magnetrons
** initial mass of sample to be dried depends on the amount of water lost during blanching

The following abbreviations for the samples in this work are used, according to the method of pre-treatment applied:
UB – unblanched chanterelle;
B70 – chanterelle water blanched at 70°C temperature;
B80 – chanterelle water blanched at 80°C temperature;
B90 – chanterelle water blanched at 90°C temperature;
B100 – chanterelle water blanched at 100°C temperature.
All dried mushroom samples prior to analyses were ground in a blender to obtain fine powder of homogenous sample.

Analyses of physical parameters
Mushroom weight loss calculation was based on sample weight before and after blanching, when excess water was drained over a strainer for 5 minutes. For analysis of mushroom microstructure the samples were cut both from caps and stems in thickness of 5 µm. The fields observed under the microscope Axioskop 40 were fixed using a digital camera at 16 × 20 (VAREL contrast) or 16 × 40. At least ten measurements of the thickness of hypha were performed using Axiovision Le Rel 4.5. Colour was detected
using Colour Tec-PCM device. Colour components L*, a*, b* of each ground sample was measured at least in fifteen various points.

**Analyses of chemical parameters**

The ash content of mushrooms was determined by incineration at 550°C (Manjunathan & Kaviyarasan, 2011). Electrical conductivity in water extract was determined to characterise the total content of mineral substances using electrode TetraCon 325 connected to a conductometer inoLab pH/Cond 720 (WTW, Germany).

The protein amount in mushroom dry matter was determined by Lowry procedure (Lowry et al., 1951) using albumin as a standard.

Water extract for determination of total phenolic content, formol number, titratable acidity and electrical conductivity 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.

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., 2007).

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 by Tanner (1987). Formol number was calculated as mmol of NaOH per 1 g of mushroom dry matter. Results of analysis were expressed per dry matter of samples.

**Evaluation of rehydration capacity**

Rehydration was carried out at controlled temperature of 55 ± 1°C. Samples were rehydrated by immersion of 5 g of each sample in 200 ml of distilled water for 7 hours. Evolution of sample weight was measured. Before weighing the sample, it was removed and allowed to drain over a mesh for 60 s in order to eliminate the superficial water. Each rehydration experiment was replicated twice; and the rehydration curves are plotted for kg moisture per kg dry matter versus time for each chanterelle sample.

**Statistical analyses**

The results are presented as the mean ± standard deviation. Data analysis was performed using in-built software of Microsoft Excel 2007. One-way analyses of variance (one-way ANOVA) were carried out to detect significant difference (P < 0.05) between the mean values that had more than two variables. For statistical analyses, ANOVA followed by t-test, Bonferroni's test or Kruskal-Wallis test followed by Dunn’s test was used where appropriate, and the results for each experimental group were compared to the results of other groups. P-values of less than 0.05 were considered to be statistically significant. Statistical calculations were performed using Prism software (GraphPad, San Diego, CA, USA).
RESULTS AND DISCUSSION

Weight loss during blanching
In the blanching process mushroom weight loss was observed due to water and soluble solids diffusion into blanching medium. Results revealed that weight loss at 70–90°C was significantly smaller compared to blanching at 100°C temperature (Fig. 1).

![Figure 1](image1.png)

**Figure 1.** Mushroom weight loss in blanching process depending on temperature of blanching medium temperature. Note: the values marked with the same letter did not significantly differ ($P > 0.05$) among each other ($n = 5$).

Mushroom weight loss can be considered as beneficial because same water is removed and this decreases the amount of water to be evaporated during drying. Nevertheless it can have adverse effect on the chemical composition due to leakage of some valuable biologically active components such as minerals and soluble proteins.

Structure and colour
Technological processes – blanching and drying – has significant effect on changes in chanterelle microstructure, which influence physical and chemical properties of dried product. Images of fresh and microwave-vacuum dried chanterelle caps and stems were used for hypha thickness measurement. Measurement results of hypha thickness in the chanterelle stems are presented in Fig. 2, but in the caps in Fig. 3.

![Figure 2](image2.png)

**Figure 2.** Hypha thickness of fresh (control) and microwave – vacuum dried chanterelle stems depending on the applied pre-treatment method: S_UB – unblanched; S_B70, S_B80, S_B90, S_B100 – blanched samples, where number of sample indicates blanching temperature.
Figure 3. Hypha thickness of fresh (control) and microwave – vacuum dried chanterelle caps depending on the applied pre-treatment method: C_UB – unblanched; C_B70, C_B80, C_B90, C_B100 – blanched samples, where number of sample indicates blanching temperature.

The significant decrease of mushroom hypha thickness was observed for all dried samples due to moisture removal and subsequent shrinkage. The highest decrease both in stems and caps was observed in chanterelle dried after blanching at 90 and 100°C.

Significant difference \( (P < 0.001) \) in colour component \( L^* \) of microwave vacuum dried chanterelle powder was detected among studied samples. The samples obtained from unblanched mushrooms or blanched at 100°C were significantly darker comparing to other samples (Table 2). However the darkest colour was found in chanterelle powder which was not blanched before drying. The same samples had more pronounced red colour component \( (a^*) \), which generally gave a little orange shade to the samples. Colour component \( b^* \), which describe yellow colour, was not significantly different among studied samples \( (P = 0.076) \).

Table 2. Colour of microwave-vacuum dried chanterelle powder depending on the applied pre-treatment method

<table>
<thead>
<tr>
<th>Samples</th>
<th>( L^* )</th>
<th>( a^* )</th>
<th>( b^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>UB</td>
<td>52.65 ± 0.57 a(^a)</td>
<td>6.64 ± 1.80 a</td>
<td>29.13 ± 0.97 a</td>
</tr>
<tr>
<td>B70</td>
<td>69.67 ± 1.04 c</td>
<td>1.78 ± 0.36 b</td>
<td>31.07 ± 0.84 a</td>
</tr>
<tr>
<td>B80</td>
<td>70.88 ± 0.82 cd</td>
<td>1.84 ± 1.82 bc</td>
<td>29.28 ± 3.55 a</td>
</tr>
<tr>
<td>B90</td>
<td>72.37 ± 0.80 d</td>
<td>1.04 ± 0.94 c</td>
<td>28.92 ± 2.64 a</td>
</tr>
<tr>
<td>B100</td>
<td>57.22 ± 0.92 b</td>
<td>4.18 ± 0.87 d</td>
<td>29.57 ± 2.54 a</td>
</tr>
</tbody>
</table>

\(^a\)Data followed by different letters in the same column are significantly \( (P < 0.05) \) different among the applied pre-treatment; \( t \)-test \( (n = 20) \).

Colour components of microwave-vacuum dried chanterelle samples blanched at temperatures 70, 80 or 90°C had similar colour values, which significantly differed from those in samples – unblanched or blanched at 100°C. Mushrooms blanched at 70–90°C better retained brightness, which could be due to enzyme inactivation resulting from blanching compared to unblanched samples. It is in agreement with Bernas & Jaworska (2014) who revealed that the level of polyphenol oxidase activity showed a moderately negative correlation with \( L^* \) and \( a^* \) colour parameters. However blanching at 100°C can cause undesirable changes due to thermal effect. It is in agreement with Kotwaliwale et al. (2007) who indicated that colour changes during drying are mostly in
the form of browning caused by enzymatic or non-enzymatic reactions between carbohydrate and amino acids at elevated temperature.

**Chemical parameters**

The content of dry matter of chanterelle was 9.5 ± 0.5%. In the blanching process mushroom weight loss was observed due to diffusion of water and soluble solids into blanching medium. The results revealed that weight loss at 70–90°C was significantly smaller compared to blanching at 100°C temperature. The results indicated the tendency of smaller changes in microstructure, weight loss and colour for samples blanched at 70–80°C temperature comparing to the samples unblanched or blanched at higher temperatures.

<table>
<thead>
<tr>
<th>Table 3. Chemical parameters of microwave-vacuum dried chanterelle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Samples</strong></td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>UB</td>
</tr>
<tr>
<td>B70</td>
</tr>
<tr>
<td>B80</td>
</tr>
<tr>
<td>B90</td>
</tr>
<tr>
<td>B100</td>
</tr>
</tbody>
</table>

Values are represented as average (n = 3; SEM values omitted for better clarity, except for protein); ᵃ indicates a significant difference from the UB group; ᵃᵇ indicates a significant difference from the B70 group; ᵃᵇᶜ indicates a significant difference from the B80 group; ᵃᵇᶜᵈ indicates a significant difference from the B90 group.

P-values of less than 0.05 considered to be statistically significant (ANOVA followed by Bonferroni’s test, Kruskal-Wallis test for protein (n = 2)). No significant difference was observed for protein content.

The protein content in microwave-vacuum dried mushroom chanterelle was from 14.4 to 18.5 g 100 g⁻¹ DW depending on the applied type of pre-treatment. At higher blanching temperatures lower protein content was observed probably due to the increased protein denaturation and increased diffusion of soluble components into blanching medium. Ash amount of chanterelle varied between 5.45 ± 0.02 g 100 g⁻¹ and 9.89 ± 0.02 g 100 g⁻¹ of mushroom dry matter, demonstrating decreasing tendency with increased blanching temperature. Electrical conductivity in water extract of microwave-vacuum dried chanterelle decreased with the increase in blanching temperature. The highest value was established for unblanched sample. The obtained result also indicates that the amount of strong electrolytes in chanterelle is high. However titratable acidity of chanterelle was significantly reduced after blanching due to leakage of soluble acids into blanching water.

**Rehydration**

The most intensive rehydration was observed within the initial period, but the rate slowed down gradually (Fig. 4) up to the saturation level. Similar results have been reported for rehydration characteristics of microwave-vacuum and convective hot-air dried mushrooms (*Agaricus bisporus*) (Giri & Prasad, 2007). Besides, it can be seen that
the rehydration ratio of blanched samples resulted in higher rehydration, compared to unblanched sample and sample blanched at 100°C.

For food preparation both the initial rehydration rate and the maximum water absorption are of importance. The maximum moisture content absorbed in rehydration process can be compared to the initial moisture content of 11.7 g moisture per gram of dry matter. Water recovery in dried samples depends on pre-treatment conditions.

![Graph showing rehydration properties of microwave-vacuum dried chanterelle](image)

**Figure 4.** Rehydration properties of microwave-vacuum dried chanterelle: UB – unblanched; B70, B80, B90, B100 – blanched samples, where number of sample indicates blanching temperature.

Blanched samples have higher rehydration ratios compared to the unblanched sample. It coincides with findings of Doymaz (2014) who established that the rehydration ratio was observed to increase with increasing broccoli blanching temperature. If the water absorption was referred to the initial moisture content then microwave-vacuum dried sample pre-treated at 90°C recovered the highest amount (33%) among studied samples. Chanterelle samples which were not blanched or those blanched at 100°C recovered 26% and 27% of the initial available water, respectively. This might be due to the thermal destruction of cells during drying process.

**CONCLUSIONS**

Considering changes in chanterelle structure, colour, and rehydration properties the blanching temperatures of 70–90°C are the most suitable for pre-treatment of mushrooms prior to microwave vacuum drying, providing the best performance of dried product. Considering the chemical composition of dried samples it was established that unblanched product has the highest content of proteins, total phenols and other parameters, followed by the sample blanched at 70°C. Thus, blanching at 70°C is considered the most appropriate among studied, although further studies on quality changes during storage of dried product would be helpful.
REFERENCES


Analysis of mechanical behaviour of *Jatropha curcas* L. bulk seeds under compression loading with aid of reciprocal slope transformation method and tangent curve method

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**Abstract.** This study is focused on the utilization of the reciprocal slope transformation method (RST) and tangent curve method (TCM) for description of mechanical behaviour of *Jatropha* bulk seeds under compression loading. The experimental data derived from a compression test was done using compression device and pressing vessel with diameter 60 mm at compression speed of 1 mm s\(^{-1}\) and compressive force between 0 kN and 100 kN. Measured dependency between compressive force and deformation was fitted by RST method and TCM method and they were statistically analysed by ANOVA. Comparison of both methods in individual pressing regions is an integral part of this manuscript. It was determined that reciprocal slope transformation method describes precisely the beginning of deformation characteristics and it can cover the reorganizing process in the bulk seeds. From the conducted study it also follows that tangent curve method is suitable for description of mechanical behaviour at bulk deformations in which the reorganization process in the bulk seeds is finished yet.

**Key words:** stress, strain, oilseed, mathematical model, deformation characteristic, oil point.

**INTRODUCTION**

Mathematical description of deformation characteristic is important to understand the mechanical behaviour of bulk oilseeds under compression loading which could be used for technological improvement. There have been already published two mathematical models describing the mechanical behaviour of *Jatropha* bulk seeds under compression loading as one pressed unit (Herak et al., 2014a; Petru et al., 2014). First model is based on the utilization of tangent curve (Herak et al., 2013b) and second model is derived from reciprocal slope transformation (Blahovec, 2011), these two models have been already deeply described in the literature and also they have been mutually compared (Herak et al. 2014b). In published studies their applications were analysed throughout deformation characteristics however it is very well known that deformation characteristic of *Jatropha curcas* bulk seeds has non-linear course and it can be divided into few regions in which the compressed bulk seeds shows different mechanical behaviour (Kabutey et al., 2013a). From practical point of view it means that the mathematical model can fit properly whole deformation characteristic although the beginning of deformation curve or other deformation regions can be described statistically insignificant. It has been already published that deformation characteristics
of Jatropha curcas bulk seeds has three general regions of mechanical behaviour: under lower oil point – the oil is not gained yet, between lower oil point and upper oil point – the leakage of oil is running, and above upper oil point – theoretically the oil leakage is stopped (Herak et al., 2013a; Kabutey et al., 2013a). The aim of this study is to compare utilization of reciprocal slope transformation (RST) and tangent curve method (TCM) for mathematical description of mechanical behaviour of Jatropha curcas L. bulk seeds under compression loading at regions under lower oil point and above lower oil point.

**MATERIALS AND METHODS**

**Samples**

Samples of bulk Jatropha curcas L. seeds, variety IPB2, obtained from North Sumatra, Indonesia were used for the experiment. The general physical properties of the oilseed crop are given in Table 1. The moisture content of the samples was determined using standard moisture measurement equipment (Farm Pro, model G, Czech Republic). The mass of sample was determined using an electronic balance (Kern 440–35, Kern & Sohn GmbH, Balingen, Germany). The porosity was calculated from the relationship between the bulk and true densities (Blahovec, 2008). The bulk density was determined from the mass of the sample divided by initial pressing volume. The true seed density was determined gravimetrically (Blahovec, 2008). The results obtained were expressed as mean of three replicates.

<table>
<thead>
<tr>
<th>$M_c$</th>
<th>$m$</th>
<th>$V$</th>
<th>$P_f$</th>
<th>$\rho_b$</th>
<th>$\rho_t$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(%)</td>
<td>(g)</td>
<td>(mm$^3$)</td>
<td>(%)</td>
<td>(kg m$^{-3}$)</td>
<td>(kg m$^{-3}$)</td>
</tr>
<tr>
<td>8.5 ± 0.2</td>
<td>87.95 ± 1.19</td>
<td>226224 ± 6340</td>
<td>59.98 ± 1.26</td>
<td>388 ± 12</td>
<td>980 ± 12</td>
</tr>
</tbody>
</table>

$M_c$ – moisture content of bulk seeds in dry basis, $m$ – mass of bulk seeds, $V$ – initial volume of bulk seeds, $P_f$ – porosity of bulk seeds, $\rho_b$ – bulk density, $\rho_t$ – true density.

**Compression test**

To determine the relationship between compressive force and deformation characteristic curves, a compression device (Labortech, model 50, Czech Republic) was used to record the course of deformation function. A single pressing vessel diameter, 60 mm with plunger (Fig. 1.) was used.

Initial pressing height 80 mm of Jatropha bulk seeds were tested with a compression speed of 1 mm s$^{-1}$ under temperature of 20 °C. The compressive force was between 0 and 100 kN. The experiment was repeated three times. Recorded deformation characteristic was divided into five regions according to the study already published by Herak et al. (2013a) and Kabutey et al. (2013a) such as shown in Fig. 2. Individual regions were defined by bulk deformations: region I. from 0 mm to 10 mm, region II. from 10 mm to 40 mm, region III. from 40 mm to 50 mm, region IV. from 50 mm to 55 mm and region V. from 55 mm to 60 mm.
Reciprocal slope transformation (RST)

Dependency between compressive force \( F_{\text{RST}} \) (N) and corresponding deformation \( x \) (mm) was described by Eq. 1 using reciprocal slope transformation method (RST) (Herak et al., 2014), where \( a \) (N\(^{-1}\) mm\(^{-2}\)), \( b \) (N\(^{-1}\) mm\(^{-1}\)), \( c \) (N\(^{-1}\)) and \( d \) (N mm) are coefficients of the RST method. The coefficients were determined by the least squares method applicable in MathCAD software.

\[
F_{\text{RST}}(x) = \frac{x}{ax^3 + bx^2 + cx + d}
\]  

\( (1) \)

Tangent curve method (TCM)

Dependency between compressive force \( F_{\text{TCM}} \) (N) and corresponding deformation \( x \) (mm) was described by tangent curve method (TCM) (Herak, et al., 2013b) which is given by Eq. 2.

\[
F_{\text{TCM}}(x) = A \cdot \left[ \tan(B \cdot x) \right]^3,
\]

\( (2) \)

where: \( A \) (N) is force coefficient of mechanical behaviour and \( B \) (mm\(^{-1}\)) is coefficient of mechanical deformation behaviour. These coefficients were determined by Marquardt Levenberg approximation process (Marquardt, 1963) using MathCAD software.

RESULTS AND DISCUSSION

The measured characteristic was fitted by Eq. 1 and Eq. 2 such as presented in Fig. 3 with determined coefficient of variation \( CV = 10\% \) and the coefficients of this function are presented in Table 2 and Table 3. ANOVA analysis of the measured data using MathCAD 14 software was statistically significant at significance level 0.05, that is, the values of \( F_{\text{crit}} \) (critical value comparing a pair of models) were higher than the \( F_{\text{rat}} \) values (value of the F – test) and values of \( P_{\text{value}} \) (significance level at which it can be rejected
the hypothesis of equality of models) (Table 4) were higher than 0.05. The validity of these equations is limited to the region where the deformation of seeds varies from zero to maximum. It is evident that both method (RST) and (TCM) can be used for appropriate description of mechanical behaviour of Jatropha bulk seeds which was confirmed by already published studies (Kabutey et al., 2013a; Divisova et al., 2014; Herak et al., 2014a; Petru et al., 2014; Sigalingging et al., 2014). From the graphical presentations (Fig. 3) and also from statistical analyses (Table 4) it follows that used methods Eq. 1 and Eq. 2 show different possibility of their utilization for description mechanical behaviour in individual regions.

**Figure 3.** Measured amounts of mechanical characteristic of Jatropha bulk seeds with displayed amounts of standard deviation and their fitted functions by Eq. 1 and Eq. 2. F is measured force, $F_{RST}$ is force determined by RST, $F_{TCM}$ is force determined by TCM, I., II., III., IV., V. are individual regions, F. CH. is full deformation characteristics.
Table 2. Determined coefficients of RST method

<p>| | | | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>b</td>
<td>c</td>
<td>d</td>
</tr>
<tr>
<td>(N^-1 mm^2)</td>
<td>(N^-1 mm^-1)</td>
<td>(N^-1)</td>
<td>(N^-1 mm)</td>
</tr>
<tr>
<td>2.14·10^-7</td>
<td>-2.85·10^-5</td>
<td>0.93·10^-3</td>
<td>16.34·10^-5</td>
</tr>
</tbody>
</table>

a, b, c, d – coefficients of reciprocal slope transformation

Table 3. Determined coefficients of TCM method

<p>| | |</p>
<table>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>(N)</td>
<td>(mm^-1)</td>
</tr>
<tr>
<td>2320</td>
<td>0.026</td>
</tr>
</tbody>
</table>

A – force coefficient of mechanical behavior, B – deformation coefficient of mechanical behavior

It is clear (Fig. 3) that for small bulk seeds deformations up to 40 mm the RST method is more appropriate than TCM method. From mechanical behaviour in regions I. (Fig. 3) it implies that RST method describes precisely origin of the bulk seeds reorganization which is in accordance to the study of Petru et al. (2014), the TCM method ignores this origin of the change of bulk seeds mechanical behaviour under small deformations which is confirmed by statistical analysis in Table 4. ANOVA shows statistical significances between measured data and both method used for description of mechanical behaviour in region II. From Table 4 and Fig. 3 follows that RST method more appropriately describes the whole course of deformation characteristics in region II. which is obvious from the amount of P_value (Table 4).

Table 4. ANOVA statistical analysis

<table>
<thead>
<tr>
<th></th>
<th>F _rat (-)</th>
<th>F _crit (-)</th>
<th>P _value (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. CH.</td>
<td>RST 0.062</td>
<td>3.919</td>
<td>0.803</td>
</tr>
<tr>
<td></td>
<td>TCM 0.005</td>
<td>3.918</td>
<td>0.942</td>
</tr>
<tr>
<td>I.</td>
<td>RST 0.001</td>
<td>4.747</td>
<td>0.971</td>
</tr>
<tr>
<td></td>
<td>TCM 24.244</td>
<td>4.747</td>
<td>0.001</td>
</tr>
<tr>
<td>II.</td>
<td>RST 0.006</td>
<td>4.170</td>
<td>0.938</td>
</tr>
<tr>
<td></td>
<td>TCM 3.748</td>
<td>4.170</td>
<td>0.062</td>
</tr>
<tr>
<td>III.</td>
<td>RST 0.281</td>
<td>4.351</td>
<td>0.602</td>
</tr>
<tr>
<td></td>
<td>TCM 0.355</td>
<td>4.351</td>
<td>0.558</td>
</tr>
<tr>
<td>IV.</td>
<td>RST 4.024</td>
<td>4.026</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td>TCM 4.015</td>
<td>4.026</td>
<td>0.036</td>
</tr>
<tr>
<td>V.</td>
<td>RST 67.324</td>
<td>5.317</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>TCM 3.376</td>
<td>5.317</td>
<td>0.103</td>
</tr>
</tbody>
</table>

F\_rat – value of the F test, F\_crit – critical value that compares a pair of models, P\_value – the significance level at which it can be rejected the hypothesis of equality of models

For bulk seeds deformations between 40 mm and 50 mm the both methods can be used without limitations such as follows from graphical presentation of region III. (Fig. 3) and from statistical analysis (Table 4). From the course of deformation characteristics (Fig. 3) implies that description of mechanical behaviour in region IV. shows differences between models given by Eq. 1 and Eq. 2 and measured data. The amounts of differences are approaching to the limit of the applicability of both models.
which is also given by very low amount of $P_{value}$ such as small difference between $F_{rat}$ and $F_{crit}$ however from the statistical point of view both methods can be used for description of mechanical behaviour in region IV. From the determined coefficient of variation of deformation curve it is clear that *Jatropha* bulk seeds show variances between mechanical behaviour of individual seeds which is given by their biological nature. Taking into account of this variability which is graphically presented by standard deviations in Fig. 3 the data determined by Eq. 1 and Eq. 2 are still in the range bounded by standard deviations. The lowness limit amount of bulk deformation (55 mm) in region IV. was determined in previously published studies as lower oil point (Willems et al., 2008; Herak et al., 2013a). In the region V. it is clear that RST method doesn’t appropriate describe mechanical behaviour above bulk deformations of 55 mm which is also evident from the statistical analysis (Table 4.) and also from Fig. 3 where the RST data are out of standard deviations. From the conducted study it follows that RST method given by Eq. 1 describes more precisely the beginning of the pressing process and it provides a detailed view of the process of reorganizing of individual seeds in the compressed bulk seeds. Deviations from deformation curve at larger deformations can be eliminated by using a multiple-member polynomial in Eq. 1. The RST method in the present form given by Eq. 1 is suitable for description of the mechanical behaviour of *Jatropha* bulk seeds under compression loading at deformation equal to or less than lowness oil point. The TCM method describes appropriately mechanical behaviour of *Jatropha* bulk seeds under compression loading at deformations in which the reorganization process of compressed bulk seeds was finished and the bulk seeds show mechanical behaviour similar to the one compressed unit. Practically it means the compression above bulk deformation of 40 mm. From this study it is also evident that using derivation of Eq. 1 and Eq. 2 the dependency between modulus of elasticity and bulk deformation can be determined (Herak et al., 2011; Sigalingging et al., 2014). Used models show that the developed mathematical equations Eq. 1 and Eq. 2 take into account the experimental boundary conditions of the linear compression of *Jatropha* bulk seeds for the mechanical behaviour description. The boundary conditions means that the origin of the deformation curve starts from zero force and zero deformation which is followed by an increasing function within the whole range of pressing process and when compressive force is approaching infinity then deformation reaches a maximum limit (Herak et al., 2013b; Kabutey et al., 2013a; Raji & Favier, 2004; Sayyar et al. 2009). It is important to mention here that both methods are suitable for mechanical behaviour description of bulk oilseeds of *Jatropha curcas* L. (Herak et al., 2014a; Herak et al., 2014b). RST method using least square method for data fitting and its coefficients can be determined by standard computer software (Blahovec, 2011; Blahovec & Yanniotis, 2009), however TCM method using Marquardt Levenberg approximation process is determined by the MathCAD software (Herak et al., 2011; Sigalingging et al., 2014). On the other hand by the integration of Eq. 1 and Eq. 2 the deformation energy of compressed bulk seeds can be determined and from mutual comparison published by Herak et al. (2014b) it is clear that determined energies are significant and both models can be also applied for deformation energy determination. The results of this study are in accordance with already published theories related to the tangent curve method (Petru et al., 2012; Herak et al., 2013b; Sigalingging et al., 2014) and to the reciprocal slope transformation (Blahovec and Yanniotis, 2009; Blahovec 2011; Herak et al., 2014a). The advantage of using both methods is that it is not conditional to resolve individual
particles and their properties and relationships between particles since both methods use the bulk seeds as a unit which is affected by constrains between the pressing vessel and bulk oilseeds and the pressing process. These theories can be used for determining the mechanical behavior of different materials under compression loading such are oil palm kernels, rapeseeds, sunflower seeds, wood chips, paper chips and other (Herak, et al., 2011; Kabutey et al., 2012; Kabutey et al., 2013b; Divisova et al., 2014, Sigalingging et al., 2014). The mechanical behaviour of bulk oilseeds can be described also by different solution methods generally based on the Darcy’s Law (Fasino & Ajibola, 1990) and fluid flow through porous media (Singh & Kulshreshtha, 1996) or by methods based on the Terzagi’s model (Shirato et al., 1986; Willems et al., 2008) or the energetic balance model (Zheng et al., 2005). In terms of the tangent curve method (Herak et al., 2013b) and reciprocal slope transformation (Blahovec 2011; Herak et al., 2014a), the bulk oilseeds are considered as a unit which is most relevant from construction engineering and economical point of view. Generally, the results of the present study were in agreement with the results published by different authors (Mrema & McNulty, 1985; Omobuwajo et al., 1998; Braga et al., 1999; Guner et al., 2003). Using of RST method as well as TCM method has its justification for description of mechanical behaviour of *Jatropha curcas* bulk seeds under compression loading and the advantage both methods is using the bulk seeds as a unit which is affected by constrains between the pressing vessel and bulk oilseeds and the pressing process.

**CONCLUSIONS**

The reciprocal slope transformation method and tangential curve method were used for mathematical description of *Jatropha curcas* L. bulk oilseeds. From the statistical analysis, it is evident that both models can be significantly used for description of mechanical behaviour of *Jatropha* bulk seeds under compression loading as well as for deformation energy determination. It was determined that reciprocal slope transformation method describes precisely the beginning of pressing process and it can cover the reorganizing process in the bulk seeds. From the conducted study it follows that tangent curve method is suitable for description of mechanical behaviour at bulk deformations in which the reorganization process in the bulk seeds is finished yet. A suitable determination of coefficients of these two mathematical models can be used for a more accurate description of deformation characteristics in individual pressing regions. Both methods consider bulk oilseeds as a unit which is important from technological and economical point of view.

**REFERENCES**


Rheological properties of whole wheat and whole triticale flour blends for pasta production

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Abstract. Whole grain flour can be considered as a good candidate for pasta fortification due to the health benefits. In the literature it is mentioned, that when pasta dough is fortified with non-traditional ingredients, it behaves differently. Therefore, the purpose of the current research was to investigate the rheological properties of the whole wheat and whole triticale flour blends for pasta production. Whole grain flour made from triticale and wheat grains was used in the present research. Wheat flour type 405 was used as a control. The blends were made from whole triticale and whole wheat flour in combination with wheat flour (type 405) in various proportions (from 10% to 100%). The following quality parameters were analysed by using standard methods: the rheological properties of dough were analysed using Brabender Farinograph-AT; moisture content of flour samples was determined according to AACC (2000) Method No. 44-15A. The results of the present research demonstrate that the rheological properties of dough become worse by increase the amount of whole grain flour in a blend. Water absorption is higher and dough development time of dough with whole grain flour addition is longer than the parameters of control wheat flour (type 405). The same results were obtained during analysing dough stability and development time. However, higher dough stability was obtained for the blends with whole wheat flour, compared to blends with whole triticale flour, what possibly is due to the higher gluten content in whole wheat flour.

Key words: whole wheat, whole triticale, flour blends, pasta, farinograph.

INTRODUCTION

Cereals and their products constitute an important part of the human diet, providing a high proportion of carbohydrates, proteins, fats, dietary fibre, B-group vitamins and minerals. More and more foods are made from whole grain (Okarter et al., 2010). 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 fibre and micronutrients (McKeown et al., 2002). Traditionally pasta products are made from wheat semolina, although more recently other cereals have been used to partially replace it. Common wheat flour also can be useful for pre-cooked pasta products, but because of the low protein content, addition of high protein components such as whole grain flour may enrich the products and result in improved functional properties and quality when the right processing conditions are used (Chillo et al., 2008).
Wheat (*Triticum* spp.) is the main cereal crop used for human consumption in many areas worldwide. Traditionally, pasta is manufactured from durum wheat (*Triticum durum* Desf.) with protein content less than 15%, which results in a product considered to be of superior quality for pasta made from cheaper common wheat (*Triticum aestivum* L.) or a blend of the two species. 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. Cooked pasta is firm and resilient with no surface stickiness and little if any cooking losses (Troccoli et al., 2000).

Triticale (*X Triticosecale* Wittmack) is a man-made cereal grain species resulting from a plant breeder's cross between wheat (*Triticum*) and rye (*Secale*). Triticale incorporates the functionality and high yield of wheat and the durability of rye. Triticale has a potential for use in bread production. However, because most of the varieties available are not suitable for leavened bread making on their own, due to the production of a weak and sticky gluten, they can only be successfully used for producing a range of unleavened products such as cakes, cookies, biscuits, waffles, noodles, pastas, and breakfast cereals. Recently, efforts to enlarge food resources have resulted in new approaches to expanding triticale's applicability for human consumption. For example, certain triticale varieties have been used to produce bread of the acceptable quality under special bread making conditions (Doxastakis et al., 2002).

Characterization of the rheological properties of dough is effective in predicting the processing behaviour and in controlling the quality of food products (Song & Zheng, 2007). When wheat flour is mixed with water, with the required amount of energy, dough is formed. The behaviour of the resulting dough when submitted to mechanical energy input is determined by dough rheological properties (Bloksma, 1990). Gliadin and glutenin are the two primary types of grain proteins which are responsible for the elastic and viscous properties, respectively, which help to form a continuous spatial network in the dough (Koehler et al., 2010). These properties derive largely from the gluten proteins, which form a continuous viscoelastic network within the dough. Gliadins are monomeric proteins that form only intra-molecular disulphide bonds, if present, whereas glutenins are polymeric proteins whose subunits are held together by inter-molecular disulphide bonds, although intra-chain bonds are also present. Among these storage proteins, glutenins (polymeric proteins) have been shown to be extremely important in determining the rheological properties (Jia et al., 1996). Gluten is the main base of the wheat dough and is the protein that only exists in wheat and rye. Wheat flour dough simultaneously exhibit characteristics of a viscous liquid and of an elastic solid and hence are classed as viscoelastic materials (Bagley et al., 1998). Dough mechanical properties depend on a large variety of factors including flour cultivar, mixing time, temperature, etc. (Bagley et al., 1998; Pastukhov & Dogan, 2014). Bran and germ particles also disrupt the continuity of the protein network, resulting in weaker, less firm pasta (Manthey & Schorno, 2002).

In the scientific literature it is mentioned, that when pasta dough is fortified with non-traditional ingredients, it behaves differently (Roda, 2013; Rayas-Duarte et al., 1996). Therefore, the purpose of the current research was to investigate the rheological properties of the whole wheat and whole triticale flour blends for pasta production.
MATERIALS AND METHODS

The study was carried out at the scientific laboratories of the Faculty of Food Technology at Latvia University of Agriculture (LLU) and at the laboratory of the JSC ‘Jelgavas dzirnavas’ (Latvia).

Conventional triticale (line ‘9405-23’), grains of 2014 cultivated at State Priekuli Plant Breeding Institute (Latvia), wheat (‘Zentos’) grain cultivated at LLU Research Centre ‘Peterlaviki’ (Latvia) were used in the experiments. For the flour blend obtaining wheat flour type 405 from JSC ‘Dobeles dzirnavnieks’ (Latvia) was used. Wheat and triticale grain was ground in a laboratory mill PLM3100/B (Perten, Sweden) obtaining fine whole grain flour. Wheat flour type 405 was used as a control. The flour blends were made from whole wheat or whole triticale flour in a combination with wheat flour (type 405) in various proportions. For the research twenty one flour blends was made: flour blend with whole wheat or whole triticale flour. Experimental flour blends were made substituting part of wheat flour type 405 with whole grain flour in proportion from 10% to 100%.

Moisture content of the flour samples was determined using an air-oven method according to AACC (2000) Method No. 44–15A.

Farinograph analyses were done for wheat flour Type 405 (control), whole triticale and whole wheat flours. For analysis of the rheological properties Brabender Farinograph-AT (GmbH & Co.KG., Germany) was used according to the international standard methods (AACC No. 54–21, ICC No. 115/1). The results of farinograph tests were analysed primarily in the aspect of the dynamics of changes in the consistency of dough during the mixing (D’Appolonia & Kunerth, 1984). For all samples the following parameters were determined: water absorption (WA) of flour and flour blends, stability of dough (S), dough development time of (DDT), degree of softening (DS). Water was added automatically from the farinograph water container to flour and mixed to form the dough. The farinograph was connected to a circulating water pump and a thermostat which operated at 25 ± 2°C. The default speed value for flukes was 63 rpm and the duration of the experiment was 20 min.

Microsoft Excel software was used for the research purpose to calculate mean arithmetical values and standard deviations of the obtained data. SPSS 20.0 software was used to determine the significance of research results, which were analysed using the two-factor ANOVA to explore the impact of factors and their interaction at the significance effect with statistical significance (α) set at \( P < 0.05 \). Pearson's \( r \) coefficient was calculated for measure of the strength and direction of the linear relationship between two variables that is defined as the covariance of the variables divided by the product of their standard deviations: close interconnection if \( 0.7 < |r| < 0.9 \); average interconnection if \( 0.50 < |r| < 0.69 \); not significant interconnection if \( 0.20 < |r| < 0.49 \).

RESULTS AND DISCUSSION

Water migration is a common problem in many food products. However, the physical structure of food materials plays a decisive role on their moisture transport properties generally characterized by overall effective moisture diffusivity. For example
in a porous product, this effective diffusivity is affected mainly by the volume fraction and distribution of both solid and gas phases (Roca et al., 2007), comparing with compressed products as pasta.

In the present experiments significantly higher \( P = 0.004 \) moisture content (Table 1) was established for wheat flour type 405, compared to whole wheat and whole triticale flour. The moisture content of the control wheat flour was by about 4% higher compared to the whole wheat flour and by about 3% compared to the whole triticale flour.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>Moisture, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Wheat flour (control)</td>
<td>14.16 ± 0.03^b</td>
</tr>
<tr>
<td>2.</td>
<td>Whole wheat flour</td>
<td>10.23 ± 0.05^a</td>
</tr>
<tr>
<td>3.</td>
<td>Whole triticale flour</td>
<td>10.96 ± 0.03^a</td>
</tr>
</tbody>
</table>

Data followed by different letters are significantly \( (P < 0.05) \) different.

Moisture content of 14% is commonly used as a conversion factor for other tests in which the results are affected by moisture content. Moisture is also an indicator of grain storability. Flour with high moisture content (greater than 14.5%) attracts mould, bacteria and insects, all of which cause deterioration during storage. Flour with low moisture content is more stable during storage. Moisture content can be an indicator of profitability in milling (Keranet al., 2009). Therefore, the moisture content in flour should not exceed 15% (Kunkulberga & Seglins, 2010).

The Farinograph results characterise dough-mixing properties which are ascribed to wheat gluten, starch, lipid and water contents as well as the amount and activity of α-amylase. Gliadins and glutenins are the two primary types of grain protein which are responsible for the elastic and viscous properties, respectively, which help to form a continuous spatial network in the dough. The cohesive strength of wheat flour dough comes from glutenin. The gliadin molecules are known to reduce the stiffness and increase the extensibility of the gluten phase (Koehler et al., 2010).

Water absorption is the most important parameter measured by farinograph, which indicates the amount of water required to develop the standard dough of 500 farinograph units (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). Water absorption is affected by the protein content of the flour, the amount of starch damaged during milling and the presence of non-starch carbohydrates (Finney et al., 1987). In the current research it was detected, that water absorption of the analysed flour samples increased with the amount of whole flour in the blend (Fig. 1). Close interconnection \( (r = 0.9586 \text{ for whole wheat flour and } r = 0.9024 \text{ for whole triticale flour}) \) was obtained between the increasing amount of whole flour additive to the wheat flour and the increase of flour water absorption. However, the smaller water absorption was obtained for a control–wheat flour sample \( (59.1 \pm 0.15\%) \), significantly \( (P = 0.012) \) higher–for whole wheat flour \( (69.2 \pm 0.17\%) \). The smaller water absorption mainly indicates smaller amount of added water for dough formation. The present results demonstrate that the value of water absorption was increased if the amount of added whole flour increased. However, the inclusion of a higher amount of bran in the dough formulation usually resulted in increased dough water absorption due to the higher levels of pentosans present in bran.
(Sanz-Penella et al. 2008) and bran from whole grain flour can interfere with water migration, increasing water retention within the pasta (Villeneuve & Gélinas, 2007).

Dough stability (DS) is defined as the time difference between the point where the top of the curve first intercepts the 500 FU line and the point where the top of the curve leaves the 500 FU line. Dough stability indicates the time when the dough maintains maximum consistency and is a good indication of dough strength. Good quality dough has stability of 4–12 min (Kulhomäki & Salovaara, 1985). Close interconnection ($r = 0.9341$ for whole wheat flour and $r = 0.8846$ for whole triticale flour) was obtained between increasing amount of whole flour additive to the wheat flour and decrease of the dough stability. In the present experiments it was obtained that stability time of control dough sample was 12:15 min. Dough stability time decreased by increased amount of whole flour (Fig. 2) additive. Such changes mainly can be explained with decrease of gluten content in the analysed whole flour samples. Short dough stability time can mainly indicate non-acceptable dough properties during pasta dough mixing and formation. The stability time is an indication of the strength of flour, a higher value signifying stronger dough. Similar results were obtained by Zhang et al. (2014), where a significant difference was found in the stability time between the two flours ($P < 0.05$), the dough of waxy wheat flour exhibiting a lower stability than normal wheat flour (1.4 min and 2.7 min, respectively).

For obtaining of dough with good properties, the additive of whole triticale flour could be 30%, but of whole wheat flour – 50% (Fig. 2). Both quality and quantity of gluten affect the flour processing quality. Gluten has viscoelastic behaviour in which gliadin and glutenin fractions represent viscous and elastic behaviour, respectively. Variation in protein content alone is not responsible for the differences in dough properties and suitability for end-products amongst the cultivars (Zhu & Khan, 2002). As a result the obtained products for example pasta could have non-acceptable quality properties. The dough stability time less than 4 min is not acceptable.
Close interconnection ($r = 0.9573$ for whole wheat flour and $r = 0.8635$ for whole triticale flour) was obtained between the increasing amount of whole flour additive to the wheat flour and the increase of dough development time. The shortest dough development time was found for the sample made exceptionally from wheat flour (2:22 min), but the longest dough development time (6:30 min) was observed for whole wheat flour and for whole triticale flour (7:41 min) (Fig. 3).

An increased dough development time of dough samples with whole wheat and triticale flour additive, comparing with control flour sample mainly can be due to differences in a chemical composition of whole flour, and its elevated dietary fibre content, especially. Similar results were found in the scientific literature, as the dough development time for wheat flour (1.5 min) was shorter than that of wheat flour
(2.1 min), an advantage for improving output during actual production (Zhang et al., 2014).

The results of the pasta dough rheological properties demonstrate, that maximum of whole flour amount to be added could be for whole wheat flour – 50%, whole triticale – 30%.

CONCLUSIONS

Water absorption of the analysed flour samples increased with the amount of whole wheat or triticale flour increase in the flour blend.

Higher dough stability was observed for the flour blends with whole wheat flour, compared to blends with whole triticale flour, possibly due to higher gluten content in whole wheat flour.

The shortest dough development time (2:22 min) was observed for wheat flour (type 405), but the longest dough development time – for whole wheat flour (6:30 min) and whole triticale flour (7:41 min).

In pasta production, 30% of wheat flour type 405 can be replaced with whole triticale flour or 50% of whole wheat flour still having acceptable characteristics of dough.

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REFERENCES


Integrated evaluation of cowpea (Vigna unguiculata (L.) Walp.) and maple pea (Pisum sativum var. arvense L.) spreads

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Abstract. The aim of this research was to develop pea spreads using local legumes and complete integrated evaluation of the spreads to find the most suitable pea spreads for shelf-life investigation. A total of twelve pea spreads were made of ground re-hydrated cooked seeds of cowpeas (Vigna unguiculata (L.) Walp.) or maple peas (Pisum sativum var. arvense L.), to which salt, citric acid, oil and different spices were added. Standard analytical methods were employed to determine overall preference and physicochemical composition (protein, fibre, ash, pH, etc.) of spread samples. Principles of integrated evaluation were used to select the most suitable spreads for pea spread shelf-life investigation. The overall preference of cowpea and maple pea spread samples ranged from 2.8 to 4.9 with significant differences among spreads (P < 0.05). Physicochemical evaluation was completed with only sensory satisfactory samples. There were no significant differences in protein, ash and dry matter content among pea spread samples (P > 0.05). Pea spreads were good sources of total dietary fibre (10.72 to 14.81 g 100 g⁻¹). Addition of spices had a significant impact on the lightness (L*) and firmness of pea spreads (P < 0.05). Cowpea spread with bruschetta spice (15.43) and maple pea spread with bruschetta spice (22.09) had the lowest integrated evaluation values among spreads from the same legume. It was concluded that shelf-life investigation should be completed with the most suitable spread (the lowest integrated evaluation value) and control sample, i.e., cowpea spread and maple spread with bruschetta spice and without spices.

Key words: cowpea, maple pea, physicochemical evaluation, sensory evaluation.

INTRODUCTION

Problem of sufficient protein supply is very acute for humans around the world as growing population requires more quantities and improved quality of protein. The need for dietary fibre is also rising due to the numerous health benefits, e.g. lower glycaemic index, increased satiation, cancer prevention, reduction in cholesterol levels, prevention or alleviation of constipation, and protection against cardiovascular diseases (Wang et al., 2010); dietary fibre consumption is typically low in the Western pattern diet which positively correlates with an elevated incidence of obesity, death from heart disease, cancer (especially colon cancer), and other Western pattern diet related diseases (McEvoy et al., 2012).

Local legumes growing in Europe – cowpeas (Vigna unguiculata (L.) Walp.) and maple peas (Pisum sativum var. arvense L.) – can be used for innovative product development to satisfy the daily needs for protein and fibre and increase legume...
consumption. Hard-to-cook phenomenon, meteorism and time consuming preparation are the main reasons for low legume consumption in Latvia (Kirse & Karklina, 2014). Nutritionally, peas 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).

Legumes constitute an important source of dietary protein for large segments of the world’s population particularly in those countries in which the consumption of animal protein is limited by non-availability or is self-imposed because of religious or cultural habits (Boye et al., 2010). However, consumption of legumes, which are one of the most reliable sources of good quality protein and dietary fibre, in the Western world remains quite low at less than 3.5 kg per capita per year while in other parts of the world annual legume consumption can range up to 40 kg per capita (Mudryj et. al., 2012). Among European countries, higher legume 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).

The concept of commercially available legume spreads as an innovative product and an alternative to traditional animal–derived spreads or pates is fairly new, however, as non-dairy and reduced fat/calorie spreads are becoming popular for health conscious people, animal product alternatives have the potential to contribute to overall public health, as well as increasing consumer choice.

Therefore, the aim of this research was to develop pea spreads using local legumes and complete integrated evaluation of the spreads to find the most suitable pea spreads for shelf-life investigation.

MATERIALS AND METHODS

Materials
For legume spread production the following materials were used: maple peas ‘Bruno’ (Pisum sativum var. arvense L.), cowpeas ‘Fradel’ (Vigna unguiculata (L.) Walp.), ‘Extra virgin’ canola oil (Ltd. Iecavnieks, Latvia), citric acid (Ltd. Spilva, Latvia), Himalayan salt (country of origin: Pakistan), onion spice ‘Zwimax’ (Ing. E. Graf KG, Germany), herb (sun-dried tomato, garlic and basil) spice ‘Bruschetta’ (P.P.H. fleisch mannschaft®-Polska Sp. z o.o., Poland), bell pepper spice ‘Paprika spice mix’ (Ing. E. Graf KG, Germany), sesame seeds (Ltd. Gemoss, Latvia), green herbs (fresh dill, dry parsley, dill, spring onions) which consists of ‘Herba Fresh DILL’ (Fuchs GmbH & Co. KG, Germany) and ‘Mieszanka Wiosenna II’ (P.P.H. fleisch mannschaft®-Polska Sp. z o.o., Poland).

Preparation of legume spreads
Legume spreads were prepared at the laboratory of Faculty of Food Technology (Latvia University of Agriculture) based on the vegetarian spread preparation technology (Latvian Republic Patent № 14705, 2014).

Maple peas (or cowpeas) were soaked in water (with added NaHCO₃, 21.5 g kg⁻¹) at 20 ± 2°C for 15 h, then rinsed and boiled in a pressure cooker (KMZ, USSR) until tender (about 35 ± 5 min plus 15 min for natural pressure release). Warm cooked peas were then grinded in a food processor (Philips HR 7761/00, Philips, The Netherlands)
together with salt and citric acid, spices were added to the pea paste (if needed); oil was added at the end of mixing in the food processor. Vegetarian pea spreads were packed in 200 ± 5 g polypropylene cups and stored at 3 ± 1°C for 12 h prior to sensory and physicochemical evaluation. For physicochemical analyses where dry product samples were required, pea spreads were dried in a conventional dryer at 45 ± 1°C for 3 h to moisture content 15 ± 2%. Recipes of pea spreads are given in Table 1.

**Table 1. Recipes of pea spreads without spices**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Cowpea spread</th>
<th>Maple pea spread</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cowpeas, g</td>
<td>940.0</td>
<td>–</td>
</tr>
<tr>
<td>Maple peas, g</td>
<td>–</td>
<td>940.0</td>
</tr>
<tr>
<td>Oil, ml</td>
<td>60.0</td>
<td>60.0</td>
</tr>
<tr>
<td>Salt*, g</td>
<td>3.2</td>
<td>3.2</td>
</tr>
<tr>
<td>Citric acid, g</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Total, g</td>
<td>1004.0 ± 2</td>
<td>1004.0 ± 2</td>
</tr>
</tbody>
</table>

* salt was not added to spreads with dry herbs and paprika spice because these spices already contained salt in respective amounts.

For maple pea and cowpea spread flavour diversification spices in the following amounts were used (g 1000 g⁻¹): onion spice – 21.0 g, bruschetta spice – 8.8 g, paprika spice mix – 33.0 g, roasted sesame seeds – 8.5 g, green herbs – 9.0 g (Herba Fresh DILL) and 4.0 g (Mieszanka Wiosenna II).

**Methods**

Sensory evaluation of pea spreads was performed during the Baltics food industry fair ‘Riga Food 2014’ (120 panellists; 62% women and 38% men, average age 35 years) using 5-point hedonic scale (5 – like very much and 1 – dislike very much) in order to determine the overall preference of the samples (ISO 4121:2003). After the sensory evaluation the samples which received higher points were subjected to physicochemical evaluation.

**Physicochemical analyses**

Physicochemical analyses including nutrients were determined according to standard methods: protein content (AACC 46-11.02), total dietary fibre content (AOAC 985.29), ash content (ISO 2171:2010). pH was determined using ISO 10523:2012, dry matter in pea spreads was calculated as 100% of product minus moisture content (ISO 24557:2009).

**Polyphenol content**

Total polyphenol content was determined spectrophotometrically using Folin-Ciocalteu reagent according to the method of Akond et al. (2011) using gallic acid as a standard phenolic compound. 0.5 ml extract sample (1 g product in 20 ml acidified (HCl) 70% ethanol and acetone blend) was placed in a test tube and mixed with 2.5 ml Folin-Ciocalteu reagent (Sigma-Aldrich Chemie GmbH, Germany) previously diluted 1:10 with deionized water. Between 1 min and 8 min, 2 ml sodium carbonate solution, prepared by dissolving 75 g in 1 L of deionized water (Sigma-Aldrich Chemie GmbH, Germany) was added to test tube and mixed thoroughly by hand. Then the test tubes with
the mixtures were allowed to stand for 1 h in the dark. Absorbance of the resulting solutions was read at 760 nm using a spectrophotometer (Jenway 6300, Bibby Scientific Limited, UK). Quantification of total phenolics was based on a gallic acid standard curve generated by preparing 0, 5, 10, 15, 20, 30 mg L\(^{-1}\) of gallic acid (Sigma-Aldrich Chemie GmbH, Germany) in deionized water. Total phenolics were expressed as mg gallic acid equivalent (GAE) per gram of pea spread using the following formula: gallic acid equivalent (mg g\(^{-1}\) GAE) = (‘x’ Coefficient from gallic acid standard curve x Absorbance at 760 nm + Slope of the gallic acid standard curve) x 40 (dilution factor).

**Colour analysis**

Colour analysis was performed using *Colour Tec PCM / PSM* with CIE L*a*b* colour system (Accuracy Microsensors Inc., USA). For integrated evaluation only L* value (lightness intensity value at the day of preparation) was taken into account. Measurements were completed in tenfold repetition.

**Texture analysis**

Texture analysis – firmness of pea spreads – was performed with *TA.XT. Plus Texture Analyser* applying *Back extrusion* (Stable Micro Systems, UK). Data collection and analysis was carried out with program *Texture EXPONENT 32* using Back Extrusion Cell with a 35 mm disc. Disc movement speed during test mode was 1 mm s\(^{-1}\) (forwards) and 5 mm s\(^{-1}\) (backwards) with distance of 20 mm.

**Pea spread assessment**

Principles of integrated evaluation were used for pea spread assessment by a set of features (Martinov, 1987). Integrated evaluation method by a set of specific features is used when different features of samples (e.g., physicochemical composition, sensory features etc.), which are to be compared as a whole, have different measurement scales (e.g., proteins are measured in grams, firmness – in newton, dry matter content – in %). Then each feature is assigned with a contribution coefficient depending on how much of a contribution each feature is (e.g., for legume spreads higher protein and fibre content is important, therefore, these two features have higher contribution coefficients than pH or colour component L*). Integrated evaluation method can be used to assess and reduce the number of samples if the initial sample count is high, in order to limit the costs of time consuming analyses when these analyses will not produce the expected outcome.

As it can be seen in Table 2, the evaluation is completed by analysing the observations made with different measurement scales and assigning each feature group and each individual feature a contribution coefficient.

For example, high contribution coefficients can be given to protein and total dietary fibre content but valuable physicochemical composition is not compatible with low hedonic assessment values considering that no consumer is interested in a product which is sensory unsatisfactory but has a high nutritional value. Therefore it is important to assess each feature by its contribution in the final product, and integrated evaluation indicates the optimal ratio between feature contributions and the final product is both sensory satisfactory and nutritionally valuable.
Integrated evaluation value \( (IN) \) is characterized by the deviation of pea spread assessment values from the optimal values, which results in a lower integrated evaluation value corresponding to the spread, which is most suitable for a particular purpose, in this case, shelf-life investigation of pea spreads.

**Initial shelf-life assessment**

Shelf-life of freshly prepared spread was evaluated according to Guidelines for the Interpretation of Results of Microbiological Analysis of Some Ready-to-eat foods Sampled at Point of Sale (Gilbert et al., 2000). According to the guidelines, pea spread
is included in savoury group (paté (meat, seafood or vegetable)) which belongs to category 3, and satisfactory microbiological safety is obtained if total plate count for ready to eat pea spread is below $10^5$ colony forming units per gram (CFU g$^{-1}$). Preparation of test samples, initial suspension and decimal dilutions for microbiological examination was carried out according to ISO 6887-1:1999. Total plate count (TPC) was determined according to the standard ISO 4833-1:2014. 90 ml 0.1% peptone water was added to 10 g sample of pea spread in a stomacher bag; then the sample was homogenized with a stomacher BagMixer400 (Interscience, USA) for 10 seconds. After preparing serial decimal dilutions of the homogenate with 0.1% peptone water, triplicate plates were prepared using pour plate method for enumeration. Total viable counts were determined on Plate Count Agar with incubation at $+30 \pm 1$ °C for 72 ± 3 h. After the specified period of incubation, colony forming units were counted with automated colony counter aCOLyte (Topac Inc., USA).

**Software and data processing**

The obtained data processing was performed using mathematical and statistical methods with statistical software ‘R 3.0.2’ and ‘Microsoft Office Excel 14.0’; differences among results were analysed using two way analysis of variance and Tukey’s test. Each sample was analysed in triplicate (unless stated otherwise), and the results were expressed as mean ± standard deviation. For the interpretation of the results it was assumed that $\alpha = 0.05$ with 95% confidence and differences among results were considered significant if $p$-value < $\alpha_{0.05}$.

**RESULTS AND DISCUSSION**

Six pea spreads from each pulse were subjected to hedonic evaluation during the Baltics food industry fair ‘Riga Food 2014’ (Table 3). The overall preference of cowpea spread samples ranges from 2.8 (‘not sure’) to 4.6 (‘like very much’) and there were significant differences among cowpea spreads. Cowpea spreads with roasted sesame seeds (D), dry herbs (E), paprika (F) and cowpea spread without spices (A) were not significantly different among themselves however they were preferred significantly less ($P < 0.05$) than cowpea spread with onions (B) or bruschetta (C).

<table>
<thead>
<tr>
<th>Table 3. Results of hedonic evaluation of pea spreads</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pea spread samples</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Control sample–without spices</td>
</tr>
<tr>
<td>With onion spice</td>
</tr>
<tr>
<td>With bruschetta spice</td>
</tr>
<tr>
<td>With roasted sesame seeds</td>
</tr>
<tr>
<td>With green herbs</td>
</tr>
<tr>
<td>With paprika spice</td>
</tr>
</tbody>
</table>

* values within a column not sharing a superscript letter are significantly different ($P < 0.05$).
The overall preference of maple pea spread samples were within a similar range–from 3.0 (‘not sure’) to 4.9 (‘like very much’). There were four spread samples that had a higher preference and did not differ significantly among themselves ($P = 0.221$): maple pea spread with onions (L), bruschetta (M), paprika (P) and without spices (K). The preference of samples N and O was significantly lower than of the previously mentioned maple pea samples ($P = 0.013$).

Preference of pea spread samples with bruschetta, onion and paprika spice (in the case of maple pea spread) was given due to more pronounced taste than in other spreads. Spreads with roasted sesame seeds and dry herbs had too mild taste for most panellists’ liking. Both spread samples with bruschetta spice were characterised as ‘very similar to traditional pate (made of meat)’ but spreads with onion spice ‘would taste excellent with a glass of kefir’. Spreads with higher hedonic value were said to have ‘good consistency’.

The results of hedonic evaluation of pea spreads showed that not all samples should be subjected to physicochemical evaluation because some were sensory unsatisfactory. Further analyses were completed with control samples and highest rated samples: cowpea spreads A, B and C, and maple pea spreads K, L, M and P.

Physicochemical analyses showed significant differences among some pea spread samples (Table 4). Protein content in pea spreads ranged from 7.05 to 7.47 g 100 g$^{-1}$ with no significant differences ($P = 0.071$) among all samples. A previous study on white bean ($Phaseolus vulgaris$ L.) spreads showed that protein content was not dependent on spices used (Kirse & Karklina, 2013).

### Table 4. Physicochemical composition of pea spreads (I): A, K – control sample (without spices), B, L – with onion spice, C, M – with bruschetta spice, P – with paprika spice

<table>
<thead>
<tr>
<th>Pea spreads</th>
<th>Protein, g 100 g$^{-1}$</th>
<th>Total dietary fibre, g 100 g$^{-1}$</th>
<th>Ash, g 100 g$^{-1}$</th>
<th>Total phenolics, mg GAE g$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cowpea</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>7.23 ± 0.06$^{a*}$</td>
<td>14.80 ± 0.20$^{a}$</td>
<td>2.78 ± 0.02$^{a}$</td>
<td>7.94 ± 0.45$^{a}$</td>
</tr>
<tr>
<td>B</td>
<td>7.14 ± 0.07$^{a}$</td>
<td>13.80 ± 0.02$^{b}$</td>
<td>2.53 ± 0.02$^{a}$</td>
<td>8.44 ± 0.49$^{a}$</td>
</tr>
<tr>
<td>C</td>
<td>7.05 ± 0.02$^{a}$</td>
<td>12.00 ± 0.15$^{c}$</td>
<td>2.52 ± 0.02$^{a}$</td>
<td>9.23 ± 0.63$^{b}$</td>
</tr>
<tr>
<td><strong>Maple pea</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>7.47 ± 0.01$^{a}$</td>
<td>14.81 ± 0.25$^{a}$</td>
<td>2.94 ± 0.02$^{a}$</td>
<td>11.67 ± 0.48$^{c}$</td>
</tr>
<tr>
<td>L</td>
<td>7.42 ± 0.03$^{a}$</td>
<td>12.69 ± 0.02$^{c}$</td>
<td>2.90 ± 0.02$^{a}$</td>
<td>12.33 ± 0.60$^{c}$</td>
</tr>
<tr>
<td>M</td>
<td>7.38 ± 0.04$^{a}$</td>
<td>11.98 ± 0.18$^{c}$</td>
<td>2.92 ± 0.05$^{a}$</td>
<td>11.64 ± 0.30$^{c}$</td>
</tr>
<tr>
<td>P</td>
<td>7.16 ± 0.07$^{a}$</td>
<td>10.72 ± 0.01$^{d}$</td>
<td>2.91 ± 0.03$^{a}$</td>
<td>10.82 ± 0.22$^{d}$</td>
</tr>
</tbody>
</table>

* values within a column not sharing a superscript letter are significantly different ($P < 0.05$).

McCarty et al. (2009) have noted that legume proteins are relatively low in the essential amino acid methionine (as are seeds and nuts), nevertheless, most plant proteins are incomplete and by combining complementary foods from two or more incomplete protein sources, a complete protein can be created. Grains (which are deficient in lysine) are commonly consumed along with legumes to form a complete diet of protein. Legume spreads are supposed to be consumed together with bread or crackers hence avoiding incomplete protein. Legumes are among the best protein sources in the plant kingdom and unlike conventional animal food sources of protein such as beef or milk, legumes are packed with hormone-free, steroid-free and antibiotic-free plant
protein (Papanikolaou & Fulgoni, 2008). Amino acid content will be analysed during legume spread shelf-life investigation to identify imbalance of essential amino acids.

Ash content did not depend on spices used ($P = 0.061$), Filipiak-Florkiewicz et al. (2011) have shown similar findings on legume ash content.

Pea spreads were good sources of total dietary fibre (10.72 to 14.81 g 100 g$^{-1}$). Products can be labelled as a ‘source of fibre’ (Commission Directive 2008/100/EC; Regulation No 1169/2011) if the product contains ≥ 3.0 g fiber 100 g$^{-1}$, and ‘high in fiber’ if the product contains ≥ 6.0 g fiber 100 g$^{-1}$. According to previously mentioned documents, pea spreads are ‘high in fibre’ and a serving (100 g) of pea spreads covers over 43% of recommended daily fibre for adolescents (Regulation No 1169/2011) which is 25 g per day (per 2,000 kcal diet). Soluble and insoluble fiber ratio in cooked cowpeas and maple peas is about 1 : 3.2 (Khan et. al., 2007) that corresponds to European Guidelines on cardiovascular disease prevention in clinical practice (Perk et al., 2012); the ratio is maintained in the new pea spreads. Maple pea spread with paprika spice (sample P) has significantly lower total dietary fibre content ($P = 0.012$). To prepare maple pea spread with paprika spice, more of the spice was used (33.0 g per 1,000 g of the spread) compared to other spreads. Paprika spice is liquid (it contains oil) and therefore practically does not contribute to total dietary fibre content, but, in fact, slightly lowers it (as the amount of legume is reduced in 1,000 g of spread).

Khan et al. (2007) have shown that total dietary fibre content in cowpeas and maple peas is 18.2 and 13.4 g 100 g$^{-1}$ (dry weight) which is less than in pea spreads. This can be due to changing climatic conditions, legume-growing region, harvesting time and legume storage conditions, as increased soil drought contributes to the increase in fiber content of legumes, and legume seed coat may account to over 10.2–19.6% of the legume seed mass (Gupta, 2011).

Siddhuraju & Becker (2007) have determined that total phenols in different varieties of cowpea (autoclaved after soaking) range from 6.45 to 9.53 mg GAE g$^{-1}$ which correspond to total polyphenol content in cowpea spreads. However, our values for total phenolic content are significantly different to those reported by Zia-Ul-Haq et al. (2013), as total phenolic content in cowpea cultivars commonly consumed in Pakistan ranged from 11.90 to 19.32 mg GAE g$^{-1}$. Nithiyananthanam et al. (2012) have shown that total phenolics in field pea seeds autoclaved after soaking ranged from 12.45 to 24.70 mg GAE g$^{-1}$ which is similar but higher than total polyphenol content in maple pea spreads. The loss of phenolic compounds could be attributed to water-soluble phenolics leaching into soaking and cooking water. According to Amarowicz et al. (2004), the total phenolic content is directly associated with antioxidant activity; the binding between phenolics and the protein matrix might account for the enhancement of antioxidant capacity in peas because a phenolic–protein interaction is able to stabilize the protein and its antioxidant capacity is increased during heating (Tsai & She, 2006). During shelf-life investigation antioxidant activity will be determined.

The measurements with CIE $L^*a^*b^*$ colour system showed that cowpea spreads were lighter than maple pea spreads and addition of spices had a significant impact on the lightness ($L^*$) of pea spreads ($P = 0.010$); samples without spices were lighter than pea spreads with spices (Table 5).

<table>
<thead>
<tr>
<th>Pea spreads</th>
<th>L* value</th>
<th>pH</th>
<th>Dry matter, %</th>
<th>Firmness, N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cowpea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>60.72 ± 0.31a*</td>
<td>5.90 ± 0.02a</td>
<td>33.00 ± 0.05ab</td>
<td>8.50 ± 0.02a</td>
</tr>
<tr>
<td>B</td>
<td>60.63 ± 0.81a</td>
<td>5.80 ± 0.03a</td>
<td>35.10 ± 0.05ab</td>
<td>12.25 ± 0.03bd</td>
</tr>
<tr>
<td>C</td>
<td>58.89 ± 0.49b</td>
<td>5.83 ± 0.03a</td>
<td>33.00 ± 0.04ab</td>
<td>11.76 ± 0.03b</td>
</tr>
<tr>
<td>Maple pea</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>57.46 ± 1.01b</td>
<td>5.91 ± 0.03a</td>
<td>33.50 ± 0.05ab</td>
<td>9.72 ± 0.03a</td>
</tr>
<tr>
<td>L</td>
<td>51.27 ± 1.42c</td>
<td>5.81 ± 0.02a</td>
<td>33.40 ± 0.03ab</td>
<td>14.02 ± 0.05c</td>
</tr>
<tr>
<td>M</td>
<td>51.84 ± 1.51c</td>
<td>5.83 ± 0.01a</td>
<td>34.00 ± 0.03ab</td>
<td>13.46 ± 0.04cd</td>
</tr>
<tr>
<td>P</td>
<td>50.38 ± 0.94d</td>
<td>5.42 ± 0.02b</td>
<td>32.30 ± 0.04a</td>
<td>9.50 ± 0.03a</td>
</tr>
</tbody>
</table>

* values within a column not sharing a superscript letter are significantly different (P < 0.05).

pH of maple pea spread with paprika spice was significantly lower than pH of other samples (P = 0.023) because lemon juice and vinegar are components of paprika spice mix. Dry matter content is similar in all pea spreads. Firmness of pea spreads ranged from 8.50 to 14.02 N and was influenced by the addition of spices (P = 0.006), more force was needed to compress samples with solid spices. Paprika spice mix is liquid; therefore less force was needed to compress the sample compared with maple pea spread without spices.

In order to determine the suitability of pea spreads for shelf-life investigation, many factors were assessed and high contribution coefficients (ωi) were given to protein content (g), total dietary fibre (g), total phenolics (mg), firmness (N) and hedonic evaluation, lower contribution coefficients were given to ash content (mg), dry matter content (%), pH and L* value.

The integrated evaluation shows that both cowpea spread with bruschetta spice (Fig. 1) and maple pea spread with bruschetta spice (Fig. 2) have lower integrated value and are most suitable for pea spread shelf-life investigation.

Figure 1. Cowpea spread suitability for shelf shelf-life investigation: A – control sample without spices, B – with onion spice, C – with bruschetta spice.
Shelf-life investigation should be completed with the most suitable spread and control sample, i.e., cowpea spread without spices (A) and with bruschetta spice (C). The integrated evaluation value of maple pea spread without spices (K) and with bruschetta spice (M) are not significantly different ($P > 0.05$). Both spreads should be subjected to shelf-life investigation as well. In pea spreads with lower integrated values there is a better balance between sensory and nutritional parameters thus suggesting these spreads have the potential to be produced for consumer consumption after shelf-life investigation.

![Integrated evaluation values](image1)

*Figure 2.* Maple pea spread suitability for shelf shelf-life investigation: K – control sample without spices, L – with onion spice, M – with bruschetta spice, P – with paprika spice.

Initial shelf-life testing was performed with maple pea spread with bruschetta spice as it was suggested for shelf-life investigation. Total plate count in freshly made maple pea spread with bruschetta spice was $4.11 \log \text{CFU g}^{-1}$ and reached the critical $5.00 \log \text{CFU g}^{-1}$ after less than six days of storage at refrigerator temperature (Fig. 3). This short term storage would not allow any manufacturer to expand the trade in further regions of Latvia or export this product.

![Total plate count dynamics](image2)

*Figure 3.* Total plate count ($\log \text{CFU g}^{-1}$) dynamics in maple pea spread with bruschetta spice during storage at $+4.0 \pm 0.5 ^\circ \text{C}$ temperature.
Heat treatment and appropriate packaging solutions must be selected to extend pea spread shelf-life, thus reducing the total number of micro-organisms and avoiding accelerated deterioration of the product.

CONCLUSIONS

Total dietary fibre, total polyphenols, colour and firmness of pea spreads depend on spices used (\(P < 0.05\)), while protein, ash and dry matter content is not significantly different among pea spreads.

The integrated evaluation of new legume spreads shows that both cowpea spread with bruschetta spice and maple spread with bruschetta spice had the lowest integrated evaluation values and are most suitable spreads for shelf life investigation.

Shelf-life of maple pea spread with bruschetta spice is five days; heat treatment and appropriate packaging solutions must be considered.

ACKNOWLEDGEMENTS. The research was supported by the FP7 Research Project No 61378 ‘EUROLEGUME–Enhancing of legumes growing in Europe through sustainable cropping for protein supply for food and feed’.

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A study on bryophyte chemical composition–search for new applications

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Abstract. Bryophytes are the taxonomic group in the plant kingdom represented with about 25’000 species. They contain a high number of biologically active compounds; however their use as a food source is negligible. The aim of this paper is to evaluate bryophyte chemical composition and new possible applications. In order to evaluate bryophyte potential usage as a raw food material, bryophyte basic chemical content and the secondary metabolite profile was determined. To obtain best results bryophyte secondary metabolite extraction options has been studied. Couple of extraction methods were used (conventional, ultrasound, microwave, supercritical CO$_2$ extraction etc.) and different solvents (ethanol, water etc.). A total concentration of polyphenols and substances determining free radical scavenging activity has been determined. The extracts obtained from bryophytes have remarkable antioxidant activity, the extent of which depends on the extraction conditions and bryophyte species. Comparison of five extraction methods and several solvents indicates that microwave assisted extraction and supercritical CO$_2$ extraction is the most promising approach to obtain highest yields of extractives.

Key words: bryophytes; extraction, polyphenols, antiradical activity, optimization, food.

INTRODUCTION

Bryophytes are the second largest group in the plant kingdom with about 25’000 bryophyte species (Asakawa et al., 2013) and they can be found in any kind of ecosystems (Glime, 2007). In comparison with higher plants use of bryophytes for human consumption is negligible due to their low caloric value (Forman, 1968) and poor organoleptic properties. Traditionally, use of bryophytes as a food source is limited for famine periods, however in Northern regions of Europe and America bryophytes are used as an ingredient of bread or soup. In circumpolar regions bryophytes are a common animal feed (Glime, 2007).

Recent research demonstrates a presence of a large number of biologically active substances in the composition of bryophytes. Bryophytes due to the presence of high number of biologically active compounds in their composition are commonly used in ethnopharmacology and as medical plants for treatment of wounds and burns (Singh et al. 2006; Cheng et al., 2012; Fu et al., 2012; Asakawa et al., 2013). More specifically bryophytes demonstrate antibacterial, antifungal, antiviral activities, antioxidant, antiplatelet, antithrombin, insecticidal, neuroprotective activities, as well as cytotoxicity in respect to cancer cells (Spjut et al., 1986; Cheng et al., 2012).
Bryophytes are mainly composed of hemicelluloses and pectin (30 to 60% respectively, cellulose content from 15 to 25%). Bryophytes also contain 5 to 10% proteins, 5 to 10% lipids and phenolic compounds (Orlov et al., 2005). The content of lignin has been found to be insignificant (Klavina, 2015), however controversies in this respect exist (Painter et al., 2003). Notably, the chemical composition of bryophytes differs depending on species, growth environment, and season (Goffinet & Shaw 2008; Glime 2007; Xie et al., 2009).

Growth of human population and a need to develop healthy diets requires looking for new, alternative food as well as for sources of important food ingredients, for example antioxidants. Further, caloric value of food considering abundance of high calory food nowadays, is by far not the main direction of activities in search for alternative food plants (Haines & Renwick 2009). In this respect bryophytes are highly relevant and prospective object of studies. Nowadays bryophyte availability for practical applications does not depend on their field sampling possibilities, but they can be grown using biotechnological approaches (in reactors) as axenic cultures, but also in large scales (ton quantities – Sphagnum farming) (Beike et al., 2012; Pouliot et al., 2014). To advance the studies of alternative food sources, it is important to study bryophyte and their extract composition.

The aim of this paper is to evaluate potential of bryophyte use in food and extraction possibilities of their secondary metabolites with a special emphasis on polyphenolic compounds as a source of natural antioxidants.

MATERIALS AND METHODS

Materials
In this study 16 bryophyte species common for Northern Europe (from moist coniferous forests, moist deciduous forests and bogs in Latvia) were selected. Bryophyte living parts were collected, identified (Strazdiņa et al., 2011), and cleaned from biotic contamination, washed with distilled water and air dried. Top parts (2–5 cm) of bryophytes were used and before analysis samples were stored at -20°C. Each bryophyte species specimen voucher is stored in Department of Environmental Science, University of Latvia.

Conventional extraction of bryophytes
Sample of Rhytidiadelphus triquetrus was dried at +40°C in an oven until constant mass. Dry sample was grinded in a mill and 0.3 g of bryophyte sample was weighed into 100 mL bottles with screw cap and 50 ml of solvent was added. Solvents such as ethanol (96, 80, 60, 40, and 20%) diluted with demineralised (Millipore) water and water. The bottles were shaken in a shaker for 24 h at 140 rpm. All extracts were filtered and stored until analysis at 4°C up to 1 month.

Ultrasound-assisted extraction of bryophytes
Solvents such as ethanol (96, 80, 60, 40, and 20%) diluted with demineralised (Millipore) water and water were used. Samples afterwards were treated with 20 and 40 min of ultrasound (100W) in ultrasound bath (Cole Parmer), temperature was regulated with regular adding of cold water to keep constant temperature of +40°C. The bottles then were shaken in a shaker for 24 h at 140 rpm.
**Extraction of bryophytes by microwave treatment**

Dry samples were ground in a mill and 0.3 g of bryophyte sample were weighted into Teflon extraction tubes and 50 ml of solvent (96%, 80%, 60%, 40%, 20% ethanol) were added and sealed using Milestone Twister. Extraction was performed using *Milestone Ethos One* microwave oven in 120°C and 150°C with power of 1500 W. Extraction took 40 min: 10 min to reach chosen temperature, 20 minutes for a steady extraction at a set temperature and 10 minutes for oven to cool down.

**Extraction of bryophytes with supercritical CO₂**

Dry sample was ground in a mill and 15 g of bryophyte sample was weighted into metallic column. Column was inserted in preheated (+102°C) oven, after CO₂ flow of 10 ml per minute was set. Extraction was done using *Separex CO₂ supercritical extractor*. After first trials it was concluded that coupled extraction was required for best results, therefore 96 % ethanol flow (5 ml min⁻¹) also was set. Extraction experiments were conducted under 20 MPa pressure for 30 minutes and 60 minutes.

**Soxhlet extraction of bryophytes**

Dry bryophyte sample (20 g) was weighted into fabric bag. Fabric bag was sealed and inserted in extraction tube. Extraction was done using Soxhlet extractor and as a solvent 96% ethanol was used. Extraction process was done in 80°C for 8 h and 24 h time period.

**Estimation of dry weight**

Prepared extracts were kept in room temperature for an hour and total amount of extract measured using pipette; afterwards 5 ml of each extracts were measured in previously prepared and weighted weight glasses. Extraitent was evaporated on a stove (70°C) until dry. After weight glasses have cooled down it was placed in exicator for 24 h. After that weight glasses were weighted in triplicate. Dry weight of extracts was expressed as mg 100 g⁻¹ dry moss weight.

**¹³C-NMR spectroscopy**

Solid-state ¹³C–NMR spectroscopy was carried out using the technique of cross-polarization with magic angle spinning (CP/MAS). The spectra were recorded on a *Bruker Avance wide-bore 600 MHz* solid state NMR spectrometer equipped with a 4 mm MAS double-resonance probe. 2 ms contact time and 2 s repetition time were used. The sample magic angle spinning was 10 kHz, and chemical shifts were referenced to adamantane at 38.48 ppm.

**Total polyphenol concentration determination in bryophyte extracts**

Before all analysis, bryophyte extracts were kept at room temperature for ~1 hour. 1 ml of bryophyte extract was added to a test tube and 5 ml of 10% Folin-Ciocalteu reagent (*Aldrich*) was added, after 5 minutes 4 ml of 7.5% sodium carbonate (*Aldrich*) was added. The test tube was shaken thoroughly and kept in a dark place at room temperature for 2 hours. Absorption was then measured using a quartz cuvette (d = 1 cm) on a spectrophotometer (*Hach-Lange DR 2800*) at 725 nm wavelength Narwal et al. 2011. Results were calculated using a standard curve (gallic acid concentration
5–150 mg l\(^{-1}\)), which was expressed as gallic acid 100 g\(^{-1}\) (GE 100 g\(^{-1}\)) dry matter (Singleton et al., 1999). Three parallel measurements were carried out.

**Radical scavenging activity determination in bryophyte extracts using DPPH**

In a test tube 0.3 ml of bryophyte extract was added and was mixed with 3.6 ml of 4% solution in 96% ethanol 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Aldrich). Mixture was incubated for 20 minutes in a dark place in room temperature. Absorption was measured using a quartz cuvette (d = 1 cm) with a spectrophotometer at 517 nm wavelength. Three parallel measurements were carried out. Results were calculated using a standard curve (gallic acid concentration 1–5 mg l\(^{-1}\)), which is expressed as gallic acid 100 g\(^{-1}\) (GE 100 g\(^{-1}\)).

**RESULTS AND DISCUSSION**

The selection of the bryophyte species was done considering their abundance in the Northern Europe, sampling and cultivation possibilities, use in traditional medicine and food, and possible presence in their composition of biologically active substances. The studied bryophytes showed a relatively low variability in their elemental composition and the ranges of basic elements in bryophyte species were: C 41–44%; O 49–52%; H 5.5–6%; N 0.4–2%; S 0% (Maksimova et al., 2013). Metal content and basic chemical characteristics of studied bryophyte species were given in previous study (Maksimova et al., 2013). To study basic chemical composition, the cross-polarization magic angle spinning \(^{13}\)C nuclear magnetic resonance spectra (CPMAS \(^{13}\)C NMR) of solid samples of the studied bryophyte species was used (Fig. 1). This method supports quantitative estimation of major structural elements of organic matter, including biota samples (Nierop et al., 2001).

![Figure 1. \(^{13}\)C NMR spectra of moss Rhytadiadelphus triquetrus.](image)

Chemical shifts in spectra were compared with literature data (Karlström et al., 1995) and they were divided into chemical shift regions, depending on different functionalities of chemical compounds.

The chemical shift region from 0 to 50 ppm corresponds to aliphatic carbon and amount of aliphatic structures on average has been ~ 10%. The chemical shift region 50 to 92 ppm corresponds mainly to the ring carbon of carbohydrates (Karlström et al., 1995). The chemical shift region from 92 to 112 ppm corresponds to the axial carbon of carbohydrates (Karlström et al., 1995; Nierop et al., 2001). CPMAS \(^{13}\)C NMR spectra
confirm that carbohydrates were the main constituents of bryophytes as it was stated already before (Maksimova et al., 2013). The chemical shift region from 112 to 136 ppm corresponds to the aromatic carbon (Karlström et al., 1995). The chemical shift region from 136 to 159 ppm corresponds to the phenolic and N-substituted aromatic carbon. Amount of aromatic compounds, including lignin and its derivatives was low in studied bryophytes. The chemical shift region from 159 to 190 ppm corresponds to the carbon in fatty acids, including free carboxylic acids, their esters, and also amides. The chemical shift region from 190 to 212 ppm corresponds to the carbonyl group carbon (Silverstein et al., 2005). Fatty acids and alcohols were present in bryophytes in minimal amounts. Composition of bryophytes partly explains low caloric value of bryophytes (3,800–5,000 cal g⁻¹ dry weight, Forman 1968, Rastorfer 1976), however accessibility of structural carbohydrates for animal consumption could be another reason (Klavina 2015) to this. So the composition of secondary metabolites thus could become a major direction of research of bryophyte composition and their application possibilities.

Optimization of extraction was performed using moss – *Rhytidiadelphus triquetrus* as representative sample of mosses common for mixed wood forest ecosystems. As criteria for the evaluation of extraction efficiency, the following parameters were used: yield of extracts (dry residue), total polyphenolic content, radical scavenging activity, carbohydrate content analysis. Amongst criteria of extraction efficiency major stress was put on the yield of extracted substances and the antioxidant activities of the extracts, considering recent interest just in this kind of activity of natural compounds (Cheynier et al., 2013) and for this purpose DPPH radical scavenging activity analysis was used. Considering interest in studies of bryophyte biologically active compounds and more broadly in the composition of bryophyte secondary metabolites, for the extraction low-cost, low-toxicity, volatile solvents and their mixtures were selected with ability to extract substances with possibly wider range of properties (water, ethanol, CO₂). Five different extraction methods ensuring possibly highest extraction yield, prospective for obtaining of preparative amounts of extracts of were used: a) conventional extraction (shaking at room temperature); b) Soxhlet extraction; c) ultrasound-assisted extraction; d) extraction using treatment with microwaves; e) extraction with supercritical CO₂. To study impact of the extraction procedures, extraction conditions (time and temperature) were changed to compare efficiency of each selected method.

The most efficient extraction method (Table 1) proved to be microwave extraction at 150°C both judging by the total polyphenol content and the radical scavenging activity. Conventional and Soxhlet extraction provided high yields, but in comparison with intensive extraction methods, required much more time. Conventional extraction consumed also much more solvent than the other studied methods. Soxhlet and extraction showed good results when extracts were tested in respect to radical scavenging activity; however total polyphenol levels were lower than, for example, in case of ultrasound assisted extraction. This implies that not only polyphenolic compounds in bryophytes were responsible for radical scavenging activity. Supercritical CO₂ extraction provided good results in total polyphenol content, but the overall yields and radical scavenging activity was relatively low.
Table 1. Extraction efficiency of different extraction methods of biologically active compounds from *Rhytadiadelphus triquetrus* (solvent 60% ethanol*). Data are average from three replicates ± SE.

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Time of extraction, h dry weight</th>
<th>Total polyphenol content, GE 100 g⁻¹ dry weight</th>
<th>Radical scavenging activity, GE 100 g⁻¹ dry weight</th>
<th>Extraction yield, mg 100 g⁻¹ dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soxhlet*</td>
<td>165.5 ± 8.3</td>
<td>116.4 ± 5.8</td>
<td>205.2 ± 10.3</td>
<td>165.5 ± 8.3</td>
</tr>
<tr>
<td>Microwave</td>
<td>239.6 ± 11.9</td>
<td>142.6 ± 7.1</td>
<td>231.5 ± 11.6</td>
<td>239.6 ± 11.9</td>
</tr>
<tr>
<td>Microwave</td>
<td>111.2 ± 5.6</td>
<td>167.8 ± 8.4</td>
<td>195.6 ± 9.8</td>
<td>111.2 ± 5.6</td>
</tr>
<tr>
<td>Microwave</td>
<td>486.9 ± 24.4</td>
<td>172.9 ± 8.6</td>
<td>150.2 ± 7.5</td>
<td>486.9 ± 24.4</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>243.7 ± 12.2</td>
<td>54.1 ± 2.7</td>
<td>195.5 ± 9.7</td>
<td>243.7 ± 12.2</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>254.6 ± 12.7</td>
<td>63.3 ± 3.2</td>
<td>150.6 ± 7.5</td>
<td>254.6 ± 12.7</td>
</tr>
<tr>
<td>Supercritical CO₂</td>
<td>230.4 ± 11.5</td>
<td>162.4 ± 8.1</td>
<td>125.1 ± 6.3</td>
<td>230.4 ± 11.5</td>
</tr>
<tr>
<td>Supercritical CO₂</td>
<td>274.9 ± 13.5</td>
<td>143.5 ± 7.2</td>
<td>124.8 ± 6.2</td>
<td>274.9 ± 13.5</td>
</tr>
<tr>
<td>Conventional</td>
<td>150.7 ± 7.6</td>
<td>25.8 ± 1.3</td>
<td>99.5 ± 4.9</td>
<td>150.7 ± 7.6</td>
</tr>
<tr>
<td>Conventional</td>
<td>194.3 ± 9.7</td>
<td>36.9 ± 1.8</td>
<td>97.2 ± 4.9</td>
<td>194.3 ± 9.7</td>
</tr>
</tbody>
</table>

* Extraction done using 96% ethanol.

For a better understanding of the extraction efficiency using ultrasound the effect of treatment time, as well as composition of extrahents, in respect to application potential, ethanol: water mixture ratio was compared (Fig. 2). Ultrasound-assisted extraction helped to improve extraction yield due to mechanical stress which cavitation induces, following cellular breakdown and release of secondary metabolites.

![Figure 2](image-url)

**Figure 2.** Effect of ethanol: water ratio, extraction and treatment with ultrasound time on total polyphenol content in extracts from bryophyte *Rhytadiadelphus triquetrus*.

The increase of the treatment time with the ultrasound helped to significantly increase yield of polyphenolics (Fig. 2). Difference of extraction efficiency with 40 min ultrasound treatment and without was approximately 20–40% in some cases, but the difference between the treatments for 20 or 40 min was less than 10%. It may be concluded that it was effective to use ultrasound to increase extraction efficiency but the sonification duration if it exceeds 20 min do not have major impact. Highest yield of polyphenolics in case of *Rhytadiadelphus triquetrus* ensured use of 20–60% ethanol.
Detected differences in the extraction yield of total polyphenolics indicated differences in the bryophyte composition. For screening of bryophyte secondary metabolite composition 60% ethanol could be suggested and were further used in this study.

As a next step in polyphenolic, radical scavenging and total mass of extracted substances extraction optimization process various types of solvents were used ensuring secondary metabolite and especially phenolic compound isolation from bryophytes (Table 2). Solvents used were water and ethanol. Selection of solvents was based considering economic reasons and toxicity of solvents. Microwave extraction was concluded to be the most effective extraction technique; nevertheless solvent optimization was done using sample treatment with ultrasound with subsequent shaking for 24 h.

Table 2. Extraction efficiency of *Rhytadiadelphus triquetrus* using different solvents and solvent mixtures (extraction conditions—ultrasound treatment for 20 min with following shaking for 24 h and repeated 20 min ultrasound treatment). Data are from three replicates ± SE

<table>
<thead>
<tr>
<th>Extrahent</th>
<th>Total polyphenol content, GE 100 g⁻¹ dry weight</th>
<th>Radical scavenging activity, GE 100 g⁻¹ dry weight</th>
<th>Extraction yield, mg 100 g⁻¹ dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>230.0 ± 11.5</td>
<td>10.5 ± 0.5</td>
<td>36.8 ± 1.8</td>
</tr>
<tr>
<td>96%</td>
<td>254.0 ± 12.7</td>
<td>11.5 ± 0.6</td>
<td>652.5 ± 32.6</td>
</tr>
<tr>
<td>80%</td>
<td>304.0 ± 15.2</td>
<td>50.4 ± 2.5</td>
<td>667.5 ± 33.4</td>
</tr>
<tr>
<td>Ethanol</td>
<td>296.0 ± 14.8</td>
<td>24.0 ± 1.2</td>
<td>195.0 ± 9.8</td>
</tr>
<tr>
<td>60%</td>
<td>334.0 ± 16.7</td>
<td>7.9 ± 0.4</td>
<td>187.5 ± 9.4</td>
</tr>
<tr>
<td>40%</td>
<td>320.0 ± 16.0</td>
<td>6.3 ± 0.3</td>
<td>96.6 ± 4.8</td>
</tr>
<tr>
<td>20%</td>
<td>320.0 ± 16.0</td>
<td>6.3 ± 0.3</td>
<td>96.6 ± 4.8</td>
</tr>
</tbody>
</table>

Higher polyphenolic concentrations were found using ethanol. Antioxidant capacity (measured with DPPH method) was also higher using ethanol, but the differences were not as significant as in case of total polyphenolics content and the total dry extract mass. The optimal yields of polyphenolics, radical scavenging substances and total dry extract mass could be obtained from mosses using aqueous ethanol in concentration range from 60% till 80%.

As mentioned in Table 1 and 2 the best results were obtained using microwave assisted extraction with ethanol as the solvent. For better understanding the controlling factors of extraction efficiency, another experiment using microwave assisted extraction was carried out in order to see how ethanol concentrations affecting extraction efficiency (Fig. 3).

Higher polyphenol content was reached when microwave treatment was used at 150°C in comparison with 120°C. Microwave assisted extraction in comparison with ultrasound assisted extraction showed similarities when it comes to differences between different optimal solvent concentrations. Optimal ethanol concentration for *Rhytadiadelphus triquetrus* at 120°C temperature was 60%. Meanwhile optimal ethanol concentrations when used ultrasound assisted extraction was 40%. The extraction conditions elaborated for *Rhytadiadelphus triquetrus* were efficiently applied for extraction of biologically active secondary metabolites from bryophytes common in Northern Europe (Table 3).
Optimized extraction conditions allowed obtaining extracts with relatively high content of polyphenolics and high radical scavenging activity, also much dependent on the studied species. In comparison with much widely studied higher vegetation species...
(Häkkinen et al., 1999; Kolesnikov and Gins. 2001), especially, berries and plants used as a source of polyphenolics and antioxidants in food, the values of total polyphenol content and radical scavenging activity in studied bryophytes was significantly lower, however, the composition of bryophyte secondary metabolites could be considered as prospective to continue studies of their composition.

CONCLUSION

Bryophytes as abundant plant group, especially in Northern regions are interesting group of plants for studies of alternative food sources, despite low caloric value of them. Elemental composition analysis and $^{13}$C nuclear magnetic resonance spectra indicate that carbohydrates are a major structural component of bryophytes. Bryophytes contain numerous secondary metabolites. This study indicates that the extracts obtained from bryophytes have remarkable antioxidant activity, the extent of which depends on the extraction conditions. The principal factors that contribute to the efficiency of extraction are the type of solvent, temperature, ratio solvent: bryophyte mass, etc. Some of these parameters have been evaluated in this work on the extraction of polyphenolics and antioxidants.

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Study of functional and technological characteristics of protein concentrates from lupin seeds

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Abstract. The purpose of the research was to study functional and technological characteristics of concentrated protein preparations produced from lupin seeds and to demonstrate a feasibility of their usage as functional ingredients in food products. The method of production of concentrated protein preparations from seeds of Lupinus angustifolius was developed based on an acidic water extraction of non-protein compounds of lupin flour in presence of an optimized multi-enzyme composition composed of 1.1 ± 0.2 unit g⁻¹ of Celluclast and 5.2 ± 0.4 units g⁻¹ of Pentopan Mono and 2.5 ± 0.2 units g⁻¹ of Amilosubtilin. It was shown that crude protein content of protein concentrate preparations obtained with enzymatic treatment of polysaccharides increased to (63.2 ± 1.3) % on m.f.b. versus (50.4 ± 1.3) % on m. f. b. in the control preparation produced without an enzymatic treatment. Process parameters of infrared drying of lupin protein concentrate wet residues in the short wavelength range up to 2.5 µm were studied. The comparative analysis of functional and technological characteristics of lupin protein preparations, lupin flour and soy flour was carried out. Water-holding capacity, fat-holding capacity and emulsifying capacity of lupin protein concentrates produced via enzymatic treatment have been increased versus the control sample. The process and the formulation of fermented dairy product with inclusion of lupin protein additives in a range of 0.5% to 2.0% (% w w⁻¹) were developed. Sensory, physico-chemical and structural-mechanical properties of a dairy product enriched with lupin protein were analyzed. It was shown that usage of lupin protein preparations as nutritional supplements was beneficial for human gastrointestinal tract. 1.5% (% w w⁻¹) inclusion level of the lupin preparation in the food formulation provided the best consumer properties of finished products. It was shown that the lupin protein concentrate preparation can be used as a functional ingredient in fermented dairy products.

Key words: lupin protein concentrate, multi-enzyme composition, functional and technological properties, enriched dairy products, functional food ingredients.

INTRODUCTION

Production of vegetable proteins from non-traditional plant sources is one of perspective directions of increase of assortment and volumes of production of valuable protein ingredients for the food industry in Russia. Protein-based preparations can be used in formulations of sports nutrition products, specialized dry mixes, in meat and milk production and in bakery and in confectionery industry. Imported soybeans are used
often for industrial production of protein products. It could result in a high cost of finished products and besides soy protein preparations could be obtained from genetically modified plants (Papavergou et al., 1999; Sipsas, 2003).

In recent years many technological solutions have been developed. It allows to produce quality high-protein products from non-traditional vegetable raw materials (Ocheme and Chinma, 2008; Domoroshchenkova et al., 2009; Iakovchenko and Silantjeva, 2014). In particular, a new technology of isolated vegetable protein has been proposed (Lqari et al., 2002). Studies of the chemical composition and properties of flax seeds for development of functional foods have been conducted (Minevich, 2009).

Usage of additives produced from narrow-leaf lupin seeds in formulations of products from blended raw materials and in bakery, meat and dairy products analogues could be regarded as an innovative direction in food industry (Krasilnikov et al., 2010; Osman and Razig, 2010; Vij et al., 2011).

Study of functional and rheological properties of lupin proteins such as solubility, gel-forming capability, water-holding capacity, fat-holding capacity, foaming capacity and stabilizing properties etc. has shown that lupin proteins are very similar to soy proteins (Hojilla-Evangelistaa et al., 2004; Khalil et al., 2006). The rheological properties of the defatted white lupin flour suspension and viscoelastic properties of lupin proteins obtained by ultrafiltration have been studied at the Illinois National Center for Agricultural Utilization Research. The behavioristic characteristics of these systems during deformation are similar to the behavior of wheat gluten proteins under similar conditions (Xu and Mohamed, 2003; Xu et al., 2006).

However, lupin protein isolate (LPI) has formed weaker and more deformable gels versus gels formed by soy protein isolate presumably due to a large amount of free sulfhydryl groups which were increased upon heated (Berghout, J.A.M. et al., 2015).

This study considers usage of additives produced from food grade lupin seeds as an import nutritional and functional substitute due to an increase of protein content and improvement of functional and technological properties of final protein preparations and due to an increase of nutritional and biological value of enriched products as well as on basis of an improvement of structural and mechanical properties of enriched dairy products.

The presence of some anti-nutritional components and a high level of structural and storage polysaccharides in lupin seeds necessitate the use of methods that reduce its amount in final products (Ferket and Middleton, 1998, Lott J et al., 2000; Yuliarti et al., 2011). This problem is solved by the use of enzyme preparations with different substrate specificities that ensure a higher solubility of lupin polysaccharide complex.

The enzymatic degradation of polysaccharides causes a modification of functional and technological properties of protein preparations, such as water-holding capacity, fat-holding capacity, fat emulsifying capacity and gel-forming capability etc., which provides an opportunity to use them as a functional agent in food media (Damodaran et al., 1989; Enujiugha et al., 2003).

The objective of the study is to develop a method of production of lupin protein concentrate including a stage of an enzymatic degradation of polysaccharides, to demonstrate possibilities of lupin protein concentrate usage as a food additive and to develop a process and a formulation of enriched fermented dairy product.
MATERIALS AND METHODS

Materials
Lupin flour was kindly provided by the Russian Research Institute of Lupin, Brynsk. It was produced by grinding of dehulled lupin seeds of Lupinus angustifolius of ‘Snezhet’ variety planted in Brynsk origin (Russia). It contained 46% of crude protein on moisture free basis (m.f.b.) and 7% of crude fat on m.f.b.

For enzymatic treatment the following enzyme preparations have been used:
- Cellolux-F – an enzyme preparation that contains complexes of cellulase with activity of 2,000 ± 200 units g⁻¹, xylanase with activity up to 8,000 units g⁻¹, glucanase with activity up to 1,500 units g⁻¹. The preparation catalyzes the breakage of cellulose, xylans, β-glucans of plant cells up to sugars. The enzyme preparation was supplied by ‘Sibbiopharm’, Russia;
- Amilosubtilin – an enzyme preparation containing a complex of amylolytic enzymes, as well as related enzymes: β-glucanase, xylanase, glucoamylase, protease. Activity: 1,500 units g⁻¹. The enzyme preparation was supplied by ‘Sibbiopharm’, Russia;
- Celluclast BG – cellulase preparation made by submerged fermentation of the selected strain of fungus Trichoderma reesei, containing 3,500 endoglucanase units gram⁻¹. Preparation was provided by Novozymes, Denmark;
- Pentopan Mono BG – xylanase preparation from fungi Aspergillus oryzae, containing 2,500 fungal xylanase units gram⁻¹, Novozymes, Denmark.

A fullfat soybean flour sample for comparative studies was provided by the All-Russia Scientific Research Institute of Fats. It contained 42% of crude protein on m.f.b., 18% crude fat on m.f.b.

The yogurt starter culture of Streptococcus salivarius ssp. thermophilus and Lactobacillus delbrueckii ssp. bulgaricus were provided by the All-Russia Scientific Research Institute of Fats, Russia.

Preparation of the lupin concentrates
A method of soy protein concentrate production via acid leaching of non-protein substances at isoelectrical pH value of proteins (Sair, L. 1959) from soybean meal or flour has been used on an industrial scale. At the first stage of the study a similar technique has been used and adjusted for obtaining of protein concentrates from lupin flour and optimal conditions of extraction of non-protein compounds of lupin flour in acidic medium have been investigated (Kuznetsova et al., 2014).

A sample of lupin flour was mixed with water in ratio of 1:15. pH of this mixture was adjusted to 4.5 with 5% HCL solution at constant stirring at temperature 55°C. Then a multi-enzyme composition composed of (1.1 ± 0.2) units g⁻¹ of Celluclast + (5.2 ± 0.4) units g⁻¹ of Pentopan Mono + (2.5 ± 0.2) units g⁻¹ of Amilosubtilin was added. The suspension was stirred at constant temperature and pH in the range 4.4-4.5 for 40 minutes. The resulted suspension was centrifuged at 3,500x g for 15 min. and separated into two phases- a solid residue and whey. Crude protein was analyzed in a solid phase and it was used as an indicator of an efficiency of bioconversion of carbohydrates of initial lupin flour. Total sugar content was determined in lupin whey.
The wet residue of lupin protein concentrate was subjected to infrared radiation at short wavelengths from 2.0 microns to 2.5 microns at the density of IR emitter heat flow of 2.84 kW (m²)⁻¹ (Demidov et al., 2011). The width of a layer of the wet lupin concentrate residue was 4–5 mm. The distance from the IR emitter to the layer varied in the range of 60 to 80 mm. The drying was performed to reach up to 10% moisture content of the product. A fine powder of lupin protein concentrate passing through a sieve No.80 (0.178 mm) was obtained. It was used in subsequent experiments as a functional ingredient.

Preparation of the enriched fermented product

The study of dairy products technology with use of lupin protein concentrate as a functional ingredient was based on a method of industrial production of dairy products.

Samples of dairy products were obtained by water dilution of powered skimmed milk up to 3% dry matter content at 45°C and at constant stirring. Then the resulted milk was cooled up to 6°C and left for three hours in order to restore the natural structure of milk (normal density to achieve a due protein swelling and to eliminate a watery taste). Then the milk was pasteurized and cooled up to the fermentation temperature. Then 0.5% to 2% w w⁻¹ additives of dry lupin protein preparation to the weight of the mixture were added with an interval of 0.5% w w⁻¹. The mixture was thoroughly stirred for 10–15 minutes to obtain a uniform distribution of particles. At the next stage a yogurt starter was added and the mixture was fermented in a thermostat. Active and titratable acidity of the mixture as well as organoleptic and structural and mechanical properties of the system were monitored every hour. When the clot achieved a well-formed structure (and the value of titratable acidity ranged from 75°T to 80°T), the fermentation process was stopped. Samples of the enriched fermented product were cooled up to 4–6°C. The water holding capacity of samples was measured and the viscosity and organoleptic properties were evaluated.

Methods

Moisture content in the lupin protein concentrate was determined by the gravimetric method (AOAC, 1998). Crude protein content was analyzed by Kjeldahl method at the automated analyzer Kjeltec Auto 1030, Sweden, according to the standard protocol of the equipment vendor. The conversion factor used to estimate protein content was N x 6.25. Changes in pH were measured with Orion 920A pH-meter (Russia). Analysis of the total content of water-soluble carbohydrates in lupin whey was conducted by the Bertran method.

An IR dryer (Vympel, Russia) was used to dry the lupin protein preparation.

For determination of the water-holding capacity the sample of protein preparation was weighted and stirred with distilled water (1 g per 30 ml of water) at 1,000 rpm then the suspension was centrifuged at 3,000 rpm. A supernatant was decanted and a tube with a wet residue was weighed. Water holding capacity (WHC) was determined by the following formula (1):
WHC = (C - B) 100/A ,  \hspace{1cm} (1)

where: W - amount of water retained by 1 g of the sample, %; A - weight of sample, g; B - weight of centrifuge tube with a dry sample, g; C - weight of the centrifuge tube with a wet residue, g.

For determination of the fat-holding capacity (FHC) the sample of protein preparation was weighted and stirred with refined bleached deodorized sunflower oil (5 g per 30 ml of oil) at 1,000 rpm for one minute and then the mixture was left for 30 minutes. Then the suspension was centrifuged for 25 minutes at 3,200 rpm. Free oil was carefully decanted and a tube with a residue was weighed. The fat holding capacity was determined by the formula (2):

\[ FHC = (A-B) \frac{100}{C}, \hspace{1cm} (2) \]

where: A - weight of centrifuge tube with sample and retained oil, g; B - weight of centrifuge tube with sample, g; C - weight of sample, g.

For determination of the fat emulsifying capacity (FEC) the sample was weighted and stirred with distilled water (7 g per 100 ml of water) at 4,000 rpm for one minute. Then 100 ml of refined bleached deodorized sunflower oil were added to the mixture and stirred in a high-speed mixer at 8,000 rpm for 5 minutes. The final mixture was poured into calibrated centrifuge tubes and centrifuged for 5 minutes at 2,000 rpm. The volume of an emulsified layer and total volume of a mixture were measured. The fat emulsifying capacity was determined by the formula (3):

\[ FEC = \frac{V_e}{V_o} \times 100, \hspace{1cm} (3) \]

where: \( V_e \) - volume of the emulsified layer, cm\(^3\); \( V_o \) - total volume of the mixture, cm\(^3\).

For determination of the critical gel concentration (CGC) 10 homogeneous suspensions of the protein preparation in distilled water were prepared with concentration of 30%, 31%, etc. at intervals of 1%. Suspensions were transferred into 10 g centrifuge tubes and placed in a water bath and incubated for 30 minutes at temperature of 90 ± 2°C. Then the mixtures were rapidly cooled with a tap water to temperature 25°C, placed in a refrigerator and kept for 16 hours at temperature 4–6°C. The lead ball (m = 0.5 g) was placed on the surface of the suspension and left for 2 hours at temperature 4–6°C (Mahotina, 2009). The concentration of the sample when there was no destruction of gel structure under the pressure of the lead ball was considered as CGC.

The dynamics of acid accumulation during fermentation milk product was determined based on the values of titratable acidity-TTA (IDF Standard 86:1981 ISO 6091:1980).

Water holding capacity of coagulates was determined by centrifuging of samples for 30 min and determining the precipitated whey volume every 5 min.

Determination of structural and mechanical properties of clots was carried out on the ‘Rheotest-2’ rotational viscometer using a measuring cylinder N. To determine the
viscosity loss coefficients (Lv), the degree of recoverability of the structure (Rs), and the coefficient of mechanical stability (MS), the following formulas were used (4; 5):

\[ Lv = 100(\eta_{\text{first}} - \eta_{\text{last}})/\eta_{\text{first}}, \]  

(4)

where \( \eta_{\text{first}} \) - initial viscosity of the intact structure (viscosity at the time of the device switching on); \( \eta_{\text{last}} \) - viscosity of the most broken structure (the last measurement);

\[ MS = \eta_{\text{first}}/ \eta_{\text{last}}; \quad Rs = 100 \eta_r/ \eta_{\text{first}}, \]  

(5)

where: \( \eta_r \) – viscosity measured after structure recovery for 15 min.

**Statistical evaluation of the data**

All experiments were performed in with three replicates at least; data were processed by methods of mathematical statistics at theoretical frequency 0.95. Statistical processing of data was carried out using computer programs Microsoft Office Excel 2010 and Mathcad 15.0.

**RESULTS AND DISCUSSION**

**Preparation of lupin protein concentrates using multi-enzyme composition**

The objective of this stage of the study was to prove the working hypothesis that the enzymatic degradation of lupin polysaccharides would result in an increased concentration of proteins in a solid phase as well as in a more dynamic transition of soluble substances into whey.

First of all, a technology was developed that allowed to produce the lupin protein concentrate using an alternative multi-enzyme composition in optimum conditions for extraction of non-protein compounds (Kuznetsova and Zabodalova, 2013). In order to produce the alternative multi-enzyme composition specific to polysaccharides of the original lupin flour individual enzyme preparations were used such as Celluclast BG, Pentopan Mono BG and Amilosubtilin. The experiment results were compared with those obtained using the Cellolux-F complex cytolytic system (Table 1).

<table>
<thead>
<tr>
<th>The name of the enzyme</th>
<th>Crude protein content of concentrate, % on m.f.b.</th>
<th>Moisture content of concentrate, %</th>
<th>Dry matter content of whey, %</th>
<th>Total sugars content of whey, % on m.f.b.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control sample</td>
<td>50.40 ± 1.30</td>
<td>75.22 ± 1.06</td>
<td>1.30 ± 0.36</td>
<td>10.10 ± 0.31</td>
</tr>
<tr>
<td>Cellolux-F</td>
<td>54.12 ± 1.10</td>
<td>78.31 ± 0.42</td>
<td>1.90 ± 0.22</td>
<td>27.00 ± 0.20</td>
</tr>
<tr>
<td>Multi-enzyme composition</td>
<td>63.17 ± 1.30</td>
<td>77.48 ± 1.10</td>
<td>2.20 ± 0.23</td>
<td>29.00 ± 0.25</td>
</tr>
</tbody>
</table>

At addition of the multi-enzyme composition the total sugar content of the lupin whey has reached maximum content of 29% on m.f.b. The crude protein content of the protein concentrate was increased to (63.2 ± 1.3) % on m.f.b., i.e. 9% higher than in case of usage of the Cellolux-F complex cytolytic system.
Protein concentrates drying

Wet paste of lupin protein concentrate was placed into the dryer chamber, applied in a thin (4–5 mm) layer on the base plate and exposed to the short-wave (2.0–2.5 microns) infrared radiation (Fig. 1).

Figure 1. Drying of lupin protein concentrate. a – a wet paste immediately after placing in the IR-dryer camera, b – after 4 h of drying, c – after 5 h of drying.

It was important to find an appropriate balance between the drying time and the distance from the IR emitter to a layer of the product preventing a thermal denaturation of proteins.

The temperature on the surface layer was held at 42°C. The drying process was stopped at an average moisture content of the product of 10%. The drying time depended on the distance between an infrared emitter to the layer of protein concentrate. It was adjusted within the range of 60–80 mm. The final product drying time was 5 hours for the selected distance from the IR-emitter to the product layer of 60 mm. In case of increasing of the distance up to 80 mm the drying time was increased up to 6.5 hours.

Study of functional and technological characteristics of lupin protein preparation (LPP)

The functional and technological properties the following protein additives were analyzed: lupin flour, fullfat soybean flour, lupin protein preparation obtained without enzymatic treatment (negative control) and lupin protein preparation obtained with enzymatic degradation of polysaccharides (Table 2).

Table 2. Functional and technological characteristics of protein concentrates, lupin flour and soybean flour

<table>
<thead>
<tr>
<th>Sample</th>
<th>Type of treatment</th>
<th>WHC, %</th>
<th>FHC, %</th>
<th>FEC, %</th>
<th>CGC, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean flour</td>
<td>-</td>
<td>171 ± 20</td>
<td>75 ± 5</td>
<td>52 ± 1</td>
<td>39 ± 1</td>
</tr>
<tr>
<td>Lupin flour</td>
<td>-</td>
<td>210 ± 15</td>
<td>97 ± 5</td>
<td>65 ± 1</td>
<td>35 ± 1</td>
</tr>
<tr>
<td>LPP</td>
<td>negative control</td>
<td>317 ± 15</td>
<td>209 ± 5</td>
<td>87 ± 2</td>
<td>35 ± 1</td>
</tr>
<tr>
<td>LPP</td>
<td>cellolux-F</td>
<td>353 ± 20</td>
<td>267 ± 7</td>
<td>92 ± 2</td>
<td>37 ± 1</td>
</tr>
<tr>
<td>LPP</td>
<td>multi-enzyme composition</td>
<td>360 ± 17</td>
<td>312 ± 7</td>
<td>97 ± 1</td>
<td>39 ± 1</td>
</tr>
</tbody>
</table>
According to Table 2 functional and technological properties of the tested sample of fullfat soybean flour were worse than of the samples of lupin flour and lupin protein preparations. On the other hand WHC of industrial samples of soy protein isolates could much higher than of lupin preparations and reach up to 500–600% (M. Domoroshchenkova et al, 2007).

WHC, FEC and FHC of the tested protein preparations were consistently increased in the following sequence: lupin flour < LPP (control) < LPP (produced with the Cellolux-F enzymic preparation) < LPP (produced with multi-enzyme composition). This may be explained by a gradual increase of crude protein content in the protein preparations according to the mentioned sequence and due to modification of spatial structure of proteins (alteration of the charge of protein globules, bounds release, hydrophobic properties modification, α-helix unfolding etc.) which leads to improvement of functional and technological properties of the preparations. However the gel-forming capability was lower for enzyme treated lupin samples which could be related with a breakage of protein-polysaccharide bonds, partial proteolysis and polysaccharide complex degradation caused by action of enzymes. As known the structural modification of proteins leads to the decrease in the ability to form the gel spatial grid (Schwenke et al., 1998). CGC values of lupin protein concentrate in the range of concentrations of 30–40% are characteristic for a weak gelling agent.

As a result of enzymatic degradation of polysaccharide complex at the stage of extraction of protein-free compounds from lupin flour the functional and technological properties of the finished protein preparation were improved as compared to those of the raw material sample as well as to those of the control sample. WHC of the concentrate preparation obtained with multi-enzyme treatment was 43% higher, FHC – 103% and FEC – 10% higher compared to corresponding functional characteristics of the control concentrate sample.

### Analysis of organoleptic and physico-chemical parameters of enriched fermented foods

Values of active and titratable acidity (TTA) of samples of fermented milk products with the additives of the lupin protein preparation were assessed immediately after addition of the starter population (0 hr), after 2 hours and thereafter every hour of the experiment. Dynamics of acid accumulation of samples of dairy products enriched with lupin protein concentrate is given in Table 3.

#### Table 3. The effect of LPP mass fraction on the acid accumulation dynamics

<table>
<thead>
<tr>
<th>LPP, %</th>
<th>Time, h</th>
<th>Titratable acidity, °T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>-</td>
<td>21 ± 1</td>
<td>42 ± 1</td>
</tr>
<tr>
<td>0.5</td>
<td>21 ± 1</td>
<td>42 ± 1</td>
</tr>
<tr>
<td>1.0</td>
<td>22 ± 2</td>
<td>44 ± 1</td>
</tr>
<tr>
<td>1.5</td>
<td>23 ± 1</td>
<td>48 ± 2</td>
</tr>
<tr>
<td>2.0</td>
<td>25 ± 1</td>
<td>50 ± 2</td>
</tr>
</tbody>
</table>
Introduction of the protein preparation in amount of 1% and above slightly increased the initial value of TTA of the mixture (at 0 hours) due to the acidic pH value of the initial protein preparation associated with the isoelectrical processing technique.

It was used the water extraction of non-protein compounds, which led to pH in an acidic range in finish product.

The total fermentation time was 6 hours, and the control production of TTA (without LPP) reached 85°T. As the concentration of LPP in the mixture increased, the value of TTA increased at the respective stages of the experiment. In the case of sample with 2% of LPP value of TTA increased to 90°T (at 6 h).

At selected stages of the experiment, organoleptic characteristics of mixtures were assessed. Addition of 0.5% of LPP did not lead to any noticeable change of values. Enriched dairy products were homogeneous of viscous consistency. But upon increasing of LPP up to 2% the whey was separated and the product color was changed from milky white to creamy. Samples of products with LPP of 1.5% and 2.0% had a slight beany flavor.

The ability of milk clots to retain moisture could provide indirect evidence of structure formation processes in the system. Thus samples with a fairly solid clot had a minimum amount of separated whey and respectively a high WHC. Control samples had the lower WHC (Fig. 2).

![Figure 2. Characteristics of water holding capacity of clots.](image)

A gradual increase of the content of LPP in the mixture resulted in a corresponding increase in water holding properties of clots. This is associated with the growing concentration of plant proteins in the systems which are characterized by high WHC.
Study of rheological properties of enriched fermented food samples

The rheometry of these food media was performed at room temperature using the ‘Rheotest 2’ rotational viscometer. On selected stages of the fermentation process (at 0, 2, 3 hours, etc.) the flow properties of fluids were measured in strictly defined conditions provided by the universal viscometer construction. An examined food medium was placed in an annular gap between the rotating inner cylinder and the outer cylinder remaining stationary. Measurements of viscosity expressed by the effective viscosity coefficient were carried out at constant shear rate of 27 s\(^{-1}\) (Fig. 3).

The initial viscosity of the samples with LPP dosage of 0.5%; 1.0% to 2.0% were measured immediately after inoculation of the starter (0 h). Then the samples were placed into a thermostatic chamber for fermenting. Spatial network of gel formed by dairy and plant proteins gradually began to develop. After 2 h of fermentation, it was possible to measure the first visible changes in the structural and mechanical properties of samples. For this purpose the samples were taken out from the thermostat and their effective viscosity at the selected constant shear rate was measured. Shear rate of 27 s\(^{-1}\) was selected from the range of available shear rates on the device (from 3 to 1312 s\(^{-1}\)), which allowed to measure the viscosity in the range required for monitoring of structure formation of clots.

Alongside with the increase of the duration of the fermentation process complex formation of clots structure took place in the samples of enriched fermented milk products together with a growth of viscosity.

Figure 3. Modification of viscous properties of samples during fermentation process.

In diagrams we can identify the main phases of the formation of spatial structure of clots: induction phase, coagulation phase and metastable equilibrium phase. Upon increasing of lupin protein concentrate dosage in the composition of clots up to 2% their density and viscosity increased as well.
After completion of the fermentation process milk products were cooled up to 4–6°C and properties of finished products were evaluated. The viscosity properties of the products were evaluated by the hysteresis loop method. For that purpose the samples were tested under different shear rates in the range from 3 to 1312 s\(^{-1}\) with a gradually increasing and decreasing velocity gradient. Addition of 0.5% of LPP didn’t influence the rheological characteristics of the product. The sample with 2% LPP had a more viscous structure; however the thixotropic recovery capacity was slightly worse than of the control sample, which was probably due to increased number of fragile irreversibly destroyed bonds. The studied food systems have expressed thixotropic properties, for numerical expressions the estimated coefficients were used. Rheometry was performed at a uniform shear area during 2 minutes at intervals of 15 s at a shear rate of 27 s\(^{-1}\) (Table 4).

Table 4. The structural and mechanical properties of enriched dairy products

<table>
<thead>
<tr>
<th>LPP, %</th>
<th>(L_v, %)</th>
<th>MS</th>
<th>Rs, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>54.7 ± 1.4</td>
<td>2.20 ± 0.15</td>
<td>70.1 ± 3.1</td>
</tr>
<tr>
<td>0.5</td>
<td>54.9 ± 1.3</td>
<td>2.20 ± 0.11</td>
<td>68.5 ± 3.2</td>
</tr>
<tr>
<td>1.0</td>
<td>54.9 ± 1.1</td>
<td>2.20 ± 0.10</td>
<td>62.0 ± 3.6</td>
</tr>
<tr>
<td>1.5</td>
<td>55.2 ± 2.1</td>
<td>2.21 ± 0.13</td>
<td>59.5 ± 3.2</td>
</tr>
<tr>
<td>2.0</td>
<td>61.5 ± 2.3</td>
<td>2.60 ± 0.14</td>
<td>57.7 ± 3.1</td>
</tr>
</tbody>
</table>

According to Table 4 the structural and mechanical properties of systems with LPP concentrations of 0.5%, 1.0% and 1.5% were the same with the properties of the control sample. However in case of the increased LPP concentrations the clots have lost the structure recovery ability. This is proved by the increase of the coefficient of mechanical stability (MS) up to 2.60 for the sample with 2.0% LPP. The sample of fermented dairy product with 2.0% LPP had the worst structural and mechanical properties, its viscosity loss had reached 6.8% compared to the control product, while MS had increased by 18% indicating the change of the ratio of coagulation-condensation bonds (more elastic) and condensation-crystallization bonds (more fragile due to the formation of chemical bonds) in the clots with the growth of the last-mentioned ones. Based on a series of experiments carried out we can conclude that the maximum amount of the protein concentrate that can be used is 1.5%, which will provide a fermented dairy product with good organoleptic and structural and mechanical characteristics, with a total protein content of 4% inclusive 1% of plant proteins.

CONCLUSIONS

A process of the effective concentration of LPP using a multi-enzyme composition was investigated. Crude protein content of the obtained lupin protein concentrate has reached \((63.2 ± 1.3)\%\) on m.f.b. while crude protein content of the control product was \((50.4 ± 1.3)\%\) on m.f.b. The infrared drying parameters for the wet paste of LPP have been set within shortwave range up to 2.5 microns. As a result, a fine beige powder has been obtained which can be used without limitations for various applications in food industry.

Functional and technological properties of the preparation have been examined and it has been shown that the process of enzymatic degradation of polysaccharides leads to
a modification of spatial structure of protein molecules accompanied by an increased ability of protein to manifest the water holding capacity, fat holding capacity and ability to emulsify fats. The critical gelling concentration of the lupin protein preparation has been determined. The functional and technological properties of the preparation have been compared with the samples of fullfat soybean flour and of lupin flour.

The technology of enriched dairy products has been proposed. The organoleptic, physico-chemical, structural and mechanical properties of clots produced with 0.5%, 1.0%, 1.5% and 2.0% LPP additives have been examined. The effective LPP concentration which ensures better organoleptic and structural and mechanical properties of the products has been determined.

Thus LPP used as a functional ingredient is able to increase the biological value of feedstock, as well as to improve its composition. Total protein content in the final fermented dairy product is increased up to 4 g per 100 g of product; fat content reaches 0.4 g fat per 100 g, carbohydrates content reaches 5.1 g per 100 g, including fiber content up to 0.1 g per 100 g. The energy value of the new developed product is increased by 26% compared to a non-enriched dairy product. At a regular use of this product dietary fibers added to a daily diet will contribute to a beneficial effect on the gastrointestinal tract of a man.

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REFERENCES


Yeast Physiological State Influence on Beer Turbidity

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Abstract. The physiological state of yeast affects the intensity of propagation and biosynthesis of secondary metabolites and determines the sensory profile of beer. Besides that, yeast with low physiological activity increases the number of colloidal particles in beer, which consist of proteins and polysaccharides. The purpose of this study was to select a method for assessing the physiological state of yeast and to study the influence of yeast physiological state on the adsorption of compounds that determine the colloidal stability of beer. As a result of comparative analysis of different methods for determining the non-viable and weakened cells with dyes a certain correlation between them was found. The highest correlation \( R^2 = 0.84 \) was set between parameters obtained by staining yeast cells by methylene blue with Safranin O, which stains both dead and weakened cells and dihydrorhodamine 123, which detects only physiologically least active yeast. Sufficiently high correlation \( R^2 = 0.83 \) was observed when cells were stained with methylene blue, which identifies only dead cells, and with methylene blue with Safranin O. The worse the physiological condition of yeast, the less they adsorb turbidity-inducing compounds, so more substances remain in beer, reducing its stability. Thus, the increase in the proportion of damaged and non-viable cells from 24.2 to 32.2% leads to increased beer turbidity from 1.5 to 3.3%. Z-potential of yeast cell walls determines their sorption properties. Physiologically active yeast cells are able to adsorb positively charged colloids due to the negative charge of their surface. Activation of the yeast’s surface potential occurs in the presence of oxygen.

Key words: yeast, Z-potential, colloids, staining methods.

INTRODUCTION

Beer represents a complex colloidal system with particles from 0.01 to 3 microns in size. Bad conditions of beer storage cause integration of colloidal particles by their condensation and polymerization. Formed deposit cause colloidal instability of beer and problems with its realization. Chemical, physical, fermentative and mechanical ways of impact on colloidal system of beverages are widely used in modern brewing production for the purpose of product stability increase. The choice direction is defined by the Specific objectives facing the brewer define the choice direction. Some chemical ways reduce oxidizing processes speed in beer. For this purpose, brew masters use antioxidants, interacting with oxygen from air and preventing oxidation of phenolic beer compounds. Physical and chemical ways are making it possible to remove colloids of

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various natures by means of adsorbents. In particular, silica gels remove the haze forming proteins and polyvinyl piralidone decreases the concentration of phenolic compounds. Besides, such technological operations as separation and filtration could increase colloidal stability of beer (Meledina, 2003). Antioxidants interact with oxygen from air and prevent oxidation of phenolic substances. Adsorption is physical and chemical way directed on removal of colloids of various nature. For extraction colloids from beer, filtration materials are used. The most popular filtering material is diatomaceous earth of various brands having wide range of particles size (Meledina, 2003). Besides at this stage various adsorbents of colloidal particles are used (Nikolashkin, et al., 2004; Rinqwaer, et al., 2010). In particular, silica gels remove proteins and polypeptides (Rehmandji, et al 2000; Evans, 2001). Brew masters use polyvinilpirrolidon (PVPP) for long storage beer production (up to 12 months) (McMurrough, et al 1997; Siebert, 1999; Mc Keown, et al., 2003). PVPP represents polyamide and contains NH-groups, interacting with phenolic substances due to formation of hydrogen bonds. As a result, part of phenolic substances removal, Protein and tannic compoundes form fewer complexes during beer storage because of part of phenolic substances removal. Besides, there are less condensation and copolymerization, leading to tannoid formation. In particular, silica gels remove haze-forming proteins and PVPP decreases the concentration of phenolic substances. Such technological operations as a separation and a filtration increase beer stability (Burrell, to et al., 1994; Meledina, 2003; Dedegkaev, 2011).

All listed processing methods of beer physical and chemical stability increase are rather well studied. However, now the role of a physiological condition of yeast in this process is not well known. Contents of reserved carbohydrates (glycogen and trehalose) which are the source of endogenous glucose used during the period log growth phase (the adaptation period), sterol and non-saturated fatty acids biosynthesis which are the most important components of cellular membranes or the speed of carbon dioxide release and oxygen consumption can determine physiological activity of yeast cells.

It is necessary to mention the test of 'the acidulation force' (Patino et al., 1993) and the modified Davydenko's method (Davydenko, 2012) among efficient practical methods of yeast physiological condition assessment. Besides, various dyes staining methods can estimate physiological activity of microorganisms (Selivanov, 2003; Smart et al 1999; Mailloux et al. 2008). Visible light or fluorescent microscopy determines the particular staining methods (Van Zandycke et al., 2003). The method of dead cells identification by means of the methylene blue is widely used. Living yeast cells reductases restored the dye to non-coloured substances; dead cells are blue (Smart, 1999). Staining by methylene blue, tannin and safranin gives fuller information on physiological condition of cells. Safranin O and some other dyes are also efficient for this purpose. The histone forming chromatin leaves the DNA surface and phosphoric groups of DNA molecules and reacts with safranin and other dyes during an active transcription of a certain site of DNA. Thus, active process of a transcription and active metabolism correlate. The methylene blue stains cytoplasm proteins (Selivanov, 2003).

Magnesium salt 1-anilino-8-naftalen of sulfonic acid (Mg-ANS) is widely used for yeast cells viability. Getting into the yeast cell, Mg-ANS forms fluorescent complexes with cytoplasm proteins (Jenkins et al., 2003; Van Zandycke et al., 2003). Dead cells have fluorescent luminescence.
It is possible to reveal the existence of the active oxygen forms in yeast cells by means of digidrorhodamine 123 (Fluka). Digidrorhodamine oxidizes to the fluorescing rhodamine (Henderson et al., 1993). Accumulation of free radicals (ROS, reactive oxygen species) in the yeast cell reflects to its aging, or stress conditions. In both cases, the cells are physiologically poorly active. The digidrorhodamine 123 method allows ROS status estimation by quantity fluorescing (red or green) cells. Unlike methylene blue, which stains already dead cells, rhodamine stains cells which are in poor physiological condition.

The purpose of this work was to choose yeast physiological condition assessment method by cells staining and microscopy and to study the yeast strains genetically based features on yeast physiology in normal and stress conditions and its influence on beer haze indicators.

**MATERIALS AND METHODS**

Research objects were lager yeast strains 34/70 from Hefebank Weihenstephan collection, strain Y-3194 selected in the research center of ‘Baltika Breweries’–Part of Carlsberg Group St Petersburg (Davydenko et al., 2008, Davydenko, et al., 2010) and the strain which is widely used in the European brewing which we designated as X.

Beer production was carried out by Mini Brewery plant system (Beraplan Harter) with cylindro conical tanks (CCTs) containing 120 l of 12° beer wort at 14°C with 100% barley malt according to classical beer production technology, including mashing, boiling, wort filtration, fermentation, yeast cropping, beer stabilization and filtration (*Hapuyucc*). Wort (12°P), contained 160 mg l⁻¹ of free nitrogen and 8.6% of sugars.

Beer turbidity was measured by Haffmans Hazemeter at red light (650 ± 30 nm) with formazin calibration standard. Particles smaller than 1µm (such as proteins, polysaccharides and polyphenols) were measured at 90°. Particles larger than 1µm (yeast cells, diatomaceous earth etc.) were measured at 25°.

Particle size of particles in beer was estimated by Nanotrac U2313ES (Microtrac Inc.).

Microscopy of yeast cultures was performed using an Axioskpo MOT Microscope (Zeiss); preparations were photographed using an AxioCam video camera.

Zeta potential measurements were performed with a Brookhaven Instruments NanoBrook ZetaPALS. For enhanced sensitivity, the motion of the charged protein is probed with so-called phase analysis light scattering (PALS). In traditional Laser Doppler electrophoresis, the frequency shift in the scattered light that arises due to the motion of the scatters determines the motion of the proteins.

Centrifugation was executed by Sigma 2-5 centrifuge.
Methods of cell's staining

In order to choose the most informative method of yeast physiological conditions assessment and existence of dead cells in yeast population, the results of cell's from different generations of strain 34/70 staining by both lifetime and fixed staining methods, were compared.

Techniques of cells staining by methylene blue

Methylene blue was prepared as follows: 0.01g methylene blue and 2.0 g dihydric citrate were dissolved in 10 ml of the distilled water, filtered and volume was adjusted to 100 ml. For definition of quantity of dead cells, one drop of yeast suspension and solution of a methylene blue (0.01%) was subjected on glass slide and in 2 min the calculation of total number of cells and quantity of the blue dead cells was carried out. We counted yeast cells in five vision fields, total counted of cell number not less than 500. Yeast cells were counted in five vision fields; total count of cell number was no less than 500. The percent of dead cells was calculated accordingly to Smart et al (1999).

Technique of cells staining by methylene blue and safranine О

Yeast was centrifuged for 5 min at 4000 rpm, washed by physiological solution and centrifuged again. Further yeast suspension concentration of $10^7$ cells ml$^{-1}$ was then prepared. The drop of yeast suspension was dried at the room temperature on the fat-free glass. After that, it was fixed in flame and filled with methylene blue solution and maintained 4 min at the room temperature. Then dye was washed away by warm water and filled in by freshly prepared 5% tannin solution for 2 min. Dye was washed away under a water stream and filled with 1% solution of safranin and maintain for 16 min and then the dye was washed away. Microscopy was carried out at 400 multiplication, non-fluorescent oil was used (Selivanov, 2003).

Technique of yeast cells staining by dihydorhodamine

2 mg of a dihydorhodamine 123 (Fluka) were dissolved in 1.0 ml 0.1M Tris buffer (2 amino-2-(hydroxy-methyl)-1,3-propanedino) pH 8.0. Yeast suspension was centrifuged within 5 min at 4000 rpm and then yeast suspension of $10^7$ cells ml$^{-1}$ was prepared (Henderson et al., 1993).

Technique of yeast cells staining by magnesium salt 1-anilino-8-naftalen of sulfonic acid (Mg-ANS)

300 mg of Mg-ANS (Sigma) were dissolved in 2 ml of ethyl alcohol and added to 98 ml of sterile water. Solution can be stored at 4°C in glass bottles (dark glass) within 6 months (Jenkins et al., 2003). 0.5 ml of 0.3% Mg-ANS solution was added to 0.5 ml of yeast suspension and incubated for 5 min at 25°C. Then dead (yellow-green fluorescence) and living cells were calculated.

RESULTS AND DISCUSSION

In current research, the investigation of various yeast strains and generations and determination of correlation between different staining methods by means of which it is possible to estimate a physiological condition of cells, was carried out. Yeast was stained
by methylene blue, methylene blue together with safranin, and digidrorhodamine. Partially weakened cells were detected using digidrorhodamine and methylene blue with safranin; dead yeast stained by methylene blue, Mg-ANS and methylene blue with safranin. As a result of comparison of different methods the highest coefficients of determination were found between methylene blue with safranin and digidrorhodamine and between methylene blue, and methylene blue with safranin O, $R^2 = 0.84$ (Fig. 1) and $R^2 = 0.83$ (Fig. 2) respectively. $R^2$ shows reliability of linear dependence between the studied indicators.

**Figure 1.** Linear relationship between Methylene blue + safranine O and digidrorodamin 123 staining methods.

**Figure 2.** Linear relationship between methylene blue + safranine O and methylene blue methods of staining.

Coefficient of determination for Mg-ANS and methylene blue with safranin O was $R^2 = 0.63$ (Fig. 3). Lowest coefficients of determination was between methylene blue and Mg-ANS, $R^2 = 0.43$ (Fig. 4). Further, methods of staining cells by methylene blue and methylene blue with safranin O were used for yeast physiological condition assessment.

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Figure 3. Linear relationship between methylene blue + safranine O and Mg-ANS methods of staining.

Figure 4. Linear relationship between Mg-ANS and methylene blue methods of staining.

Adsorption of the colloidal particles on yeast surface and their sedimentation with flocculating cells makes the main changes of fermenting wart colloidal system during fermentation are connected with. Zeta-potential of yeast cellular wall defines sorption properties of yeast. Physiologically active yeast due to negative charge of their surface is capable to adsorb positively charged colloids, such as proteins and phenolic substances (Table 1).

Table 1. Change of quantity of the colloidal particles of a different chemical composition during beer fermentation

<table>
<thead>
<tr>
<th>Duration of fermentation process, days</th>
<th>Quantity of particles, % of total of particles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>proteins (B)</td>
</tr>
<tr>
<td>0</td>
<td>55 ± 4</td>
</tr>
<tr>
<td>3</td>
<td>33 ± 5</td>
</tr>
<tr>
<td>10</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>14 (ready beer)</td>
<td>29 ± 3</td>
</tr>
</tbody>
</table>
The reduction of their concentration in the fermenting wort in parallel increases a share of particles partly formed by dextrins. As a result, in the course of fermentation zeta-potential value of cells falls from 46 mV to 23 mV (Table 2), thus concentration of 0.01–1.0 µm colloidal particles (Fig. 5), i.e. those particles, which influence beer turbidity, decreases. In general, the number of small and big colloidal particles decreases during fermentation.

In parallel, cells zeta-potential decreases and the superficial potential of colloidal particles increases. Thus, the potential of 0.01–0.10 µm particles increased from 37 to 49 mV and of 0.1–1.0 µm particles potential changed from 33 to 41 mV.

When using yeast with low physiological activity adsorption of the proteins and phenolic substances, which are the reason of low colloidal stability of beer decreases.

Table 2. Superficial potential of yeast (strain 34/70) and colloidal particles change in the course of wort fermentation

<table>
<thead>
<tr>
<th>Fermentation time, days</th>
<th>Superficial potential of particles, mV</th>
<th>Range of the colloidal particles, microns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.01–0.1</td>
</tr>
<tr>
<td>0</td>
<td>46 ± 2.3</td>
<td>37 ± 1.8</td>
</tr>
<tr>
<td>2</td>
<td>37 ± 1.5</td>
<td>37 ± 1.5</td>
</tr>
<tr>
<td>4</td>
<td>31 ± 0.3</td>
<td>39 ± 1.5</td>
</tr>
<tr>
<td>6</td>
<td>29 ± 0.5</td>
<td>41 ± 1.8</td>
</tr>
<tr>
<td>8</td>
<td>26 ± 0.02</td>
<td>43 ± 2.0</td>
</tr>
<tr>
<td>10</td>
<td>24 ± 1.2</td>
<td>45 ± 1.5</td>
</tr>
<tr>
<td>12</td>
<td>23 ± 0.5</td>
<td>47 ± 2.3</td>
</tr>
<tr>
<td>14</td>
<td>23 ± 1.0</td>
<td>49 ± 2.3</td>
</tr>
</tbody>
</table>

The physiological condition of cells decrease, if yeast has any stresses: osmotic and hydrostatic pressure, sharp fluctuations of temperature etc. It is possible to judge it based on trehalose segregation from cells (Attfield et al., 1992; Eleutherio et al., 1993), glycerol content in beer (Dedegkaev et al., 2005) and concentration increase of dead and weakened cells. Data showing decrease of the number of colloidal particles during wort fermentation (Table 3).

Table 3. Change of concentration of 0.01 to 1.0 µm colloidal particles during wort fermentation of yeast strain 34/70 |p|≤ 0.15.

<table>
<thead>
<tr>
<th>Fermentation time, days</th>
<th>Number of particles</th>
<th>Range of the colloidal particles, microns</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.01–0.1</td>
</tr>
<tr>
<td>0</td>
<td>24500</td>
<td>137</td>
</tr>
<tr>
<td>2</td>
<td>24300</td>
<td>120</td>
</tr>
<tr>
<td>4</td>
<td>23000</td>
<td>105</td>
</tr>
<tr>
<td>6</td>
<td>22500</td>
<td>87</td>
</tr>
<tr>
<td>8</td>
<td>22000</td>
<td>85</td>
</tr>
<tr>
<td>10</td>
<td>21700</td>
<td>80</td>
</tr>
<tr>
<td>12</td>
<td>21200</td>
<td>74</td>
</tr>
<tr>
<td>14</td>
<td>20000</td>
<td>73</td>
</tr>
</tbody>
</table>
In order to study the influence of yeast cells physiological condition on adsorption of colloids defining beer turbidity, 7 samples of the yeast were received after beer fermentation. In these samples by means of staining cells by methylene blue and safranin the maintenance of partially weakened and dead cells was estimated (Table 4). Further, subsequent to centrifugation yeast was washed 3 times with water. After each centrifugation, turbidity of washing water was measured by Haffmans Hazemeter. Then total relative turbidity was counted in terms of 1 g of biomass with a mass fraction of solids 22% (damp yeast), for this purpose, we summarized values of a turbidity of all three washouts and divided into amount of the washed-out yeast.

Table 4. Influence of a physiological condition of yeast on a turbidity of beer and adsorption of colloids

| Sample | Beer turbidity, EBC (|p|≤ 0.10) | Relative turbidity of washing waters, biomass EBC g\(^{-1}\) (|p|≤ 0.10) | Results of cells staining by methylene blue and safranin, % (|p|≤ 0.20) |
|--------|-------------------------------|-------------------------------------------------------------|---------------------------------------------------------------|
|        |                               |                                                              | good shape | partially weakened | dead |
| 1      | 1.5                           | 42.0                                                         | 75.8       | 20.5             | 3.7  |
| 2      | 3.3                           | 11.9                                                         | 67.8       | 19.6             | 12.6 |
| 3      | 2.0                           | 17.5                                                         | 73.1       | 18.6             | 8.3  |
| 4      | 1.9                           | 51.0                                                         | 75.1       | 20.7             | 4.3  |
| 6      | 1.91                          | 47.6                                                         | 75.5       | 21.0             | 3.5  |
| 7      | 2.32                          | 22.7                                                         | 74.5       | 19.0             | 7.5  |

The less normal physiological conditions of yeast, the less they adsorb the haze coursing substances and as a result, more substances remain in beer that raises its turbidity. Least of all cells in a good physiological shape in the second sample (67.8%) and this beer has maximum turbidity – 3.3 units of EBC. However, not only a physiological condition of yeast, but also features of various strains in certain conditions can influence adsorption of colloids. Influence of colloidal particles concentration in beer depending on particles size was investigated in case of two yeast strains. Y-3194 and X strains were used for 12% wort fermentation with 15 ± 2 million cells ml\(^{-1}\).

Table 5. The characteristic of colloidal particles in beer, using different yeast strains (|p|≤ 0.10)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Hydrostatic stress</th>
<th>Average size of particles, µm</th>
<th>Interval of fluctuation</th>
<th>Concentration of particles, million ml(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y-3194</td>
<td>no</td>
<td>5.6</td>
<td>4–9</td>
<td>0.24</td>
</tr>
<tr>
<td>X</td>
<td>no</td>
<td>4.7</td>
<td>4–10</td>
<td>0.22</td>
</tr>
<tr>
<td>Y-3194</td>
<td>yes</td>
<td>8.2</td>
<td>4–15</td>
<td>1.10</td>
</tr>
<tr>
<td>X</td>
<td>yes</td>
<td>8.3</td>
<td>3–13</td>
<td>2.30</td>
</tr>
</tbody>
</table>

There was no difference in number and in size of colloidal particles in beer fermented by two strains (Table 5). However, increase of pressure during fermentation up to 2 bars affected adsorption of colloids by various strains differently. As a result, the quantity of the colloid particles in beer when using strain X was twice higher, than in the beer made by Y-3194 yeast. It testifies that yeast X, is less resistant against hydrostatic stress. Under the influence of various factors hydrophilic colloids that are present in beer, coagulate. Large colloidal particles start reflecting light, causing opalescence. Then particles integrate with each other, so that become visible, and beer becomes turbid.
Different substances form haze in beer – proteins, polysaccharides, polyphenols, etc., as different sorbents – absorb colloidal complexes from beer.

CONCLUSIONS

It is possible to draw a conclusion that all methods of staining have rather exact correlation among themselves, and if necessary, they can substitute each other or be used in total to obtain accurate results. During beer production, it is possible to use only methylene blue method to determine yeast cell’s physiological activity. However, staining with methylene blue with safranin gives an opportunity to divide cells on physiological activeness–weakened and dead. This is important from practical point of view, since it is possible to activate the weakened cells, for example, by addition of wart. When using only methylene blue one estimates both weakened and not viable cells as dead.

Falling of zeta-potential of cells causes, on one hand the reduction of colloidal particles concentration during fermentation and on the other hand – increase in their charge. Decrease of physiological status of cells leads to colloids adsorption reduction and increase of beer turbidity.

In the absence of stress (during fermentation), the concentration of colloidal particles depends on the strain used in beer production. However, in stressful conditions, for example at elevated pressure, different strains are capable to adsorb various colloidal particles in different quantity. Thus, yeast stress resistance influence the turbidity of beer.

REFERENCES


Stability of vitamin A and E in powdered cow's milk in relation to different storage methods

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Abstract. In this article, the influence of different ways of storage on the content of vitamin A and E in powdered cow’s milk was studied. The cow’s whole milk powder was taken directly from the manufacturer and stored for one year in 4 different ways – in the light at room temperature, in the dark at room temperature, in a refrigerator at 8°C and in a freezer at -20°C. The content of vitamins was measured 4 times during the first month and then once a month. The samples were stored for one year. Vitamins A and E were determined by HPLC using DAD and FLD detectors. Vitamin A was identified in all samples but only α-tocopherol (out of various forms of vitamin E) was detected in all samples. In all cases steeper decline of both vitamins in first 14 days of storage was identified. The highest losses of vitamin A and E in powdered milk occurred during storage in the light at room temperature. The value decreased by 91 resp. 95% of the original value.

Key words: stability, milk powder, storage, vitamin A, vitamin E.

INTRODUCTION

Milk in human nutrition has a nutritional, protective and detoxification function. It represents inter alia an important dietary resource of minerals and vitamins (Pánek, 2002). Convenient way to long-term storage of surplus milk is drying. Minimum durability extends this way up to two years.

Drying is a proven successful method of preserving with success also applicable to dairy products. Using this technological procedure obtain a product enabling economic transport of milk for a larger distance to areas, which are milk deficient. This product is easy to store and to recover. Powdered milk is stable due to the low water content, it is very slow in microbiological spoilage, oxidation and enzymatic processes. Dried milk products must be protected during storage, shipment and distribution from wetting or secondary contamination by microorganisms. Raw milk is considered a good source of vitamins A and E, but little information is known about the content of these vitamins in the long term storage of dried milk. In addition, the information is different (Ramalho et al., 2012, Yasmin et al., 2012, Michlová et al., 2015). The sustainability of these products depends on the type of product, quality, packaging, storage temperature and oxygen
content. It is known that, while the mineral content is stable over time, the content of vitamins is lost. Powdered milk is also an important commodity in international trade.

Vitamin A plays an important role in the biochemical pathways related to visual perception. It affects also the growth, differentiation and maturation of gametes, and is important for growth, fetal and bone development (Debier et al., 2005). It plays a crucial role in the synthesis of proteins, nucleic acids, and lipoproteins. Vitamin A is also an effective antioxidant. Vitamin deficiency is associated with vision disturbances (i.e. night blindness), inhibition of growth and deformities of bone and reproductive organs. High doses of vitamin A cause increased hepatic reserve. In pregnant women it may have teratogenic effects (Miller et al., 1998). According to Capita & Calleja (2006) the recommended daily dose of vitamin A ranges for an adult from 0.8 to 1 mg (2,600–3,300 IU) and for a child from 0.4 to 0.6 mg (1,300–2,000 IU).

Vitamin E is a very important antioxidant. It has a significant function in protecting the body against free oxygen radicals, which can lead to DNA damage. It is also a factor that slows down the ageing of the body and plays a role in the prevention of cardiovascular diseases and cancer (Eitenmiller & Junsoo, 2004). Vitamin E is present in food, being dissolved in fats, and is released and subsequently absorbed during its cleavage in the intestine. The recommended daily dose of vitamin E is reported as 10–15 mg (15–22 IU) for adult. This value is around 5–8 mg (7–12 IU) for child (Monsen, 2000). Vitamin E deficiency is often associated with disorders of fat absorption or distribution or with cystic fibrosis (Pekmezci, 2011).

Factors that affect the stability of vitamins in powdered milk vary depending on the monitored vitamin. As for the vitamins A and E the most important factors are heat, moisture, oxygen, light and pH (Ottaway, 2010).

The aim of our study was to determine the stability of these vitamins in powdered milk depending on different methods of storage during one year period and its subsequent use in nutrition.

**MATERIALS AND METHODS**

**Experimental material**

The vitamin A and E content was monitored in cow’s whole milk powder, which had been obtained directly from the manufacturer (26% fat). The sample was divided into 4 x 16 parts. The first four of them were immediately analysed for the vitamin A and E content, and others were stored for one year in 4 different ways - in the light at room temperature, in the dark at room temperature, in a refrigerator at 8°C and in a freezer at -20°C. The content of both vitamins were analysed on the 7th, 14th, 21st, 28th, 59th, 90th, 120th, 151st, 181st, 212nd, 243rd, 274th, 304th, 335th, 365th days after storage. To ensure the homogeneity of the sample the reconstituted milk was thoroughly shaken for 2 minutes prior to the measurement.

**Measurement of vitamin E and A content in milk samples**

Vitamin A and vitamin E (or the individual tocopherols (T) and tocotrienols (TKT) were determined by high performance liquid chromatography with spectrophotometric and fluorescence detection, respectively.
For the preparation of the analytical samples, the following standards and chemicals were used: DL-\(\alpha\)-tocopherol, 98.2\% (CALBIOCHEM, Canada), tocopherol set (CALBIOCHEM, Canada), retinol, > 99\% (Sigma-Aldrich, Germany), pyrocatechol, > 99.5\% (Sigma-Aldrich, Germany), potassium hydroxide, min. 85\% (Lachema, Czech Republic), methanol, p.a., content 99.5\% (Lachner, Czech Republic), hexane, clean min. 95.0\%, Penta, Czech Republic, methanol, super gradient, content min. 99.9\% (Lachner, Czech Republic) and treated distilled water (Milipore, France).

The content of both vitamins was extracted by the method of Sánchez-Machado et al. (2006) with minor modification. Approximately 1 g of reconstituted homogenized sample was weighted in a plastic tube with a lid. 200 ml of methanolic pyrocatechol (0.2 g ml\(^{-1}\)) and 5 ml 1M KOH was added. The mixture was vortexed for 20 seconds. The sample was saponified for 10 minutes on ultrasound. Then the mixture was vortexed again 20 seconds. 5 ml of hexane and 1 ml of distilled water were added to the mixture. The mixture was vortexed for 1 minute. Subsequently, 3 ml were taken from the upper hexane layer and evaporated until dry using a Büchi rotovapor R-215 (Büchi Labortechnik GmbH, Essen, Germany). The residue was dissolved in 0.5 ml of methanol and an aliquot was transferred through a nylon filter into 1 ml Eppendorf, which was kept for 30 minutes in the freezer (-20\°C). The sample was centrifuged for 2 minutes (Eppendorf miniSpin plus, by 14.4 rpm) and drained off into a dark vial. Analysis was carried out using a High Performance Liquid Chromatograph Ultimate 3000 (Thermo Fisher Scientific, Dionex, Sunnyvale, CA, USA) with a quarternary pump, refrigerated autosampler, column heater, and FLD and DAD detector. Tocols and tocopherols in the sample were determined by HPLC-FLD under the following conditions: analytical column Develosil 5\(\mu\)m RP AQUEOUS (250 × 4.6 mm) (Phenomenex, Torrance, CA, USA), which allows the separation of all forms of tocopherols and tocotrienols (Fig. 1); pre-column Develosil 5\(\mu\)m C30 UG-100A (10 × 4 mm) (Phenomenex, Torrance, CA, USA); mobile phase methanol : dionized water (97:3, v v\(^{-1}\)), HPLC super gradient methanol Lach-ner, Ltd. (Neratovice, Czech Republic) and water Milli-Q water, isocratic elution; flow rate 1 ml min\(^{-1}\); injection 10 \(\mu\)l, column temperature 30\°C; detection FLD (excitation 292 nm, emission 330 nm).

![Chromatogram of vitamin E (compared to the standard).](image)

**Figure 1.** Chromatogram of vitamin E (compared to the standard).
Retinol was determined by the same chromatographic conditions using DAD detector (λ = 325 nm) (Fig. 2).

Figure 2. Chromatogram of vitamin A (compared to the standard).

The detection limit for vitamin A and each tocopherol and tocotrienol, expressed as a ratio of three times the value of the signal-to-noise ratio, were as follows: vitamin A 0.025 µg ml⁻¹, δ-tocotrienol and δ-tocopherol 0.01 µg ml⁻¹, β-tocotrienol, γ-tocotrienol, β-tocopherol and γ-tocopherol 0.025 µg ml⁻¹, α-tocotrienol and α-tocopherol 0.05 µg ml⁻¹ respectively. The results were processed with Chromeleon and MS Excel. All results were expressed as mean value (mg kg⁻¹) of three replicates.

RESULTS AND DISCUSSION

The content of vitamin E in freshly produced milk powder is about 1.5 times higher than the level of vitamin A. During storage, there was a decrease in the amount of both monitored vitamins. In addition, differences were observed in the decrease of particular vitamins.

Vitamin A

The decrease of vitamin A was almost identical with various ways of storage. The highest decrease occurred at the beginning of storage (about 14 days). In about 6–7 months of storage there was decline about 39% when stored in dark, 43% when stored in refrigerator and in freezer, and about 63% when stored in light. At the end of the storage period (1 year) there was a steep decrease in vitamin A. However, the swiftest decrease was detected by the storage in the light (about 51% during 28 days against 29–34% in the other cases). After one year of storage the value of vitamin A content in milk in all four cases of the storage was very similar and averaged 0.40 mg kg⁻¹ (decrease by about 91%) (Fig. 3).
The effect of different storage conditions on the content of vitamin A is given by various authors. Frias et al. (2009) confirms that the storage period has considerable influence on vitamin A content. In their study value of vitamin A in milk powder decreased during 6 months of storage at 30°C by 68%. Their values are in good agreement with the data found in this study. Losses were determined by Chávez Servín et al. (2008). They reported that vitamin A in milk powder at room temperature falls within the range from 5.4 to 28.9% after 70 days storage at room temperature (25°C). On the other hand, Duarte Fávaro et al. (2011) argue that vitamin A can be stored without significant losses when certain conditions are observed. The stability of vitamin A is dependent on production and storage conditions, e.g. absence of oxygen in contact with the packaged product or safe storage in the absence of light and at a temperature not exceeding 30°C. Boer et al. (2010) investigated the stability of vitamin A in powdered milk for 16 weeks at different temperatures in the dark and with exposure to fluorescent light. Samples stored in the dark lost 20–38% of the original value of vitamin A. The loss in the samples exposed to light was 70%. The highest vitamin loss occurred in this study during the first 10 weeks of storage. These data are consistent with the values reported in the present study.

As no significant differences were observed during storage of milk powder at room temperature neither in the dark nor in the refrigerator and the freezer, it is possible to store the milk powder in business and at home in conventional shelves. However, it should be stored in the dark and not in transparent plastic containers, since the influence of light occurs with the exception of end-stage monitoring significantly higher losses of vitamin A in powdered milk.

![Figure 3](image-url)  
**Figure 3.** Average content of vitamin A in whole milk powder during different ways of storage.
**Vitamin E**

The amount of vitamin E also varies according to ways of storage. The highest decrease in the content of vitamin E occurs during storage of milk powder in the light at room temperature. In this way of storage of milk powder the vitamin E content dropped from the initial 6.61 mg kg\(^{-1}\) to 0.19 mg kg\(^{-1}\), which means an overall decrease of 97.1%. After six months of storage powdered milk contained about 57% of the original value of vitamin E when stored in dark, 48% when stored in refrigerator, 42% when stored in freezer and only 34% when stored in the light. In case of studied storage methods the level of vitamin E was at the end of the storage period approximately 0.50 mg kg\(^{-1}\) (loss 92.4%) (Fig. 4).

![Figure 4](image-url)

**Figure 4.** Average content of vitamin E in whole milk powder during different ways of storage.

The decrease in vitamin E during storage of powdered milk was also recorded by other authors. Valverde et al. (1993) claimed that loss of vitamin E occurs during the first month when stored at 30°C. After increasing the temperature from 30°C to 40°C the content of vitamin decreases even more. According to author, short time (60 days) of storage in the freezer does not cause any loss of vitamin E (resp. α-tocopherol). Losses occur in the course of 4 to 8 months of storage in the freezer. Their findings correspond with the results in this study, where the storage began at 25°C and the decrease of vitamin E up to 42% depending on the method of storage was found out. Lower losses were recorded by Chárvez Servín et al. (2008) who reported that vitamin E content in powdered milk decreases at room temperature in the range from 2.2 to 17.7% after 70 days storage at room temperature (25°C). Discussed results almost agree with the main findings of the study of Frias et al. (2009), who reported a reduction of vitamin E during the six-month storage from 3.98 ± 0.077 mg kg\(^{-1}\) to 0.149 ± 0.004 mg kg\(^{-1}\), which represents a decrease of 96% of the original value.

Decrease of the amount of vitamin E is slightly higher than the decrease of vitamin A under described conditions. A large drop in storage of vitamin E in the light may be
caused by considerable sensitivity to light of vitamin E. From the viewpoint of maintaining the quantity of vitamin E it is appropriate that the milk powder is stored in dark containers to prevent presence of light.

**CONCLUSION**

Significant differences in the content of vitamin A and E were found when four different ways of storage of whole cow’s milk powder were used. The most rapid decrease in almost all cases was measured within the first 14 days of storage. The highest losses of vitamin A and E in powdered milk occurred during the storage in the light at room temperature. In other examined methods of storage (in the dark at room temperature, in the refrigerator and the freezer at -20°C) the content of vitamin A has declined almost identically, in contrast to the decline of vitamin E. The average reduction in vitamin A and E was 91%, resp. 95% after one year storage period. Powdered milk is a relatively good source of vitamin A and E about 5–6 months after production, but after 1 year of storage period, regardless of the storage method, is the content of both vitamins very low and this milk product cannot be recommended as a source of these vitamins in the diet.

To maintain the largest possible amounts of vitamins A and E, the powdered milk should be stored in dark containers and consumed before the expiry date of minimum durability, preferably within six months.

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**REFERENCES**


Development of cheese product with hydrolyzed soybean emulsion

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Abstract. The expediency of production of food products based on complex raw material compositions is currently proved. According to the modern concept of a healthy nutrition it is important to optimize the composition of the product considering the content of biologically valuable substances in raw materials during a development of such products. This paper deals with the studies demonstrating an option of usage of soybean emulsion as a raw material of plant origin for development of a high-protein food product similar to a soft cheese. Soybean food emulsion (SFE) was developed and produced by All-Russian Scientific Research Institute of Fats. For minimizing activity of antigenic factors of soybeans (such as glycinin and β-conglycinin) SFE was subjected to partial proteolysis by a complex enzyme preparation. At the Department of Milk Technology and Food Biotechnology of ITMO University recipes and technology of a cheese product with HSFE were developed. Experimental samples were prepared with different doses of HSFE in recipes. The degree of hydrolysis of the HSFE was varied from 6% to 12% with intervals of 2%. Amino acid score for essential amino acid was calculated, the absence of limiting amino acids in developed products confirms their high biological value.

Key words: cheese product, soybean emulsion, hydrolyzed soybean emulsion, degree of hydrolysis, biological value of protein, amino acids.

INTRODUCTION

Nowadays more attention is paid to development and production of food products based on scientific requirements to human diets. However a balanced diet of a modern man is a compromise between limited possibilities of a society and/or a person in the supply of food and of the theory based optimal nutrition. Most often it could be explained by a limited availability of some foods or their high cost. It’s especially true for proteins which demand could be satisfied by consumption of meat, fish and in many cases–of dairy products.

Special attention should be paid to high-protein dairy products–cheeses. According to the recommendations of the Ministry of Health and Social Development of the Russian Federation (August 2, 2010, N 593n) per capita cheese intake should be 6 kg per annum (for all types of cheeses including processed cheeses and cheese products). The cheese demand in the RF is 876,000 tons (based on the country's population in 2014). Russian producers release to the market up to 350,000 tons of cheeses per year
Therefore physiologically recommended level of cheese consumption in Russia could be satisfied up to 40% only by the domestic cheese production not taking into account export volumes of these products. Thus the share of imported cheese exceeds 50%

Currently volumes of production of soft cheeses in Russia are higher than volumes of hard cheeses. This is connected with a lower dosage of milk per unit of finished product and an absence of a long cheese ripening period which results in an accelerated turnover of funds and in a reduction of labor expenditure while lowering production expenses. Soft cheeses can be sold without ripening and they have a high biological value (Yakovchenko & Silantjeva, 2014).

However it is known that in any cheese manufacturing process the smaller part of the initial raw material mixture (about 20% of milk) is converted to a cheese and the majority of mixture (about 80%) is converted to byproducts—namely a whey. Thus raw materials expenses for the production of high-protein foods are essential. Therefore the purpose of this study was to select a combination of raw materials of animal or vegetable origin for development of a cheese product. A process of selection of perspective ingredients was associated with a number of objectives including the optimization of the biological value of the product and reaching of the economic efficiency of its industrial processing.

Soybeans are reaching in proteins and contain a number of biologically active compounds which could contribute to nutritional value and health benefits of finished products (Medic et al., 2014). It encourages food manufacturers to develop new soy products and to incorporate more soy additives in food formulations. Over the years the All-Russian Scientific Research Institute of Fats was conducting complex studies on development of processing technologies of various soy products in particular of soybean emulsions for edible applications (TS 9146–166–00334534–97) (Domoroshchenkova et al., 2006).

Soybean food emulsion (SFE) is a product of processing of whole soybeans. The quality parameters of the SFE must meet the following requirements according to TS:
- dry matter content min. 15.0%;
- crude protein content min. 6.0%;
- crude fat content min. 2.3%;
- pH 6.5–7.0.

SFE is rich in vitamins and minerals as it contains in average: 41.55 mg calcium, 105.60 mg phosphorus, 0.27 mg potassium, 0.74 mg zinc, 0.26 mg ascorbic acid, 0.24 mg niacin, 0.13 mg thiamine, 0.13 mg riboflavin, 0.06 mg vitamin B6 per 100g of SFE et al. Besides, SFE contains other important dietary components such as dietary fibre in amount of 0.9 g per 100g product. SFE also contains isoflavones which are known to have anti-cancer properties.

Oligosaccharides of soybeans causing flatulence (raffinose, stachyose, verbascose) are removed in process of soaking and draining of soybeans. Trypsin inhibitors detrimental for protein digestion are inactivated by a heat treatment in process of manufacturing of SFE. Lipoxygenase and lipase enzymes causing lipids oxidation and hydrolysis are heat inactivated as well too.
Hydrolyzed soybean food emulsion (HSFE) is a product of enzymatic hydrolysis of SFE. The studies of All-Russian Research Institute of Fats report on the increase of biological value of HSFE as a result of enzymatic treatment. This is explained by a generation of low molecular weight peptides during the hydrolysis which are easily absorbed by the body and by a decrease of total antigenicity of HSFE (Domoroshchenkova et al., 2003; Domoroshchenkova et al., 2014). The use of hydrolysed soybean food emulsion (HSFE) with a high degree of hydrolysis in food formulations is particularly important as hydrolysed proteins improve the digestibility of the product. HSFE is characterized by a high biological value due to the inactivation of anti-nutritional factors of soybeans and removal of physiologically undesirable components.

**MATERIALS AND METHODS**

The test samples were prepared by combining vegetable and animal raw materials. The control sample was manufactured from fresh cow milk meeting the requirements of the Russian State Standard GOST R 52054–2003 and of Technical Regulations of the Customs Union ‘On the safety of milk and dairy product’ (TR CU 033/2013).

**Production of HSFE**

SFE with 12% dry matter content meeting requirements of TS 9146–166–00334534–97 was a used as a substrate for enzyme treatment. SFE was hydrolysed in a pilot reactor equipped with automatic titration unit and temperature control at agitation rate 150 rpm. The initial SFE was treated with proteolytic enzyme preparation Flavourzyme TM type A (Novo Nordisk A/S, Bagsvaerd, Denmark) with an activity of 1,000 Leucine Aminopeptidase Units (LAPU) g−1 at E/S – 40 LAPU/g, t – 50°C, pH – 6.5. The degree of hydrolysis (DH) of hydrolysed soybean emulsion was varied from 6% to 12% with an interval of 2%. DH was determined by changes of amino nitrogen content (Novo Nordisk’s Analytical Method AF 298/1). The reaction was stopped by elevation of reaction mixture temperature to 85–90°C.

**Production of clots**

Coagulation of test samples was performed with the following reagents: dairy starter cultures of *Lc. lactis, Lc. cremoris, Lc. diacetilactis* (TS 49559); rennet powder (TS 10–02–824); calcium chloride (TS 6–09–4711).

In experiment with the acid coagulation method of cheese production a bacterial starter was used as a coagulant, in case of the acid-rennet coagulation method of cheese production – a rennet and a bacterial starter, in case of the rennet coagulation method of cheese production – a rennet. To optimize the coagulation process calcium chloride solution was added.

The reagents were added to the mixture in following quantities: starter – 2% v v−1; rennet powder – on the basis of calculation of the clotting mixture at 32–35°C for 30–35 min; solution of calcium chloride on the basis of addition of 40 g of anhydrous salt per 100 kg of mixture.

The samples were kept at the same conditions during the process of study. Final analysis of raw materials, coagulates and finished products were performed according to the following procedures.
Sensory evaluation
Organoleptic characteristics of the product were examined according to ISO 22935–3:2009 Milk and milk products – Sensory analysis – Part 3: Guidance on a method for evaluation of compliance with product specifications for sensory properties by scoring (IDT). The samples were evaluated by a trained panel of 12 members. Twelve panellists (age 22–38 years) qualified for sensory evaluation techniques and regular consumers of products estimated the sensory properties of the samples.

Quality parameters analysis
The titratable acidity was analysed according to AOAC (1998).
Crude protein content was analysed by Kjeldahl method at the automated analyser Kijltec Auto 1030, Sweden, according to the standard protocol of the equipment vendor (GOST R 54662–Cheeses and processed cheeses).
Aminoacid content of proteins was analysed by HPLC method at the analyser DJEOL, Japan, at the department of biochemistry and molecular biology of All-Russian Institute of Plants, St. Petersburg. Samples were hydrolysed by 6N hydrochloric acid during 24 hours at temperature of 110°C in a nitrogen atmosphere in sealed glass tubes and then subjected to HPLC analysis.
All experiments were performed with at least three replicates; data was processed by methods of mathematical statistics at theoretical frequency 0.95.

RESULTS AND DISCUSSION
The previous research has demonstrated positive results of development of fermentation process of dairy-soy mixtures and of coagulating of proteins with a mixed composition. Therefore a possibility of usage of the known methods of coagulation of proteins of a dairy-soybean mixture in production of cheese product was proposed.
At the initial stage of the current research we’ve studied the process of the joint coagulation of proteins at 2:1 ratio of cow milk and SFE in the initial mixture (based on the data of preliminary studies).
Organoleptic characteristics of samples obtained by different coagulation methods were analysed. In studies of coagulation process of combined protein mixtures the best results were achieved for the acid and acid-rennet coagulation methods. In the experiment with the rennet coagulation of proteins of dairy-soybean mixture the small-size flocculated clot was formed and the resulted whey was poorly separated. It is known that milk coagulation ability caused by the rennet and the quality of the clot are affected by several factors such as a ripeness of milk, a pasteurization temperature, a temperature of coagulation and clot processing. But in our case usage of the higher ripeness milk (22–25°C), of the optimal temperature of mixture pasteurization (70–72°C), of the optimal temperature of coagulation (33–35°C) and processing of clot (40–45°C) in order to reach a firmer clot haven’t resulted in the formation of a clot of an acceptable quality. It could be caused by a lack of casein level required for the rennet coagulation. Thus samples obtained by the rennet coagulation were not used in further studies.
The study showed that it was possible to receive a plastic and high quality clot with a homogenous consistency in process of production of the acid-rennet coagulated cheese product with SFE additive apparently due to the presence of fibre in SFE.
In experiments with 1/3 ratio substitution of milk by SFE the resulted clots were characterized by a weak taste of soybeans. Combined clots had a white colour with a creamy shade inherent to soy products.

One of the main reasons limiting the widespread usage of soyfoods and soy additives in European diets is its low consumer appeal. Soy products often have specific odour and flavour which are unusual for a Russian consumer. Usage of soy additives by the Russian dairy industry is relatively limited (Domoroshchenkova et al., 2006; Domoroshchenkova & Lishayeva, 2010). Therefore there is an objective to develop new food products containing dairy and plant components with consumer properties equal or close to the properties of traditional foods. In the circumstances a serious attention was paid to the evaluation of organoleptic characteristics of cheese products with a different composition.

Sensory tests were performed with 3 samples with a different ratio of dairy and plant components in the mixture: 2:1 (sample 1), 1:1 (sample 2), and 1:2 (sample 3). Organoleptic characteristics of acid coagulated samples are presented in Fig. 1. Acid-rennet coagulated samples showed a similar dynamics in organoleptic score. The studied characteristics of sample 1 are the most close to the control.

![Figure 1. Organoleptic parameters of acid coagulated samples.](image)

Thus the positive indicators of clots were achieved for the acid and acid-rennet method of coagulation of proteins for milk and soy mixture with ratio 2:1.

A positive effect of partial controlled hydrolysis of soybean food emulsions for usage in formulations of cheese products was proposed. The influence of the degree of hydrolysis (DH) of soybean emulsion used as a part of the initial mixture on fermentation of samples was studied. Control samples were prepared from milk and from milk and SFE mixture. On the basis of preliminary results of the study the ratio of dairy and plant components in the starting mixture was adjusted to 2:1.

The coagulation process of the mixture is one of the most important processes in manufacturing of cheeses. Its success depends on a number of factors which are described by many authors (Smirnova & Ostroumova, 2006). The most indicative effect of influence of DH of HSFE on an acid accumulation process of samples could be demonstrated for an acid coagulation method of dairy-soybean mixture. The titratable
acidity of the mixture, the duration of the fermentation process and the ability of a clot to separate whey were used as evaluation criteria.

Fig. 2 shows changes of the titratable acidity of samples with different DH during the fermentation process. The increase in acidity during fermentation of the mixtures of different composition was uneven. Changes of the acidity of the control sample were small during the first four hours, then after 6 hours of fermentation process the acidity started to increase quickly and after 14 hours it reached the acidity of the finished clot (90°T), and after 24 hours - the maximum 120°T. The increase of the titratable acidity of the combined mixture was more intensive at the beginning of fermentation process. It was observed that for the higher DH of HSFE in the starting mixture the fermentation process proceeded more intensively. However the intensity of the fermentation process of dairy-soy mixture dropped greatly after 10 hours of fermentation time versus a control sample. The clot with HSFE with 6% DH was ripened in 16 hours of maturation of the mixture, with 8% DH in 12 hours, with 10% DH in 10 hours and with 12% DH in 8 hours.

Figure 2. Changes of titratable acidity of the samples during fermentation process.

This can be explained by the positive influence of protease treatment of soybean emulsion associated with an increase of hydrophobicity of proteins and their ability to emulsify fats.

The ability of clots to separate whey during self-pressing process is equally important in the production of soft cheeses. Fig. 3 shows the influence of DH of HSFE in the dairy-soy raw materials mixture on the syneresis properties of the test clots obtained by acid coagulation. It is obvious that the control samples had a higher ability to separate whey. It was observed that the clots with HSFE have separated whey better than the samples with SFE. The samples with HSFE with DH of 12% were more similar to the control clots by the ability to separate the whey. The volume of the separated whey during self-pressing was decreased by 2–2.5% with a decrease of DH of HSFE per each 2%.
The varying water-holding capacities of clots have affected quality of finished products mainly the consistency of cheeses. A total score of products with HSFE with different DH was evaluated (Fig. 4).

Many authors consider the issues of improving of the nutritional value of foods in particular by an increase of a biological value of protein components (Orlova & Nasonova, 2014; Zabodalova et al., 2014; Nadtochii & Koryagina, 2014). For evaluation of biological value of protein components of products the content of amino acids should be determined. The amino acid composition of cheese products based on a raw material mixture of milk and SFE in a ratio of 2:1 is presented in Tables 1 and 2.

### Table 1. Amino acid content of cheese products

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>acid-rennet cheese product with SFE g per 100 g of product</th>
<th>% to protein</th>
<th>acid cheese product with SFE g per 100 g of product</th>
<th>% to protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential amino acids, total</td>
<td>3.110</td>
<td>41.4</td>
<td>7.170</td>
<td>43.0</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.250</td>
<td>3.3</td>
<td>0.470</td>
<td>2.8</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.453</td>
<td>6.0</td>
<td>0.994</td>
<td>6.0</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.258</td>
<td>3.4</td>
<td>0.657</td>
<td>3.9</td>
</tr>
<tr>
<td>Valine</td>
<td>0.431</td>
<td>5.7</td>
<td>0.952</td>
<td>5.7</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.163</td>
<td>2.2</td>
<td>0.369</td>
<td>3.4</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.315</td>
<td>4.2</td>
<td>0.636</td>
<td>3.8</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.623</td>
<td>8.3</td>
<td>1.288</td>
<td>7.7</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.112</td>
<td>1.5</td>
<td>0.232</td>
<td>1.4</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.307</td>
<td>4.1</td>
<td>0.647</td>
<td>3.9</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.448</td>
<td>6.0</td>
<td>1.195</td>
<td>7.2</td>
</tr>
<tr>
<td>Non-essential amino acids, total</td>
<td>4.416</td>
<td>58.6</td>
<td>9.509</td>
<td>57.0</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.345</td>
<td>4.6</td>
<td>0.650</td>
<td>3.9</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.186</td>
<td>2.5</td>
<td>0.332</td>
<td>2.0</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0.642</td>
<td>8.5</td>
<td>1.509</td>
<td>9.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.258</td>
<td>3.4</td>
<td>0.453</td>
<td>2.7</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>1.216</td>
<td>16.1</td>
<td>2.811</td>
<td>16.9</td>
</tr>
<tr>
<td>Proline</td>
<td>1.105</td>
<td>14.7</td>
<td>2.276</td>
<td>13.6</td>
</tr>
<tr>
<td>Serine</td>
<td>0.315</td>
<td>4.2</td>
<td>0.780</td>
<td>4.7</td>
</tr>
<tr>
<td>Cysteine</td>
<td>0.099</td>
<td>1.3</td>
<td>0.228</td>
<td>1.4</td>
</tr>
<tr>
<td>Total</td>
<td>7.526</td>
<td>100</td>
<td>16.679</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 2. Amino acid score of the cheeses with SFE

<table>
<thead>
<tr>
<th>Essential amino acids</th>
<th>FAO/WHO, 2007, g 100 g⁻¹ of protein</th>
<th>Amino acid content of cheese products, %</th>
<th>Amino acid score of cheese products, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>by acid-rennet coagulation</td>
<td>by acid coagulation</td>
<td>by acid-rennet coagulation</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.5</td>
<td>3.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>3.0</td>
<td>4.2</td>
<td>3.8</td>
</tr>
<tr>
<td>Leucine</td>
<td>5.9</td>
<td>8.3</td>
<td>7.7</td>
</tr>
<tr>
<td>Lysine</td>
<td>4.5</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Methionine+ Cysteine</td>
<td>2.2</td>
<td>3.5</td>
<td>4.8</td>
</tr>
<tr>
<td>Phenylalanine + tyrosine</td>
<td>3.8</td>
<td>10.1</td>
<td>11.1</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.3</td>
<td>3.4</td>
<td>3.9</td>
</tr>
<tr>
<td>Valine</td>
<td>3.9</td>
<td>5.7</td>
<td>5.7</td>
</tr>
</tbody>
</table>
Amino acid score of essential amino acids was calculated by a comparison of the amino acid content of the test protein with its recommended pattern:

\[
Amino\ acid\ score = \frac{g\ of\ amino\ acid\ in\ 100\ g\ test\ protein}{g\ of\ amino\ acid\ in\ 100g\ protein\ FAO/WHO,2007}
\] (1)

To refine the indicators of biological value of protein component we used basic indicators and criteria: such as biological value of protein component (BV) and coefficient of differences of amino-acid scores (CDAAS) (Lipatov, 1995).

CDAAS shows the average differences of essential amino acids score (DAAS) as compared to the minimum level of an essential amino acid. The coefficient of differences of amino-acid scores (CDAAS) is calculated as follows:

\[
CDAAS = \frac{\sum C_i - C_{min}}{n}, \quad (2)
\]

where: \( \Delta DAAS = C_i - C_{min} \),

\[
\Delta DAAS = C_i - C_{min}, \quad (3)
\]

where: \( C_i \) – score of \( i \)-essential amino acid; \( C_{min} \) – minimum amino-acid score.

\[
BV = 100\% - CDAAS, \quad (4)
\]

Calculated quality indicators of protein component of cheese products are presented in Table 3.

**Table 3. Quality indicators of the protein component of the cheese products**

<table>
<thead>
<tr>
<th>Quality indicators</th>
<th>Cheese product by acid-rennet coagulation</th>
<th>Cheese product by acid coagulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum amino acid score, %</td>
<td>133</td>
<td>127</td>
</tr>
<tr>
<td>( \Delta DAAS ), %</td>
<td>286</td>
<td>390</td>
</tr>
<tr>
<td>CDAAS, %</td>
<td>36</td>
<td>49</td>
</tr>
<tr>
<td>BV, %</td>
<td>64</td>
<td>51</td>
</tr>
</tbody>
</table>

Quality indicators confirm a relatively high BV of protein component of cheese products. Moreover, estimate indicators of cheese product by acid-rennet coagulation slightly higher on indicators of cheese product by acid coagulation.

**CONCLUSIONS**

On the basis of the conducted studies we’ve reached the following conclusions:

1. The possibility of production of cheese products by acid and acid-rennet coagulation of mixture of cow’s milk and soybean food emulsion was demonstrated.
2. The ratio of milk and SFE of 2:1 in raw materials mixture allowed obtaining the product with acceptable quality.
3. DH of HSFE influenced the clot formation process and its quality. The best results were obtained for HSFE with DH 8–10%.
4. Finished cheese products obtained by acid and acid-rennet coagulation were characterized by a relatively high BV of protein component.

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The role of cyclic amides in the formation of antioxidant capacity of bakery products

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Abstract. This paper discusses the possibility of using of additives of vegetable raw materials in the manufacture of bakery products with antioxidant capacity. The optimal doses of additives in the recipe of bakery products were established. These were 3% for blueberry powder and 6% for pine nuts flour. Antioxidant capacity of hydrophilic fractions of blueberries powder, pine nuts flour, bakery crusts and crumbs were studied in vitro by chemiluminescence technique. Cyclic amides (lactams) were also identified. The antioxidant capacity of hydrophilic fraction of crust of bakery products with pine nuts flour was in 1.7 times higher than that obtained by calculation, for crumb in 1.5 times. For crust of bakery products with blueberry powder the antioxidant capacity of hydrophilic fraction was in 2.2 times higher, and for crumb in 1.3 times higher. The antioxidant capacity of hydrophilic fraction of crust of bakery products with blueberry powder was in 1.2 times higher and for bakery products with pine nuts flour, conversely was higher the antioxidant capacity of the hydrophilic fraction of crumb – in 1.3 times. The antioxidant capacity of hydrophilic fraction of crumb of bakery products with pine nuts flour was in 1.1 times higher in comparison with bakery products with a blueberry powder. The amount of mono-heterocyclic γ-lactams in the crumb of bakery products with pine nuts flour was higher than in the product with blueberries powder, but less than in the control sample. Thus, the formation of antioxidant capacity of hydrophilic fractions of crumb and crust occurs due to Maillard reaction intermediates.

Key words: bakery products; blueberry powder; pine nuts flour; antioxidant capacity; cyclic amides (lactams).

INTRODUCTION

The creation and launching of functional food products is one of the directions of the human nutrition program initiated by the UN (Nadtochii et al., 2014). One of the trends of healthy food development is creating products with antioxidant properties. Such products can minimize an oxidative stress, or prevent ‘breaking’ in the body, which occur as a result of negative effects of free radicals.

Natural antioxidants give a preventive effect only through regular consumption with food. Therefore, it is necessary to enrich the products of mass consumption that are used every day, which include bakery products. It is known, that in order to create
Antioxidant properties of bakery products it is necessary to use wheat and rye flour with high extraction rate (Horszwald et al., 2010) or whole-grain flour (Belyavskaya et al., 2014). Buckwheat, corn and amaranth flour (Antiochia et al., 2012, Chlopicka et al., 2012), vegetable powders (Belyavskaya et al., 2013), buckthorn (Nilova, 2012) and other derivatives of vegetable origin which are used in the recipes of bakery products create and improve their antioxidant capacity. Antioxidant capacity of enriched bakery products is associated with introduction of botanical derivatives to recipes. They have increased levels of antioxidant substances such as phenolic compounds, tocopherols and tocotrienols, carotenoids, ascorbic acid. At the same time there is evidence that products of the Maillard reaction (melanoidins), which are cyclic amides (lactams) formed in the process of bread baking also have antioxidant properties (Borelli & Fogliano, 2005, Chawla et al., 2009). The experiment proved that the dark brown crusts ethanol extracts have high antioxidant capacity. In crusts ethanol extract cyclic amides – pyrrolinone-reductones are formed. Among them the highest antioxidant capacity has the pyrrolinone reductonyl-lysine (shortly pronyl-lysine) (Lindenmeyer et al., 2002). Cyclic amides which are formed during baking, have a structure similar to the flavonoids. They contain a system of conjugated double bonds in the heterocyclic and quinoid links (Fig. 1). Flavonoids which have in their composition fragments of quinoid and sugar residues are also reductones (Selemenev et al., 2008). Molecules of these substances have one or more aromatic (benzene) ring bearing one or more hydroxyl groups. Antioxidant capacity of phenolic compounds is based on their ability to give an electron or proton and to pass into form of stable phenoxy radical, which are capable to delocalize the unpaired electron (Korulkin et al., 2007). Due to the presence of flavonoids rings and cyclic amides in the structure of generalized π-electron system, occurs the displacement of the negative charge on the oxygen or nitrogen. As a result of this hydrogen atom quite easily separates with formation of radicals.

Cyclic amides divided into two groups: soluble in alcohol and soluble in water. In order to exclude the influence of alcohol-soluble cyclic amides formed during bread baking, and fat-soluble antioxidants of pine nuts flour on antioxidant capacity, it is necessary to determine the antioxidant capacity of aqueous extracts of bakery products. There are various methods of determination of antioxidant capacity: photometric, electrochemical, volumetric, fluorescent, chemiluminescent, and others. Methods are

![Figure 1. Comparison of the structural formulas of flavonoids and intermediate products of Maillard reactions.](image-url)
based on chemiluminescence, are widely used in biological systems in Russia. These methods are known as highly informative and are used for investigation of the kinetics of antioxidants and radicals interaction (Izmailov et al., 2011).

The aims of this research were:

- the study of the antioxidant capacity of aqueous extracts of blueberry powder and pine nuts flour additives and crumbs and crusts of bakery products;
- the study of formation of cyclic amides in bakery products by IR spectroscopy;
- definition of relation between antioxidant capacity and the formation of cyclic amides (lactams).

**MATERIALS AND METHODS**

For the development of bakery products from patent wheat flour (fancy white wheat flour) with antioxidant capacity we used natural sources of antioxidants – the powder of wild blueberry from the North-West of Russia and pine nuts flour (a content of fat was 20%) produced by LLC (Limited liability company) ‘Specialist’, Biysk, Altai Territory. Blueberries were picked up in the forest area, dried at 50–55°C to 6% of moisture and floured to a powder.

Optimization of doses of blueberry powder and pine nuts flour in the recipe was carried out by a laboratory test baking. The formulation of control bakery products was: wheat flour (ash content was 0.55%; gluten was 28.9%); sugar 5%; refined deodorized sunflower oil 4%; salt 1.5%. Enrichment of bakery products is achieved by adding blueberry powder and nuts flour instead of wheat flour. The content of additives varied from 1 to 5% with the increment of 1% for blueberry powder and from 2 to 8% with the increment of 2% for pine nuts flour. All test samples were prepared by using the straight dough method. Test bakery products weighing 100 g were baked in laboratory at the temperature of 220°C during 23 minutes (Nilova, 2012).

Assessment of bakery products quality was carried out by following properties:

- organoleptic – appearance (shape, surface, crust color), condition of crumb (elasticity, porosity), taste and smell;
- physico-chemical and physical - mass proportion of moisture was determined by drying at a temperature of 13°C during 40 minutes, acidity was determined by titration, using 0.1 n. solution of NaOH, porosity – was determined as the ratio of pore volume to the total volume of products, pore volume – as the difference between the volume of product and the volume of non-porous mass, specific volume – as the ratio of product volume to 100 g of flour; dimensional stability – as the ratio of height of the product to diameter.

Obtained samples were compared with control sample of bakery products without additives.

The antioxidant properties of the crust and crumb of bakery products with additives were determined in water extracts by using chemicoluminescence method with biochemiluminometer BCL-06M (Nizhny Novgorod, Russia).

After baking, bakery products were cooled to room temperature during 4 hours. Then crumb was separated of crust and both were dried at a temperature of 40°C to a moisture content of 6.5–7.0%.
For the research was taken 1 ml of the extract, made of: milled sample in an amount of 250 mg and 10 ml of distilled water, which were centrifuged for 10 minutes at 3,500 rev min⁻¹.

Antioxidant capacity was determined in a model system in vitro, comprising riboflavin, hydrogen peroxide and a divalent iron. Chemiluminescence reaction of riboflavin (substrate for oxidation) was used in the presence of ferrous ions and hydrogen peroxide. 610 μl of potassium phosphate buffer solution (pH 7.5), 40 μl of 10 mM solution of riboflavin, 100 μl of physiological solution, 25 mM of iron sulfate solution (II) (model system) were introduced into the measuring cuvette of biochemiluminometer BKL 06M. In order to intensify the process, 0.1% solution of H₂O₂ was introduced to the mixture. In determining of antioxidant capacity of the samples, physiological solution was replaced by relevant extracts of different concentrations (Putilina et al., 2006). The calibration curve was built up for ascorbic acid (AA). It was chemically pure, obtained from ‘Spectrum-Chem’, Russia. An aqueous solution of ascorbic acid 100 μg ml⁻¹ was used; each succeeding dilution was 1:2.

Calculation of theoretical antioxidant capacity of crumb and crust of enriched bakery products was based on the number of additives in the formulation (in percentage terms).

Cyclic amides (lactams) were studied by FTIR spectroscopy in 1,680–1,800 cm⁻¹ (Bellami, 1971, Silverstein, 2011). Study of the spectral curves was carried out by FT-IR spectrometer ‘FSM 1202’ LLC ‘Infraspektr’ (Russia), with automatic calculation of absorption intensity. The absolute error in calibration of the scale of wave numbers is no more than ± 0.1 cm⁻¹. The deviation of 100% transmission line from the nominal value (1,950–2,050 cm⁻¹, definition 4 cm⁻¹, 20 scans), is no more than ± 0.5 (in percentage terms). Standard deviation of 100% transmission line (1,950–2,050 cm⁻¹, definition 4 cm⁻¹, 20 scans), is no more than, 0.025 (in percentage terms). Parameters of recording spectra were: spectral range – 400–4,000 cm⁻¹; number of scans – 20; resolution of 4 cm⁻¹; mode – the interferogram. The resulting interferograms were converted into absorption spectra. In order to prepare the samples for analysis powders were compressed with potassium bromide. To prepare the tablets, potassium bromide and powder (1.5 g) were triturated in the agate mortar. 100 mg of a mixture were selected and pressed in moulds for 15 minutes for each side.

Identification of heterocyclic compounds was carried out by peak areas in relative standard units, calculated in relation to the base line, carbonyl absorption bands: γ-lactam monocyclic – 1,700 cm⁻¹; polycyclic in the range between 1,700–1,750 cm⁻¹; β-lactams monocyclic in the range between 1,760–1,730 cm⁻¹; polycyclic lactams, which condensed with other rings – in the range between 1,770–1,800 cm⁻¹; cyclic amides (lactams), with large rings – near 1,680 cm⁻¹ (Bellami, 1971).

Baking of bakery products, evaluation of their quality, antioxidant capacity and removal of the IR spectra were carried out in the laboratory of examination of consumer goods, St. Petersburg State University of Trade and Economic. All bakery products were baked three times. Each bakery product sample been researched 3 times. The accuracy of experimental data was evaluated by using methods of mathematical statistics in Microsoft Excel. These data are presented with a confidence coefficient of 0.95.
RESULTS AND DISCUSSION

For the production of bakery products with antioxidant capacity blueberries powder as a source of water-soluble antioxidants (anthocyanins, phenolic acids, ascorbic acid) (Wang et al., 2009, Gupta-Elera et al., 2012) and pine nut meal as a source of fat-soluble antioxidants (tocopherol) were taken (Nilova, 2012).

Using a method of test laboratory baking we established the optimal doses of additives of natural raw materials in the compositions of bakery products, it was 3% for blueberry powder and 6% for pine nuts flour (by weight of flour).

Addition of blueberry powder to wheat dough changed organoleptic characteristics, particularly the colour of finished products. The colours of crusts changed from golden-brown in bakery products with 1% of powder to a brown, and even with small particles of blueberry powder in products with 5% of powder, crumb colour was from light grey to dark brown with a purple tinge. But blueberry taste and flavour in bakery products was felt only with introduction from 3% of powder. With introduction of 4% of blueberry powder products crumb began to lose its elasticity and became more rubbery and porosity was uniform, but pore wall thickness increased. Blueberry powder had an impact on the physicochemical properties of bakery products, especially on the porosity and specific volume. With introduction up to 3% of powder, these parameters were gradually increased, and when the doses were 4, and 5% parameters started to decline. Most of all, these changes were expressed in determination of the specific volume. Thus, for the production of bakery products from wheat flour the most optimal dose of blueberry powder was 3% provided the use of 4% of vegetable (sunflower) oil in the recipe.

The use of pine nut flour had no effect on the shape and surface of bakery products of various recipes. Bakery products with pine nut flour became higher, rounded, with a convex crust. Pine nut flour oil and sunflower oil included in the formulation of products, made the dough more elastic, and contributed to a more equal distribution of carbon dioxide in the process of maturation of dough and its knock-back. As a result, finished products had a better structure of porosity; it was more uniform and thin walled. However, with the addition of pine nut flour in the amount of 7% crumb of bakery products became jammed. The products crumb colour had a cream tint. Gentle cedar smell was felt only at introduction into recipe of bakery products 5% or more of pine nut flour. It was found that bakery product with 6% of pine nuts flour had the best quality. This product had a specific volume of 79.6 cm³ 100g⁻¹ higher than the product with blueberry powder and of 109.5 cm³ 100g⁻¹ higher than control sample. The porosity of the sample with pine nut flour was higher of 4.9% than the product with blueberry powder and of 7% in comparison with the control sample. Shape stability of bakery products with pine nuts flour was the highest in comparison with other samples.

The results of research of physical and chemical parameters of obtained enriched bakery products are presented in Table 1.
Table 1. Physical and chemical quality of enriched bakery products

<table>
<thead>
<tr>
<th>Chemical components</th>
<th>Bakery products</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>with blueberry</td>
<td>with pine nuts flour</td>
<td></td>
</tr>
<tr>
<td>Mass fraction of moisture, %</td>
<td>40.1</td>
<td>39.8</td>
<td>40.4</td>
<td></td>
</tr>
<tr>
<td>Acidity, deg.</td>
<td>2.1</td>
<td>2.0</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>Porosity, %</td>
<td>69.7</td>
<td>71.8</td>
<td>76.7</td>
<td></td>
</tr>
<tr>
<td>Specific volume, cm³ 100g⁻¹</td>
<td>375.6</td>
<td>405.5</td>
<td>485.1</td>
<td></td>
</tr>
<tr>
<td>Shape stability (h d⁻¹)</td>
<td>0.53</td>
<td>0.58</td>
<td>0.63</td>
<td></td>
</tr>
</tbody>
</table>

Water extracts of additives had the following antioxidant capacity (mg of ascorbic acid (AA) per g dry basis (DB)): wild blueberry powder – 14.47; pine nut flour – 1.74. A high blueberries powder antioxidant capacity is provided by using of soft modes of drying. All antioxidants of blueberry powder, in particular phenolic compounds retain their activity. In contrast pine nut flour does not substantially contain water-soluble antioxidants. Therefore, its water extract has low antioxidant capacity.

Determination of the antioxidant capacity of water extracts of bakery products crust and crumb showed that even products with traditional composition (without additives) had an antioxidant capacity. Moreover the antioxidant capacity of the crumb of control sample is in 1.4 times higher as compared with the crust. (Table 2). It can be connected with the fact that the heating temperature of crust reaches 180°C and for crumb does not exceed 100°C. (Auerman, 2005) Therefore, the reaction of melanoids formation has a greater rate in the crust and it is forming final products without antioxidant properties.

Table 2. The antioxidant capacity of 1 g of bakery products in a model system in vitro, mg AA per g DB

<table>
<thead>
<tr>
<th>Type</th>
<th>Crust</th>
<th>Crumb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bakery products (control)</td>
<td>9.69</td>
<td>13.91</td>
</tr>
<tr>
<td>Bakery products with blueberry:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>theoretical</td>
<td>9.83</td>
<td>13.93</td>
</tr>
<tr>
<td>actual</td>
<td>21.27</td>
<td>18.08</td>
</tr>
<tr>
<td>Bakery products with pine nuts flour:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>theoretical</td>
<td>9.21</td>
<td>13.18</td>
</tr>
<tr>
<td>actual</td>
<td>15.50</td>
<td>20.09</td>
</tr>
</tbody>
</table>

Antioxidant capacity of hydrophilic fractions of crumb and crust of enriched bakery products exceeded the data obtained by calculation. The hydrophilic fraction of crust of bakery products with pine nut flour had an antioxidant capacity in 1.7 times more than that obtained by calculation, and the crust – in 1.5 times; crust of bakery products with blueberry powder – in 2.2 times, crumb – in 1.3 times. Bakery products with blueberry powder had antioxidant capacity of hydrophilic fraction of crust in 1.2 times higher in comparison with the crumb, and conversely bakery products with pine nut flour had higher antioxidant capacity of crumb hydrophilic fraction, it was in 1.3 times higher in comparison with the crust. Antioxidant capacity of hydrophilic fraction of crumb of bakery products with pine nuts flour was in 1.1 times higher in comparison with bakery
products with blueberry powder. Increase of antioxidant capacity of the crumb can be associated with the formation of intermediate products of the Maillard reaction (lactams).

Formation of the products of the Maillard reaction (melanoidins) can be studied by IR spectrum. Melanoidins in crust and crumb of enriched bakery products give a large number of sharp characteristic bands during IR spectroscopy (Fig. 2).

The structure of aromatic type is best recognized by the presence of bands of stretching vibrations of the C = C in the 1,600–1,500 cm⁻¹ (Bellami, 1971). However, the heterocyclic aromatic compound with six members also give two bands at 1,600 cm⁻¹ corresponding to C = C and C = N bonds. It is impossible to separate them in this field. Identification of heterocyclic compounds (lactams) carried out by the intensity of the
absorption bands of the carbonyl group in 1,680–1,800 cm\(^{-1}\). Reducing of number of members in the ring leads to displacement of bands towards to high frequencies. The results of research of melanoidins formation by IR spectroscopy as an example of mono- and polycyclic γ- and β-lactams in the crust and crumb of enriched bakery products are presented in Table 3. Peak area was calculated automatically in relative standard units relative to the baseline.

**Table 3.** Characteristics of cyclic amides (lactams) of enriched bakery products (relative standard units)

<table>
<thead>
<tr>
<th>Lactams</th>
<th>Absorption lines, cm(^{-1})</th>
<th>Bakery products</th>
<th>Type (component)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>with blueberry</td>
</tr>
<tr>
<td>with large rings</td>
<td>Near 1,680</td>
<td>crust</td>
<td>11.264</td>
</tr>
<tr>
<td>mono</td>
<td>1,700</td>
<td>crust</td>
<td>17.849</td>
</tr>
<tr>
<td>γ-lactams</td>
<td></td>
<td>crumb</td>
<td>19.831</td>
</tr>
<tr>
<td>poly</td>
<td>1,700–1,730</td>
<td>crust</td>
<td>29.723</td>
</tr>
<tr>
<td>γ- lactams</td>
<td></td>
<td>crumb</td>
<td>44.520</td>
</tr>
<tr>
<td>mono</td>
<td>1,750–1,760</td>
<td>crust</td>
<td>65.004</td>
</tr>
<tr>
<td>β- lactams</td>
<td></td>
<td>crumb</td>
<td>29.489</td>
</tr>
<tr>
<td>poly</td>
<td>1,770–1,800</td>
<td>crust</td>
<td>43.367</td>
</tr>
<tr>
<td>β- lactams</td>
<td></td>
<td>crumb</td>
<td>45.718</td>
</tr>
</tbody>
</table>

The nature of formation reaction of heterocyclic compounds depended on both components of bakery products (crust and crumb) and used products of processing of plant raw materials. The structure of bakery products with a powder of blueberry includes anthocyanins and the number of lactams in the crust was more than in the crumb. In bakery products with flour of pine nuts and traditional recipe (control) was noted the predominance of lactams in the crumb. The flow rate of melanoidins formation associate with the influence of temperature during baking, which leads to different moisture crumb and crust. Warming of the surface layers of dough at a temperature of 200°C (baking chamber) leads to formation of crusts in the first few minutes of baking and accompanies by a dehydration of the surface layer of dough. (Auerman, 2005). In these conditions the caramelization reaction and melanoidins formation proceed rapidly, and further increase of baking temperature can cause partial pyrolysis of polyphenol of herbal supplements and lactams (Steele, 2006). Warming of crumb is slow towards the end of baking at 95°C and it creates favourable conditions for the formation of heterocyclic compounds (intermediates melanoidins). Perhaps, the higher intensity of the absorption lines of lactams in the crust bakery products with a blueberry powder associated with the presence in them of pentoses (ribose, arabinose and xylose) (Selemenev et al., 2008). As a result, bakery products with blueberry powder have the largest number of mono γ-lactams in the crusts. But despite the fact that there was a great intensity of the process in crust compared to the crumb, the largest number of polymer γ and β-lactams was in bakery products of traditional recipes, which indicates a favourable course of melanoidins formation.

The intensity of stretching vibrations, which are inherent to four-membered monoheterocyclic compounds (β-lactams), in the crumb of bakery products with pine nuts flour was higher than in the control sample and in bakery products with blueberry
powder. In the crumb bakery products with pine nuts flour has more than in the product of a blueberries powder, and less than in the control sample.

Crumb of bakery a product with pine nut flour has more monoheterocyclic γ-lactams, than products with blueberry powder, but has less than in the control sample.

In bakery products crumb formation of heterocyclic compounds proceeded rapidly in the control sample and in enriched with pine nuts flour sample. But the ratio of mono- and poly-lactams fractions was different. Poly γ-lactams predominated in the control sample, they were in 2 times higher in comparison with mono γ-lactams and almost in 1.5 times more than the poly β-lactams. In bakery products with pine nuts flour mono β-lactams predominated and the number of polymer γ and β-lactams was approximately the same. Blueberry powder did not have significant effect on the ratio of different lactams fractions, although it slowed their formation compared to the control sample.

CONCLUSION

Antioxidant capacity of aqueous extracts of blueberry powder and pine nuts flour additives and crumbs and crusts of bakery products was established. Antioxidant capacity of water extracts of blueberry powder (AA mg per DB g) was 14.47; for pine nuts flour – 1.74. Antioxidant capacity of enriched bakery products was more than antioxidant capacity of bakery products without additives. Aqueous extracts of crust of bakery products with blueberry powder had in 2.2 times higher antioxidant capacity in comparison with the control, aqueous extracts of crumb – in 1.3 times. Aqueous extracts of crust of bakery products with pine nuts flour had in 1.6 times higher antioxidant capacity in comparison with control, crumb aqueous extracts – in 1.4 times;

Cyclic amides (lactams) have been studied by IR spectroscopy. The largest amount of polymer γ- and β-lactams, without antioxidant capacity was in bakery products without additives (control), especially in the crumb. In aqueous extracts of the crumb of these products were in 1.91 times more poly γ-lactams in comparison with aqueous extracts of bakery products with blueberry powder and in 1.86 times higher in bakery products with pine nuts flour. In bakery products without additives are formed more polycyclic amides, which haven’t antioxidant capacity.

In bakery products with pine nuts flour are formed more monocyclic amides with antioxidant capacity. In aqueous extracts of crumb observed in 1.15 times more mono β-lactams in comparison with the control. Enrichment of bakery products with blueberry powder reduces the formation of cyclic amides, especially in the crumb. Aqueous extracts of the crumb of bakery products with blueberry powder contain in 1.14 times less mono γ-lactams and in 1.72 times mono β-lactams in comparison with the crust.

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Automatic control and maintaining of cooling process of bakery products

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Abstract. During the development of automation control of the bakery products cooling process using a ‘cooler’ it is necessary to build an integrated control system, which allows to optimize the process parameters in case of a variety of work situations that may occur in the bakery enterprise. The main task for control of such multifactorial object is to reach a certain temperature in the center of the product by maintaining the temperature of the cooling air near the surface of the loaf set in the limits \( t \pm \Delta t \), its velocity \( v \pm \Delta v \) and humidity \( \varphi \pm \Delta \varphi \), and maintain the speed of the conveyor within \( V_c \pm \Delta V_c \) using the frequency converters. Simultaneous exposure to multiple channels of control allows selecting the optimal combination of the cooling process control commands, yielding the product with necessary temperature and mass. Automatic control and regulation of the cooling process is based on the fact that the current value of the adjustable parameters in mismatch block is compared with predetermined values of the corresponding parameters, and the mismatch signals are formed, which are amplified to a value sufficient to trigger actuators that provide executive regulators. Novelty of the paper consists in the development of the process control system based on the mathematical model which allows calculating the temperature at the center of freshly baked products by the surface temperature of the product at any time of location in the cooling zone. Experiments were carried out on the bread made in the laboratory and cooled after baking, by natural and forced convection. Baking was conducted in a special parallelepiped tins with lids. Thus, the output product had a parallelepiped shape. Process of cooling by natural convection was implemented in a cooling chamber, which allowed to measure temperature and relative humidity. Temperatures changing in the center of the loaf and weight loss were recorded during the experiments. Cooling time was determined by the time required to achieve the temperature in the center of the loaf 30°C. On the basis of the experimental data a mathematical model and the computer system, allowing calculating the parameters of cooling and in time to make a regulatory influence on one of the control channels and thus optimize the process parameters were built.

Key words: heat transfer, mass transfer, automation, spiral conveyor.

INTRODUCTION

The question of cooling bakery products has been widely discussed in scientific and industrial circles during last 10 years. The main purpose for cooling freshly baked products is to prepare the product for processing in the subsequent steps of cutting and packing, and the reduction of weight loss which occurs due to shrinkage and can reach up to 4%. Conditionally two periods of shrinkage are distinguished. The first period is characterized by rapid change in velocity of shrinkage from the maximum value at the
The most effective way to reduce weight loss of bakery products is to reduce the duration of the first period of shrinkage. In industrial practice, such reduction is achieved by accelerated cooling of the bread which is just taken out of the oven to the temperature of the warehouse.

There are different methods and technical solutions implemented for cooling bakery products, but the spiral-conveyor system which is called ‘cooler’ is considered to be the most effective in terms of productivity and the occupied area.

The advantages of applying spiral conveyor systems have been widely discussed for over 5 years (Pastukhov & Danin, 2011). However, today the problem of the use of spiral conveyor devices for reducing the duration of the cooling process and shrinkage of bakery products remains actual in the scientific community.

‘Cooler’ (Fig. 2) is a multitier spiral conveyor system positioned in the chamber made of insulating material. Due to the action of the cooling air flow of the required humidity in the working area of ‘cooler’ the cooling of freshly baked bread takes place. The product goes through all tiers from lower to upper. Thereafter, the cooled loaves are dropped from the upper tier into a hopper or fed by an additional transporter to the weight table, slicing machine or packaging machine.
In order to maintain the bakery products cooling automation scheme, it is necessary to develop an integrated management system, which allows optimization of the process parameters in the case of performance of different technological situations that may arise in the bakery enterprise, and ensuring efficient operation equipment without excessive energy consumption (Pastukhov, 2013).

A standard block diagram of a system for automatic monitoring and control of the cooling process is shown on a Figure 3. Transducer (sensor SE) –thermometer provides the conversion process of variable parameter $Y$ (product temperature) in the signal, convenient for further processing in the secondary converter (SC). Setter (Set.) evaluates and gives the required value of the controlled parameter $Y$, and it forms the set point signal $X_{set}$ that in the mismatch block (MB) is compared with the current value of the controlled parameter $X_m$, then the mismatch signal enters to the input of the forming unit (FU): $\Delta X = |X_{set} - X_m|$. Depending on the requirements for quality of regulation the signal outputs from the forming unit (FU) to the power amplifier (PA) in order to strengthen the signal to the value which is required to trigger the actuator (Act.), which, through the regulatory body (RB) provides a change of flow of energy or material flow in the object of regulation (OR) in the form of a regulatory impact $X_R$. 

![Figure 3. Block diagram of automatic regulation and control system.](image-url)
In other words, the system of automatic control and regulation of the cooling process is based on the fact that the current value of the adjustable parameters in mismatch block is compared with predetermined values of the corresponding parameters, and the mismatch signals are formed, which are amplified to a value sufficient to trigger actuators that provide the job of the regulatory bodies. The aim of this study is to compute the set of parameter values used in the cooling set point adjuster – setter (Set.). The computer system for calculating the temperature at the center of the loaf, depending on the temperature of its surface is developed. The system takes into account the thermal properties (Jarny & Maillet, 1999), shape and mass of the freshly baked product, and therefore can be used for calculating of the cooling parameters of various assortments of bakery products and other food products. The calculations are based on experimental studies (choice of initial and boundary conditions for modelling), literature data (Zueco et al, 2004) and mathematical modelling.

The development of autonomous regulation systems begins with parametric and structural analysis of the control object.

MATERIAL AND METHODS

Fig. 4 shows a parametric diagram of a spiral conveyor as an object of control. The process is represented in the form of a ‘black box’, which receives unmanaged, disturbing and control actions and outputs formed indicators of the quality of the cooled bakery products. Different types of bakery products have different thermal properties, shape and weight. Therefore, to achieve a temperature of 30°C in the center of the crumb, different cooling parameters are required.

Figure 4. Parametric diagram of the control object.

The input parameters are the thermal properties, the shape and weight of freshly baked products. Cooling process controlled parameters include temperature, humidity and velocity of the cooling air, the velocity of the conveyor belt moving.
Revolting influences $\nu_j$ lead to changes in the control parameters. These influences can be external, internal and parametric. External perturbations are heat income through the outer and inner fences of spiral conveyor. Sources of internal disturbances are heat income from the hot fresh bread, illumination, workers and electric motors of air fan coolers.

Parametric perturbations occur as changes in the heat transfer coefficients due to lubrication of inner surface evaporators, appearance of a water stone in a condenser unit, the outer surface rust, etc. Output parameters are the temperature of the cooled product and its weight.

Table 1. Ranges of technological parameters

<table>
<thead>
<tr>
<th>Technological parameters</th>
<th>Measurement units</th>
<th>Mean value</th>
<th>Technological interval deviation from the mean value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air temperature</td>
<td>°C</td>
<td>22.0</td>
<td>-2.0 to +4.0</td>
</tr>
<tr>
<td>Air speed</td>
<td>m s$^{-1}$</td>
<td>0.5</td>
<td>-0.5 to +2.5</td>
</tr>
<tr>
<td>Relative humidity</td>
<td>%</td>
<td>50.0</td>
<td>-10.0 to +10.0</td>
</tr>
<tr>
<td>Conveyor belt speed</td>
<td>m min$^{-1}$</td>
<td>3.0</td>
<td>-1.0 to +1.0</td>
</tr>
</tbody>
</table>

The main task for control of such multifactorial object is to reach a certain temperature in the center of the product by maintaining the temperature of the cooling air near the surface of the loaf set in the limits $t \pm \Delta t$, its velocity $v \pm \Delta v$ and humidity $\varphi \pm \Delta \varphi$, and maintaining the speed of the conveyor within $V_c \pm \Delta V_c$, with shrinkage for each type of product should not exceed the specified in normative documents.

Simultaneous exposure to multiple channels of control allows selecting the optimal combination of the cooling process control commands, yielding the product with necessary temperature and mass.

Experiments were carried out with white pan bread which was baked from scratch in a laboratory and then cooled by natural and forced convections.

Dough was prepared based on the straight-dough bread making recipe (Finney, 1984) which is given in Table 2.

Table 2. Bread formula

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Ingredients, %</th>
<th>Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread Flour</td>
<td>100</td>
<td>1160</td>
</tr>
<tr>
<td>Water</td>
<td>62</td>
<td>742</td>
</tr>
<tr>
<td>Yeast(fresh)</td>
<td>2.0</td>
<td>24</td>
</tr>
<tr>
<td>NFDM</td>
<td>3.0</td>
<td>35</td>
</tr>
<tr>
<td>A.P. Shortening</td>
<td>3.0</td>
<td>35</td>
</tr>
<tr>
<td>Gran. Sugar</td>
<td>6.0</td>
<td>70</td>
</tr>
<tr>
<td>Salt</td>
<td>2.0</td>
<td>23</td>
</tr>
<tr>
<td>Total Weight</td>
<td></td>
<td>2085 (approx.)</td>
</tr>
</tbody>
</table>

Mixing was done in VMI spiral mixer (France). Dough was rounded and left for rest for 7 minute at ambient temperature and final moulded using a Sorensen 'New Universal. Mark 2' moulder (England) and then loaded into slightly greased unlidded rectangular tins (100 x 100 x 300 mm). Tins were placed in an Acrivan Proving Cabinets (UK) for final proofing (40°C, 70% RH) for 1 hour. Baking was performed in a
Frederick Bone gas-fired reel oven (UK) set at 244°C for 30 min without steam injection with leads covered tins to achieve rectangular shape of final loaf.

Process of cooling by natural convection was implemented in a cooling chamber, which allowed to measure temperature and relative humidity. During the experiments the temperature change in the center of the loaf and weight loss were recorded (weight loss was determined by calculating the difference between weight of the loaf before and after cooling. Mettler PE 3600 precision scale weights were used for measuring the mass of loaves.

Cooling by forced convection was conducted in a climate chamber Votsch VC 7018 (Votsch-Lindenstrath, Germany) equipped with temperature and relative humidity controllers. Air velocity was measured using an anemometer VelociCalc 8355 (TSI, MN, USA), and ranged from 1.5–2.5 m s⁻¹.

Cooling time was determined by the time required to achieve the temperature in the center of the loaf 30°C. Temperature measurement in the middle of the crumb and crust surface temperature was conducted during 12,000 seconds every second using thermocouples connected to a data acquisition system Grant data-acquisition system (UK).

Values of temperature and relative humidity were recorded during cooling and processed by data acquisition system DataLog 20 (AOIP, Orangis, France), Type-K thermocouples and data logger Hygrolog (Rotronic, Bassersdorf, Switzerland).

Crumb and crust moisture was determined gravimetrically after cooling, by sampling them, and heating for 1.5 hours at 130°C.

RESULTS AND DISCUSSION

Modelling is based on the Fourier’s second law for heat transfer and the Fick’s second law for the mass transfer (devVries et al, 1988; Simpson & Cortes, 2004). The heat diffusion equation (1):

\[
\frac{\partial T}{\partial t} = \frac{\lambda(T)}{\rho_b C_{pb}(T,W)} \left( \frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} \right),
\]

where: \(\lambda(T)\) - thermal conductivity (W m⁻¹·K⁻¹); \(\rho_b\) - density of bread (kg (m³)⁻¹); \(C_{pb}\) - specific heat of bread (J (kg K)⁻¹)

As a boundary condition, \(\lambda(T)\) is defined taking into account heat transfer by convection, radiation and evaporation (2):

\[
-\lambda(T) \left( \frac{\partial T}{\partial x} \right)_{x=L} = -\lambda(T) \left( \frac{\partial T}{\partial y} \right)_{y=L} = \alpha \left( T_s - T_{air} \right) + \alpha \sigma \left( T_s^4 - T_{air}^4 \right) + \\
\beta H_{lg}(W) \left( p_s(T,W) - p_{ext}(T,W) \right),
\]

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where $\alpha$ - heat transfer coefficient (W m$^{-2}$K$^{-1}$); $\varepsilon$ - emissivity of bread surface; $\sigma$ - Stefan–Boltzmann constant; $T_{\text{air}}$ - air temperature near the bread surface (K); $T_s$ - temperature of bread surface (K); $\beta$ - mass transfer coefficient (m s$^{-1}$); $p_s, p_{\text{ext}}$ - partial vapour pressure on bread surface and near the bread surface (3), (Pa)

$$ p_s = a_{ws} p_s^g, \quad (3) $$

where: $a_{ws}$ - water activity at the surface; $p_s^g$ - saturating vapour pressure at the bread surface and can be found from Antoine’s law as (4):

$$ p_s^g = 133.3 e^{\frac{183036 - 381644}{T_s - 46.13}}, \quad (4) $$

Partial vapour pressure in the air near the bread surface $p_{\text{ext}}$ depends on relative humidity of the ambient air (5):

$$ H_{\text{ext}}, p_{\text{ext}} = H_{\text{ext}}^g p_{\text{ext}}^g, \quad (5) $$

where: $p_{\text{ext}}^g$ - saturating vapor pressure in the ambient air (6)

$$ p_{\text{ext}}^g = 133.3 e^{\frac{183036 - 381644}{T_{\text{ext}} - 46.13}}, \quad (6) $$

The mass transfer equations (7; 8):

$$ \frac{\partial W}{\partial t} = D(W)\left(\frac{\partial^2 W}{\partial x^2} + \frac{\partial^2 W}{\partial y^2}\right), \quad (7) $$

where: $W$ - local moisture content ($W$ surface = 0.1); $D(W)$ - mass diffusion coefficient (m$^2$ s$^{-1}$).

$$ -D(W) \left(\frac{\partial W}{\partial x}\right)_{x=L} = -D(W) \left(\frac{\partial W}{\partial y}\right)_{y=L} = \beta(p_s(T,W) - p_{\text{ext}}(T,W)), \quad (8) $$

The initial condition concerned mainly the temperature distribution in the crumb at the end of baking. The experimental temperature distribution was implemented in the code. The initial temperature was constant and equal to $T_0$ in the sample, except close to surface where it was decreasing from $T_{s0}$ to $T_0$ with a linear relationship through a 1 cm thickness layer. The local water content was decreasing with a linear evolution from $W_{\text{max}}$ in the center to $W_0$ at 1 cm under the surface, and then from $W_0$ to $W_{\text{min}}$ until the surface. The initial conditions and parameters are given in Table 3.
Table 3. Initial Conditions Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_0$</td>
<td>372</td>
<td>K</td>
</tr>
<tr>
<td>$T_0$</td>
<td>415</td>
<td>K</td>
</tr>
<tr>
<td>$W_0$</td>
<td>0.7</td>
<td>g water/ g dry matter</td>
</tr>
<tr>
<td>$W_{\text{max}}$</td>
<td>0.78</td>
<td>g water/ g dry matter</td>
</tr>
<tr>
<td>$W_{\text{min}}$</td>
<td>0.1</td>
<td>g water/ g dry matter</td>
</tr>
</tbody>
</table>

The solution of equations (1 and 2) are based on the boundary and initial conditions derived from field experiments have allowed us to obtain a mathematical model of convective bread cooling.

On the basis on modelling a computer system (Fig. 5) allowing calculating the parameters of cooling bakery various assortments were developed.

Figure 5. Computing system for calculating the cooling parameters.

The comparison between calculated temperatures and experimental data is shown on Fig. 6. The curves have some distinction, but we can admit that there is a good agreement of model and experiment.

Figure 6. Experimental and computed temperature history during natural cooling at ambient temperature 20.6°C.
CONCLUSIONS

Conducted structural and parametric analysis of a complex multifactorial control object, which is ‘cooler’, allowed to develop an algorithm for computing the values of temperature, humidity and air velocity which is used for solving the problem of automatic control and process control of cooling the bakery of various assortments. Applying the appropriate set of parameters for the particular kind of product system allows conserving some energy which in the opposite case is just wasted.

To assess the degree of disturbances and control actions influence on the determination of the object control range, selecting the control law and control settings as further work the structural-parametric object diagram will be considered, with the assessment of the functional dependency of units and carried out work on the definition of static and dynamic characteristics of the object.

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Effect of imported Duroc boars on meat quality of finishing pigs in Estonia

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Abstract. The objective of this study was to evaluate the carcass and meat quality characteristics of Duroc-sired progeny marketed in May and June 2014. Carcasses of the following genotypes were evaluated: purebred Landrace (LxL), crossbred Large White x Landrace (LWxL) and LWxL crosses with Duroc (DxLW/L) and Duroc x Landrace (D/LxLW/L) terminal boars. Carcass characteristics recorded: hot carcass weight, carcass length, backfat thickness and loin eye area (LEA). The following physicochemical parameters determined in the Longissimus thoracis muscle were pH value, colour, electroconductivity, water-holding capacity, drip loss, cooking loss, and dry matter, protein, fat and ash content. Duroc-sired pigs were slaughtered at the older age, but at about the same live weight as those of other genotypes. The study revealed that genotypes incorporating Duroc breed had significantly shorter carcasses (D/LxLW/L – 95.38 ± 0.98 cm and DxLW/L – 96.88 ± 0.95 cm; P < 0.01), but a larger LEA (D/LxLW/L – 51.75 ± 1.44 cm² and DxLW/L – 52.24 ± 1.39 cm²; P < 0.05) compared to white-coloured genotypes (carcass length: LxL – 101.12 ± 0.95 cm and LWxL – 101.82 ± 0.98 cm; LEA: LxL – 46.35 ± 1.39 cm² and LWxL – 47.04 ± 1.44 cm²). Duroc sire had a significant effect on the muscle protein and intramuscular fat (IMF) content. DxLW/L genotype had the greatest IMF level (2.71 ± 0.21%; P < 0.05), while it was the lowest in the LxL and LWxL (1.23 ± 0.21% and 1.71 ± 0.22%, respectively). Genotype combinations had no effect on carcass fat deposition. The differences that exist between the breeds of pigs make it possible to modify breed-specific traits such as growth performance, leanness and meat quality. It can therefore be concluded that Duroc boars provide Estonian pig breeders with a valuable source of genetic material for improving the carcass and meat quality of finisher pigs.

Key words: Duroc, Landrace, Large White, crossbreeding, carcass quality, meat quality, leanness, backfat.

INTRODUCTION

Growing consumer demand for healthier and enhanced meat products forces breeders to develop new practices to improve meat quality of pigs. It encourages the pig breeders to use different breeds to utilize them in commercial pig production. Production of crossbred finisher pigs is extensively used to improve farm efficiency (Bennet et al., 1983). Meat quality of pigs has become increasingly essential for the pig industry. Many factors affect meat quality of pigs, including nutrition, slaughter management, breed etc. According to Josell et al. (2003), most of the meat quality parameters are affected by breed. It is thus essential to consider that carcass and meat quality traits depend on the genotype when selecting animals for crossbreeding scheme (Jiang et al., 2012).

The number of different breeds of pigs imported to Estonia has increased over the past two decades. Beside foreign white-coloured Landrace (L) and Large White (LW) breeds, several coloured pig genotypes were introduced, which were used to improve meat quality of local finishers. The first Hampshire boars were imported from Sweden in 1999, whereas four years later they were replaced with the Pietrain breed imported from Austria. As the meat flavour and colour characteristics of the crossbred progeny genotypes above did not satisfy local consumers, the Estonian Pig Breeding Association decided to introduce another new breed from Canada. Hence the Duroc (D) boars were introduced to Estonia twenty years after the first sire breed was imported from Sweden. Several studies have shown that meat from the pigs of Duroc-sired genotypes has a higher intramuscular fat (IMF) level (McGloughlin et al., 1988; Edwards et al., 1992; Oliver et al., 1994), which affects the sensory quality of meat. Kriauzienė & Rekštys (2003) and Klimas et al. (2007) demonstrated that the crossbreeding scheme, in which a terminal Duroc boar was utilized, had superiority over other genotypes.

To choose the best crossbreeding strategy for pork production, it is important to understand, that pig carcass and meat quality characteristics depend on the breeds used. Therefore, a study was conducted to evaluate the carcass and meat quality characteristics of Duroc-sired progeny utilized in commercial pig production.

MATERIAL AND METHODS

Animals and sample collection

A total of 40 marketed pigs (20 gilts and 20 barrows) of four different genotypes (ten animals in each group) were evaluated from May to June 2014. The control scheme included purebred Estonian Landrace breed and its cross with Estonian Large White terminal boar. White coloured combinations were opposed to DxL and purebred Duroc-sired progeny, the maternal side of which contained LWxL genotypes. White coloured pigs were born and reared in a top nucleus, and both genotypes Duroc-sire in it in a well-managed commercial herd. The pigs were penned in groups and had ad libitum access to oat-corn-soybean meal based diet (Table 1).
Table 1. Chemical composition of pig diets

<table>
<thead>
<tr>
<th>Component</th>
<th>Growers 25–60 kg</th>
<th>Finishers &gt;60 kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter, %</td>
<td>87.6</td>
<td>87.9</td>
</tr>
<tr>
<td>Metabolisable energy, MJ kg⁻¹</td>
<td>12.6</td>
<td>12.4</td>
</tr>
<tr>
<td>Crude protein, %</td>
<td>16.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Crude fat, %</td>
<td>3.4</td>
<td>3.0</td>
</tr>
<tr>
<td>Crude fibre, %</td>
<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td>Crude ash, %</td>
<td>5.1</td>
<td>4.8</td>
</tr>
<tr>
<td>Lysine, g kg⁻¹</td>
<td>9.8</td>
<td>9.8</td>
</tr>
<tr>
<td>Methionine, g kg⁻¹</td>
<td>2.8</td>
<td>2.6</td>
</tr>
<tr>
<td>Ca, g kg⁻¹</td>
<td>7.0</td>
<td>6.4</td>
</tr>
<tr>
<td>P, g kg⁻¹</td>
<td>6.3</td>
<td>6.7</td>
</tr>
<tr>
<td>Na, g kg⁻¹</td>
<td>1.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Ten pigs of each genotype, aged 160–182 days, and of 98–133 kg live weight, were randomly selected. Live weight was recorded immediately prior to slaughter. Animals reared under similar conditions were slaughtered in local abattoirs. The carcasses were trimmed and bisected lengthwise along the vertebral column. Hot carcass halves were weighed after final trimming with the accuracy of 0.1 kg. Carcass measurements were taken 45 minutes after slaughter on the right side of the carcasses using a tape measure (Fig. 1) and the Intrascope device (Alt, 2006). Intrascope records were used to calculate the lean meat content of the carcasses according to the approved methodology (Alt, 2006).

Figure 1. Location of measurement sites on a carcass. Backfat thickness: BF1 – thickest spot in the shoulder; BF2 – above the 6th and 7th rib; BF3 – thinnest spot in dorsum; BF4 – highest spot above Gluteus medius muscle. Carcass length: CL – from the cranial edge of the first neck segment to the anterior edge of Symphysis pubis.

Carcasses were bisected between the 13th and 14th ribs perpendicular to the Longissimus thoracis muscle to take digital photos of the surface of the loin eye and the layer of fat on the above (Fig. 2) with Scan-STAR CPU device (Ingenieurbüro R. Matthäus, 2011a). Images were processed using Scan-STAR K software for PC to measure the loin eye area (LEA) and fat layer area (FLA) (Ingenieurbüro R. Matthäus, 2011a). These two areas were used to calculate the leanness index (1). Additionally, fat thickness was recorded at two separate spots (Fig. 2).
Figure 2. Parameters estimated with the Scan-STAR K imaging system. LEA – loin eye area, FLA – fat layer area, FT1 – fat thickness at the thinnest spot, FT2 – fat thickness above Serratus dorsalis muscle.

\[
\text{Leanness index} = \frac{\text{Fat layer area (cm}^2\text{)}}{\text{Loin eye area (cm}^2\text{)}},
\]

Samples (200 g) were taken from the Longissimus thoracis muscle on the right side of the hot carcasses to estimate meat quality parameters. All samples were harvested at the same location on the loins and placed into plastic bags for transport to the laboratory. The physicochemical characteristics of meat were estimated 24 hours after slaughter by the meat laboratory at the Estonian University of Life Sciences.

**pH measurements**

The initial and ultimate pH values of Longissimus thoracis muscle were measured 45 minutes and 24 hours after slaughter, respectively, by using a Testo 205 pH electrode (Testo AG, 2006). The electrode was calibrated with a standard buffer solution at 25 °C for the measurements. The PSE meat usually has an initial pH value less than 5.8 while DFD meat has an ultimate pH value above 6.0 (Warriss, 2000). Table 2 shows pH levels used in current study to determine stress-induced muscle damage.

<table>
<thead>
<tr>
<th>Category</th>
<th>$\text{pH}_{45\text{min}}$</th>
<th>$\text{pH}_{24\text{hr}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSE</td>
<td>&lt;5.8</td>
<td>&lt;5.3</td>
</tr>
<tr>
<td>Normal</td>
<td>5.8–6.4</td>
<td>5.3–6.0</td>
</tr>
<tr>
<td>DFD</td>
<td>&gt;6.4</td>
<td>&gt;6.0</td>
</tr>
</tbody>
</table>

**Colour**

The colour of muscle tissue samples was measured using an Opto-STAR optometer, which measures light intensity reflected from the muscle surface (Ingenieurbüro R. Matthäus, 2011b). Measurements were standardised by yellow and blue calibration blocks at room temperature.
**Electroconductivity**
An LF-STAR CPU conductivity probe (Ingenieurbüro R. Matthäus. 2011c) was used to measure the electrical conductivity of muscle tissue. Two parallel steel electrodes were inserted into the muscle tissue and the electrical current between the electrodes was recorded. The results reflect the degree of muscle tissue damage, which is directly related to the water-holding capacity of muscle.

**Water-holding capacity (WHC)**
The WHC of muscle was determined using the Grau and Hamm (1952; 1957) method, with minor modifications (Volovinskaja and Kel’man, 1961). Samples (0.3 g) were placed on an ash-free filter paper (No. 43, MN 640m) with the diameter of 150 mm, and exposed to 1 kg pressure between two glass plates. The surface area of flat squashed meat and wet stain areas were photographed with a Scan-STAR CPU device and measured with Scan-STAR K software (Ingenieurbüro R. Matthäus. 2011a). WHC was calculated according to the formula 2:

\[ B = \left( \frac{A - 8.4 \cdot V}{A} \right) \cdot 100 \tag{2} \]

where: B – proportion of the water emerged from the sample, %; A – total content of the water in the sample, mg; 8.4 – constant (1 cm\(^2\) of filter paper area contains 8.4 mg water); V – area of the water emerged from the sample in the filter paper, cm\(^2\).

WHC (%) characterizes the ability of muscle to retain naturally occurring moisture even though external pressures are applied to it.

**Drip and cooking loss**
Drip loss was measured according to the method described by Honikel (1998). Meat samples were placed on a non-absorbent mesh and dangled in a plastic bag. After storage of 24 hours at 4°C, the sample was weighed and drip loss calculated as percentage.

Cooking loss results from the loss of liquid and soluble substances from meat during thermal treatment. Muscle samples (100 g) were sealed into a plastic bag supplied with a thermometer. The bag was placed into hot water (95°C) and heated until the internal temperature of the sample increased up to 72°C. The sample was cooled down and weighed, and cooking loss was calculated as a percentage of the precooking weight.

**Biochemical composition**
The dry matter content of muscle was determined according to the Estonian standard EVS-ISO 1442:1999 (EVS, 1997). The protein content was measured according to ISO 937:1978 (EVS, 1978) by using a Kjeltec device. The fat level of muscle was analysed with the Soxtec apparatus according to EVS-ISO 1444:1996 method (EVS, 1996). Ash content of the samples was determined by incineration in electric muffle furnace according to EVS-ISO 936:1998(E) methodology (EVS, 1998).
**Statistical analysis**

General Linear Model procedure (3) of the SAS statistical package (SAS, 1999) was used to estimate the effect of the genotype on carcass and meat quality variables. All results are presented as least squares means ± SEM.

\[ Y_{ijk} = \mu + T_i + S_j + \epsilon_{ijk} \]

where: \( Y_{ijk} \) – dependent variable; \( \mu \) – model intercept; \( T_i \) – fixed effect of the pig genotype (LxL, LWxL, D/LxLW/L and DxLW/L; \( i = 1–4 \)); \( S_j \) – fixed effect of the gender (gilt and barrow; \( j = 1, 2 \)); \( \epsilon_{ijk} \) – random residual effect.

Pearson correlation coefficients were calculated to assess the relationship between carcass and meat quality variables (not all results presented). Differences were considered statistically significant at the level of \( P < 0.05 \).

Data visualization was aided by Daniel’s XL Toolbox Add-In for MS Excel, version 6.53, by Daniel Kraus, Würzburg, Germany.

**RESULTS AND DISCUSSION**

**Field tests**

Fast-growing animals make pig farming more profitable. The study showed that purebred Landrace and its cross with Large White breed achieved slaughter age 5.4–8.4 days earlier than both genotypes of the Duroc-sired finishers (D and DxL) (Table 3). The pigs crossed with the purebred Duroc sire reached slaughter weight significantly later (175.80 days, \( P < 0.05 \)) compared to purebred Landrace and LWxL crossbred pigs (167.40 and 167.70 days, respectively). Tänavots et al. (2011a) demonstrated in their earlier study that white coloured pigs and their Duroc-sired crosses reached the desired slaughter weight at the same age. Yet, they found that the pigs reached slaughter weight later (at 182.92–191.76 days of age) than in the current study. While Tänavots et al. (2011a) observed a significantly higher live weight at the same slaughter age in Duroc crosses compared to white-coloured genotypes, a prolonged fattening period did not resulted in the increase of the live slaughter weight of the finishers in the current study. On the contrary, both Duroc-sired genotypes were 1.16–3.82 kg lighter than those of the white-coloured pigs, but this difference was not statistically significant.

There were only small differences in carcass weight between genotype combinations, whereas the heaviest carcasses were found in white-coloured crossbred pigs and their cross with a purebred Duroc sire (80.45 kg, both), which also corresponds to the higher slaughter yield (69.22% and 70.55%, respectively). Čandek-Potokar et al. (2002) found also only small differences between genotype groups, although carcass weight was significantly higher in Duroc-sired crossbred animals.

Better growth performance was observed in white-coloured genotype combinations with daily gains over 690 g, while both crossbred genotypes with Duroc sire showed more modest results (651.33–652.24 g). Klimienè & Klimas (2013) demonstrated that growth performance may influence the lean meat content and backfat thickness of pigs with different genotypes, but not the size of the loin area. As the key to successful pig production is efficient feed conversion, the carcass daily gain can be considered an important quality parameter. Purebred Landrace pigs and crossbred animals with Large White and DxL terminal sires
showed slightly higher carcass daily gain (472.05, 474.47 and 475.23 g, respectively) than crossbreds with purebred Duroc sire (443.25 g). These findings contrast with those of Hurnik (2004) and Tänavots et al. (2011), both revealing better performance in Duroc-sired finishers. Nevertheless, the fattenig performance of the local pigs has improved over recent years.

Carcass length expressed significantly \( (P < 0.01) \) in white-coloured pigs, being 4.24–6.44 cm longer than in both of the Duroc-sired genotypes. While four years ago the average carcass length of white-coloured genotypes was 94.98 cm (Tänavots et al., 2011), the current study showed that the length of carcasses of these genotypes has increased, exceeding 100 cm (Table 3). On the contrary, Berg et al. (2003) did not find statistically reliable difference in carcass length between purebred Landrace and Duroc pigs.

### Table 3. Least square means of fattenig performance and carcass quality traits of finishers (n = 40, 10 of each genotype)

<table>
<thead>
<tr>
<th>Traits</th>
<th>LxL</th>
<th>Genotype ((\delta \times \delta))</th>
<th>LWxL</th>
<th>D/LxLW/L</th>
<th>DxLW/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at slaughter, d</td>
<td>167.40(^a)</td>
<td>2.05</td>
<td>167.70(^a)</td>
<td>2.11</td>
<td>173.10(^ab)</td>
</tr>
<tr>
<td>Live weight, kg</td>
<td>115.48(^a)</td>
<td>3.05</td>
<td>116.31(^a)</td>
<td>3.15</td>
<td>112.49(^a)</td>
</tr>
<tr>
<td>Hot carcass weight, kg</td>
<td>78.96(^a)</td>
<td>2.11</td>
<td>80.45(^a)</td>
<td>2.18</td>
<td>77.06(^a)</td>
</tr>
<tr>
<td>Slaughter yield, %</td>
<td>68.34(^a)</td>
<td>1.09</td>
<td>69.22(^a)</td>
<td>1.12</td>
<td>68.58(^a)</td>
</tr>
<tr>
<td>Daily gain, g</td>
<td>691.87(^a)</td>
<td>16.16</td>
<td>697.26(^a)</td>
<td>16.67</td>
<td>652.24(^a)</td>
</tr>
<tr>
<td>Carcass daily gain, g</td>
<td>472.05(^a)</td>
<td>16.96</td>
<td>474.47(^a)</td>
<td>17.49</td>
<td>475.23(^a)</td>
</tr>
<tr>
<td>Carcass length, cm</td>
<td>101.12(^a)</td>
<td>0.95</td>
<td>101.82(^a)</td>
<td>0.98</td>
<td>95.38(^b)</td>
</tr>
<tr>
<td>Lean meat content, %</td>
<td>58.45(^a)</td>
<td>0.63</td>
<td>58.96(^a)</td>
<td>0.65</td>
<td>58.73(^b)</td>
</tr>
<tr>
<td>Loin eye area, cm(^2)</td>
<td>46.35(^a)</td>
<td>1.39</td>
<td>47.04(^a)</td>
<td>1.44</td>
<td>51.75(^b)</td>
</tr>
<tr>
<td>Fat layer area, cm(^2)</td>
<td>19.41(^a)</td>
<td>1.48</td>
<td>17.31(^a)</td>
<td>1.52</td>
<td>17.43(^a)</td>
</tr>
<tr>
<td>Leanness index</td>
<td>0.42(^a)</td>
<td>0.03</td>
<td>0.37(^ab)</td>
<td>0.03</td>
<td>0.33(^b)</td>
</tr>
</tbody>
</table>

\(^a\) – least square mean values in the same row with different superscript letters differ significantly \((P < 0.05)\); D – Duroc, L – Landrace, LW – Large White.

Even though the lean meat content (58.45–58.96%) did not differ significantly between genotype groups, the Duroc-sired crossbred pigs had significantly larger average LEA. Crossbred pigs sired by purebred Duroc boars had the largest loin eye (52.24 cm\(^2\)), followed by pigs sired by crossbred Duroc and Landrace terminal boars (51.75 cm\(^2\)). The LEA of purebred Landrace and crossbred LWxL pigs was 46.35 and 47.04 cm\(^2\), respectively (Table 3). The Duroc breed is generally used in crossbreeding programmes to improve meatiness traits, while the white-coloured genotypes exhibit stronger maternal effects. Hurnik (2004) concluded that loin eye size depends on the genetics of pig, as it was found that Duroc-sired genotypes had a larger loin eye than Landrace-sired animals. His study demonstrated a linear relationship between carcass weight and loin eye size. Similarly, in the current study a moderate correlation \((r = 0.468; \ P < 0.01)\) was found between LEA and carcass weight of the pigs. A moderate correlation \((r = 0.406; \ P < 0.01)\) was also found between the FLA and carcass weight, but there was no relationship \((r = 0.074; \ P > 0.05)\) between the LEA and that of the FLA. The FLA above loin eye was slightly but not significantly smaller (0.58–2.68 cm\(^2\)) in the pigs sired with purebred Duroc boars, being the largest (19.41 cm\(^2\)) in purebred Landrace animals. These two traits showed that the FLA to LEA ratio was significantly \((P < 0.05)\)
higher in Duroc-sired genotypes compared to purebred Landraces (Table 3). Similar results were obtained also by Tänavots et al. (2011a), although they detected a larger average LEA and a smaller FLA in all genotype combinations compared to current study, whereas the lower leanness index demonstrates the relatively faster increase in the backfat thickness over recent years.

Tänavots et al. (2011a) measured a slightly thicker backfat in the pigs sired with purebred Duroc terminal boars. Also, the Estonian farmers had prejudice that Duroc-sired finishers have thicker backfat. On the contrary, Berg et al. (2003) reported that purebred Duroc pigs had significantly ($P < 0.05$) thinner backfat measured at the 10th rib (20.3 mm) than that of Landrace animals (23.7 mm). This study, however, did not confirm these results as none of the fat thicknesses measured at different locations did not differ significantly between genotype combinations (Fig. 3). Except, fat thickness at the thinnest spot above the Longissimus thoracis muscle (recorded by Scan-STAR) was significantly thinner in the pigs sired with purebred Duroc terminal boars (10.09 mm; $P < 0.05$) compared with purebred Landrace (14.51 mm) and crossbred LWxL (13.51 mm) pigs. Similar results were also observed in DxL sired animals (10.29 mm), but a significant difference was found only with purebred Landrace pigs. Tänavots et al. (2011a) earlier study show a slight increase in backfat thicknesses in all genotype combinations.

**Figure 3.** Least square means (± SEM) of backfat thickness measured on the carcass of finishers at different locations ($abc$ – least square mean values in the same row with different superscript letters differ significantly ($P < 0.05$); D – Duroc, L – Landrace, LW – Large White).

Carcass backfat layer measured by tape measure was distributed rather unevenly across the body, being the thickest on the shoulder and the thinnest on the loin. Selection for leanness by using certain measuring locations may lead to excessive fat in other parts of the body (D’Souza et al., 2004; Suster et al., 2005), which indicates that deposition of
fat in the carcasses may vary. However, fat deposition in local genotypes was similarly distributed in all genotype combinations.

**Laboratory analysis**

The muscles from Duroc-sired genotypes showed lower initial pH values than that from the white-coloured genotypes, whereas the pH of the muscles from DxL sired pigs differed significantly \((P < 0.05)\) (Table 4). One Duroc and two DxL sired pigs showed signs of PSE meat as the initial pH of muscle was below 5.8. In fact, the ultimate pH reached its normal level \((\geq 5.3)\) after 24 hours. The level of ultimate pH remained lower in the muscles from both Duroc-sired genotypes, but differed significantly \((P < 0.05)\) only from that of the crossbred white coloured animals. Eggert et al. (1998) and Brewer et al. (2002) concluded that the difference between the lean meat ultimate pH values of the finishers of different sire genotypes is insignificant. Some authors, however, have detected considerably higher initial (Jeleníková et al., 2008) and ultimate (Gjerlaug-Enger et al., 2010; Li et al., 2013) pH values in the muscle from purebred Duroc pigs compared to that of white-coloured animals. The ultimate pH affects traits such as colour and the ability of muscle to retain water. The current study showed that muscles with a lower ultimate pH was paler \((r = 0.429; P < 0.01)\) and with slightly lower WHC \((r = 0.155; P > 0.05)\).

Table 4. Least square means of the quality traits of *Longissimus thoracis* muscle of finishers \((n = 40, 10 of each genotype)\)

<table>
<thead>
<tr>
<th>Traits</th>
<th>Genotype (♂ × ♀)</th>
<th>LxL</th>
<th>LWxL</th>
<th>D/LxLW/L</th>
<th>DxLW/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH45min</td>
<td>(\bar{x})</td>
<td>SEM</td>
<td>(\bar{x})</td>
<td>SEM</td>
<td>(\bar{x})</td>
</tr>
<tr>
<td>LxL</td>
<td>6.05</td>
<td>0.06</td>
<td>6.14</td>
<td>0.07</td>
<td>5.84</td>
</tr>
<tr>
<td>LWxL</td>
<td>5.50</td>
<td>0.03</td>
<td>5.62</td>
<td>0.03</td>
<td>5.44</td>
</tr>
<tr>
<td>D/LxLW/L</td>
<td>73.58</td>
<td>1.51</td>
<td>75.25</td>
<td>1.46</td>
<td>75.25</td>
</tr>
<tr>
<td>DxLW/L</td>
<td>73.16</td>
<td>1.17</td>
<td>73.94</td>
<td>1.07</td>
<td>73.01</td>
</tr>
<tr>
<td>pH24hr</td>
<td>(\bar{x})</td>
<td>SEM</td>
<td>(\bar{x})</td>
<td>SEM</td>
<td>(\bar{x})</td>
</tr>
<tr>
<td>LxL</td>
<td>74.94</td>
<td>1.17</td>
<td>76.91</td>
<td>1.20</td>
<td>72.89</td>
</tr>
<tr>
<td>LWxL</td>
<td>5.94</td>
<td>0.30</td>
<td>5.61</td>
<td>0.41</td>
<td>4.77</td>
</tr>
<tr>
<td>D/LxLW/L</td>
<td>72.89</td>
<td>1.20</td>
<td>72.89</td>
<td>1.20</td>
<td>72.89</td>
</tr>
<tr>
<td>DxLW/L</td>
<td>73.01</td>
<td>1.17</td>
<td>73.01</td>
<td>1.17</td>
<td>73.01</td>
</tr>
<tr>
<td>Colour45min</td>
<td>(\bar{x})</td>
<td>SEM</td>
<td>(\bar{x})</td>
<td>SEM</td>
<td>(\bar{x})</td>
</tr>
<tr>
<td>LxL</td>
<td>83.95</td>
<td>1.46</td>
<td>82.72</td>
<td>1.51</td>
<td>73.58</td>
</tr>
<tr>
<td>LWxL</td>
<td>74.94</td>
<td>1.17</td>
<td>76.91</td>
<td>1.20</td>
<td>72.89</td>
</tr>
<tr>
<td>D/LxLW/L</td>
<td>5.94</td>
<td>0.30</td>
<td>5.61</td>
<td>0.41</td>
<td>4.77</td>
</tr>
<tr>
<td>DxLW/L</td>
<td>72.89</td>
<td>1.20</td>
<td>72.89</td>
<td>1.20</td>
<td>72.89</td>
</tr>
<tr>
<td>Colour24hr</td>
<td>(\bar{x})</td>
<td>SEM</td>
<td>(\bar{x})</td>
<td>SEM</td>
<td>(\bar{x})</td>
</tr>
<tr>
<td>LxL</td>
<td>61.70</td>
<td>0.70</td>
<td>61.69</td>
<td>0.72</td>
<td>60.57</td>
</tr>
<tr>
<td>LWxL</td>
<td>4.00</td>
<td>0.50</td>
<td>3.52</td>
<td>0.51</td>
<td>3.28</td>
</tr>
<tr>
<td>D/LxLW/L</td>
<td>54.00</td>
<td>0.64</td>
<td>43.99</td>
<td>0.66</td>
<td>43.32</td>
</tr>
<tr>
<td>DxLW/L</td>
<td>3.72</td>
<td>0.40</td>
<td>3.61</td>
<td>0.41</td>
<td>4.77</td>
</tr>
<tr>
<td>Electroconductivity45min, mS</td>
<td>(\bar{x})</td>
<td>SEM</td>
<td>(\bar{x})</td>
<td>SEM</td>
<td>(\bar{x})</td>
</tr>
<tr>
<td>LxL</td>
<td>7.99</td>
<td>1.03</td>
<td>7.25</td>
<td>1.06</td>
<td>8.11</td>
</tr>
<tr>
<td>LWxL</td>
<td>4.00</td>
<td>0.50</td>
<td>3.52</td>
<td>0.51</td>
<td>3.28</td>
</tr>
<tr>
<td>D/LxLW/L</td>
<td>54.00</td>
<td>0.64</td>
<td>43.99</td>
<td>0.66</td>
<td>43.32</td>
</tr>
<tr>
<td>DxLW/L</td>
<td>3.72</td>
<td>0.40</td>
<td>3.61</td>
<td>0.41</td>
<td>4.77</td>
</tr>
<tr>
<td>Drip loss, %</td>
<td>(\bar{x})</td>
<td>SEM</td>
<td>(\bar{x})</td>
<td>SEM</td>
<td>(\bar{x})</td>
</tr>
<tr>
<td>LxL</td>
<td>4.00</td>
<td>0.50</td>
<td>3.52</td>
<td>0.51</td>
<td>3.28</td>
</tr>
<tr>
<td>LWxL</td>
<td>54.00</td>
<td>0.64</td>
<td>43.99</td>
<td>0.66</td>
<td>43.32</td>
</tr>
<tr>
<td>D/LxLW/L</td>
<td>3.72</td>
<td>0.40</td>
<td>3.61</td>
<td>0.41</td>
<td>4.77</td>
</tr>
<tr>
<td>DxLW/L</td>
<td>4.00</td>
<td>0.50</td>
<td>3.52</td>
<td>0.51</td>
<td>3.28</td>
</tr>
<tr>
<td>Cooking loss, %</td>
<td>(\bar{x})</td>
<td>SEM</td>
<td>(\bar{x})</td>
<td>SEM</td>
<td>(\bar{x})</td>
</tr>
<tr>
<td>LxL</td>
<td>26.04</td>
<td>0.18</td>
<td>26.15</td>
<td>0.19</td>
<td>26.53</td>
</tr>
<tr>
<td>LWxL</td>
<td>23.60</td>
<td>0.16</td>
<td>23.23</td>
<td>0.16</td>
<td>23.11</td>
</tr>
<tr>
<td>D/LxLW/L</td>
<td>1.23</td>
<td>0.21</td>
<td>1.71</td>
<td>0.22</td>
<td>2.19</td>
</tr>
<tr>
<td>DxLW/L</td>
<td>1.21</td>
<td>0.02</td>
<td>1.20</td>
<td>0.02</td>
<td>1.22</td>
</tr>
</tbody>
</table>

\(\bar{x}\), \(\bar{x}\), \(\bar{x}\) – least square mean values in the same row with different superscript letters differ significantly \((P < 0.05)\); D – Duroc, L – Landrace, LW – Large White.

Muscle tissue contains 26% dry matter and 25% protein (Warriss, 2000). Insignificantly higher dry matter level (0.35–0.49%) was found in both genotypes of Duroc-sired genotypes compared with white-coloured finisher groups (Table 4). In an earlier study, Tännavots et al. (2011\(^b\)) observed that the *Longissimus thoracis* muscle of the Duroc-sired finishers contained 1.23% \((P < 0.05)\) more dry matter than that of white-coloured breed combinations.
While the protein content of muscle was strongly, but negatively related with the IMF content ($r = -0.736$; $P < 0.001$), the lean meat from the pigs of both Duroc-sired finisher groups that had a low protein level (DLxLW/L - 23.11%; DxLW/L - 22.58%), showed a remarkably high IMF level (D/LxLW/L - 2.19%; DxLW/L - 2.71%) in the Longissimus thoracis muscle. On the contrary, the highest protein level (23.60%) and the lowest IMF level (1.23%) were found in the muscle of purebred Landrace pigs, whereas the IMF content was 0.36% lower compared to an earlier study by Somelar et al. (2001). A similar tendency was observed also in Poland (Daszkiewicz et al., 2005), where 84% of the crossbred pigs under investigation had an IMF content of less than 2%. The difference may be caused by intensive selection for leanness and decrease in the backfat thickness in pigs. IMF level can vary considerably, from 1.1–7.0% (Fischer, 1994). DeVoll et al. (1988) and Fischer et al. (2000) concluded that increased IMF content improves the eating quality of meat, whereas the optimal fat level of muscle is 2.5–3.5% (Bejerholm & Barton-Gade, 1986; Fernandez et al., 1999; Font-i-Furnols et al., 2012). However, according to Rincker et al. (2008), the IMF content only slightly affects the flavour, juiciness and tenderness of meat, or does not affect these qualities at all. Wood et al. (2004) suggested that the easiest way to optimise the IMF level is to use special breeds or crosses, such as Duroc, whose backfat is relatively thin. Berg et al. (2003) concluded, having studied various genotypes, that meat from Duroc pigs had higher WHC, IMF content and ultimate pH, and showed lower lightness value of the Longissimus thoracis muscle compared to the meat from Landrace and Yorkshire pigs.

CONCLUSIONS

Producers have the possibility to use the boars with a higher lean meat content in breeding programme if they intend to improve that aspect in the finishers. The results of this study demonstrated that the genotype combination can affect carcass and meat quality traits. Carcass traits such as carcass length, LEA and leanness index were significantly affected by the Duroc sire line. Even in case of shorter carcasses, the weight of the carcass and slaughter yield was comparable with those of white-coloured genotypes. This is why we can presume that along with the significantly larger Longissimus thoracis muscle, other muscles of Duroc-sired pigs are also larger. Furthermore, Duroc sire had a consistent effect on meat quality traits such as protein and IMF content. Higher IMF content may positively affect the quality (taste and eatability) of pork that attracts consumers. Genotype combination had no effect on carcass fat deposition in different locations, which should refute breeders’ fears about the negative effect of Duroc sires. Methodical investigation of the relationships between carcass and meat quality traits could help breed pigs for improved meat quality.
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Calculation model for the assessment of animal by-product resources in Estonian meat industry

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Abstract. Aim of current study was the elaboration of a calculation model for monitoring system which makes it possible to assess the animal by-product (ABP) resources in cooperation with companies and state authorities. Data about quantities of processed animals by species were collected from existing public databases, Estonian Animal Waste Processing Plant and meat processing enterprises of Estonia. Data from scientific literature and available statistics as well as data about the quantities of meat and slaughtering products observed in slaughterhouses was used for estimation the average of ABP quantities per animal by species. Based on these two datagroups – number of animals (by species) and yield of ABP per animal during meat processing, functionality of the general calculation model for monitoring was tested. Inputs for this model are numbers of animals by species and outputs accordingly the quantities of ABP by risk-categories and types. During evaluation of the calculation model it was estimated that nearly 22 thousand tons of ABP are generated in the recognized slaughterhouses of Estonia annually. 1,900 tons of it consists of 1st category ABP, 3,400 tons 2nd category and over 17,000 tons 3rd category ABP. On the other hand quantities of ABP delivered from meat industry to the reprocessing as a 1st category was 4,900 tons which exceeded the estimated (by calculation model) amounts about 3.5 times. Thus a great deal of other ABP categories had also been sent for reprocessing as the most dangerous waste. This fact indicates to the insufficient use of 2nd and 3rd category ABP as raw material in Estonian meat industry. Existence of an efficient monitoring system will promote the management of ABP recourses in slaughterhouses and meat processing companies. For this the relevant databases, procedures and methods should be worked out.

Key words: animal by-products, meat processing, monitoring system.
INTRODUCTION

The deficit of protein, including protein of animal origin, is particularly high. Animal proteins suitable for human consumption comprise less than one-third of the total food protein ‘fund’ available globally (FAOSTAT 2013). On the other hand, there is also an increasing need in protein sources for animal feed production (Boland et al., 2013). At the same time, the amount of protein lost during meat processing is unreasonably large – up to 30% protein suitable for human consumption (Sannik et al. 2013). This is caused by the methods commonly used for industrial meat processing, especially in treatment of the animal by-products (ABP). Latter are usually utilized for fat production, for technical purposes or destroyed for safety reasons (Pearl, 2004).

According to EU regulations (Regulation EC. 2001, 2009) ABP has been categorized into three main categories according to the possible health risk. 1st and 2nd categories of ABP are classified as high risk materials and 3rd category as low risk materials. So the use of the 1st category ABP in food- or feed-chain have to be eliminated totally, due to the TSE risk. A limited use of the 2nd category ABP for the composting or technical purposes is allowed. Use of the 3rd category ABP is allowed for many purposes: for composting, for technical purposes, for feeding and in limited forms for food. At the same time some authors (Ockerman & Hansen, 2000; Arvanitoyannis & Ladas, 2008) suppose that getting all of requirements together at one time in one place is not always an easy task. Detailed principle of ABP categorization etc. is given in EU regulations.

Relevant institutions have settled strict rules for handling of different ABP categories, how these can be collected, stored and transported, and how these can be treated, used and disposed in EU member countries (Juste, 2010). Especially by-products that are unsuitable for human consumption may pose at wrong handling serious risk to the health of people and animals. This has been confirmed by several earlier crises: the outbreaks of foot-and-mouth disease, the spread of the transmissible spongiform encephalopathy and the dioxin compounds in fodder (Segarra & Rawson, 2001). These crises were caused by the incorrect use or handling of certain ABP, which resulted in re-entering those into food chain. ABP may also harm the environment and biodiversity due to handling problems.

Many papers describe a variety of assurance initiatives, and explore how targeted research and development can be used to provide the successful managing of food safety and quality risks in meat production (Ockerman & Basu, 2004; Olgo, 2011; Lehto, et al., 2012). At the same time, a fully integrated assurance system, with effective control applied at all stages in the production chain is not at present achievable in all but a few operations (Toldra et al., 2012; Sannik et al., 2013). Constant monitoring of ABP will definitely help in solving the problems of food safety assurance.

The utilization of ABP is becoming an increasingly critical issue because of rising consumption of meat and growing quantities of ABP generated by slaughterhouses as well as the increasingly strict requirements and prohibitions established for the handling and use of ABP. Use of ABP as a raw material for biogas production seems to be promising at present, as gas can be utilized to generate energy (heat and electricity or fuel) locally (Marcos et al., 2010). This enables also co-use of plant resources for bioenergy production in a more efficient way (Juste, 2010). However, it should be emphasized that use of pure ABP as raw material for biogas production without specific knowledge is likely to cause a drastic drop in the efficiency of the process and an
unpleasant odor problem (Pitk et al., 2012). On the other hand, there would be an active market and high demand for the 3rd category material produced by slaughterhouses, because many components of this ABP category are as valuable as pure meat, which is why an in-depth analysis of types, quantities and processing possibilities of these by-products should be of special concern in corresponding studies (Sannik et al., 2013a, Sannik et al., 2013b).

The official requirements of the European Union do not follow the principle that ABP must be valued as fully as possible and to the maximum level. Prerequisite for that is the existence of a relevant monitoring system. Currently no official pressure exists on establishment of ABP monitoring in EU countries. Estonia also lacks any kind of system for ABP monitoring (excluding ABP registration of the first category in slaughterhouses) now. Therefore, at present, there is no overview available about the quantities of ABP categories that could be transformed into products via separate handling or into bioelectricity and heat via anaerobic fermentation.

At the same time from these enterprises where ABP is processed the data about the ABP quantities are not available or have the limited access (Sannik, 2010). An efficient monitoring system would make it possible to check and control the recourses of ABP in Estonia (Sannik et al., 2013a). The existence of a core calculation model for estimation of ABP quantities by categories and types will be the first step in monitoring system design.

Object of current study was the elaboration and evaluation the functionality of a calculation model for monitoring system, which makes it possible to assess the ABP resources in Estonian meat industry in cooperation with companies and state authorities.

**METHODS**

Calculations based on number of slaughtered animals (by species) and yield of ABP per animal during meat processing has been proposed. The following data from 2010 to 2012 were used.

1. Data from the existing databases, including the Commercial Register, the Ministry of Agriculture and the Estonian Institute of Economic Research. The Commercial Register provided data concerning the animal species and the number of animals processed in meat processing plants. The Ministry of Agriculture provided data concerning the number of live animals produced and sold to slaughterhouses by animal species during these years. The Estonian Institute of Economic Research provided data concerning the quantities of meat produced in Estonia during the same years by animal species, including data about imported meat and live animals. The Institute also provided data concerning the consumption of meat (including feed, forage, losses) and the export thereof (including live animals).
1. Check-list for the monitoring provided in slaughterhouses

<table>
<thead>
<tr>
<th>Row no.</th>
<th>Characteristics of slaughter-dressing</th>
<th>Data from slaughtering of cattle, pigs, sheep or poultry, kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Average slaughter weight of carcass</td>
<td>DFS</td>
</tr>
<tr>
<td>2</td>
<td>Average live weight</td>
<td>DFS</td>
</tr>
<tr>
<td>3</td>
<td>Meat and edible products</td>
<td>Sum of rows 4–5</td>
</tr>
<tr>
<td>4</td>
<td>Meat and meat-products</td>
<td>DFS</td>
</tr>
<tr>
<td>5</td>
<td>Edible products from slaughtering</td>
<td>Sum of rows 6–11</td>
</tr>
<tr>
<td>6</td>
<td>Small fat, skirt, trimmings (edible)</td>
<td>DFS</td>
</tr>
<tr>
<td>7</td>
<td>Lungs, kidneys (edible)</td>
<td>DFS</td>
</tr>
<tr>
<td>8</td>
<td>Heart, liver (from poultry also crop, neck and partly legs)</td>
<td>DFS</td>
</tr>
<tr>
<td>9</td>
<td>Bones (edible)</td>
<td>DFS</td>
</tr>
<tr>
<td>10</td>
<td>Blood (edible)</td>
<td>DFS</td>
</tr>
<tr>
<td>11</td>
<td>Losses from slaughtering</td>
<td>DFS</td>
</tr>
<tr>
<td>12</td>
<td>3rd category ABP</td>
<td>Sum of rows 13–23</td>
</tr>
<tr>
<td>13</td>
<td>Leaf fat, omentum, fatty trimmings (inedible)</td>
<td>DFS</td>
</tr>
<tr>
<td>14</td>
<td>Lungs, heart, kidneys (inedible)</td>
<td>DFS</td>
</tr>
<tr>
<td>15</td>
<td>Blood (inedible)</td>
<td>DFS</td>
</tr>
<tr>
<td>16</td>
<td>Trachea, throat, esophagus</td>
<td>DFS</td>
</tr>
<tr>
<td>17</td>
<td>Stomachs and intestine (no cattle) cleaned</td>
<td>DFS</td>
</tr>
<tr>
<td>18</td>
<td>Bladder, genitals, spleens (no sheep), pig brains, hide-trims</td>
<td>DFS</td>
</tr>
<tr>
<td>19</td>
<td>Legs, horns/bristle/hoofs, feathers, partly poultry legs</td>
<td>DFS</td>
</tr>
<tr>
<td>20</td>
<td>Hides</td>
<td>DFS</td>
</tr>
<tr>
<td>21</td>
<td>Bones from cutting, chicken heads, partly pork heads</td>
<td>DFS</td>
</tr>
<tr>
<td>22</td>
<td>Vessels, tendons, cartilage, glands</td>
<td>DFS</td>
</tr>
<tr>
<td>23</td>
<td>Other ABP of the 3rd category</td>
<td>DFS</td>
</tr>
<tr>
<td>24</td>
<td>2nd category ABP</td>
<td>Sum of rows 25–26</td>
</tr>
<tr>
<td>25</td>
<td>Intestinal and stomachs content</td>
<td>DFS</td>
</tr>
<tr>
<td>26</td>
<td>Other ABP of the 2nd Category (perished animals etc.)</td>
<td>DFS</td>
</tr>
<tr>
<td>27</td>
<td>1st category ABP</td>
<td>Sum of rows 28–31</td>
</tr>
<tr>
<td>28</td>
<td>Cattle and sheep heads</td>
<td>DFS</td>
</tr>
<tr>
<td>29</td>
<td>Cattle and sheep spinal cord</td>
<td>DFS</td>
</tr>
<tr>
<td>30</td>
<td>Beef intestine (cleaned), intestine fat, sheep ileum</td>
<td>DFS</td>
</tr>
<tr>
<td>31</td>
<td>Sheep spleen</td>
<td>DFS</td>
</tr>
<tr>
<td>32</td>
<td>Sum of ABP</td>
<td>Sum of ABP = Rows 12+24+27</td>
</tr>
</tbody>
</table>

DFS - data from slaughterhouses.

2. A survey by means of pilot monitoring, on-site interviews and observations was conducted (Sannik 2010) in eight meat processing plants that produce approximately 75% of the ABP resources. The monitored plants and companies were the Rakvere Meat Processing Plant Ltd, Saaremaa Lihatööstus Llc, Rey Ltd, Linnamäe Lihatööstus Ltd, Aruküla Lihatööstus Llc, Märjamaa Lihatööstus Llc, Pandivere Lihatööstus Llc and Maag Lihatööstus Ltd. Electronic surveys were additionally conducted in four meat processing plants and companies: Atria Valga Lihatööstus Ltd, Otepää Lihatööstus Edgar Llc, Tallegg Ltd and Lihakarn Llc. The following data were collected from the meat processing plants and companies: a) the number of processed animals by species, b) the live weight and slaughter weight of the processed animals, c) the quantities of meat, meat products, slaughter sub-products and by-products by animal species, and d) the quantities of by-products
and waste by categories. Special form was worked out for conducting the monitoring (Table 1).

3. Data provided by the Estonian Animal Waste Processing Plant in Väike-Maarja (Vireen Ltd), which is the only plant in Estonia authorized to process animal by-products of 1st category (cf. Table 2). Data concerning ABP of the 1st category were collected by means of interviews and observations conducted in the course of repeated visits, and the accounting records of the company were also examined. Estimated ABP quantities were compared with the calculated ABP quantities of various risk categories generated in Estonian meat processing plants. The ABP yield percentages of the live weight were calculated by animal species on the basis of the quantities of meat, meat products, slaughter sub-products and by-products. For processing of collected data, an MS Excel database was created, which further was elaborated into calculation model for determination the quantities of ABP by types and categories. Using that calculation model, ABP quantities by categories and types were estimated and assessed for Estonian meat processing plants.

**RESULTS AND DISCUSSION**

From gathered data the average live and slaughter weight of the processed animals the quantities of meat, meat products, slaughter sub-products and other by-products were estimated, and the percentages of the outputs of the various ABP categories and types regarding live weight were calculated by animal species. These data were used for model calculation of ABP quantities by types and categories during given period and for a certain company (or region). The core of this model calculation for the year 2012 is presented in Table 2.

Based on model calculations, quantities of ABP generated in Estonian recognized slaughterhouses and meat processing plants in 2012 were the following: 21.8 thousand tons of ABP in total, of which 1.4 thousand tons constituted waste of the 1st category, 3.4 thousand tons were by-products of the 2nd category and 17.0 thousand tons (77% of the total volume of ABP) belonged to by-products of the 3rd category.

Monitoring carried out in 2012 revealed that most of animals were slaughtered in few larger production units. For example, 95% of the pigs utilized in Estonian meat processing plants were slaughtered in four slaughterhouses (Rakvere Meat Processing Plant, Atria Eesti Valga Production Unit and Saaremaa Lihatööstus) and 75–80% of the respective quantities of bovine animals were slaughtered in same three slaughterhouses. Also Estonian ABP derives mostly from these bigger representatives of the meat industry. Sheep were an exception – only 5% of the sheep farmed in Estonia were slaughtered in these plants that year. Table 3 presents an example of calculated ABP quantities generated in Estonian meat processing plants by risk categories according to proposed calculation model. An important enterprise considering ABP treatment is Vireen Ltd (Väike-Maarja Animal Waste Processing Plant). It processes (destroys) all animals that have perished on farms, ABP delivered by meat processing plants classified as waste of 1st category and other animal based materials (goods confiscated by customs, animals perished in traffic, zoos and for other reasons) etc.
Table 2. Average yield percentage and quantities (kg) of ABP from various types of animals slaughtered in Estonia in 2012 (per carcass).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cattle</th>
<th>Pigs</th>
<th>Sheep</th>
<th>Poultry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average weight of carcass, kg</td>
<td>275</td>
<td>85</td>
<td>15</td>
<td>1.9</td>
</tr>
<tr>
<td>Average live weight, kg</td>
<td>550</td>
<td>110</td>
<td>30</td>
<td>2.25</td>
</tr>
</tbody>
</table>

| Meat and edible products                     | 42.97  | 239.23 | 80.20 | 88.23 | 36.15 | 10.78 | 78.89 | 1.78 |
| Meat and meat-products                       | 40.75  | 224.10 | 66.83 | 73.51 | 34.80 | 10.44 | 75.64 | 1.70 |

| Edible products from slaughtering            | 2.22   | 15.13 | 13.38 | 14.72 | -0.65 | 0.34  | 3.33  | 0.08 |
| Small fat, skirt, trimmings (edible)         | 2.12   | 11.66 | 1.95  | 2.14  | 0.00  | 0.00  | 0.00  | 0.00 |
| Lungs, kidneys (edible)                      | 0.13   | 0.70  | 2.19  | 2.41  | 0.00  | 0.00  | 0.00  | 0.00 |
| Heart, liver (from poultry also)             | 0.36   | 1.97  | 0.26  | 0.29  | 0.63  | 0.19  | 3.33  | 0.08 |
| Bones (edible)                               | 0.01   | 2.94  | 9.98  | 10.98 | 0.00  | 0.00  | 0.00  | 0.00 |
| Blood (edible)                               | 1.61   | 8.86  | 2.00  | 2.20  | 0.72  | 0.22  | 0.00  | 0.00 |
| Losses from slaughtering                     | -2.00  | -11.00| -3.00 | -3.30 | -2.00 | -0.07 | 0.00  | 0.00 |

| 3rd category ABP                             | 39.70  | 218.35| 16.77 | 18.45 | 43.58 | 13.07 | 19.33 | 0.44 |
| Leaf fat, omentum, fatty trimmings (inedible)| 4.19   | 23.02 | 1.17  | 1.29  | 2.70  | 0.81  | 0.00  | 0.00 |
| Lungs, heart, kidneys (inedible)              | 0.74   | 4.21  | 0.54  | 0.59  | 1.43  | 0.43  | 0.00  | 0.00 |
| Blood (inedible)                              | 1.61   | 8.86  | 1.64  | 1.81  | 3.70  | 1.11  | 0.00  | 0.00 |
| Trachea, throat, esophagus                    | 0.70   | 3.85  | 0.62  | 0.68  | 1.04  | 0.31  | 0.00  | 0.00 |
| Stomachs and intestine (no cattle cleaned)    | 3.00   | 16.50 | 3.77  | 4.15  | 5.37  | 1.61  | 0.67  | 0.02 |
| Bladder, genitals, spleens (no sheep)         | 2.92   | 16.05 | 1.10  | 1.21  | 0.84  | 0.25  | 0.00  | 0.00 |
| Legs, horns/bristle/hoofs, feathers, poultry legs (partly) | 4.82 | 26.50 | 2.13 | 2.34 | 2.00 | 0.60 | 8.44 | 0.19 |
| Hides                                         | 7.63   | 41.97 | 0.00  | 0.00  | 6.56  | 1.97  | 0.00  | 0.00 |
| Bones from cutting, chicken heads, pork heads (partly) | 10.68 | 58.75 | 3.33 | 3.66 | 16.50 | 4.95 | 3.11 | 0.07 |
| Vessels, tendons, cartilage, glands           | 2.01   | 11.08 | 1.52  | 1.67  | 1.00  | 0.30  | 0.00  | 0.00 |
| Others of the 3rd category ABP               | 1.37   | 7.56  | 0.95  | 1.05  | 2.43  | 0.73  | 7.11  | 0.16 |

| 2nd category ABP                             | 9.31   | 51.23 | 3.03  | 3.33  | 14.46 | 4.34  | 1.78  | 0.04 |
| Intestinal and stomachs content              | 8.93   | 49.13 | 2.93  | 3.22  | 14.26 | 4.28  | 0.00  | 0.00 |
| Others of the 2nd category ABP (perished animals etc.) | 0.38 | 2.10 | 0.10 | 0.11 | 0.20 | 0.06 | 1.78 | 0.04 |

| 1st category ABP                             | 8.02   | 44.13 | 0.00  | 0.00  | 5.81  | 1.74  | 0.00  | 0.00 |
| Cattle and sheep heads                       | 3.00   | 16.50 | 0.00  | 0.00  | 4.73  | 1.42  | 0.00  | 0.00 |
| Cattle and sheep spinal cord                 | 0.02   | 0.13  | 0.00  | 0.00  | 0.08  | 0.02  | 0.00  | 0.00 |
| Beef intestine (cleaned), intestine fat, sheep ileum | 5.00 | 27.50 | 0.00 | 0.00 | 0.77 | 0.23 | 0.00 | 0.00 |
| Sheep spleen                                 | 0.00   | 0.00  | 0.00  | 0.00  | 0.23  | 0.07  | 0.00  | 0.00 |

| Sum of ABP                                   | 57.04  | 313.70| 19.80 | 21.78 | 63.85 | 19.16 | 21.11 | 0.48 |

It should be emphasized that the technology of Vireen Ltd has been built upon the basis of the safety principles and designed for processing of the 1st category ABP only. Current study indicates that the average quantities processed by Vireen Ltd are approximately 10 thousand tons per year, which is the planned capacity of the plant also.
The yearly dynamics of the ABP quantities processed in Vireen Ltd is presented in Table 4. These data were used in ABP calculation model assessment for analyses of 1\textsuperscript{st} category generation and reprocessing in Estonia.

**Tabel 3.** Quantities of ABP by risk-categories of major slaughterhouses and meat processing plants in Estonia in 2012

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cattle (x1,000)</th>
<th>Pigs (x1,000)</th>
<th>Sheep (x1,000)</th>
<th>Poultry (x1,000)</th>
<th>Total ABP (tons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slaughtered animals in the approved enterprises, thousands a year, incl.</td>
<td>30.8</td>
<td>428.3</td>
<td>6,910,096</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rakvere Meat Processing Plant, tons</td>
<td>10</td>
<td>235</td>
<td>0</td>
<td>0</td>
<td>7,336</td>
</tr>
<tr>
<td>Atria Estonia, tons</td>
<td>8</td>
<td>110</td>
<td>0</td>
<td>0</td>
<td>4,371</td>
</tr>
<tr>
<td>Saaremaa Lihatōöstus, tons</td>
<td>5</td>
<td>36</td>
<td>4</td>
<td>0</td>
<td>2,092</td>
</tr>
<tr>
<td>Others in total, tons</td>
<td>8</td>
<td>48</td>
<td>3</td>
<td>0</td>
<td>3,182</td>
</tr>
<tr>
<td>Tallegg, tons</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10,096</td>
<td>4,796</td>
</tr>
</tbody>
</table>

3\textsuperscript{rd} category ABP average quantities of a single animal, kg

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cattle (x1,000)</th>
<th>Pigs (x1,000)</th>
<th>Sheep (x1,000)</th>
<th>Poultry (x1,000)</th>
<th>Total ABP (tons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rakvere Meat Processing Plant, tons</td>
<td>1,721.82</td>
<td>3901.64</td>
<td>0.00</td>
<td>0.00</td>
<td>5,623</td>
</tr>
<tr>
<td>Atria Estonia, tons</td>
<td>1,411.04</td>
<td>1,830.11</td>
<td>0.00</td>
<td>0.00</td>
<td>3,241</td>
</tr>
<tr>
<td>Saaremaa Lihatōöstus, tons</td>
<td>846.62</td>
<td>598.94</td>
<td>44.42</td>
<td>0.00</td>
<td>1,490</td>
</tr>
<tr>
<td>Others in total, tons</td>
<td>1,411.04</td>
<td>798.59</td>
<td>32.21</td>
<td>0.00</td>
<td>2,242</td>
</tr>
<tr>
<td>Tallegg, tons</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>4,391.76</td>
<td>4,392</td>
</tr>
</tbody>
</table>

Total 3\textsuperscript{rd} category ABP, tons in a year | 5,390.52 | 7,129.29 | 76.63 | 4,391.76 | 17,048 |

2\textsuperscript{nd} Category ABP average quantities of a single animal, kg

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cattle (x1,000)</th>
<th>Pigs (x1,000)</th>
<th>Sheep (x1,000)</th>
<th>Poultry (x1,000)</th>
<th>Total ABP (tons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rakvere Meat Processing Plant, tons</td>
<td>500.09</td>
<td>781.97</td>
<td>0.00</td>
<td>0.00</td>
<td>1,282</td>
</tr>
<tr>
<td>Atria Estonia, tons</td>
<td>409.83</td>
<td>366.79</td>
<td>0.00</td>
<td>0.00</td>
<td>777</td>
</tr>
<tr>
<td>Saaremaa Lihatōöstus, tons</td>
<td>245.90</td>
<td>120.04</td>
<td>17.35</td>
<td>0.00</td>
<td>383</td>
</tr>
<tr>
<td>Others in total, tons</td>
<td>409.83</td>
<td>160.06</td>
<td>12.58</td>
<td>0.00</td>
<td>582</td>
</tr>
<tr>
<td>Tallegg, tons</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>403.84</td>
<td>404</td>
</tr>
</tbody>
</table>

Total 2\textsuperscript{nd} category ABP, tons in a year | 1,565.64 | 1,428.87 | 34.27 | 403.84 | 3,428 |

1\textsuperscript{st} category ABP average quantities of a single animal, kg

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cattle (x1,000)</th>
<th>Pigs (x1,000)</th>
<th>Sheep (x1,000)</th>
<th>Poultry (x1,000)</th>
<th>Total ABP (tons)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rakvere Meat Processing Plant, tons</td>
<td>430.77</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>431</td>
</tr>
<tr>
<td>Atria Estonia, tons</td>
<td>353.02</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>353</td>
</tr>
<tr>
<td>Saaremaa Lihatōöstus, tons</td>
<td>211.81</td>
<td>0.00</td>
<td>6.96</td>
<td>0.00</td>
<td>219</td>
</tr>
<tr>
<td>Others in total, tons</td>
<td>353.02</td>
<td>0.00</td>
<td>5.05</td>
<td>0.00</td>
<td>358</td>
</tr>
</tbody>
</table>

Total 1\textsuperscript{st} category ABP, tons in a year | 1,348.61 | 0.00 | 12.01 | 0.00 | 1,361 |

Total ABP a year, tons | 8,364.76 | 8,558.15 | 122.91 | 4,791.60 | 21,838 |

For example in 2012 the quantities of ABP delivered from meat industry to the reprocessing as 1\textsuperscript{st} category was 4.9 thousand tons, which exceeded the predicted (by calculation model) amounts 3.5 times. Explanation to this contradiction is that great deal of other ABP categories were also sent for reprocessing as the most dangerous waste. Given example indicates to the insufficient use of 2\textsuperscript{nd} and 3\textsuperscript{rd} category ABP as raw material in Estonian meat industry. Thus, data about processed ABP in Vireen Ltd could be used for estimation of insufficient sorting of ABP into risk categories at meat processing plants.
Proposed monitoring system would become a powerful instrument for prediction of ABP daily (or for longer periods) quantities generated in meat processing plants by categories and types. Authors of current study did not found any reference in literature about up-to-date ABP monitoring systems set into praxis elsewhere. Therefore, Estonian system, if implemented, may become an example for ABP monitoring in other countries too. The existence of a monitoring system based on objective data should encourage meat processing plants to sort ABP into the various risk categories more thoroughly to allow treating these as valuable raw materials and to increase the added value of meat production chain accordingly. There is a large variety of applications for human and animal foods, rendered fat for cosmetics and chemistry products etc. Innovative proposals have been published concerning wider use of ABP proteins with better technological or nutritional properties for goods production (Toldra, 2012). At the same time it should be pointed out that regulatory requirements in many countries restrict broad use of ABP for food safety and quality reasons (Jayathilalan et al., 2012).

**Tabel 4. ABP reprocessed in Vireen Ltd during 2006–2012 (tons yearly)**

<table>
<thead>
<tr>
<th>Year</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABP purchased from meat-companies</td>
<td>4,388</td>
<td>4,424</td>
<td>4,532</td>
<td>3,551</td>
<td>3,630</td>
<td>4,320</td>
<td>4,888</td>
</tr>
<tr>
<td>Perished animals from farms</td>
<td>5,487</td>
<td>6,057</td>
<td>6,199</td>
<td>5,729</td>
<td>5,362</td>
<td>5,162</td>
<td>5,312</td>
</tr>
<tr>
<td>Other ABP</td>
<td>410</td>
<td>128</td>
<td>142</td>
<td>122</td>
<td>80</td>
<td>119</td>
<td>148</td>
</tr>
<tr>
<td><strong>Total ABP</strong></td>
<td><strong>10,285</strong></td>
<td><strong>10,609</strong></td>
<td><strong>10,873</strong></td>
<td><strong>9,402</strong></td>
<td><strong>9,072</strong></td>
<td><strong>9,601</strong></td>
<td><strong>10,348</strong></td>
</tr>
</tbody>
</table>

The following variables must be monitored and analyzed with sufficient frequency in order to ensure efficient processing of ABP with optimal economic impact:
1. The number of animals and poultry processed in slaughterhouses during a respective period (may base on existing datasets).
2. The division of animal carcasses processed in the same period on the basis of the SEUROP classification.
3. The quantities of raw materials of 3rd category ABP usable for human consumption (may be calculated with the appropriate prediction model).

The continuous monitoring would help to establish economically reasonable ABP processing in Estonia. The basic scheme of the initial monitoring system and the activities required for the introduction of it are presented on Fig. 1.

The database, into which the data about slaughtered animals will be gathered, forms the core part of the system. ABP quantities by various categories and types may be estimated using similar procedures given in the calculation model created during current research. The data will be accessible for ABP users via queries through computer networks and internet.
During the applied monitoring system elaboration, the following questions must also be answered:

- Who will administrate the system?
- How the system will be operated (entering, processing and analyzing the data)?
- What will be the reasonable intervals for the data entry?
- Who will have access to the data?
- Who and how will be involved in product development concerning ABP processing and producing information about the possibilities for the utilization various types of ABP in the most efficient way?

CONCLUSIONS AND SUGGESTIONS

Since there is no efficient monitoring system at present which makes it possible to control and manage the resources of ABP in slaughterhouses and meat processing companies in Estonia, the relevant databases, procedures and methods should be worked out during the following studies. In development of the monitoring system, the simplicity, legal aspects, sufficient complexity and free access to required data must be taken into consideration.

Model presented in current research could be used as a base for ABP calculations. Official datasets of the Ministry of Agriculture of the Republic of Estonia, the Estonian Animal Recording Centre, the Estonian Veterinary and Food Laboratory, the Estonian Agricultural Registers and Information Board can be used as data sources for ABP estimation. Respective system for ensuring complex monitoring brings about legal issues and imposes a burden on the companies. Therefore, further activities should proceed from the principle that as little data as possible is to be gathered for monitoring from meat processing plants.
General plan for commercialization of ABP products should be worked out in which the aspects how to stimulate industries in ABP use for human consumption, pharmaceutic, cosmetic and other purposes. The mentioned commercialization plan should take into account the local and world markets demand and also give suggestions to all meat processors about the most optimal way in ABP sorting into several types and product-groups, and handling of these.

REFERENCES


The effect of packaging type on quality of cereal muesli during storage

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Abstract. Cereal-derived breakfast products (cereal flakes, bread, and muesli) are increasingly consumed because they are an important source of energy for adults and children. Shelf-life of foods is highly dependent on the permeability characteristics of the packaging materials, which emphasize the importance of packaging design. The aim of this study was to evaluate changes of physically-chemical, microbiological and sensory parameters of cereal muesli with chocolate and apricots during storage. Samples of muesli with chocolate and apricots were packaged in 3 different types of packaging—paper bag, paper tubes and Doypack (stand-up pouches) and stored for 9 months (at 20 ± 2°C temperature and relative air humidity – 55 ± 3%). During the storage moisture content, water activity, total plate count of mesophilic aerobic and facultative anaerobic microorganisms (MAFAm), mould and yeast, volatile compounds and sensory quality were evaluated in analysed muesli samples. The lowest moisture content after 9 months storage was determined for muesli samples stored in paper bag. Total number of mesophilic aerobic facultative anaerobic microorganisms (MAFAm), mould and yeast, volatile compounds and sensory quality were slightly increased. After 9 months storage muesli with chocolate and apricots in the Doypack had the best sensory properties. In muesli with chocolate and apricots there were identified 18 volatile compounds. The results indicated that paper bags were the least suitable for packaging of cereal muesli with chocolate and apricot, because of essential quality changes of samples during their storage. The shelf-life of 9 months can be recommended for cereal muesli with chocolate and apricots packaged in paper tube or Doypack.

Key words: cereals, muesli, packaging, storage time.

INTRODUCTION

Cereals are crop plants from the grass family (Poaceae) and produce seeds (fruits) with high starch contents which are used for human consumption, animal feed production and industrial purposes. Among the many cultivated species of cereals, an increasingly important role is played by barley, rye and oats (Perkowski et al., 2012). Cereals provide a very substantial proportion of the needs of the world's population for dietary energy, protein, and micronutrients. The major cereal crops are wheat, rice, and maize, but sorghum, millets, barley, oats, and rye are important only in some regions. Unprocessed cereals are low in fat, and a good source of fibre and phytochemicals. Cereal grains are made into a very wide range of cereal-based foods using traditional
and technologically more advanced processes, which can result in changes in nutritional value (Price & Welch, 2013).

Muesli is a mixture of grain flakes and dried fruits, where can be also added seeds and nuts. It is traditionally consumed for breakfast together with milk, yogurt or hot water.

Albertson et al. (2008) showed that ‘cereal itself contains high level of healthful micronutrients and macronutrients, compared to foods consumed during non-cereal breakfasts; cereal tends to facilitate consumption of other healthful foods at breakfast and replace consumption of less healthful foods; and cereal consumption may be a marker for a pattern of behaviour that includes healthful eating and high levels of physical activity throughout the day’.

Oats, maize, rye or wheat can be primarily used for the preparation of breakfast cereal and muesli. However, there are relatively few studies where the muesli cereals are triticale, barley and other cereals (Senhofa et al., 2014). Triticale, oats and barley belong to the group of crops with high energy and nutritional value arising from a high content of biologically valuable proteins, high portion of lipids compared to other cereals, favourable saccharide composition as well as from significant levels of dietary fibre, vitamins and mineral substances (Demirbas, 2005; Gajdosova et al., 2007).

Extra additives with elevated nutritive value as dried fruits, nuts and others contain more moisture than flakes thus creating additional microbiological hazard during muesli storage. Some of the microorganisms present in cereals constitute a potential hazard since their development may alter the properties of the grains, and the mycotoxins produced by some moulds could potentially pose a health risk. It has been reported, that these microorganisms are located close to the surface of the grain, but the real thickness affected by microbial contamination has not been determined (Laca et al., 2006). Contamination of the grains during storage, transportation and processing further affects the microflora of developed new product as, for example, muesli.

Muesli and breakfast cereal shelf-life is limited by chemical and physical changes and the rate of deteriorative reactions depends on its composition as well as environmental factors. Moisture content is identified as the critical quality parameter and relative air humidity as the most influential environmental factor (Macedo et al., 2009; Macedo et al., 2013).

The use of packaging in the food supply chain is very important and is an essential part of food processing. Cereal products are usually packaged in paper/polyethylene packaging. Packaging materials for flakes can be combined using 2 different material types—the first is foreseen for product protection and the other for consumer for ease use (Robertson, 2006). Significant developments in food packaging materials have provided the means to suppress microbial growth as well as protect foods from external microbial contamination (Cutter, 2002). Packaging materials have been developed specifically to prevent the deterioration of foods resulting from exposure to air, moisture, or pH changes, retaining sensory properties.

The aim of this study was to evaluate physically-chemical, microbiological and sensory parameters changes of cereal muesli with chocolate and apricots during storage.
MATERIALS AND METHODS

Experiments were carried out at the Latvia University of Agriculture, Faculty of Food Technology. During storage (0, 2, 4, 6 and 9 months) the moisture content, water activity \((a_w)\), microbiological parameters, sensory properties and volatile compounds were determined for muesli with chocolate and apricots.

**Characterisation of muesli with chocolate and apricots**

Muesli with chocolate and apricots contains:
- 45% roasted \((200 \pm 10^\circ C \text{ for } 10 \text{ min})\) whole grain triticale flakes;
- 22% roasted \((200 \pm 10^\circ C \text{ for } 10 \text{ min})\) whole grain oat flakes;
- 15% dried apricots;
- 10% dark chocolate pieces;
- 8% roasted linseeds.

**Characterisation of packaging materials**

Samples of muesli with chocolate and apricots were packaged in three different multi-layered packaging materials, which provide light and moisture impermeability:
- Paper tubes with lid (Fig. 1, a) – cardboard paper tubes with aluminium layer inside and low density polyethylene (LDPE) black cover (Visican Ltd., Netherlands);
- Paper bag (Fig. 1, b) – brown kraft paper 90–100 g m\(^{-2}\) with polypropylene (PP) window (UAB Eltaka, Lithuania);
- Sealable Doypack (stand-up pouches,) (Fig. 1, c) – Pap50g/Alu7/Pe60 material (Cor Rijken Verpakkingen B.V., Netherlands).

![Figure 1. The packaging used in the research (a – paper tube with lid, b – paper bag with transparent window, c – sealable Doypack).](image)

The amount of muesli in each package was \(250 \pm 10\) g, the muesli was stored for 9 months at \(20 \pm 2^\circ C\) temperature and relative air humidity \(– 55 \pm 3\%\).

For determination of moisture content, water activity, and volatile compounds in muesli with chocolate and apricots, approximately 100 g of sample was ground in laboratory mill Knifectec Mill 1095 (AB Foss Analytical, Sweden) and immediately used for analysis.
Moisture content
Moisture content of muesli with chocolate and apricots was determined by drying 5.00 ± 0.03 g of sample in the oven (GmbH Memmert, Germany) for 1 hour at 110 ± 1°C (LVS EN ISO 712:2010A). The analyses of moisture content were done in triplicate.

Water activity ($a_w$)
Water activity ($a_w$) was determined using LabSwift-aw (AG Novasina, Switzerland) equipment. Ground muesli sample was filled in the sample dish and placed in the measurement chamber according to producer’s recommendations. The analyses of water activity were done in triplicate.

Microbiological parameters
Total plate count of mesophilic aerobic and facultative anaerobic microorganisms (MAFAm) were determined in conformity with the standard LVS EN 4833:2003 ($n = 6$); yeasts and moulds—the standard ISO 21527-2:2008 ($n = 6$).

Sensory evaluation
The experts were selected and trained according to the recommendations described in ISO 8586-1 (1993). Training was completed at the Latvia University of Agriculture. It was done in two sessions—training and selection of panellists, and specific training for evaluation of muesli sensory properties (8 selected assessors).

Descriptive test was used for detection and description of sensory quality of muesli. Descriptors for evaluation of muesli sensory properties (overall appearance, texture, aroma, and taste) were determined by consensus of all selected assessors. Each sensory property was evaluated in the range from 5 (very good quality) to 1 (unsatisfactory quality, serious defects). There was used quality number (QN) for sensory properties evaluation of muesli with chocolate and apricots. The quality number was calculated according to the following equation (Straumite et al., 2012):

$$QN = \frac{Ap + Te + Ar + Ta}{4},$$

where: $Ap$ – muesli overall appearance; $Te$ – muesli texture; $Ar$ – muesli aroma; $Ta$ – muesli taste.

Based on QN muesli with chocolate and apricots quality was classified as follows: 5.00–4.80 – very good quality (performance of quality parameters); 4.79–4.00 – good quality (inessential deviations); 3.99–3.50 – average quality (pronounced deviations, insignificants defects); 3.49–2.50 – satisfactory quality (significant defects); 2.49–1.00 – unsatisfactory quality (serious defects).

Overall appearance was evaluated for dry muesli samples. Muesli samples (15 g) for texture, aroma and taste assessment were poured with hot water ($t = 95 \pm 2°C$), stirred and assessed after 5 min.
Volatile compounds
Volatile compounds were extracted using solid-phase microextraction (SPME) in the combination with gas chromatography/mass spectrometry. SPME fibre 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 a 20 ml vial. Volatile compounds were detected according to methods described by Sabovics et al. (2014). Compounds were identified by comparing their mass spectra with mass spectral library Nist98.

Statistical analysis
The results (mean, standard deviation, \( P \) value) were processed by mathematical and statistical methods using Microsoft Office Excel 2007 software; significance was defined at \( P < 0.05 \).

RESULTS AND DISCUSSION

Moisture content
The moisture content of freshly prepared muesli is 14.42 ± 0.18% (Fig. 2). It is relatively high comparing with cereal flakes because muesli contains various ingredients with different moisture content, such as dried apricots, chocolate pieces, and seeds. As a result the moisture content of several compounds is not equal; however, all compounds can interact each to other until moisture balance in the package is achieved. Experimental results revealed that moisture content of muesli with chocolate and apricots is significantly \( (P = 0.0004) \) affected by packaging material type during its storage.

![Figure 2. The moisture content changes of muesli with chocolate and apricots during storage.](image)

The results of present experiment demonstrate, that the highest water loss after 9 months storage was observed for muesli samples packed in paper bag (56% water loss) and paper tube (51% water loss). There was no significant difference between moisture content of muesli with chocolate and apricots stored in paper bag or paper tube \( (P > 0.05) \).
After 9 month storage muesli moisture content was 6.48–9.20%, what coincides with results of Aigster et al. (2011), who found that granola muesli moisture content is in the range of 5.7–7.1%. It can be concluded that the muesli sample moisture content during storage is adequate, compared to other authors findings on flakes moisture content. The smallest moisture loss was observed in the sample of muesli with chocolate and apricots packaged in Doypack. Wherewith, it can be concluded that the most suitable packaging among studied for long-term storage is the Doypack.

**Water activity**

Reduced water activity (a$_w$) does not provide a complete inactivation of microorganisms, but partially restrict their activity in the product, which helps to ensure a longer shelf-life. In all types of studied packages water activity of the samples decreased during storage (Fig. 3), thus demonstrating significant ($P < 0.05$) effect of packaging material on water activity of the product.

![Figure 3. Changes in water activity (a$_w$) of muesli with chocolate and apricots during storage.](image)

In Latvia does not exist regulation on water activity of muesli and cereal products, but it is important parameter determining the product shelf life. Beuchat (1981) has carried out a study which found that dry food products like flakes have water activity below 0.6. This indicates that the product is microbiologically safe and it slows the development of various microorganisms. After 9 months storage in different packages water activity of muesli samples was below 0.6. During muesli storage for 9 months water activity (a$_w$) of muesli with chocolate and apricots packaged in paper bag decreased 3.6 times from 0.62 to 0.17. The water activity of muesli in paper bag decreased most rapidly, what could be explained with the packing material and its moisture permeability. Paper bag is recommended for muesli storage up to 2 months, but in case of longer storage time (up to 9 months) – Doypack packaging, in which a$_w$ decreased only 1.2 times.
Microbiological parameters

Microorganisms in food are a serious problem that can lead to product spoilage and deterioration in the quality (Celiktas et al., 2007). Traditionally grain microflora is composed of bacteria, yeasts and moulds; however, flakes and grains containing grain husks have increased risk of microbial contamination.

During muesli storage experiment it was detected, that the total count of mesophilic aerobic and facultative anaerobic microorganisms (MAFAm) increases in all three types of packaging materials (Table 1). The highest total colony count after 9 months of storage was observed in the samples packed in paper bags – $2.5 \times 10^5 \pm 3.4 \times 10^2 \text{ CFU g}^{-1}$.

Table 1. Changes of muesli microbiological parameters during storage

<table>
<thead>
<tr>
<th>Parameters / Storage time, months</th>
<th>0</th>
<th>2</th>
<th>6</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAFAm CFU x g$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paper tube</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$1.4 \times 10^3 \pm 2.1 \times 10^1$</td>
<td>$2.2 \times 10^4 \pm 3.4 \times 10^2$</td>
<td>$3.7 \times 10^4 \pm 3.1 \times 10^2$</td>
<td></td>
</tr>
<tr>
<td>paper bag</td>
<td>$1.4 \times 10^3 \pm 2.1 \times 10^1$</td>
<td>$2.7 \times 10^4 \pm 3.6 \times 10^2$</td>
<td>$2.4 \times 10^4 \pm 2.1 \times 10^1$</td>
<td>$3.1 \times 10^4 \pm 3.4 \times 10^2$</td>
</tr>
<tr>
<td>Doypack</td>
<td>$1.4 \times 10^3 \pm 2.1 \times 10^1$</td>
<td>$2.7 \times 10^4 \pm 3.6 \times 10^2$</td>
<td>$2.6 \times 10^4 \pm 3.1 \times 10^2$</td>
<td>$3.2 \times 10^4 \pm 1.9 \times 10^2$</td>
</tr>
<tr>
<td>Yeasts CFU x g$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>paper tube</td>
<td>$1.3 \times 10^2 \pm 1.0 \times 10^1$</td>
<td>$2.6 \times 10^3 \pm 2.1 \times 10^1$</td>
<td>$3.2 \times 10^3 \pm 3.4 \times 10^3$</td>
<td>$3.8 \times 10^3 \pm 1.2 \times 10^1$</td>
</tr>
<tr>
<td>paper bag</td>
<td>$1.3 \times 10^2 \pm 2.8 \times 10^3$</td>
<td>$4.3 \times 10^3 \pm 5.2 \times 10^3$</td>
<td>$3.4 \times 10^3 \pm 5.8 \times 10^3$</td>
<td>$3.6 \times 10^3 \pm 3.0 \times 10^2$</td>
</tr>
<tr>
<td>Doypack</td>
<td>$1.3 \times 10^2 \pm 2.5 \times 10^4$</td>
<td>$3.2 \times 10^3 \pm 1.8 \times 10^3$</td>
<td>$3.4 \times 10^3 \pm 3.6 \times 10^3$</td>
<td>$3.2 \times 10^3 \pm 1.8 \times 10^3$</td>
</tr>
<tr>
<td>Moulds CFU x g$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>paper tube</td>
<td>$1.0 \times 10^2 \pm 0.8 \times 10^4$</td>
<td>$2.4 \times 10^3 \pm 1.0 \times 10^2$</td>
<td>$3.6 \times 10^3 \pm 5.5 \times 10^3$</td>
<td>$3.8 \times 10^3 \pm 1.2 \times 10^2$</td>
</tr>
<tr>
<td>paper bag</td>
<td>$1.0 \times 10^2 \pm 2.4 \times 10^3$</td>
<td>$3.2 \times 10^3 \pm 4.9 \times 10^3$</td>
<td>$3.5 \times 10^3 \pm 5.5 \times 10^3$</td>
<td>$3.7 \times 10^3 \pm 1.8 \times 10^2$</td>
</tr>
<tr>
<td>Doypack</td>
<td>$1.0 \times 10^2 \pm 1.1 \times 10^4$</td>
<td>$1.3 \times 10^3 \pm 2.7 \times 10^3$</td>
<td>$3.5 \times 10^3 \pm 3.7 \times 10^3$</td>
<td>$3.7 \times 10^3 \pm 1.6 \times 10^2$</td>
</tr>
</tbody>
</table>

Increase of yeast cell count occurs in the first 4 months of storage. The smallest yeast cell growth was detected after 9 months of storage in muesli with chocolate and apricots packaged in Doypack ($3.6 \times 10^3 \pm 1.8 \times 10^1 \text{ CFU g}^{-1}$).

FS (International Commission for the microbiological specific actions for food products) has indicated that the limit for the number of moulds in dry products such as cereals could vary from $10^2$ to $10^4 \text{ CFU g}^{-1}$ (ICMFS, 2005). Freshly prepared samples of muesli with chocolate and apricots contain $1.0 \times 10^2 \pm 0.8 \times 10^1 \text{ CFU g}^{-1}$, however after 9 months of storage the amount of microorganisms increased up to $5.5 \times 10^3 \pm 1.8 \times 10^2 \text{ CFU g}^{-1}$ in paper bag.

Mesophilic aerobic and facultative anaerobic microorganisms (MAFAm), yeast and moulds growth dynamics in muesli samples during storage is influenced by the presence of air and its diffusion through packaging material, contributing to development.
of microorganisms. Paper bags are the most permeable packaging material among studied materials, therefore microflora development in a package can be observed.

**Sensory evaluation**

Sensory evaluation of muesli samples was realised for muesli with chocolate and apricots after 9 months of storage. Obtained results demonstrate that the highest evaluation received muesli packaged in *Doypack* (QN = 5.00) and paper tubes (QN = 5.00) (Table 2).

**Table 2. Sensory evaluation results of muesli with chocolate and apricots**

<table>
<thead>
<tr>
<th>Package</th>
<th>Overall appearance</th>
<th>Texture</th>
<th>Aroma</th>
<th>Taste</th>
<th>QN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper tube</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Paper bag</td>
<td>5.00</td>
<td>5.00</td>
<td>4.00</td>
<td>3.00</td>
<td>4.25</td>
</tr>
<tr>
<td>Doypack</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
</tbody>
</table>

Sensory evaluation of stored muesli samples with chocolate and apricots in paper tubes and *Doypack* revealed that product along with cereal taste and aroma has typical chocolate aroma and taste. However, in the same muesli sample stored in paper bags, chocolate and apricots taste and aroma became less pronounced and it was dominated by cereal taste and aroma.

**Volatile compounds**

Changes of qualitative and quantitative composition of volatile compounds in the end product, may affect not only the packaging material but also the storage temperature and the interaction between volatile compounds of the various ingredients (Figiel et al., 2010).

![Figure 4. Changes of total amount of volatile compounds during storage.](image-url)
In the first 2 months of storage there was observed small decrease in volatile compounds peak areas, but the rapid decline of volatile substances was observed from 2 to 9 months of storage (Fig. 4). It should be noted that the most rapid reduction of volatile compounds was found in samples packaged in paper tubes, quantitative composition of the volatile compounds after 9 months storage has decreased 38.3 times. However, the least decrease of volatile compounds peak areas in products stored for 9 months in Doypack.

In chocolate and apricot muesli there were totally identified 18 volatile compounds, of which 7 compounds were identified in all samples irrespective of packaging materials. The identified volatile compounds in muesli with chocolate and apricots from the aroma profile, where dominant aromas are malty, whiskey (3-methyl-butanal), green (hexanal), almond, bread (furfural), citrus (D-limonene), grape (ethyl caprate), fruit (4-penten-2-ol) and acid (acetic acid). The other identified 12 compounds were less than 2%.

In a study of chocolate and apricot muesli samples there were identified volatile compounds belonging to different classes: alcohols, aldehydes, ketones, terpenes, esters, and carboxylic acids. The most dominating volatile compounds class in muesli with chocolate and apricots was carboxylic acids (28%), but the least have been identified alcohols and terpenes.

CONCLUSIONS

The results indicated that paper bags were the least suitable for packaging of cereal muesli with chocolate and apricots, because of essential quality changes of samples during their storage. The shelf-life for cereal muesli with chocolate and apricots packaged in paper tube or Doypack for 9 months could be recommendable.

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REFERENCES


Upgrading the technology of functional dairy products by means of fermentation process ultrasonic intensification

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Abstract. Intensification of milk fermentation without negative influence on product quality is a priority research direction in dairy industry. One of the perspective tools for solving this problem is usage of ultrasound. Careful selection of ultrasonic treatment regimens allows to activate lactic-acid bacteria metabolic activity and to improve the efficiency of dairy production. A number of cultivations were carried out for ultrasonic processing effect estimation on Lactococcus mixed culture, Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus symbiotic cultures that are often used for dairy fermentation. Milk with added starter culture was treated with ultrasound by means of ultrasonic homogenizer at a frequency of about 30 kHz. Processing duration varied from 1 to 3 minutes and ultrasound power varied from 2 to 8 W. Ultrasonication regimens of fermenting milk allowed accelerating of fermentative process by 10% and improving the quality of final product.

Key words: ultrasound, ultrasonic processing, dried skim milk, reconstitution, intensification, fermentation, Lactococcus lactis, Lactococcus cremoris, Lactococcus diacetylactis, Streptococcus thermophilus, Lactobacillus bulgaricus.

INTRODUCTION

Fermented dairy products are one of the most important sources of essential amino acids, vitamins, trace metals and other biologically active compounds in a human diet maintaining activity of immune system and reducing influence of environmental harmful factors. Nowadays fermented milk is widely used as basis for functional food products.

Fermentation is the longest and one of the most resource-consuming stages during fermented dairy products processing. It demands large floor spaces and high energy consumption for temperature conditions maintenance. Therefore intensification of fermentative processes without negative influence on a final product quality became the priority direction of our research.

Nowadays high consumer demand is observed for natural functional products of high quality which are not containing preservatives and any other chemical additives. So, various non-chemical methods of raw material processing for production improvement become more and more researched (Barba et al., 2012; Chandrasekaran et al., 2013; Kiprushkina & Baranenko, 2014). Ultrasound treatment is one of the most perspective methods for improvement some of the food processes (Rastogi, 2011).
Ultrasound is the sound wave with frequencies above 18–20 kHz, inaudible for human ear. Ultrasonic treatment is often classified by frequency and the energy amount of the generated sound field measured as sound power (W) or sound intensity (W m\(^{-2}\)) (McClements, 1995). Most of the sonochemical processes demand application of low-frequency (20–100 kHz) and high–power ultrasound (Chandrapala et al., 2012).

The main effects of high power ultrasound in liquids are the mechanical vibration of medium and moving of the solid particles contained in it; acoustic streaming which can increase mass transfer in medium (Tho et al., 2007) and acoustic cavitation which is one of the most important processes for the ultrasonic treatment (Akopyan & Ershov, 2005).

Ultrasound wave propagate through a medium as a series of compressions and rarefactions. When the rarefaction exceeds the attractive forces between liquid medium molecules, it leads to formation, oscillation and collapse of microbubbles filled with dissolved gases and vapours of this medium. Collapsing bubbles release shock waves (Chisti & Moo-Yong, 1986) which cause intense local heating up to 4,000 K and to increase the pressure up to 1,000 atm (Mason, 1998). Because of that molecules of different compounds dissolved in medium can break apart and form the free radicals which can induce various chemical effects.

When the acoustic energy applied to medium is more than 1 W cm\(^{-2}\), it exceeds the cavitation threshold and formation of gas bubbles becomes continuous (Hmelyov & Popova, 1997). This process refers to transient or stable cavitation (Ashokkumar & Mason, 2007). Cavitation threshold value widely varies depending on the media viscosity and configuration of ultrasonic equipment (Chandrapala et al., 2012) and in the common sonoreactors the ultrasound energy is not exceeded cavitation threshold in most of the reactor volume (Chisti, 2003).

At low frequencies acoustic cavitation can generate very strong physical forces, but the amount of free radicals formed is insignificant (Ashokkumar & Mason, 2007). However, for the dairy processing short time ultrasound treatment is preferred because of pyrolysis reactions inside of the cavitating bubbles. Free radicals induce lipid oxidation that generate various volatile organic compounds in trace amounts and might cause a rubbery flavour and aroma (Riener et al., 2009).

Effects of vibration, acoustic streaming and cavitation induced by ultrasound made it very useful tool for many food production processes. Ultrasound has been used in the food industry since the 1960s for food characterization and cleaning (Mason et al., 1996). Nowadays ultrasonic processing has more applications on dairy factories in such different operations as homogenization, pasteurization, drying and reconstitution of dried milk (Hmelyov & Popova, 1997; Mason, 1998; Villamiel et al., 2000; Ertugay et al., 2004; Makeev et al., 2006; Dolatowski et al., 2007; Dergachyov & Bliadze, 2009; Ashokkumar et al., 2010; Chandrapala et al., 2012).

The most common devices used for the generation of ultrasound are piezoelectric transducers. They change their geometrical sizes under the influence of the alternating high-frequency voltage and convert electrical energy to acoustic energy. Transducers can be mounted directly on the walls of sonochemical reactor (ultra-sonic bath) or can be used as separate submerged device (ultrasonic probe). Ultrasonic baths often used for low power ultrasound processing in order to avoid cavitational damage to the reactor and their sound intensity is highly depends on reactor volume (Mason, 1998). The ultrasonic

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probes are preferable for the continuous stable high-power ultrasound processing (Rastogi, 2011).

The most widespread ultrasonic devices in the dairy industry nowadays are ultrasonic homogenizers. They are represented by piezoelectric transducers mounted in a steel pipe. That kind of construction allows applying ultrasound to various purposes at any technological stage of dairy production (Makeev et al., 2006).

It is known that using power ultrasound processing at low frequencies for milk homogenization can increase viscosity and enhance texture characteristics of fermented products (Sfakianakis & Tzia, 2010). It also allows receiving very fine emulsions and altering size distribution of the fat globules in milk by changing ultrasonic power and length of sonication (Zverev & Lobanov, 2005; Ashokkumar et al., 2010). That effect can be useful in combined functional foods production. Another advantage of the ultrasonic homogenisers is the ease of their cleaning relative to traditional homogenizers (Ertugay et al., 2004).

Application of the ultrasonic treatment can also intensify dissolution of dried milk which is very useful and valuable dairy raw material for fermented dairy production. Sonication of dried milk dissolved in water increases its solubility due to breaking of its agglomerates and, thereby, reduces the optimum dissolution temperature to 25°C. Ultrasound treatment also promotes proteins swelling and changes a ratio of free and bound water (Popova, 2013) that is especially important for fermentation process efficiency.

One more positive effect of ultrasound that can be used at dried milk restoration process is foam destruction. It results from gas bubbles pulsation and impact on their surface by the turbulent acoustic streaming (Dergachyov & Bliadze, 2009). The 5 minutes of 20 kHz ultrasound treatment can remove gas bubbles from mixture and prevent the reducing of final product yield and its oxidative degradation (Villamiel et al., 2000).

The low-frequency high-power ultrasound can also cause different effects on metabolic activity of bacteria cells, including changes to organelles within cells, altering of enzyme stability and cell growth properties, breakage of extracellular polymer substances, enhancing mass transport inside and outside of the cell, alteration of cell surface charge and even rupture of cell membranes and cell lysis (Rokhina et al., 2009). For years in food industry high-power ultrasound mostly has been used for cell disruption to release intracellular organelles and enzymes (Chisti & Moo-Yong, 1986; Akopyan & Ershov, 2005) and for pathogens inactivation in food products (Mason et al. 1996; Knorr et al., 2004). In dairy products power ultrasound treatment can be used to inactivate such pathogenic bacteria as Escherichia coli, Staphylococcus aureus and Listeria monocytogenes (Gera and Doores, 2011; Herceg et al. 2012) in whole and skim milk.

The one of the most perspective and quickly developing directions of sonobiochemistry is application of ultrasound for intensification of cell metabolism and growth (Kwiatkowska et al., 2011). In the liquid media microbial cells always surrounded by liquid film, which can reduce transfer of nutrients and cell by-products (Chisti, 1999). Acoustic streaming, cavitation and other ultrasound effects can thin this film and enhance mass transfer inside and outside of the cells. Thus, controlled ultrasound with suitable sonication regimens which differ for various kinds of
microorganisms can cause beneficial effects on metabolic activity of microbial systems (Chisti, 2003).

High-power ultrasound treatment at low frequencies (about 20–24 kHz) during fermented milk products processing can cause reducing the fermentation time of yogurt (Masuzawa & Ohdaira, 2002), reducing amount of lactose in fermented product (Tobai et al., 1990) and increasing the metabolic activity of Lactobacillus delbrueckii and various strains of Bifidobacteria in milk (Wang & Sakakibara, 1997; Nguyen et al., 2009). Although, higher frequencies application and continuous sonication upon cultivation of Lactobacillus delbrueckii causes cells deactivation and intracellular enzymes leakage (Sakakibara et al., 1994; Wang et al., 1996) and can led to longer fermentation process duration (Sfakianakis & Tzia, 2010).

Thus, careful selection of the ultrasonic treatment modes allows achieving activation of lactic-acid bacteria metabolic activity and reduction of fermentation duration. This effect can be used for improving the efficiency of functional dairy production by means of fermentation process acceleration and final product enrichment with native functional compounds produced by microorganisms.

So, ultrasonic processing of fermented mixture after starter culture adding allows combining dried skim milk reconstitution and fermentative process ultrasonic intensification. This can be corresponded with the operating modes of industrial high-power and low-frequency ultrasonic homogenizers and probes which already used on various dairy factories. This technique can allow applying the same equipment for a number of different operations and reducing necessary floor spaces and capital costs, which is especially important for small dairy factories. Besides that, most of the ultrasonic beneficial effects appear over short times and at low frequencies. So, using mid-power short-time ultrasonic treatment also allows minimize some of the ultrasound negative effects and energy costs (Ashokkumar et al., 2010).

A number of cultivations were carried out for estimation an effect of ultrasonic processing on lactic-acid bacteria technological cultures that are often used for milk fermentation. Various milk components such as milk fat and lactose can reduce ultrasound influence on the bacteria cells (Chandrapala et al., 2012). For minimizing the milk components protective effect reconstituted skim milk standardized to lactose amount of 4.5% was used for cultivation.

**MATERIALS AND METHODS**

Lyophilized mesophilic mixed culture of Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, and Lactococcus lactis subsp. cremoris (biovar diacetylactis) applied in production of sour milk and sour cream and thermophilic symbiotic culture of Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus applied for production of yoghurts were used for the study. All cultures were obtained from the All–Russian Research Institute of Fats collection.

High quality dried skim milk (Latvia) was used as a basis for nutrient media. Dried milk was reconstituted to lactose concentration of 4.5% and pasteurized at the temperature of 76 ± 2°C within 20 seconds. Then lyophilized starter cultures were added in quantity of 0.02 g (0.1 activity unit) per liter of reconstituted milk and 22 samples of reconstituted milk with added starter culture were prepared. The volume of each sample was 25 ml.
Laboratory ultrasonic homogenizer SonoPuls mini20 (Bandelin, Germany) with 2.5 mm piezoelectric probe transducer operating at a frequency of about 30 kHz was applied for the ultrasound treatment.

Each sample was treated by ultrasound at the beginning of cultivation and two hours after the beginning of cultivation. Processing time and ultrasound power varied from 1 to 3 minutes and from 2 to 8 W for different samples. The temperature of cultivation was 32 ± 2°C and 40 ± 2°C for the *Lactococcus* mixed culture and for *Streptococcus* and *Lactobacillus* symbiotic culture, respectively.

Titratable acidity (Turner degrees, ºT) and pH of the samples were monitored during the cultivation as the key indicators of a product readiness. Cultivation was finished when pH of untreated sample was below 4.4 and the strong casein coagulum was formed. For the *Lactococcus* mixed culture and for *Streptococcus* and *Lactobacillus* symbiotic culture fermentation durations were 9 hours and 6 hours, respectively. Lactose content in the fermented product samples was determined after cultivation for bacteria metabolic activity estimation.

Viscosity depending on shear rate (flow curve) was studied in the final product samples for evaluation of the ultrasound influence on fermented products structure properties (Krus et al., 2000). Flow curves were measured by rotary rheometer RN 4.1 (Rheotest, Germany).

Water activity of reconstituted milk after the first sonication was studied for evaluation of milk proteins condition. High precision dew point water activity meter AVK-4 (SPbSAU, Russia) was used for water activity measurement in the fermented milk samples.

Concentrated sulfuric acid, 5% phenol solution, and 1 M sodium hydroxide solution (Vekton, Russia) were used for the photometric definition of lactose content according to Lawrence (Krus et al., 2000). An optical density of lactose and fermented samples solutions was measured on UV-1800 spectrophotometer (Shimadzu, Japan).

Visual counting of microorganisms and taking the microphotographs of treated and untreated samples were made with Axio Lab.A1 microscope (Carl Zeiss, Germany).

Water activity, titratable acidity and lactose concentration t-confidence intervals were calculated at the confidence level of 95% based on four measured values for each ultrasonic treatment regimen (Vasilinets & Kolodyaznaya, 2001). All calculations were made in Microsoft Excel.

**RESULTS AND DISCUSSION**

Cultivation cycle was repeated four times for the purpose of minimizing random deviations (Gvozdev, 2013). After each cultivation cycle the titratable acidity and lactose content of fermented samples were measured. Average values of fermented samples titratable acidity and t-confidence intervals are summarized in Tables 1 and 2.
Table 1. Titratable acidity of reconstituted milk samples fermented by *Lactococcus* mixed culture under various ultrasound treatment regimens after 9 hours of cultivation

<table>
<thead>
<tr>
<th>Ultrasound power, W</th>
<th>Treatment duration, min</th>
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<tbody>
<tr>
<td>2</td>
<td>62 ± 1</td>
</tr>
<tr>
<td>3</td>
<td>62 ± 2</td>
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<td>4</td>
<td>63 ± 2</td>
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<td>5</td>
<td>65 ± 2</td>
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<td>6</td>
<td>69 ± 1</td>
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<td>7</td>
<td>66 ± 2</td>
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<tr>
<td>8</td>
<td>64 ± 2</td>
</tr>
<tr>
<td>Untreated sample</td>
<td>60 ± 1</td>
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</tbody>
</table>

Table 2. Titratable acidity of reconstituted milk samples fermented by symbiotic *Streptococcus* and *Lactobacillus* culture under various ultrasound treatment regimens after 6 hours of cultivation

<table>
<thead>
<tr>
<th>Ultrasound power, W</th>
<th>Treatment duration, min</th>
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<tbody>
<tr>
<td>2</td>
<td>67 ± 2</td>
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<tr>
<td>3</td>
<td>68 ± 1</td>
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<tr>
<td>4</td>
<td>68 ± 2</td>
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<td>5</td>
<td>71 ± 2</td>
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<td>6</td>
<td>66 ± 2</td>
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<td>7</td>
<td>68 ± 1</td>
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<tr>
<td>8</td>
<td>70 ± 2</td>
</tr>
<tr>
<td>Untreated sample</td>
<td>65 ± 1</td>
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</tbody>
</table>

Average values of fermented samples lactose content and t-confidence intervals are summarized in Tables 3 and 4. Water activity, titratable acidity and lactose solution optical density of each sample were defined as average value of three parallel measurements. Titratable acidity in all treated samples was higher and lactose content was lower than in the untreated sample. Obtained results also show that minimum average lactose content correlate with the maximal titratable acidity of fermented media for both cultures.

Table 3. Average lactose content of reconstituted milk samples fermented by *Lactococcus* mixed culture under various ultrasound treatment regimens after 9 hours of cultivation

<table>
<thead>
<tr>
<th>Ultrasound power, W</th>
<th>Treatment duration, min</th>
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<tbody>
<tr>
<td>2</td>
<td>3.32 ± 0.06</td>
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<tr>
<td>3</td>
<td>3.26 ± 0.05</td>
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<tr>
<td>4</td>
<td>3.23 ± 0.06</td>
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<tr>
<td>5</td>
<td>3.16 ± 0.06</td>
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<tr>
<td>6</td>
<td>3.01 ± 0.06</td>
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<tr>
<td>7</td>
<td>3.04 ± 0.08</td>
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<tr>
<td>8</td>
<td>3.08 ± 0.06</td>
</tr>
<tr>
<td>Untreated sample</td>
<td>3.37 ± 0.08</td>
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</tbody>
</table>
Table 4. Average lactose content of reconstituted milk samples fermented by symbiotic *Streptococcus* and *Lactobacillus* culture under various ultrasound treatment regimens after 6 hours of cultivation

<table>
<thead>
<tr>
<th>Ultrasound power, W</th>
<th>Treatment duration, min</th>
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<tr>
<td>2</td>
<td>3.37 ± 0.08</td>
<td>2.96 ± 0.05</td>
<td>3.04 ± 0.04</td>
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<tr>
<td>3</td>
<td>3.32 ± 0.06</td>
<td>3.12 ± 0.08</td>
<td>2.96 ± 0.05</td>
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<tr>
<td>4</td>
<td>3.34 ± 0.06</td>
<td>2.93 ± 0.08</td>
<td>2.72 ± 0.05</td>
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</tr>
<tr>
<td>5</td>
<td>3.31 ± 0.04</td>
<td>2.90 ± 0.05</td>
<td>2.65 ± 0.04</td>
<td></td>
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<tr>
<td>6</td>
<td>3.25 ± 0.05</td>
<td>3.04 ± 0.06</td>
<td>2.79 ± 0.06</td>
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<tr>
<td>7</td>
<td>3.05 ± 0.07</td>
<td>3.00 ± 0.07</td>
<td>2.76 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3.02 ± 0.08</td>
<td>2.86 ± 0.06</td>
<td>2.72 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Untreated sample</td>
<td></td>
<td></td>
<td></td>
<td>3.49 ± 0.05</td>
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</table>

These results allow suggesting that mid-power short-time 30 kHz ultrasonic treatment can increase primary metabolic activity of both *Lactococcus* mixed culture and *Streptococcus* and *Lactobacillus* symbiotic culture and thus can shorten fermentation time. Visual counting of the microorganisms has shown that for the most part of the ultrasound treated samples overall amount of microbial cells was higher than in the untreated sample. Microphotographs of treated and untreated samples are shown on Figs. 1 and 2.

**Figure 1.** Microphotographs at 900x magnification of *Lactococcus* mixed culture after 9 hours of cultivation: a) untreated sample; b) 6 W ultrasonic treatment within 1 minute.

**Figure 2.** Microphotographs at 900x magnification of symbiotic *Streptococcus* and *Lactobacillus* culture after 6 hours of cultivation: a) untreated sample; b) 5 W ultrasonic treatment within 3 minutes.
It was also noticed that the ratio of various bacteria species also changes with changing of ultrasound treatment power and duration. The tendency for *Streptococcus* to form the longer chains under ultrasound influence was observed (Fig. 2). These effects need further investigation. Water activity of reconstituted milk untreated samples with addition of *Lactococcus* mixed culture and symbiotic *Streptococcus* and *Lactobacillus* culture was 0.9930 ± 0.0003 and 0.9924 ± 0.0003, respectively. After the first sonication one of the lowest water activity values also were 0.9925 ± 0.0003 under the treatment regimen of 6 W and 1 min for *Lactococcus* and 0.9919 ± 0.0003 under the treatment regimen of 5 W and 3 min for *Streptococcus* and *Lactobacillus* culture. These treatment regimens also showed the minimum lactose content and maximum titratable acidity of fermented samples. This shows maximal intensification of the fermentative processes studied. This effect can be associated with ultrasonic intensification of lactose leaching from the surface of dried milk proteinaceous particles and increase of its availability to microorganisms developed in restored milk (Popova & Potoroko, 2014). Rheological analysis of fermented samples has shown that ultrasound treatment of fermented media at all regimens causes increase of final product viscosity and enhance its thixotropic properties and structure characteristics. These changes were also very significant in the samples treated under the regimens providing fermentation processes highest intensification (Figs. 3, 4).

![Flow curves of reconstituted milk samples fermented by *Lactococcus* mixed culture after 9 hours of cultivation: a) untreated sample; b) 6 W ultrasonic treatment within 1 minute.](image)

The shelf life of treated samples was also increased. After a week of the samples cold storage at the temperature of 5°C titratable acidity of all samples did not exceed 80 °T and firmness of treated products was proven to be higher than untreated by means of syneresis reduction.

The specific regimens of ultrasound treatment by means of ultrasonic homogenizer were obtained summarizing all data. For the lyophilized mesophilic mixed culture of *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, and *Lactococcus lactis* subsp. *cremoris* (biovar *diacetylactis*) the regimen of 6 W ultrasonic treatment within 1 minute at the beginning of cultivation and 2 hours after the beginning of cultivation is recommended.
**Figure 4.** Flow curves of reconstituted milk samples fermented by symbiotic *Streptococcus* and *Lactobacillus* culture after 6 hours of cultivation: a) untreated sample; b) 5 W ultrasonic treatment within 3 minutes.

For the lyophilized thermophilic symbiotic culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus* the regimen of 5 W ultrasonic treatment within 3 minute at the beginning of cultivation and 2 hours after the beginning of cultivation is recommended. Increasing of titratable acidity of fermented samples treated by ultrasound on these regimens is shown on Fig. 5.

**Figure 5.** Increasing of titratable acidity in treated and untreated reconstituted milk samples during the fermentation: a) by *Lactococcus* mixed culture; b) by symbiotic *Streptococcus* and *Lactobacillus* culture.
Thus, these regimens provide increasing of the efficiency of fermented dairy production based on reconstituted skim milk by means of reducing the duration of lyophilized microorganisms’ revival, accelerating fermentation stage by about 10% and enhancing the texture properties of final products.

CONCLUSIONS

Ultrasonication is a relatively new method in dairy industry and most of the laboratory researches were not approved in industrial scale processes. Meanwhile, ultrasonic equipment can be rarely found on dairy factories, but it is just a matter of time.

Most of developed ultrasound techniques are more safe, energy efficient and economic than their common alternatives (Rastogi, 2011). Due to its high universality such ultrasound techniques can be directly moved from laboratory into fully operational commercial food processes using the industrial ultrasonic equipment providing the required sonication regimens. Ultrasonic equipment can also be adapted to existing processing lines for upgrading different industrial operations (Ashokkumar et al., 2010). It has a good payback on capital investment (Patist & Bates, 2008).

As a result of our research it is possible to make a conclusion that application of fermentation ultrasonic intensification technique in industrial scale will allow reducing production duration and increasing quality of different types of traditional and innovative functional dairy products on the basis of powdered skim milk. Such products can also be naturally enriched with functional substances and have less demand on special additives. This technique can reduce their prime cost and can increase availability of some types of specialised foods for the ordinary consumer. Determined regimens of ultrasonic processing can be applied on some dairy factories that already use ultrasonic homogenizers.

ACKNOWLEDGEMENTS. This work was partially financially supported by Government of Russian Federation, Grant 074-U01.

REFERENCES


Optimization of the recipe of yoghurt with additives and control of some quality attributes of new yoghurt recipe

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Abstract. According to the data and the results of clinical trials received in Nutrition Institute of ‘Russian Academy of Medical Sciences’, it was found that 80% of Russians suffer from lack of selenium. Saint-Petersburg University Innovation Company ‘Littoral’ has developed a biologically active food supplement ‘Selenium Alga plus’. The aim of this research was to investigate the possibility of using dietary supplement ‘Selenium Alga Plus’ in yoghurt manufacture. Almost all groups of the population consume such fermented milk drink as yogurt, so that it is considered that this method increasing selenium as the most effective. People suffering from diabetes may have lack of selenium in the organism, as well as healthy people. Since traditional yogurt contains 11% of sucrose, it is necessary to choose sweetening components with vegetable origin. Selecting sweetening components of vegetable origin we pursued the dual purpose. Firstly, we created a sweet product, which would be a source of organic selenium. Therefore the sucrose was replaced with the plant origin sweeteners such as syrup of Jerusalem artichoke and stevioside. Secondly, it was the development of technology and composition of functional food product, intended not only for mass consumption, but also for people suffer from diabetes. It becomes possible due to the absence of sucrose, the presence of selenium and Jerusalem artichoke, which are able to reduce the blood sugar level.

Key words: nutrition, yogurt, selenium, stevioside, Jerusalem artichoke.

INTRODUCTION

Recent years in Russia and abroad functional food is used widely. New promising food industry trend improves the structure of nutrition and the human health maintenance. Research and development of functional foods enriched with dietary fiber, antioxidants, vitamins and minerals, are relevant.

According to the Institute of Nutrition ‘RAMN’ scientists, data approximately 80% of Russia's population suffers from a deficiency of selenium. Particularly severe selenium deficiency is observed in elderly people aged 45–55 years and after 70 (‘selenium pit’), pregnant women and children fed infants food. (Volkotrub et al., 2001)

The main route of selenium in the body is alimentary. 90% of selenium intake is driven into human organism with nourishment and 10% with water. Assimilation of soluble forms of selenium (most part of selenium is absorbed in the duodenum and other sections of the intestine) is determined by the nature of the food.
Retention indices (inclusion metabolism) and potential toxicity play important role. Available data confirms the advantage of organic forms of selenium (selenomethionine, selenocysteine) as dietary sources of selenium. Selenomethionine is absorbed five to ten times better than other chemical compounds (Volkotrub et al., 2001).

Organic form (selenomethionine, selenocysteine) is the most preferable for the body to digest as 95–98%, whereas the animal form is absorbed at 30%, and nonorganic form is absorbed only at 10% (however, just mineral form of selenium requires the greatest prudence since its excess leads to producing and accumulating of the ‘ gidroselenidanion’ toxin in the body (Gmoshinsky et al., 2006).

Humans need a little quantity of selenium to maintain health. The World Health Organization believes that women need about 55 micrograms, man – 70 micrograms, and children – 15 micrograms of the substance per day.

Saint-Petersburg University innovative company ‘Litoral’ developed a biologically active food supplement ‘Selenium Alga plus’.

Dietary supplement ‘Selenium Alga Plus’ contains an organic form of selenium (sources – selenium yeast, garlic, wheat bran). The selenium is included in the composition more than 200 hormones and enzymes and thus it regulates work of all organs and systems of our body. Selenium is incorporated into proteins to make selenoproteins, which are important antioxidant enzymes. Selenium is found in glutathione peroxidase, thioredoxins, and selenoprotein P (Fraga, 2005). Selenium plays an important role in many biological functions: antioxidant defense, formation of thyroid hormones, DNA synthesis, fertility and reproduction. Some, like methylselenol, play a role in cancer prevention (Mehdi et al., 2013).

Brown algae one part of selenium supplement contains more iodine than any other maintaining product. Iodine and selenium are the most important trace elements for the health of the thyroid gland and normal production of the hormone. Numerous medical researches about the role of trace elements in the development of iodine deficiency disorders confirmed close metabolic relationship of iodine and selenium in the body, as well as experiments on rats have shown that the saturation of the selenium in a lack of iodine leads to the aggravation of iodine deficiency diseases (Gromova, 2004). The composition of the supplement also includes garlic, wheat bran, brewer's yeast, artichoke, E, C, B vitamins. Vitamin C improves the absorption of selenium, promotes the stabilization and recovery of vitamin E. Selenium is required for the action of vitamin E. Together, they create a full-fledged ‘trap’ for the fat–soluble oxidants. Selenium and Vitamin E can work only in conjunction, so that ingestion of one substance requires supplementation with another substance. Moreover, the lack of these vitamins can prevent the body to assimilate selenium (Thomson, 2004).

Jerusalem artichoke, which is a part of supplement ‘Selenium Alga Plus’, lowers blood sugar level, that beneficially affects the efficiency of the complex. Wheat bran promote the formation of the nutritive bolus, thus nutrients pass through the gastrointestinal tract with optimal speed that promotes assimilation by the body (Kochnev et al., 2002).

It should be noted that there is another no less important problem in Russia. People suffering from this disease, may suffer from selenium deficiency in an organism as well as healthy people. In addition, because of diabetes is often accompanied by intestinal dysbiosis, such people need of daily use of dairy products. Therefore, at the Department of Technology of milk and food biotechnology, it was decided to develop a fermented
milk product, available not only for general populations, but also for people with diabetes (Wotkins, 1997).

In recent years, there was a trend towards the production of functional foods (Gmoshinsky et al., 2006). They differ in various compositions, but the unity of the assortment based on the purposeful usage of milk and non-dairy origin raw-stuff imparting protein, lipid, carbohydrate, vitamin or mineral orientation of new products. Based on the above, the purpose of this study was to develop a composition of yogurt, using supplement ‘Selenium Alga Plus’ containing selenium in organic form, with the addition of sugar substitutes – syrup of Jerusalem artichoke and stevia.

MATERIALS AND METHODS

Dry skim milk was obtained from a local market. One party dry skim milk was used to provide reliability of the experimental results. Technological process was performed by the known traditional technology, thermostatic method. Required quantity of selected according to GOST 52791–2007 dry skim milk was dissolved in water (SanPin 2.1.4.1074–01) heated to a temperature 40–45°C until complete dissolution, and held at this temperature for 1 hour to intumescence proteins. Then milk was pasteurized at the temperature 90–95°C having been delayed 2–8 min, was cooled to 45°C, added starter culture, were stirred and thermostated during 4 hours.

Standard starter culture was used to ferment yogurt (Lactobacillus delbrueckii subsp. Bulgaricus and Streptococcus thermophilus) Termophilic Yoghurt Culture by firm Yo–FlexR CHR HANSEN party YF–L811 was used.

Same batch dietary supplement ‘Selenium Alga Plus’ from Company ‘Litoral’ was used. Supplement was added in dry form, after pasteurization. Dietary supplement was packaged in a vacuum packaging, thus additional heat treatment was not required to provide product safety.

Stevioside powder (31834003920000; manufactured by ‘Rudolf Wild GmbH & Co KG’) was scaled and added in dry form.

Syrup of Jerusalem artichoke was manufactured by LLC ‘Terra’ (Specification – 9185–003-56857055-05). The required amount of syrup was measured by laboratory pipette according to GOST 29227–91.

In the first stage of researching, the effect of the concentration of the studied supplement on quality yogurt and dynamics of accumulation of acidity was determined. Supplement was added in an amount of from 0.1 to 0.8%, which is from 13 to 100% of the daily requirement for selenium for adult human by eating 200 g of the product. Supplement concentration was ranged from 0.1% to 0.8% with the increments of 0.1%. Control pattern was a sample without supplement. Based on literature data, selenium has a property to be destroyed by heat treatment of over 50°C. Therefore the moment of addition supplement after pasteurization before fermentation and in the finished product before bottling was investigated.

To determine the dosage of stevioside in the product it was added from 0.01 to 0.1% in increments of 0.01%. The sample that was produced from reconstituted skim milk with a sucrose content of 11%, served as control. Organoleptic evaluation of samples was conducted. To determine the effect of Jerusalem artichoke syrup concentration in the product it was added from 1 to 5% of the dose of syrup of Jerusalem artichoke in increments of 1%.
In the second stage research the moment of entering of sweetener was determined. To determine the moment of entering the sweeteners two samples were prepared. In the first case, the stevioside and syrup of Jerusalem artichoke were introduced into normalized mixture before pasteurize but in the second case, they were added after pasteurization with starter culture. The control sample was generated by the addition 11% of sucrose. The technological process was performed in the traditional way. Dynamic of accumulation of titratable acidity was investigated, titratable and active acidity were estimated.

Samples were evaluated for organoleptic properties by a taste panel of the 11 staff members and students of the Technology of Milk and Food Biotechnology Department. The participants were selected and trained in accordance with the ISO 8586-1 standard (1993). Requirements for the work of the group of assessors were according to ISO 8589 standard (2007). Sensory evaluation of the yogurt samples was carried out using quantitative descriptive (profile) method of analytical evaluation of foods modified for yogurt, containing in its composition Supplements ‘Selenium Alga Plus’. Each indicator was evaluated on a 5-point scale (Table 1).

**Table 1. Evaluation of samples**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taste</td>
<td>Odour</td>
</tr>
<tr>
<td>sweet enough, off-flavour</td>
<td>feebly marked strange odour</td>
</tr>
<tr>
<td>TTF, excessive or insufficiently pronounced, FF inadequate sweet</td>
<td>insufficiently precipitate</td>
</tr>
<tr>
<td>TTF, moderately sweet</td>
<td>moderate, FF</td>
</tr>
<tr>
<td>TPF, sweet</td>
<td>brightly-expressed FF</td>
</tr>
</tbody>
</table>

TPF – taste pure, fermented, without foreign flavour; FF – fermented flavour; HVD – homogeneous, viscous, dense consistency; CCF – the corresponding colour filler.

pH was measured using a pH meter (pH–410 with a glass combination electrode). Titratable acidity determination was carried out by titrimetric method using phenolphthalein indicator according to GOST 3624–92.

The value of the relative viscosity was determined using a viscometer VZ 246 GOST 9070–75 (see Fig. 1).
Investigation of qualitative indexes of the experiment pattern finished product was performed in comparison with the control. In the control and experimental samples was determined organoleptic characteristics, titratable and active acidity, relative viscosity, water holding capacity of the bunch. Microscopic sample was prepared.

**RESULTS AND DISCUSSIONS**

Influence of the concentration of Supplement ‘Selenium Alga Plus’ on organoleptic properties of yogurt is shown in Table 2. As seen from the data presented in Table 2, increasing the dose over 0.4% Supplement being felt specific taste with a pronounced odour of algae, which prevents increase the concentration Supplements over 0.4%.

**Table 2. Effect of the supplement concentration on the yoghurt organoleptic quality**

<table>
<thead>
<tr>
<th>Concentration of supplement</th>
<th>Quality indicators</th>
<th>Consistency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control sample</td>
<td>pure fermented milk</td>
<td>homogeneous, viscous</td>
</tr>
<tr>
<td>0.1%</td>
<td>pure fermented milk</td>
<td>homogeneous, viscous, there are small particles of brown colour</td>
</tr>
<tr>
<td>0.2%</td>
<td>pure fermented milk, typical filler tabled</td>
<td>homogeneous, viscous, there are small particles of brown colour</td>
</tr>
<tr>
<td>0.3%</td>
<td>pure fermented milk, typical filler tabled</td>
<td>homogeneous, viscous, there are particles of brown colour</td>
</tr>
<tr>
<td>0.4%</td>
<td>pure fermented milk, typical filler tabled</td>
<td>homogeneous, viscous, there are particles of brown colour</td>
</tr>
<tr>
<td>0.5%</td>
<td>fermented milk, taste with a specific taste and smell of algae</td>
<td>homogeneous, viscous, there are particles of brown colour</td>
</tr>
<tr>
<td>0.6%</td>
<td>fermented milk, taste with a pronounced taste and smell of algae</td>
<td>homogeneous, viscous, there are particles of brown colour</td>
</tr>
<tr>
<td>0.7%</td>
<td>fermented milk, specific taste, with a pronounced taste and smell of algae</td>
<td>homogeneous, viscous, there are particles of brown colour</td>
</tr>
<tr>
<td>0.8%</td>
<td>fermented milk, unpleasant specific</td>
<td>homogeneous, viscous, there are particles of brown colour</td>
</tr>
</tbody>
</table>
Thus, based on organoleptic features – the taste and smell – sample at a concentration of 0.4% supplement was selected, which corresponds to 50% of the normal intake of selenium per day by eating 200 g of product. Influence of concentration of stevioside on quality findings of yogurt are shown in Table 3.

Table 3. Effect of the concentration of stevioside on quality yogurt

<table>
<thead>
<tr>
<th>Concentration of stevioside</th>
<th>Taste and smell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control sample</td>
<td>pure fermented milk, moderately sweet</td>
</tr>
<tr>
<td>0.01</td>
<td>Pure fermented milk</td>
</tr>
<tr>
<td>0.02</td>
<td>Pure fermented milk</td>
</tr>
<tr>
<td>0.03</td>
<td>pure fermented milk, not sweet enough</td>
</tr>
<tr>
<td>0.04</td>
<td>pure fermented milk, not sweet enough</td>
</tr>
<tr>
<td>0.05</td>
<td>pure fermented milk, not sweet enough</td>
</tr>
<tr>
<td>0.06</td>
<td>pure fermented milk, not sweet enough with a specific flavour</td>
</tr>
<tr>
<td>0.07</td>
<td>pure fermented milk, not sweet enough with a specific flavour</td>
</tr>
<tr>
<td>0.08</td>
<td>pure fermented milk, not sweet enough with a specific flavour</td>
</tr>
<tr>
<td>0.09</td>
<td>fermented milk, overly sweet with a specific flavour</td>
</tr>
<tr>
<td>0.10</td>
<td>fermented milk, overly sweet with a specific flavour</td>
</tr>
</tbody>
</table>

As seen from the data presented in Table 3, a sample with a stevioside concentration of 0.05% has insufficient sweetness, but increasing concentration gives explicit presence of a specific after-taste. Therefore, to increase the sweetness of the product the syrup of Jerusalem artichoke was chosen. Therefore, it was decided to make further Jerusalem artichoke syrup. Findings of quality yogurt with stevioside and syrup of Jerusalem artichoke are shown in Table 4.

Table 4. Effect of concentration of syrup of Jerusalem artichoke on quality yogurt

<table>
<thead>
<tr>
<th>Concentration of stevioside</th>
<th>Concentration of syrup of Jerusalem artichoke</th>
<th>Concentration of Selenium Supplements Alga Plus</th>
<th>Taste and smell</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Pure fermented moderately sweet</td>
</tr>
<tr>
<td>0.05</td>
<td>0</td>
<td>0.4</td>
<td>fermented milk, not sweet enough</td>
</tr>
<tr>
<td>0.05</td>
<td>1.0</td>
<td>0.4</td>
<td>fermented milk, not sweet enough</td>
</tr>
<tr>
<td>0.05</td>
<td>2.0</td>
<td>0.4</td>
<td>fermented milk, not sweet enough</td>
</tr>
<tr>
<td>0.05</td>
<td>3.0</td>
<td>0.4</td>
<td>fermented milk, moderately sweet, pleasant sweet</td>
</tr>
<tr>
<td>0.05</td>
<td>4.0</td>
<td>0.4</td>
<td>fermented milk, unpleasantly sweet</td>
</tr>
<tr>
<td>0.04</td>
<td>3.0</td>
<td>0.4</td>
<td>fermented milk, not sweet enough</td>
</tr>
<tr>
<td>0.04</td>
<td>4.0</td>
<td>0.4</td>
<td>fermented milk, not sweet enough</td>
</tr>
<tr>
<td>0.04</td>
<td>5.0</td>
<td>0.4</td>
<td>Fermented milk, unpleasantly sweet</td>
</tr>
</tbody>
</table>
As can be seen from the data presented in Table 4, experiment sample with syrup of Jerusalem artichoke doses 0.05% had fermented sweet taste without foreign tastes and odours. Thus the following concentrations of sweeteners were selected: stevioside – 0.05% and Syrup of Jerusalem artichoke – 3%. The effect of Supplement concentration on the dynamic of accumulation acidity presented in Fig. 2.

![Figure 2](image1.png)

**Figure 2.** The influence of the concentration of supplement on the dynamic of the accumulation of acidity.

As seen from the data presented in Fig. 2, supplement, regardless of the application dose, no effect on titratable and active acidity. Dynamic accumulation of acidity in the experimental and control samples was similar. During 4 hours ripening titratable acidity and active pH reached $76 \pm 2^\circ T$, $4.62 \pm 0.05$, respectively.

Supplement ‘Selenium Alga plus’ must be added, together with the starter culture in the pasteurized milk as during heat treatment over 50°C, are losing 50% of selenium. Dietary supplement ‘Selenium Plus Alga’ packaged in a vacuum packaging, thus it does not require additional treatment to provide the product safety.

Influence of addition moment of sweeteners on the acidity accumulation dynamic is presented in Fig. 3.

![Figure 3](image2.png)

**Figure 3.** Acidity accumulation dynamics in yogurt depending on the insertion of additives.
As could be seen from the data presented in Fig. 1 significant differences between dynamics accumulation titratable acidity, test samples with additives and control sample was not observed. Next, sensory evaluation of yogurt samples was realized. These organoleptic characteristics of yogurt samples and control sample are displayed in Fig. 4.

![Sensory evaluation of samples](image)

**Figure 4.** Sensory evaluation of samples.

Test samples with additives introduced before and after pasteurization, did not have significant differences from the organoleptic quality of control sample. Additionally, there was no difference between dynamics of the acidity accumulation. Thus both sweeteners may be applied before and after pasteurization. However, in terms of the product microbiological safety, sweeteners should be added in the standardized milk before pasteurization. Microscopic control and experiment yogurt samples are presented in Fig. 5.

![Microscopic samples of yogurt](image)

**Figure 5.** Microscopic samples of yogurt: a) without supplement; b) with supplement.

At the time of visual estimation of microscopic sample of yogurt, lactic streptococcus and rods were discovered. The presence of foreign microorganisms (*Oidiumlactis, thermotolerant yeast* and *lactic acid bacillus*) was not observed. Characteristics of the finished product are presented in Table 5.
### Table 5. Quality characteristic of yogurt enriched Supplements ‘Selenium Alga Plus’

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Control sample</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taste</td>
<td>pure fermented milk</td>
<td>pure fermented milk, with a smack of filler, sweet</td>
</tr>
<tr>
<td>Flavour</td>
<td>pronounced aroma of fermented milk</td>
<td>pronounced aroma of fermented milk, characteristic tabled filler</td>
</tr>
<tr>
<td>Colour</td>
<td>white</td>
<td>corresponding to the colour of the filler, homogeneous throughout the mass</td>
</tr>
<tr>
<td>Consistence</td>
<td>homogeneous, moderately viscous, moderately dense</td>
<td>homogeneous, moderately viscous, moderately dense</td>
</tr>
<tr>
<td>Appearance</td>
<td>a glazy surface without whey separation</td>
<td>a glazy surface without whey separation, there are inclusions of supplement, evenly distributed throughout the mass</td>
</tr>
<tr>
<td>Acidity, °T</td>
<td>78 ± 2</td>
<td>79 ± 2</td>
</tr>
<tr>
<td>Active acidity, pH</td>
<td>4.62 ± 0.05</td>
<td>4.58 ± 0.05</td>
</tr>
<tr>
<td>Relative viscosity, seconds</td>
<td>41 ± 2</td>
<td>43 ± 2</td>
</tr>
</tbody>
</table>

**CONCLUSIONS**

1. The study concluded that the supplement ‘Selenium Alga Plus’ company Litoral can be used for the production of yogurt.
2. The dose entering of 0.4% of supplement ‘Selenium Alga Plus’ by weight of the mixture was established, that corresponds to 50% of normal selenium consumption per a day by eating 200g of the product. Based on these studies it can be concluded that the enrichment of the yogurt allows to obtain a product, which is a source of organic selenium.
3. Yogurt has a sweet flavour without foreign tastes and odours and it contains 0.05% stevioside and 3% artichoke syrup. Based on these studies it can be concluded that yogurt, enriched with selenium and sweeteners, is available for people suffering from diabetes.
4. It has revealed that the stage of the sweeteners entering before pasteurization in normalized mixture and after pasteurization with starter culture has not any effect on the quality of the finished product. Choosing the moment of the sweeteners entering before pasteurization provides safe product manufacturing.

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**REFERENCES**


Investigation of beaver meat obtained in Latvia

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Abstract. There is a high interest on the part of consumers to obtain meat from animals which have been reared as close to natural conditions as possible. Game meat, characterised by high nutritional value and specific organoleptic qualities, complies also to this claim. Game animals, including beaver, meat provide an excellent investment, diversification of many consumer meals. The meat of wild animals is more favourable for human health because it has lower saturated fatty acids. Investigations about biochemical composition of beaver meat are not very common worldwide.

The aim of study was evaluate biochemical composition of beaver meat hunted in Latvia. Therefore protein, amino acids, fat, ash, cholesterol content and fatty acid composition of beaver meat samples were done.

Conclusion was made that beaver meat samples protein content was 20.07–22.68% and fat content 3.31–5.27%. The sum of essential amino acids in beaver meat samples was determined from 0.99 mg 100 g⁻¹, less than other game meat. While the content of polyunsaturated fatty acids in meat samples of beaver (42.54%) was significantly higher than content of saturated (26.80%) or monounsaturated (27.42%) fatty acids. Ratio of polyunsaturated fatty acids n–6 : n–3 in beaver meat samples were 1.26, PUFA : SFA in beaver meat samples were 1.60 and cholesterol content 49.51 mg 100 g⁻¹ was lower in comparison of domestic or wild animals meat. From this point of view beaver meat is very healthy source of fat.

Key words: wild animals, beaver, meat, biochemical composition.

INTRODUCTION

Over the recent years, the popularity of wild game meat has increased and game is considered an important addition for human consumption. Currently, among consumers, there is increased interest in meat from animals kept in conditions as close as possible to the natural ones and beaver meat one of them. There are several reasons behind this fact: the high biological value of game meat has high protein content with broad spectrum of essential amino acids, low fat content with rich composition of poly-unsaturated fatty acids in comparison with meat of domestic animals. The game meat is an important source of B-group vitamins, micro- and macro-nutrients, and considerably high iron content; moreover it is free from drug residues and growth hormones (Serratosa et al., 2006).
It is clear that protein plays a role in promoting optimal health. New research reveals increasingly complex roles for protein and amino acids in regulation of body composition and bone health, gastrointestinal function and bacterial flora (Millward et al., 2008). The amino acid profile is important because some amino acids cannot be synthesized by human organism and therefore must be supplied by the diet. Since the amino acid composition of proteins from food animals is similar to human muscle, muscles are an excellent source of the amino acids needed for growth, repair, and human body after maintenance. Meat is one of the key proteins and essential amino acids sources (Tudor et al., 2009). Average game meat protein content reported 21–25% and contains all essential amino acids (Paleari et al., 2003).

Iron is an essential mineral, which plays a role in a variety of body functions. It is important in the early stage of students cognitive development (Lozoff et al., 2000), and for women in childbearing age (Ferguson et al., 2000). The iron deficiency can cause symptoms such as fatigue, decreased immunity (Ministry of Health, 2006). Many foods in the diet contain iron, but this iron is not always easily absorbed by your body. Heme iron is found in meat, fish and poultry, and is the form of iron that is most readily absorbed from stomach and taken up into your body after you eat it. Eating meat generally boost your iron levels far more than eating non-heme iron. It is concluded that the red meat is only source of zinc in the diet (Groff & Gropper, 2000).

The beavers (Castor fiber) are the largest rodents in Latvia. They are herbivorous (Zalewski et al., 2009). Beaver carcass meat fat characterise the high concentration of unsaturated fatty acids, ranging from 68.3–79.2% and that polyunsaturated fatty acids were dominant (Jankowska et al., 2005). However, wild beaver fat contains twice as much polyunsaturated fatty acid as the fat of farm beavers (Korzeniowski et al., 1999). This is likely because the main composition of the wild beaver diet (aquatic flora) is rich in polyunsaturated fatty acids. Polyunsaturated fatty acids are not synthesized by human organism therefore these must be committed with products of animal origin, mostly fish, but wild animal’s meat is also good source, in particular, the beaver meat.

The current limited consumption of beaver meat may be partly due to the lack of public knowledge of its nutritional quality. Several papers have reported the composition of this mammal meat in Lithuania and Poland and underlined its high quality protein and high percentage of polyunsaturated fatty acids (Jankowska et al., 2005; Razmaite et al., 2011). Only small number of studies on biochemical composition of game meat in Latvia has been done (Jemeljanovs et al., 2012; Strazdina et al., 2013). An evaluation of beaver meat biochemical composition could increase the interest in wider consumption and hunting of the beavers in Europe.

Therefore aim of our investigation was to analyse the compositional characteristics and to evaluate the nutritional quality of beaver meat.

**MATERIALS AND METHODS**

Overall there 16 meat samples of beaver were analysed. Research object was the biceps femoris (as the largest and most valuable cut) obtained from beavers.

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

For amino acid analysis dried, defatted meat samples were treated with constant boiling 6N hydrochloric acid in an oven at around 110°C for 23 h. Amino acids were
detected by using reversed-phase HPLC/MS (Waters Alliance 2695, Waters 3100, column XTerra MS C18 5 μm, 1x100 mm). Mobile phase (90% acetonitrile: 10% deionized water) 0.5 ml min⁻¹, column temperature 40°C.

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

For fatty acid analysis 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, an Alltech AT–FAME analytical column (fused silica 30 m and i.d. 0.25 mm), carrier gas He (2 mL min⁻¹) 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.

Cholesterol content was detected by Blur colorimetric method using spectrometer (Shmanenkov & Alijeva, 1973).

The ash content was determined after incineration at 525 ± 15°C 5 h¹ (ISO 936:1996).

Micronutrient amount of meat was measured according to ISO 6869:2002. Methods are based on comparison of radiation absorption emitted by free metal atoms that are forming by spraying incinerated sample and the concentrations of certain metal solutions in the flames. The atomic absorption was measured by using spectrometer AAnalyst 200.

**RESULTS AND DISCUSSION**

Biochemical composition of analysed beaver meat samples hunted in Latvia, Lithuania and Poland are presented in the Table 1.

<table>
<thead>
<tr>
<th>Source</th>
<th>Dry matter, %</th>
<th>Protein, %</th>
<th>Fat, %</th>
<th>Crude ash, %</th>
<th>Fe, mg 100 g⁻¹</th>
<th>Zn, mg 100 g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current study</td>
<td>24.93 ± 1.65</td>
<td>21.39 ± 1.32</td>
<td>4.29 ± 0.98</td>
<td>1.13 ± 0.00</td>
<td>0.84 ± 0.86</td>
<td>10.64 ± 0.79</td>
</tr>
<tr>
<td>Lithuania¹</td>
<td>23.50 ± 0.23</td>
<td>21.60 ± 0.12</td>
<td>0.51 ± 0.08</td>
<td>1.09 ± 0.00</td>
<td>~</td>
<td>~</td>
</tr>
<tr>
<td>Poland²</td>
<td>27.56 ± 1.58</td>
<td>21.70 ± 0.61</td>
<td>3.90 ± 1.59</td>
<td>1.29 ± 0.10</td>
<td>~</td>
<td>~</td>
</tr>
</tbody>
</table>

¹ Razmaite et al., 2011; ² Jankowska et al., 2005; average ± standard deviation; ~ not specified

The research demonstrated that the protein content is same in beaver meat samples harvested in different places (21–22%). The intramuscular fat content of investigated samples was 4.29% being higher than in Lithuanian (0.51%) or Polish (3.9%) study. It is known that the fat content of meat most affected by nutrition. The nutrition basis of the Eurasian beaver is composed of about 200 plant species and the composition of the diet is dependent on the availability of the food resources in their living habitat and season (Razmaite et al., 2011). Although the intramuscular fat content differed among investigations, beaver muscles can be considered as low-fat meat.

Lean red meat is an excellent source of trace elements, especially of iron and zinc (Li et al., 2005). The human system has an excellent capacity of processing the iron
The average content of iron and zinc in beaver meat samples detected respectively $10.84 \pm 0.86 \text{ mg 100 g}^{-1}$ and $10.64 \pm 0.79 \text{ mg 100 g}^{-1}$. Fimreite et al. (*electronic resource*) reported that content of zinc ranged from $15.20$ to $62.20 \text{ ppm (ppm ww)}$ in beaver muscles hunted in Norway.

The nutritional value of meat is determined mainly by amino acid content and composition. The composition of amino acids and the sum of essential amino acids determined in beaver meat harvested in Latvia was presented in Table 2.

**Table 2. The amino acid content in the beaver meat samples**

<table>
<thead>
<tr>
<th>Amino acids, g 100 g$^{-1}$</th>
<th>Mean content, g 100 g$^{-1}$ raw meat</th>
<th>Amino acids, g 100 g$^{-1}$</th>
<th>Mean content, g 100 g$^{-1}$ raw meat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valine</td>
<td>0.93 $\pm$ 0.15</td>
<td>Aspartic acid</td>
<td>1.61 $\pm$ 0.31</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.09 $\pm$ 0.06</td>
<td>Serine</td>
<td>0.67 $\pm$ 0.09</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.52 $\pm$ 0.22</td>
<td>Glutamine</td>
<td>2.95 $\pm$ 0.56</td>
</tr>
<tr>
<td>Lysine</td>
<td>1.71 $\pm$ 0.34</td>
<td>Proline</td>
<td>0.82 $\pm$ 0.19</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.08 $\pm$ 0.02</td>
<td>Glycine</td>
<td>0.85 $\pm$ 0.14</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.33 $\pm$ 0.03</td>
<td>Alanine</td>
<td>1.36 $\pm$ 0.08</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.10 $\pm$ 0.02</td>
<td>Tyrosine</td>
<td>0.61 $\pm$ 0.09</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.39 $\pm$ 0.09</td>
<td>Oxyproline</td>
<td>0.86 $\pm$ 0.08</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.42 $\pm$ 0.20</td>
<td>The sum of essential AA*</td>
<td>7.63</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.00 $\pm$ 0.05</td>
<td>Tryptophan : Oxyproline</td>
<td>3.8</td>
</tr>
</tbody>
</table>

* $\Sigma$ essential amino acids (AA), g 100 g$^{-1}$ = Thr + Val + Met + Ile + Leu + Trp + His + Lys.

The results of investigation show that the sum of essential amino acids calculated $7.63 \text{ g 100 g}^{-1}$ wet meat. The recommended intake of total irreplaceable or essential amino acids is $83.5 \text{ mg 100 g}^{-1}$ on kg of body weight per day (WHO/FAO/UNU, 2007), it is $5.8 \text{ g per human with body weight 70 kg}$. The ratio of essential to non-essential amino acids was 0.63.

As shown in Table 2, beaver muscle protein contained a high level of glutamic acid, which is main amino acid responsible for taste of meat – $2.95 \text{ g 100 g}^{-1}$ raw meat. It is lower than showed results of investigations in Lithuania – $3.3 \text{ g 100 g}^{-1}$ raw meat (Razmaite et al., 2011). The sequence of other amino acids is similar to other meats, namely aspartic acid, lysine, leucine, arginine, isoleucine, alanine, histidine, valine, glycine, threonine and methionine in decreasing amounts.

Tryptophan is one of valuable amino acid. The content of tryptophan is used as full value protein measure in product. Content of oxyproline is major as measure of connective tissue protein. Therefore ratio Tryptophan : Oxyproline is one of major indicators of nutrition value of product, this ratio in beaver meat samples calculated 3.8, it is similar with beef (3–4) but lower than pork (7.2) (Jemeljanovs et al., 2012).

When compared essential amino acids of beaver meat samples to the reference amino acid pattern of adults (Fig. 1) almost all detected scores were $> 100$ (except threonine).
Nowadays, histidine is considered to be an essential amino acid because of the detrimental effects on haemoglobin concentrations (Report of a Joint WHO/FAO/UNU Expert Consultation; WHO/FAO/UNU, 2007). Results of study show that beaver meat histidine amount determined three times higher in comparison with reference.

This comparison confirms that beaver muscle protein is of high quality and well-balanced in amino acid composition. The assumed amino acid score showed that biological value or the anticipated ability absorbed protein from beaver meat to fulfil human amino acid requirements is high.

Nutritional quality of meat is highly dependent on fatty acid composition of intramuscular fat. Composition of fatty acids, especially ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) is more significant for human health than total fat content. The composition of fatty acids, ratio n-6 : n-3 and PUFA : SFA, level of cholesterol in beaver meat samples assumed in Table 3.

Fatty acids more of all influenced cholesterol level in human blood are myristic, lauric and palmitic acid. From results of investigation we can establish that myristic and lauric acid drawn up less than 2% of all fatty acids and the average content of palmitic acid in beaver meat samples detected 11.09% from all fatty acids. Razmaite et al. (2011) reported that 23.05% palmitic acid was detected in beaver ham and 12.96% of all fatty acids. From nutritionists point of view ‘ideal fat’ SFA : MUFA : PUFA ratio is 33.33% : 33.33% : 33.33% (Medeiros et al., 2002). From results of investigation shown in Fig. 2, must be concluded that the sum of MUFA in beaver meat (27.42% of all fatty acids) is quite close to ‘ideal’.

The ratio polyunsaturated fatty acids to saturated was calculated 1.60, which was high. WHO/FAO (2003) and Wood et al. (2003) reported that recommended ratio PUFA : SFA must be higher than 0.4 and that domestic animals it has too low (Wood et al., 2003). Medeiros et al. (2002), reported that PUFA : SFA ratio of beef samples is 0.38. The results of beaver meat evaluation in Lithuania showed that this ratio 1.28 in thigh and 2.37 in tail (Razmaite et al., 2011).

Recently, nutritionists have focused not only to level of PUFA, but also to balance between them, therefore the n-6 : n-3 ratio lower than 4 was suggested by World health
Organization (WHO/FAO 2003). As it is shown in the Table 3, in the fatty acids of beaver meat this ratio was 1.26, which was significantly lower than the reference n–6 : n–3 ratio pattern.

**Table 3. Fatty acid composition of beaver meat samples (% of total fatty acids)**

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Fatty acid</th>
<th>Mean value</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saturated fatty acids (SFA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C 12:0</td>
<td>Lauric</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>C 14:0</td>
<td>Myristic</td>
<td>1.43</td>
<td>0.01</td>
</tr>
<tr>
<td>C 15:0</td>
<td>Pentadecylic</td>
<td>0.76</td>
<td>0.09</td>
</tr>
<tr>
<td>C 16:0</td>
<td>Palmitic</td>
<td>11.09</td>
<td>0.52</td>
</tr>
<tr>
<td>C 17:0</td>
<td>Margaric</td>
<td>0.11</td>
<td>0.03</td>
</tr>
<tr>
<td>C 18:0</td>
<td>Stearic</td>
<td>13.36</td>
<td>1.85</td>
</tr>
<tr>
<td></td>
<td>Monounsaturated fatty acids (MUFA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C 14:1</td>
<td>Myristoleic</td>
<td>1.43</td>
<td>0.01</td>
</tr>
<tr>
<td>C 15:1</td>
<td>cis-10-Pentadecenoic</td>
<td>0.16</td>
<td>0.00</td>
</tr>
<tr>
<td>C 16:1</td>
<td>Palmitoleic</td>
<td>6.37</td>
<td>0.52</td>
</tr>
<tr>
<td>C 17:1</td>
<td>Heptadecenoic (cis-10)</td>
<td>0.92</td>
<td>0.06</td>
</tr>
<tr>
<td>C 18:1 n-9cis</td>
<td>Oleic</td>
<td>15.02</td>
<td>1.02</td>
</tr>
<tr>
<td>C 18:1 n-9tr</td>
<td>Elaidic</td>
<td>3.30</td>
<td>0.12</td>
</tr>
<tr>
<td>C 20:1 n-9</td>
<td>Eicosenoic</td>
<td>0.06</td>
<td>0.01</td>
</tr>
<tr>
<td>C 20:1 n-11</td>
<td>Gadoleic</td>
<td>0.04</td>
<td>0.00</td>
</tr>
<tr>
<td>C 22:1 n-9</td>
<td>Erucic</td>
<td>0.12</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Polyunsaturated fatty acids (PUFA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C 18:2 n-6</td>
<td>Linoleic</td>
<td>18.83</td>
<td>2.61</td>
</tr>
<tr>
<td>C 18:3 n-3</td>
<td>α-Linolenic</td>
<td>17.86</td>
<td>1.32</td>
</tr>
<tr>
<td>C 18:3 n-6</td>
<td>γ-Linolenic</td>
<td>0.07</td>
<td>0.01</td>
</tr>
<tr>
<td>C 20:2</td>
<td>Eicosadienoic</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>C 22:2 n-6</td>
<td>Cis-13,16-docosadienoic</td>
<td>0.24</td>
<td>0.02</td>
</tr>
<tr>
<td>C 20:3 n-6</td>
<td>Dihomo-γ-linolenic</td>
<td>0.18</td>
<td>0.03</td>
</tr>
<tr>
<td>C 20:4 n-6</td>
<td>Arachidonic</td>
<td>4.49</td>
<td>0.52</td>
</tr>
<tr>
<td>C 20:5 n-3</td>
<td>Eicosapentaenoic</td>
<td>0.84</td>
<td>0.07</td>
</tr>
<tr>
<td>C 22:6 n-3</td>
<td>Docosahexaenoic</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>n-3*</td>
<td></td>
<td>18.71</td>
<td>2.13</td>
</tr>
<tr>
<td>n-6**</td>
<td></td>
<td>23.81</td>
<td>0.86</td>
</tr>
<tr>
<td>n-6 : n-3</td>
<td></td>
<td>1.26</td>
<td>0.90</td>
</tr>
<tr>
<td>PUFA : SFA</td>
<td></td>
<td>1.60</td>
<td>0.03</td>
</tr>
<tr>
<td>Cholesterol,</td>
<td></td>
<td>49.51</td>
<td>4.21</td>
</tr>
<tr>
<td>mg 100 g⁻¹</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* – Σ_n-3 = 20:3_n-3 + C22:6_n-3 + C22:5_n-3 + C20:5_n-3 + C18:4_n-3 + C18:3_n-3

** – Σ_n-6 = C22:2_n-6 + C20:2_n-6 + C18:3_n-6 + C22:4_n-6 + C20:3_n-6 + C18:2_n-6 + C20:4_n-6

The average cholesterol content of beaver meat samples was 49.51 mg 100 g⁻¹, which was significantly lower than cholesterol content of beef – 76.31 mg 100 g⁻¹ (Jemeljanovs et al., 2012). Nowadays, the food quality concept includes not only safety and technological quality, but also nutritional value and food diversification is considered as quality. Therefore consumption of beaver meat should suggest as meat diversification, is high quality protein source, lower cholesterol content and a better PUFA : SFA; n–6 : n–3 ratios for human health.
Figure 2. The sum of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in beaver meat (% of total fatty acids).

CONCLUSIONS

Beaver muscle protein is good in quality and well-balanced in amino acid composition as the level of essential amino acids determined 7.63 g 100g\(^{-1}\) raw meat. The amino acid score showed that biological value or the anticipated ability absorbed protein from beaver meat to fulfil human amino acid requirements is high.

In beaver meat, the content of monounsaturated (27.42%) saturated (26.63%) and polyunsaturated (42.54% of all fatty acids) are well balanced. The ratio polyunsaturated fatty acids to saturated was calculated 1.60 and ratio n–6 : n–3 – 1.26 are considered as advantageous for human health.

REFERENCES


Pigments in mint leaves and stems

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Abstract. Mint is the genus belonging to the Labiatae family and includes a huge diversity of varieties with different sensory properties. An important quality parameter is its colour, and the compounds responsible for it are pigments such as chlorophyll a and b, carotenoids etc. The aim of the current research was to determine the pigment content in the leaves and the stems of different mint varieties grown in Latvia. Mint of nine varieties (Mentha suaveolens ‘Apple mint’, Mentha suaveolens ‘Variegata’, Mentha spicata ‘Marokko’, Mentha piperita ‘Swiss’, Mentha piperita ‘Granada’, Mentha piperita f. citrate ‘Grapefruit’, Mentha piperita ‘Chocolate’, Mentha piperita ‘Almira’, Mentha piperita ‘Bavarian’) collected in Latvia was analysed. Chlorophyll a, chlorophyll b and carotenoids were determined spectrophotometrically in the acetone extracts of fresh leaves and stems at various wavelength 470, 645 and 662 nm. To analyse a relationship between chlorophyll a and b in the leaves and the stems a calculation of ratio a/b was performed. Additionally the colour of samples was measured in CIE L*a*b* system. For the statistical analysis – linear correlation, analysis of variance, hierarchical cluster analysis was performed. The differences were considered significant at P < 0.05. Among studied mint leaves the highest content of chlorophyll a and b, carotenoids was determined in the ‘Bavarian’ mint. The colour component L* value for variety ‘Bavarian’ leaves was one of the lowest among studied samples (showing a darker colour intensity). The stems of the mint variety ‘Bavarian’ had a high content of chlorophyll especially chlorophyll b. The highest content of carotenoids was determined in Mentha spicata ‘Marokko’. Analysing a relationship between the colour components L*a*b* and the content of pigments no significant correlations were determined.

Key words: mint varieties; chlorophyll a; chlorophyll b; colour.

INTRODUCTION

Peppermint (Mentha × piperita L.), belonging to the Labiatae family, is a large family of annual or perennial, herbaceous plants of 30–100 cm height, which is cultivated in temperate climates, in America, Europe and Asia (Arslan et al., 2010). Peppermint (Mentha piperita L.) is a natural hybrid of water mint (Mentha aquatica L.) and spearmint (Mentha spicata L.) and is cultivated globally for its use as a flavouring in foods and also in some shampoos and soaps (Tarhan et al., 2010). Mint is one of the most important and common flavours in the world coming after vanilla and citrus flavours (Arslan et al., 2010). Peppermint oil and some of its constituents is known for antimicrobial and antioxidant properties. Peppermint is one of the most widely consumed single ingredients in herbal teas, and the essential oil of peppermint is used in traditional medicine (Lv et al., 2012). Peppermint has significant antimicrobial and
antiviral activities, strong antioxidant and antitumor actions, and exhibits some antiallergenic potential (Skalicka-Woźniak & Walasek, 2014).

Among the major components found in peppermint leaves are fatty acids such as linoleic, linolenic, and palmitic acid. A variety of volatile compounds, mainly menthol, menthone and isomenthone have also been identified along with β-carotene, chlorophyll, α- and γ-tocopherols and ascorbic acid (Figueroa Pérez et al., 2014).

Plant pigment is a generic expression used to designate a large number of coloured molecules. On the basis of their chemical structure, they can be classed into 5 families i.e. tetrapyrroles (e.g. chlorophyll), carotenoids (e.g. β-carotene), phenolic compounds (e.g. teafavin) and N-heterocyclic compounds (e.g. betalains) (Schoefs, 2002). Appreciable amounts of carotenoids are present in fresh tea leaves, but this value is greatly decreased during tea processing, leading to various degradation products (Ravichandran, 2002). In a Japanese study on green teas there were detected 38 carotenoids (6 of them were unknown) (Suzuki & Shioi, 2003). Among these tea pigments, pheophytins a and b were abundant, followed by chlorophylls a and b, and carotenoids such as β-carotene and lutein in lower concentrations. All these pigments exhibited significant antioxidant activities against hydroperoxide generation, in the order chlorophyll a > lutein > pheophytin a > chlorophyll b > β-carotene > pheophytin b (Loranty et al., 2010). Chlorophylls and carotenoids are frequent organic food components because they are naturally present in plants, giving their specific colouration. In vivo, these pigments play key roles in photosynthesis (Schoefs, 2002). Chlorophyll is the principal photoreceptor in photosynthesis, the light-driven process by which carbon dioxide is fixed to yield carbohydrates and oxygen. While carotenoids are a class of natural fat-soluble pigments found principally in plants, algae and photosynthetic bacteria, where they play a critical role in the photosynthetic process (Ong & Tee, 1992) and also protect chlorophyll from a photooxidative destruction (Siefermann-Harms, 1987; Giri et al., 2013). An important quality parameter of peppermint is its colour, and the compounds responsible for it are pigments such as chlorophyll a and b, carotenoids etc. There are only few papers about pigments in herbs and teas (Loranty et al., 2010).

The aim of the current research was to determine the pigment content in the leaves and the stems of different mint varieties grown in Latvia.

**MATERIALS AND METHODS**

**Plant materials**

Determination of chlorophyll $a$, chlorophyll $b$ and carotenoids

A 0.4 g sample of plant material was homogenized, extracted with 10 ml of acetone in a conical flask with a magnetic stirrer at 700 rpm for 15 minutes at room temperature. The supernatant was separated and the extraction was repeated. The extraction process was done in triplicate. The solution was analysed for chlorophyll $a$ (Ch $a$), chlorophyll $b$ (Ch $b$) and carotenoids content by a spectrophotometer JENWAY 6300 at various wavelengths 470, 645 and 662 nm using a glass cuvette. The equations used for the quantification are given in Table 1. Results were expressed as mg g$^{-1}$ of plant material.

Table 1. The equations for calculation of chlorophyll $a$, chlorophyll $b$ and the total carotenoids content (Sumanta et al., 2014)

<table>
<thead>
<tr>
<th>Parameter, units</th>
<th>Equations*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll $a$, mg ml$^{-1}$</td>
<td>$C_{Cha} = 12.25 A_{662} - 279 A_{645}$</td>
</tr>
<tr>
<td>Chlorophyll $b$, mg ml$^{-1}$</td>
<td>$C_{Chb} = 21.5 A_{645} - 5.1 A_{662}$</td>
</tr>
<tr>
<td>Total chlorophyll, mg ml$^{-1}$</td>
<td>$C_{cht} = C_{Cha} + C_{Chb}$</td>
</tr>
<tr>
<td>Ratio between chlorophyll $a$ and $b$</td>
<td>$R_{a/b} = \frac{C_{Cha}}{C_{Chb}}$</td>
</tr>
<tr>
<td>Total carotenoids, mg ml$^{-1}$</td>
<td>$C_{Ca} = \frac{1000A_{470} - 1.82 C_{Cha} - 85.02 C_{Chb}}{198}$</td>
</tr>
</tbody>
</table>

* In column Equations following abbreviations are used: $C_{Cha}$ – concentration of chlorophyll $a$ in extract; $C_{Chb}$ – concentration of chlorophyll $b$ in extract; $C_{cht}$ – concentration of total chlorophyll in extract; $C_{Ca}$ – concentration of carotenoids in extract; $A_{662}$ – absorbance of the extract at wavelength 662 nm; $A_{645}$ – absorbance of the extract at wavelength 645 nm; $A_{470}$ – absorbance of the extract at wavelength 470 nm.

Colour analysis of mint leaves

Colour of mint was measured in CIE L*$a*$b* colour system using a colorimeter ColorTec PCM (Accuracy Microsensors Inc., USA). Before the measurement, the colorimeter was calibrated using a white reference tile and a light trap (black tile). Ten random leaves were measured and the mean values were reported for each sample. In colour measurement, CIE L*$a*$b* coordinates show the degree of brightness (L), the degree of redness (+a), or greenness (–a), and the degree of yellowness (+b), or blueness (–b), respectively (Tarhan et al., 2010).

Statistical analysis

The results (mean, standard deviation, $P$ value) were processed by mathematical and statistical methods. For the statistical analysis - linear correlation, analysis of variance, hierarchical cluster analysis was performed. Significance was defined at $P < 0.05$. Hierarchical cluster analysis was performed to distinguish similar or close species. The method used was linkage between groups. The distances between samples were calculated using square Euclidean distances. As the pre-treatment of data, transformation of values of variables (average zero and standard deviation 1) called Z scores was carried out. The dendrogram similarity scales generated by the SPSS program ranged from zero (greater similarity) to 25 (lower similarity).
RESULTS AND DISCUSSION

Chlorophyll a and chlorophyll b analysis
Chlorophyll a and b differed between all the tested mint samples (Table 2). Other scientists also reported that changes in the colour and the content of chlorophylls were related to the genotype but not to the growing conditions (Bekhradi et al., 2015). The highest content of chlorophyll a, chlorophyll b and total chlorophyll in the ‘Bavarian’ mint leaves was observed 0.849, 0.179 and 1.028 mg g\(^{-1}\) respectively.

Other authors reported chlorophyll content in dry tea of *M. piperita* 75.2 \(\mu\)g g\(^{-1}\) (Loranty et al., 2010), but there was not more detailed description available about analysed *Mentha* species. For the fresh samples similar results were obtained for dill that contains 144 mg 100 g\(^{-1}\) chlorophyll (Lisiewska et al., 2004). The major chlorophylls in plants include chlorophyll a and chlorophyll b, which are usually present at a ratio of 3 (Chen & Chen, 1993). In the tested mint leaves a ratio between chlorophyll a and chlorophyll b ranged from 3.79–7.64, meaning that chlorophyll a is the main form of chlorophyll in the leaves. In *M. officinalis* tea significant levels of chlorophyll b were also present (72.9 \(\mu\)g g\(^{-1}\) dry tea from Tetley) and intra-species variation in chlorophyll b levels was seen to occur depending on the source company of tea (Loranty et al., 2010). In the stems chlorophyll content was significantly lower than in the leaves.

In the stems a ratio between chlorophyll a and chlorophyll b was lower and ranged from 0.48–1.48, meaning that chlorophyll b is more significant pigment in the stems. Analysing relationships between the composition of chlorophylls in the leaves and the stems several trends were observed. A strong correlation between the content of chlorophyll b in the tested leaves and stems was observed \((r = 0.89)\), whereas for chlorophyll a such tendency was not observed \((r = 0.06)\).

Table 2. Chlorophyll in mint leaves and stems

<table>
<thead>
<tr>
<th>Plant material</th>
<th>Chlorophyll, mg g(^{-1})</th>
<th>Ratio a/b*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ch a</td>
<td>Ch b</td>
</tr>
<tr>
<td><em>Mentha suaveolens</em> ‘Apple mint’</td>
<td>0.525 ± 0.001</td>
<td>0.071 ± 0.001</td>
</tr>
<tr>
<td>Stems</td>
<td>0.042 ± 0.002</td>
<td>0.054 ± 0.006</td>
</tr>
<tr>
<td><em>Mentha suaveolens</em> ‘Variegata’</td>
<td>0.351 ± 0.006</td>
<td>0.075 ± 0.002</td>
</tr>
<tr>
<td>Stems</td>
<td>0.077 ± 0.004</td>
<td>0.068 ± 0.002</td>
</tr>
<tr>
<td><em>Mentha spicata</em> ‘Marokko’</td>
<td>0.582 ± 0.009</td>
<td>0.153 ± 0.004</td>
</tr>
<tr>
<td>Stems</td>
<td>0.050 ± 0.001</td>
<td>0.105 ± 0.009</td>
</tr>
<tr>
<td><em>Mentha piperita</em> ‘Swiss’</td>
<td>0.507 ± 0.021</td>
<td>0.066 ± 0.001</td>
</tr>
<tr>
<td>Stems</td>
<td>0.084 ± 0.002</td>
<td>0.074 ± 0.005</td>
</tr>
<tr>
<td><em>Mentha piperita</em> ‘Granada’</td>
<td>0.518 ± 0.022</td>
<td>0.087 ± 0.001</td>
</tr>
<tr>
<td>Stems</td>
<td>0.106 ± 0.001</td>
<td>0.093 ± 0.004</td>
</tr>
<tr>
<td><em>Mentha piperita</em> f. citrate ‘Grapefruit’</td>
<td>0.519 ± 0.013</td>
<td>0.073 ± 0.005</td>
</tr>
<tr>
<td>Stems</td>
<td>0.063 ± 0.002</td>
<td>0.091 ± 0.008</td>
</tr>
<tr>
<td><em>Mentha piperita</em> ‘Chocolate’</td>
<td>0.361 ± 0.013</td>
<td>0.076 ± 0.003</td>
</tr>
<tr>
<td>Stems</td>
<td>0.077 ± 0.001</td>
<td>0.078 ± 0.006</td>
</tr>
<tr>
<td><em>Mentha piperita</em> ‘Almira’</td>
<td>0.321 ± 0.005</td>
<td>0.072 ± 0.001</td>
</tr>
<tr>
<td>Stems</td>
<td>0.108 ± 0.007</td>
<td>0.073 ± 0.002</td>
</tr>
<tr>
<td><em>Mentha piperita</em> ‘Bavarian’</td>
<td>0.849 ± 0.020</td>
<td>0.179 ± 0.008</td>
</tr>
<tr>
<td>Stems</td>
<td>0.107 ± 0.004</td>
<td>0.157 ± 0.006</td>
</tr>
</tbody>
</table>
**Carotenoids analysis**

The basic pigments of green plants are chlorophylls, always accompanied by carotenoids. The highest content of carotenoids was observed in *Mentha spicata* ‘Marokko’ stems and leaves, 16.9 and 10.3 mg 100 g\(^{-1}\) respectively (Fig.1). According to various authors the content of carotenoids ranges from 7.2 to 36.4 mg per 100 g of fresh matter of leafy vegetables (Ben-Amotz & Fischler, 1998; Kmiecik & Lisiewska, 1999). Carotenoids content in other herbs like dills are significantly higher 30.3 mg 100 g\(^{-1}\) (Lisiewska et al., 2004).

![Figure 1. Carotenoid content in mint leaves and stems.](image)

Carotenoid content differed between all the tested mint samples. In leafy *Lactuca sativa* salads also large differences were observed in carotenoids concentrations among the different cultivars with an almost identical phenotype (Reif et al., 2013). In part of samples significantly higher (*P < 0.05*) concentration of carotenoids in the stems was observed (*Mentha suaveolens* ‘Apple mint’, *Mentha suaveolens* ‘Variegata’, *Mentha spicata* ‘Marokko’, *Mentha piperita* ‘Bavarian’). Significantly higher (*P < 0.05*) content of carotenoids in the leaves only in *Mentha piperita* f. citrata ‘Grapefruit’ was determined. For other samples differences between the leaves and the stems were not significant (*P > 0.05*).

**Colour analysis of mint leaves**

Colour parameters of the mint samples presented the significant differences between species (Table 3). *Mentha suaveolens* ‘Apple mint’ and *Mentha piperita* ‘Swiss’ mint is lighter (higher L* value) more green (a* parameter is more negative) and more yellow (higher value parameter b*) compared to other samples. The darkest mint samples were *Mentha piperita* ‘Granada’, *Mentha piperita* ‘Almira’, *Mentha piperita* ‘Bavarian’ (lower L* parameter).
Table 3. Colour characteristics of the mint samples

<table>
<thead>
<tr>
<th>Plant material</th>
<th>Colour parameter values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L*</td>
</tr>
<tr>
<td><strong>Mentha suaveolens ‘Apple mint’</strong></td>
<td>39.44 ± 3.59</td>
</tr>
<tr>
<td><strong>Mentha suaveolens ‘Variegata’</strong></td>
<td>38.60 ± 2.97</td>
</tr>
<tr>
<td><strong>Mentha spicata ‘Marokko’</strong></td>
<td>38.33 ± 2.13</td>
</tr>
<tr>
<td><strong>Mentha piperita ‘Swiss’</strong></td>
<td>40.04 ± 2.86</td>
</tr>
<tr>
<td><strong>Mentha piperita ‘Granada’</strong></td>
<td>32.36 ± 1.80</td>
</tr>
<tr>
<td><strong>Mentha piperita f. citrata ‘Grapefruit’</strong></td>
<td>36.88 ± 2.84</td>
</tr>
<tr>
<td><strong>Mentha piperita ‘Chocolate’</strong></td>
<td>35.23 ± 2.94</td>
</tr>
<tr>
<td><strong>Mentha piperita ‘Almira’</strong></td>
<td>32.80 ± 1.49</td>
</tr>
<tr>
<td><strong>Mentha piperita ‘Bavarian’</strong></td>
<td>32.44 ± 1.35</td>
</tr>
</tbody>
</table>

Correlation analysis was performed to determine the relationship between colour parameters and pigment concentration. Carotenoids are in colour range from yellow to dark red. In mint there was weak \((r = 0.25)\) correlation between the content of carotenoids and colour b* parameter. There was also weak negative correlation between the lightness and the concentration of chlorophyll a, chlorophyll b and total chlorophyll, similarly also between the a* colour parameter and the chlorophyll content.

Hierarchical cluster analyses

Hierarchical cluster analysis was applied to a data set of three variables (chlorophyll a, chlorophyll b, and carotenoids) and nine mint species. According to the hierarchical cluster analysis, mints can be grouped as follows:

- the cluster A – *Mentha spicata* ‘Marokko’ with the highest content of carotenoids and also high content of chlorophyll a, chlorophyll b;
- the cluster B – *Mentha piperita* ‘Bavarian’ with the highest content of chlorophyll a, chlorophyll b, and average content of carotenoids;
- the cluster C – all other studied mint samples.

Additionally hierarchical cluster analysis was applied to a data set of colour parameters (L*, a*, b*) and according to these parameters mints can be classified in three clusters:

- the cluster A – *Mentha piperita* ‘Almira’ with darker colour and more yellow and green colour intensity;
- the cluster B – *Mentha piperita* ‘Granada’, *Mentha piperita* ‘Chocolate’, *Mentha piperita* ‘Bavarian’ with darker colour and less intense yellow and green colour;
- the cluster C – all other studied mint samples.

CONCLUSIONS

The content of pigments and the colour of analysed mint species differed significantly. The highest content of chlorophyll a, chlorophyll b and total chlorophyll in *Mentha piperita* ‘Bavarian’ mint leaves was observed, whereas *Mentha spicata* ‘Marokko’ has the highest content of carotenoids. There was not established correlation between the concentration of pigments and the parameters characterizing colour.
Hierarchical cluster analysis can be used to differentiate the mint species according to the pigment concentration and the colour parameters.

ACKNOWLEDGEMENTS. The authors are thankful to Lolita Duge (farm ‘Tereni’), who has contributed to the studies with advice, suggestions and research materials.

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Evaluation of size distribution of fat globules and fat and protein content in Estonian Goat milk

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*COrrespondence: vilma.tatar@emu.ee

Abstract. The objectives of this study were to investigate size distribution of fat globules, fat and protein content in Estonian goat milk. The bulk milk samples were collected from three different crossbreed goat herds. These herds consist of 30% of the Saanen breed and 70% did not belong to any certain breed. Lactation of goats was scattered over the year. Goat milk samples were examined weekly during a 10 month period. Fat and protein content in goat milk ranged from 3.09% to 5.04% and from 2.74% to 3.96% respectively. Fat content in cow milk ranged from 3.77% to 4.75% and protein content ranged from 3.14% to 3.75%. The average fat content in goat milk (3.88%) was less than the mean fat content in cow milk (4.0%). The average protein content in goat milk (3.41%) was higher than the mean protein content in cow milk (3.38%). Depending on the season, fat and protein content in goat milk varied by as much as 0.38% and 0.28% accordingly. The diameter of milk fat globules (MFG) was estimated using microscope Nikon SMZ 1000, equipped with the digital camera Nikon DS-U2/L2 USB and the software NIS-Elements D3.1. The average diameter of fat globules was 2.22 μm, ranging from 0.34 to 6.99 μm. The average size distribution of MFG had unimodal and slightly right skewed shape: 5.7% of globules were in range 0.5–1.0 μm, 15.9% in range 1.0–1.5 μm, 22.1% in range 1.5–2.0 μm, 21.0% in range 2.0–2.5, 16.1% in range 2.5–3.0 μm, 10.0% in range 3.0–3.5 μm, 4.3% in range 3.5–4.0 μm, 0.9% in range 4.5–5.0 μm.

Key words: goat milk, fat, protein, fat globules size distribution.

INTRODUCTION

The percentage of goat milk in total milk consumption has significantly increased during recent decades in Estonia. Therefore, more in-depth knowledge about the composition and properties of it is needed, especially in context of dairy production (Haenlein. 2004; Sanz Ceballos et al., 2009).

According to studies, carried out in different countries, fat content in goat milk ranges from 2.75% (Jandal, 1996) to 5.23% (Sanz Ceballos et al., 2009) and protein content from 2.98% to 3.66% (Strzałkowska et al., 2009) in average. Fat and protein content of goat milk depends on feeding, breed, individuals, parity, season, management, environmental conditions, locality, stage of lactation and health status of the udder (Park et al., 2007; Rewati Raman Bhattarai, 2012).

Depending on the stage of lactation and goat breeds, an average content of casein in goat milk varies from 1.06–3.01g 100g⁻¹, lactose content from 3.85–5.46 g 100g⁻¹, total solids from 9.8–15.9 g 100g⁻¹ (Salem et al., 2004; Strzałkowski et al., 2009).
average pH in goat milk ranges from 6.6–6.9 (Salem et al., 2004), density from 1025.7–1029.8 g L⁻¹ (Strzałkowski et al., 2009). Microstructure of goat milk has been studied less although the size of milk fat globules (MFG) significantly affects the valorisation of milk (Sanz Ceballos et al., 2009), especially into fat-rich products. Size of MFG has an important impact on the smoothness or hardness of cheese also (Park et al., 2007).

While goat milk has been investigated quite profoundly in many countries (Pisanu et al., 2013; Attaie and Richtert, 2000; Strzałkowska et al., 2009), no information about the content and microstructure of it can be found concerning Estonia. The aim of current research was to study the size distribution of MFG, fat and protein content in Estonian goat milk.

**MATERIALS AND METHODS**

The bulk milk samples were collected weekly from three Estonian goat herds during 10 months. Samples were cooled down and stored at 5°C after milking and all analyses were performed in the same day. Milk fat content and the size distribution of MFG were analysed in Estonian University of Life Sciences, Department of Food Science and Technology.

For estimation of the particle size in milk indirect methods basing on dynamic light scattering (DLS) and laser light scattering (LLS) have been used mainly (Attaie & Richtert, 2000; Michalski et al., 2003; Mootse et al., 2014; Sats et al., 2014). Results of these reflect the hydrodynamic diameter of particles which differs from real size to a certain extent. Fat globules can be measured directly also using light microscopy. In our experiments MFG size distribution was examined by the Microscope Nikon SMZ 1000, equipped with the Digital Camera (DC) Nikon DS-U2/L2 USB. For image processing software NIS-Elements D3.1 was used.

To estimate MFG size distribution, 10 μl of goat milk was diluted in 2 ml of distilled water. Diluted milk (1:200) was inserted into a 0.004 μl volume chamber and 40 times enlarged images of it were recorded under the microscope by the digital camera DC. Photographing was carried out with three different focusing depths on the volume chamber (Fig. 1). The diameter of fat globules was determined from these pictures later on.

![Figure 1. Photographing a unit volume chamber with Microscope Nikon SMZ 1000.](image)
MFG size of individual milk samples was described by average, standard deviation, minimum and maximum values. To derive size distribution of MFG, the results were grouped into 0.5 μm range groups and tabulated accordingly (cf. Table 2).

Protein content was estimated in Estonian Animal Recording Centre with Analysers (FOSS Electric, Denmark), using international standard methods. Milk fat content was estimated by the standard Gerber method. For comparative analysis the average fat and protein content of Estonian cow milk estimated in Estonian Animal Recording Centre was used.

Data were analysed and according illustrations were constructed using statistical package R and MS Excel.

RESULTS AND DISCUSSION

Fat content of the studied goat milk varied from 3.09% to 5.04%. An average fat content was 3.88% (Table 1), which is somewhat higher than in a study conducted in the US (Park et al., 2007). Protein content of Estonian goat milk ranged between 2.74% and 3.96%. The average protein content was 3.41%, which is similar to references in literature (Park et al., 2007). Differences in the results can be attributed to regional peculiarities, breeding conditions, feeding, etc. (Salem et al., 2004; Park et al., 2007; Sanz Ceballos et al., 2009; Rewati Raman Bhattarai, 2012). Comparing average fat and protein content in Estonian goat milk with corresponding parameters of cow milk in Estonia, it turned out that the average fat content in goat milk was a bit lower and average protein content a little higher than that in cow milk.

Table 1. Ranges in level, average fat and protein content, standard errors (SE) of goat and cow* milk

<table>
<thead>
<tr>
<th>Component, %</th>
<th>Ranges in level, %</th>
<th>Average, %</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>goat (cow)</td>
<td>goat (cow)</td>
<td>goat (cow)</td>
</tr>
<tr>
<td>Fat</td>
<td>3.09–5.04 (3.77–4.75)</td>
<td>3.88 (4.00)</td>
<td>0.46 (0.23)</td>
</tr>
<tr>
<td>Protein</td>
<td>2.74–3.96 (3.14–3.75)</td>
<td>3.41 (3.38)</td>
<td>0.27 (0.43)</td>
</tr>
</tbody>
</table>

* Estonian Animal Recording Centre, 2013.

Seasonal variation existed in the fat and protein content (Fig. 2). The lowest average fat and protein content (3.65%, 3.22%) was during spring-summer season, between April and June. The highest average fat and protein content were in October-December and in July-September accordingly.

The average diameter of Estonian goat MFG size was 2.22 μm, ranging from 0.34 μm to 6.99 μm. This range exceeded results presented by El-Zeini (2006) who investigated MFG size in the buffalo, sheep, cow, camel and goat milk. His study showed that the size of buffalo fat globules was in between 0.1–4.0 μm which was less than that of sheep (by 55.3%), cow (by 68.4%), goat (by 73.3%) and camel (by 80.6%). Differences in goat MFG size (0.14–5.70 μm) with our results (0.34–6.99 μm) could be explained by different methods used during the study.
Figure 2. Average goat milk fat and protein content during different periods of the year.

Most fat globules (22.11%) in Estonian goat milk had a diameter of 1.5–1.99 μm, followed by 21.02% with diameter of 2.00–2.49 μm, and 16.05% of globules had diameter of 2.5–2.99 μm (Table 2). The smallest number of globules (0.03%) had diameter of 6.5–6.99 μm. Mean size of fat globules and their distribution did not considerably differ of these parameters in cow milk presented by El-Zenini (2006).

Table 2. Fat globules mean size distribution of goat milk compared to it in cow milk

<table>
<thead>
<tr>
<th>Goat milk</th>
<th>MFG Diameter (μm)</th>
<th>Distribution, %</th>
<th>Cow milk*</th>
<th>MFG Diameter (μm)</th>
<th>Distribution, %</th>
<th>Goat milk**</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00–0.49</td>
<td>0.20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.50–0.99</td>
<td>5.74</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00–1.49</td>
<td>15.85</td>
<td>1–2</td>
<td>19.01</td>
<td>37.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.50–1.99</td>
<td>22.11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.00–2.49</td>
<td>21.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.50–2.99</td>
<td>16.05</td>
<td>2–4</td>
<td>49.40</td>
<td>51.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.00–3.49</td>
<td>9.86</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.50–3.99</td>
<td>4.27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.00–4.49</td>
<td>2.62</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.50–4.99</td>
<td>0.89</td>
<td>4–6</td>
<td>19.61</td>
<td>4.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.00–5.49</td>
<td>0.38</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.50–5.99</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.00–6.49</td>
<td>0.08</td>
<td>6–8</td>
<td>3.59</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.50–6.99</td>
<td>0.03</td>
<td>≥8</td>
<td>8.36</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*El-Zenini (2006)
** Distribution % according to El-Zenini (2006) MFG diameter

Table 3 presents the descriptive statistics of MFG size characteristics. The average MFG diameter in different samples varied between 1.38 and 2.73 μm. The minimum and maximum MFG diameter was on an average 0.86 μm and 5.05 μm respectively. The total range of MFG size considering all milk samples exceeded from 0.34 μm to 6.99 μm.
Table 3. Descriptive statistics of MFG size characteristics

<table>
<thead>
<tr>
<th>MFG size distribution characteristics</th>
<th>Average</th>
<th>Standard deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>2.22</td>
<td>0.388</td>
<td>1.38</td>
<td>2.73</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.77</td>
<td>0.178</td>
<td>0.48</td>
<td>1.11</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.86</td>
<td>0.283</td>
<td>0.34</td>
<td>1.38</td>
</tr>
<tr>
<td>Maximum</td>
<td>5.05</td>
<td>0.967</td>
<td>3.29</td>
<td>6.99</td>
</tr>
</tbody>
</table>

On an average over all milk samples the MFG size distribution was unimodal and lightly right skewed (Fig. 3). But distributions of individual samples were quite variable – there existed unimodal MFG size distributions with small variability as well multimodal MFG size distributions with large variability plus different kind of intermediate variants.

Our analyses revealed only some relationships between MFG size distribution characteristics and other traits. There was statistically significant intermediate positive correlation between average and standard deviation of MFG size distribution – the variability of MFG size was bigger if the average MFG size was bigger (correlation coefficient $r = 0.38$, $p = 0.037$; Fig. 4). Similar positive relationship was discovered between average MFG size and minimum and maximum MFG size.

There was only a weak negative and statistically non-significant correlation between average MFG diameter and milk fat percentage ($r = -0.10$, $p = 0.59$). The relationships of average MFG diameter with other milk parameters were even weaker. The correlations of minimal MFG diameter with milk fat and protein content were negative, intermediate and statistically significant ($r = -0.41$, $p = 0.023$ and $r = -0.46$, $p = 0.016$, respectively) – if the milk fat and protein percentage was higher, MFG-s were smaller.
There was a negative intermediate statistically significant relationship between average MFG size and sampling month ($r = -0.46, p = 0.011$; Fig. 5). As this relationship may be caused also by the different sampling times in different farms and the number of samples in present study was quite small, the future research is needed.

The average MFG diameter variation among farms was statistically significantly different ($p < 0.001$; analysis of variance), whereas this difference remained also after considering the effect of sampling month. The average MFG diameter in Farm 1 samples was on an average 0.78 and 0.61 µm smaller than the average MFG diameter in Farm 2 and Farm 3 samples ($p < 0.001$ and $p = 0.011$, respectively; Tukey post-hoc test; Fig. 6). Differences in MFG mean diameter may be influenced primarily by the structure of herds, especially concerning breed.
Differences in composition between Estonian goat and cow milk are quite small. The average total fat content in cow milk is higher and total protein content is less than that in goat milk a little bit. Range of fat and protein content (in comparison with cow's milk) is more extensive in goat milk. The average size of MFG and the size distribution range of particles in goat milk are quite similar to these parameters in cow’s milk although some differences do exist also. There was a small negative statistically not relevant link between fat content and the diameter of MFG in goat milk. However, some statistically relevant negative relations were revealed between the minimum size of milk fat globules and the content of fat and protein. The higher was fat and protein content in milk, the higher was the amount of minor fat globules.

REFERENCES


Stability of rapeseed oil with horseradish *Amorica rusticana* L. and lovage *Levisticum officinale* L. extracts under medium temperature accelerated storage conditions

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**Abstract.** This study examined the antioxidant activity of horseradish leaves and lovage leaves and stems extracts added to crude rapeseed oil, under medium temperature accelerated storage conditions. To evaluate efficiency of plant extracts they were added to oil in different concentrations (0.25, 0.5, 1.0 and 1.5%). As a control rapeseed oil without extracts where analysed. For comparison 0.01% butylatedhydroxytoluene (BHT) were added to oil. Efficiency of extracts in oil where tested at +60 ± 1°C in the dark for 22 days. For all samples peroxide value, acid value and 2,2-diphenyl-1-picrylhydrozyl (DPPH˙) activity were determined. In all steps of the experiments for samples with extract peroxide value was significantly (P < 0.05) lower comparing to the control. The control sample without extract reached 15meq O₂ kg⁻¹ oil (maximal allowed value in Latvian legislation) in 3 days. The best results showed the horseradish leaves extract (1%) and the lovage leave extract (1.5%) reaching this value in 8.3 days and 7 days, respectively. DPPH˙ activity of the oil was compared after 3 days (when blank sample reached maximal allowed a peroxide value) and it shown that for all samples it was higher compared to the control sample. The highest activity showed the samples with horseradish leave extracts. A acid value in oil samples changed slightly. Lovage leave as stem and horseradish leave extracts could be successfully used for retarding of oxidation of rapeseed oil and in further experiments their activity in meat products will be tested.

**Key words:** horseradish, lovage, extract, rapeseed oil, oxidation.

**INTRODUCTION**

Antioxidants are used as additives in food for centuries. They are mainly used to prevent accumulation of the free radicals, strengthening the food oxidative stability (Halliwell, 1995; Giese, 1996). From the viewpoint of the nutrition science and the production benefits, it is highly desirable to control the oxidation by the addition of the antioxidants, providing appropriate food quality. Antioxidants are added to foods to control lipid oxidation and reduce the free radical concentration in human diet, as well as the consumption of the free radicals into the body (Pokorny, 2007).

In the recent years are a growing interest in plant origin supplements (Brielman et al., 2006; Naczk & Shahidi, 2006), and efforts have been dedicated to the research for finding the new sources of antioxidants, that could work as effectively as synthetic ones (Michiels et al., 2012). Substances occurring in nature could be used for enrichment of
foods and creation of functional foods that could prevent many diseases (Crozier et al., 2006). The phenolic compounds are widely distributed class of the biologically active substances in the plants that are known to be very effective natural antioxidants (Shahidi & Wanasundara, 1992; Tapeiro et al., 2002; Shahidi & Naczk, 2004).

The horseradish (Armoracia rusticana L.) and the lovage (Levisticum officinale L.) are perennial plants which belong to Brassicaceae and Umbelliferae families, respectively and are cultivated in temperate climate zone. For the horseradish the main culinary value has for roots due to its particularly pungent flavour (Raghavan, 2000). But for the lovage all plant parts (seeds, leaves, stems and roots) are strongly aromatic with a characteristic earthy, celery-like flavour and smell (Szebeni-Galambosi et al., 1992). Both plants contain the compounds that can act as natural antioxidants (Raghavan, 2000).

Since the phenolic compounds have antioxidant and anti-microbial properties, they can be used for the technological purposes as an alternative to the synthetic food additives. The foods phenol compounds show a protective and stabilizing effect on the lipids, the food colors and the flavors (Kammerer et al., 2011). The anticarcinogenic, antithrombotic, anti-inflammatory, antimicrobial, antidiabetic and antioxidant properties of the phenolic compounds are widely described, making these compounds very valuable. Structural diversity of the phenolic compounds is in accordance with the differences in their biofunctional properties, thus, the plants extracts differing in their phenolic profile may have different physiological effects (Valls et al., 2009; Kammerer et al., 2011).

The aim of this study was to evaluate the natural antioxidant effectiveness of the lovage leaves and stems and horseradish leaves extracts in unrefined rapeseed oil for the shelf life extension.

**MATERIALS AND METHODS**

**Plant material**

All samples were grown in Latvia and were harvested in September 2012. The horseradish (Armoracia rusticana L.) leaves were collected at the Pure Horticultural Research Centre collection field (latitude – 57° 03’ N; longitude – 22° 91’ E) and the lovage (Levisticum officinale L.) stems and leaves were grown in Jelgava, Latvia (latitude – 56° 39’ N; longitude – 23° 44’ E).

**Herb extracts**

From the fresh plants extracts were prepared using Soxhlet extraction method, and as a solvent ethanol was used. After extraction ethanol was evaporated and samples were kept at temperature +5 ± 1°C.

**Rapeseed oil with extracts**

In the study SIA ‘Iecavnieks’ (Latvia) crude canola oil was used. Initially oil peroxide value was not higher than 7 microequivalents active oxygen in kilogram of oil (meq O₂ kg⁻¹) and an acid value ranging from 1.27 to 1.32 milligrams of potassium hydroxide in gram of oil (mg KOH g⁻¹).

Requirements for food quality schemes, their implementation, operation, monitoring and control arrangements, Latvia Republic Cabinet of Ministers Regulation
Nr.461 states that in unrefined cold-pressed rapeseed oil peroxide number may not exceed 15 meq O$_2$ kg$^{-1}$, and acid number – 4 mg KOH g$^{-1}$ oil (MKN Nr.461, 2014).

Three different extracts: the lovage leave and stem extracts and the horseradish leaves extract were added to the oil in four concentrations: 0.25, 0.5, 1.0 and 1.5% (w w$^{-1}$). As a control the unrefined rapeseed oil with no additives (control sample) were analyzed. Efficiency of natural antioxidants was compared with the oil sample containing 0.01% butylatedhydroxytoluene (BHT) in maximal allowed concentration for the oils (Regulation (EC) No. 1333/2008).

The unrefined rapeseed oil with added extract was placed in a glass conical flask sealed with a cork and was stirred with a magnetic stirrer (4.0 × magnets 0.5 cm) for 30 min in the dark at room temperature (20 ± 1°C). Prepared oil samples were stored in the glass bottles (50 mL) with HDPE cork (diameter 2.4 cm, material thickness 2 mm) and kept for 22 days at temperature 60 ± 2°C. In each step of experiment new bottle of oil was tested. Sample codes used in a study of added herbal extract efficiency in crude rapeseed oil are summarized in Table 1. Control sample without extract was marker with C, sample with BHT 0.01%–O_BHT.

Table 1. Sample codes used in a study of added herbal extract efficiency in crude rapeseed oil

<table>
<thead>
<tr>
<th>Concentration of added extracts in oil, %</th>
<th>The added plant extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>lovage leaves</td>
</tr>
<tr>
<td>0.25%</td>
<td>O_LL_0.25%</td>
</tr>
<tr>
<td>0.5%</td>
<td>O_LL_0.5%</td>
</tr>
<tr>
<td>1.0%</td>
<td>O_LL_1%</td>
</tr>
<tr>
<td>1.5%</td>
<td>O_LL_1.5%</td>
</tr>
</tbody>
</table>

**Chemicals**

2,2-diphenyl-1-picrylhydrazyl (DPPH') were purchased from Sigma-Aldrich (Switzerland). All other chemicals used in the research were obtained from Acros Organic (USA).

**Determination of peroxide value**

Peroxide value represents primary reaction products of lipid oxidation, which can be measured by their ability to liberate iodine from potassium iodide. Peroxide value of rapeseed oil samples was determined by the method as described in LVS EN ISO 3960:2010 (2010).

**Determination of acid value**

Acid value indicates the proportion of free fatty acid present in oil or fat and may be defined as the number of milligrams of caustic potassium required to neutralize the acid in 1 g of the sample. Acid value of unrefined rapeseed oil samples was determined by the method as described in LVS EN ISO 660:2009 (2009).
**Determination of antiradical activity of oil**

Antiradical activity of oil was measured by DPPH˙ free radical scavenging method (Ha, et al., 2012). Oil samples (0.15 mL) were dissolved in 3.75 mL of isooctane. Then 1 mL of solution was mixed with 3 mL of a freshly prepared solution of 0.1 mM DPPH˙ solution in isooctane. After storage for 30 minutes in the dark the results were read using spectrophotometer ‘JENWAY 6300’ (‘Baroworld Scientific’ Ltd., UK) at a wavelength of 509 nm. The following formula (1) was used for calculation of the oil antiradical activity sample.

\[
ARA,\% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100, \tag{1}
\]

where: \(A_{control}\) – absorption of control sample (isooctane with DPPH˙); \(A_{samples}\) – absorption of analysed sample.

**Statistical analysis**

Experimental results were means of three parallel measurements and were analyzed by Microsoft Excel 2010 and SPSS 17.00. Analysis of variance (ANOVA) and Tukey test were used to determine differences among samples.

**RESULTS AND DISCUSSION**

**Peroxide value**

The peroxide value describes peroxide and hydroperoxide concentration during the first stages of lipid oxidation. An analysis of the peroxide value is one of the most widely used methods for determination of the degree of the oil and fats oxidation (Gertzetal, 2000). In the current study the degree of oxidation of the rapeseed oil with and without antioxidant additives stored at elevated temperature in the dark was determined. The initial rapeseed oil peroxide value was 6.14 meq active O\(_2\) kg\(^{-1}\) oil (or 3.07 mmol O\(_2\) kg\(^{-1}\) oil), which is in accordance consistent with other studies of the unrefined rapeseed oil with a peroxide value 3.9 mmol O\(_2\) kg\(^{-1}\) oil (Kleinová et al., 2013). Requirements for food quality schemes, their implementation, operation, monitoring and control arrangements, Latvia Republic Cabinet of Ministers Regulations Nr.461 states that unrefined cold-pressed rapeseed oil, peroxide value may not exceed 15 meq active O\(_2\) kg\(^{-1}\) (MKN Nr.461 from 2014). The effect of the added antioxidant on rapeseed oil peroxide value during the experiment in accelerated storage conditions are shown in Figure 1. The control sample maximal allowed peroxide value reached during the period from the first to third days of storage, while the samples with added plant extracts at a concentration of 1% during the period from 5th to 8th storage day, suggesting that the added antioxidants retard oil oxidation.

Under the medium temperature and as well as due to the relatively high number of unsaturated fatty acids with double bonds, the peroxide value in all samples increased rapidly. The analyzed rapeseed oil samples under thermal stress conditions reached maximal peroxide number after 22 days of storage and the highest peroxide value for control sample was determined (103 ± 2 meq O\(_2\) kg\(^{-1}\) of oil).
After 5 days of storage the peroxide value of the control was 64% higher compared to the sample with lovage leaves extract (1.5%), and it was the biggest difference between the analyzed samples in this experiment. Also tendency that higher concentration of the extracts better retard oxidation process showing positive impact of the extracts on the stability of the oil by preventing oxidation was observed.

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**Figure 1.** Changes of rapeseed oil peroxide value stored in dark at 60 ± 1°C temperature.
The oil samples with added the lovage stem and the horseradish leaf extracts, as well as synthetic antioxidant BHT, showed the biggest difference with the control after 8 days of storage, reaching up to 72% in sample with 1% horseradish leaf extract. In the following days of storage, the difference significantly ($P < 0.05$) decreases, and after 22 days of storage the rises again reaching a difference of 23%. The peroxide value of rapeseed oil with BHT after 22 days of storage was 98.87 meq active O$_2$ kg$^{-1}$ of oil which is only 4.09% less than the control sample. About similar trends also reported Pukalskas et al. (2010) who found that BHT added to rapeseed oil and kept at 80°C did not prevent oxidation significantly ($P < 0.05$). After 22 days storage of the rapeseed oil samples with the plant extracts, common trend was observed: oil samples with 1% natural antioxidant extracts had lower peroxide values. Additionally, efficiency of plants was not significantly different ($P < 0.05$).

**Acid value**

The acid value of the analyzed oil samples ranged from 1.27 mg KOH g$^{-1}$ oil at the first day of experiment to 4.17 mg KOH g$^{-1}$ oil after 22 days of storage (Fig. 2.).

During the all storage period (22 days) acid value in analyzed oil samples increased for three times, but at the end of this period, only one half of the analyzed samples exceeded the allowed limit of the Latvian legislation - 4 mg KOH g$^{-1}$ (Requirements for food quality schemes, their implementation, operation, monitoring and control arrangements, MKN Nr.461, 2014).

During accelerated storage conditions for all samples the acid value increased steadily. Also, in peanut and sunflower oil samples with the added natural antioxidants stored for 40 days acid value increased slightly (Miguel et al., 2005). After 22 days of storage in the dark at +60 ± 1 °C temperature, the lowest acid value was found in the oil sample with BHT (3.84 mg KOH g$^{-1}$ oil), while the highest for the control sample (4.17 mg KOH g$^{-1}$ oil). Also comparing the oil samples with added the plant extracts in the same concentration, no significant differences ($P < 0.05$) throughout the storage period were observed.

In samples with higher concentration of the plant extracts, lower acid value was detected. This means that the added plant extracts affects the inhibition of fat decomposition and the formation of free fatty acids.

Compared the effectiveness of added natural antioxidants, it could be concluded that after 22 days of storage, the lowest acid value (3.87 mg KOH g$^{-1}$ oil) was in the oil with 1.5% lovage stem extract. Also the oil samples with added 1.5% horseradish leaves and 1.5% lovage leaf extracts acid value were lower than the in control sample. It could be concluded that in the dark under the accelerated storage conditions, natural antioxidants significantly ($P < 0.05$) reduced free fatty acid formation. However, the experiments showed that the most effectively the formation of free fatty acid prevented synthetic antioxidant (BHT).
a (lovage leaves)

b (lovage stems)

c (horseradish leaves)

The maximum allowed acid value (4 mg KOH g⁻¹ oil) in Latvian legislation

**Figure 2.** Changes of rapeseed oil acid value stored in dark at 60 ± 1 °C temperature.
**Antiradical activity of oil**

In present antiradical activity assessment method nonpolar solvent isooctane was used. The extracts added to the oil were rich in polar compounds therefore, in these experiments activity of the added compounds possible, could not be seen.

Crude rapeseed oil already contains compounds that are able to scavenge DPPH radicals. One of the most important compounds are tocopherols, that are found in most cold-pressed, fruit and nut oils and can act as a natural antioxidant and the synergist (Rodrigues et al., 2015).

Presence of these compounds may approve fact that in the control sample at the beginning of the experiment DPPH radicals scavenging activity was 80.30% (Fig.3). Activity during storage in the dark at the +60 ± 1°C temperature decreased sharply and after 22 days of storage was only 26.95% (for control).

![Figure 3](image-url)  
**Figure 3.** Changes of scavenging activity (SA) of rapeseed oil stored in dark at 60 ± 1 °C temperature.
In the oil samples with the added lovage stems, lovage leaves and horseradish leaves extracts and also BHT DPPH radicals scavenging activity during storage significantly \((P < 0.05)\) decreased. After 22 days of storage, in the oil sample with 1% lovage leaves extract antiradical activity reduced for 1.92 times, and it was 1.58 times higher compared with the control. Also, activity of oil samples with added 1.5% horseradish leaves and 0.5% lovage stem extract after 22 days of storage decreased by 1.93 and 1.98 times, respectively.

The results shows that during almost all experiment the highest antiradical activity demonstrates oil with added 1% of lovage leaves (Fig.3.a) as well as 0.5% lovage stem (Fig.3.b) and 1.5% horseradish leaf extract in oil (Fig.3.c).

In the unrefined rapeseed oil with BHT antiradical activity decreased 2.13 times, and compared to other samples it is average result.

Comparing analyzed oil samples with sample with synthetic antioxidant, it can be seen that better results demonstrated oil samples with added 1.5% and 1% of horseradish leaves, 0.5% and 1% of lavage stems, as well as 1% lovage leaves extract.

**CONCLUSIONS**

Addition of the lovage leaves, lovage stems or horseradish leaves extracts to unrefined rapeseed oil can prevent the oxidation of unrefined rapeseed oil in accelerated storage conditions in the dark. Increased efficiency was for oil samples with added 1% of the natural antioxidant extract and there were not significant differences \((P < 0.05)\) between lovage leaves, lovage stems or horseradish leaves extracts.

In oil samples with added natural antioxidant extracts acid value was less than the control sample. The lovage leaves, lovage stems and horseradish leaves extracts added to unrefined rapeseed oil during storage significantly \((P < 0.05)\) increased the oils ability to scavenge DPPH free radicals. Besides in the oil samples with 1.5% and 1% horseradish leaves extract, 0.5% and 1% with lovage stem extract and 1% lovage leaves extract antiradical activity was higher compared to synthetic antioxidant BHT.

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Effect of age on composition and quality of *Longissimus thoracis* muscle of the moose (*Alces alces* L.) harvested in Estonia

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Abstract. The aim of this study was to determine the biochemical composition and technological parameters of muscle (*Longissimus thoracis*) from adult and calf moose (*Alces alces* L.) hunter-harvested in the forest of southern Estonia. The experiment was based on 13 hunted moose, of which seven were adults (two males and five females) and six calves (two males and four females). The highest intramuscular fat (IMF) level was found in adult female moose muscles (1.50%), and the lowest in adult male moose muscle (0.46%). Adult moose muscles had higher IMF level (1.14%) than calves (0.98%) (*P* = 0.451). The protein content found in the muscle samples of adult moose was 0.64% higher than that in calves (21.80%) (*P* = 0.045). The moisture content of muscle from adult moose was lower (75.30%) and varied more than that of calves (76.07%) (*P* = 0.051). The initial (5.00–5.59) and ultimate (5.40–5.64) pH in muscle samples were within the normal range, both in adults and calves, except in one of the hunted female calf that had high pH values (pH<sub>45min</sub> = 6.60 and pH<sub>72hr</sub> = 6.90), obviously because of stress. The WHC of moose muscles was considerably high (60.50–75.20%), and cooking loss for thermally processed moose muscle ranged between 19.10% and 33.39%. Muscle sample from adult moose had the highest cooking loss (29.69%) while that from the calves was the lowest (26.42%) (*P* = 0.191). More force (32.54 N) was needed to share muscle samples from adult moose compared to cutting the samples from calves (23.92 N) (*P* = 0.374). Based on the results of the experiments it can be concluded that the meat from younger moose had better technological quality and tenderness.

Key words: moose, *Longissimus thoracis*, meat quality, age, meat composition.

INTRODUCTION

Markets for venison and other cervid meats have traditionally been strong and stable in Europe. Germany alone consumes hundreds of thousands of tons of meat from different cervids (Thorleifson & Church, 2004). Over the past years, the high-quality meat obtained from wild cloven-hoofed animals (elk, deer, and moose) has become increasingly popular. The amounts of meat from hunted moose has increased almost six times during the last two decades (Veeroja & Männil, 2014), which allows meat
processors to diversify their production. There are five large scale meat processors in Estonia, which market moose meat mostly in the processed form. Bedilo et al. (2010) consider, that moose meat and meat products increase exotic, and, at the same time, low-calorie meat assortment.

According to the Estonian Environment Agency (Keskkonnateabe Keskus, 2013) there were 13,540 hunters in Estonia in 2011, whereas there is access to 38,880 km² hunting grounds (86% of the country’s total land area). The estimated moose population was 11,650 in spring, 2014, which means that the average population density is less than five animals per 1,000 ha of habitat (Veeroja & Männil, 2014). Although the number of animals has increased over the last two decades, a 12% decline was observed in 2014 compared to year 2013. A total of 6,532 moose were harvested during the hunting season of 2013, which was the highest volume ever (Statistikaamet, 2010; Veeroja & Männil, 2014).

Meat quality can be defined in a number of ways, whereas technological, nutritional, hygienic and sensory aspects are taken into consideration (Konarzewski, 2004). The nutritional value and overall quality of meat depend on the animal species, age of the animal and animal feeding (Semple, 2011). Moose meat is fibrous, there are no fat layers in the cross-sectional surface of muscle, which refer to marbling, such as in beef. The connective tissue is underdeveloped being uniform in colour, tender and palatable (Bedilo et al., 2010).

Only a small number of studies on moose meat quality are available (Ponamareva, 1997; Taylor et al., 2002; Bedilo et al., 2010; Strazdiņa et al., 2011). The latest study on moose meat quality in Estonia dates back to the 1970s (Evendi & Tüür, 1976).

The aim of the present study was to determine the biochemical composition and technological parameters of muscle (Longissimus thoracis) from both adult and calf moose (Alces alces L.) hunter-harvested in the forests of southern Estonia.

**MATERIALS AND METHODS**

**Animals**

Thirteen moose were harvested with a hunting rifle during the hunts from 27 October till 8 December 2013 in southern Estonia, whereas the average air temperature was from -3 to +10°C. Dissection and skinning started 15 minutes to five hours after harvesting, whereas delays were due to the different locations of the harvest. Skinning and dissection of a carcass took 45–80 minutes (Table 1), whereas the duration depended on the size of the animal, e.g. skinning and dissection of calves took less time. The age of the animals was estimated by the wear of mandibular premolars and molars, whereas the work was performed by an expert. The age of adult moose varied between 30 and 90 months. The calves were born in spring, i.e. they were about six months old. Hot carcass weight was determined, and carcass yield calculated according to the method used in meat processing plants. In adult moose, the hot carcass weight was, on average, 107.82 kg and red meat yield 79.43 kg higher than those in the calves, whereas both characteristics showed more variability in the adult animal group (Table 1).

Samples from *Longissimus thoracis* between the 11th and 12th ribs were obtained from six calves and seven adult moose (Fig. 1). The calf group comprised four and the adult group five female animals, whereas both groups included two male animals. Muscle samples (500 g) were taken from the carcasses within 90 minutes after skinning.
in abattoir, and transferred directly to the chilling box. All muscle samples were packed into plastic bags and stored at +5°C until the analysis.

**Figure 1.** Location of *Longissimus thoracis* samples (500 g) obtained in carcasses between the 11\textsuperscript{th} and 12\textsuperscript{th} ribs.

**Table 1.** Descriptive statistics of the adult and calf groups of animals

<table>
<thead>
<tr>
<th>Traits</th>
<th>Adult group (n = 7)</th>
<th>Calf group (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, months</td>
<td>Mean: 54, Std. Dev: 22.8</td>
<td>Mean: 6, Std. Dev: –</td>
</tr>
<tr>
<td>Hot carcass weight, kg</td>
<td>Mean: 192.30, Std. Dev: 20.88</td>
<td>Mean: 84.48, Std. Dev: 8.40</td>
</tr>
<tr>
<td>Carcass yield, %</td>
<td>Mean: 72.79, Std. Dev: 0.39</td>
<td>Mean: 71.50, Std. Dev: 1.76</td>
</tr>
<tr>
<td>Pre-processing time, min</td>
<td>Mean: 65, Std. Dev: 8.66</td>
<td>Mean: 45.83, Std. Dev: 2.04</td>
</tr>
<tr>
<td>Red meat yield, kg</td>
<td>Mean: 139.93, Std. Dev: 14.61</td>
<td>Mean: 60.50, Std. Dev: 6.75</td>
</tr>
</tbody>
</table>

**Biochemical analysis**

The analyses were carried out using acknowledged methods in the meat laboratory of the Department of Food Science and Food Technology at the Estonian University of Life Sciences. The chemical composition of *Longissimus thoracis* was determined three days after harvesting, whereas fat, protein, ash and moisture content were recorded. Muscle samples were homogenized in a micro grinder for 20 seconds per a preparation, and placed into plastic containers, which were covered with a lid.

The moisture content of muscle was determined according to the Estonian Centre for Standardisation (EVS) standard EVS-ISO 1442:1999 (EVS, 1997). The protein content was measured according to ISO 937:1978, the method of EVS (EVS, 1978) by using a Kjeltec device. The fat level of muscles was determined by using the Soxtec apparatus according to EVS–ISO 1444:1996 method (EVS, 1996). Ash content of the samples was determined by incineration in electric muffle furnace according to EVS–ISO 936:1998 methodology (EVS, 1998).
**Technological characteristics**

The following characteristics were determined in moose muscle: pH, WHC (WHC), electroconductivity, colour, cooking loss and shear force.

The initial pH-value (pH_{45min}) of *Longissimus thoracis* was measured by using pH-meter Testo 205 (Testo AG, 2006) 45 minutes after the muscle samples were taken. The ultimate pH (pH_{72hr}) was measured using pH-STAR CPU (Ingenieurbüro R. Matthäus, 2011) within 72 hours after skinning and dissection of the carcasses.

The WHC of muscle was determined using the Grau & Hamm (1952; 1957) method, modified by Volovinskaja & Kel'man (1962).

To determine the electrical conductivity of muscle tissue the LF-STAR CPU apparatus was used (Ingenieurbüro R. Matthäus, 2011). The equipment has two parallel steel electrodes, which were pressed into the tissue, and the electrical current between the electrodes was recorded. The result shows the degree of damages in cell structure, which is directly related to the WHC of muscle.

The colour of muscle tissue was determined using optometer Opto-STAR, which uses a light source to radiate muscle surface (Ingenieurbüro R. Matthäus, 2011), that enables researchers to determine quality defects according to the intensity of emanated light from the muscle surface.

Cooking loss is the loss of liquid and soluble substances from meat during thermal treatment. Each muscle sample (100 g) was cut from *Longissimus thoracis* and sealed into a plastic bag with a thermometer. The bag was placed into hot water (95°C) and heated until the internal temperature of the sample increased up to 72°C. The sample was cooled down and weighed. Cooking loss was calculated according to the initial weight.

The shear force of moose muscles was determined by using texture-analyser TMS-Pro, which was equipped with force element TMS-PRO LOAD CELL 1 kN and cutting blade TMS-PRO Light weight blade set (Food Technology Corporation, 2011). The specifications of the TMS-Pro equipment were as follows: 60 degree V-shaped 1.016 mm cutting-blade; cutting speed of the blade 500 mm min^{-1}, and the maximum force applied ≤ 1000 N. Preparation of the samples and determination of shear force were performed according to Warner-Bratzler methodology (Savell et al., 2013). Muscles were aged 72 hours before testing in a freezer at 5°C. Samples were sheared perpendicularly to muscle fibres, whereas abnormal records (connective tissue detected) were excluded from the final analysis.

**Statistical analysis**

Data management and statistical analysis were performed by using a spreadsheet program MS Excel 2010. To determine the statistical difference between age groups the student’s *t*-test was used. Data visualization was aided by Daniel’s XL Toolbox addin for MS Excel, version 6.53, by Daniel Kraus, Würzburg, Germany.

**RESULTS AND DISCUSSION**

**Biochemical analysis**

Strazdiņa et al. (2011) analysed game (deer, roe deer, moose and wild boar) muscle samples and determined that intramuscular fat (IMF) content varied from 1.31% to 2.82%, being the lowest in moose muscles and the highest in wild boar muscles.
According to Anderson et al. (1989) from USDA, the fat content of raw moose meat was 0.74%. Bedilo et al. (2010) found that moose meat was low in fat: 0.68% in *Longissimus thoracis* and 1.45% on average in the red meat, whereas the study showed that fat content depends on the type of muscle used for analyses. On the contrary, Reede (personal communication, 15 December 2013) noted, that they found high IMF level (3.85%) in *Longissimus thoracis* from moose. Ponamareva (1997) studied trimmed moose meat used in making sausage, and found that this kind of meat also had a higher fat level (1.95%).

The fat content in the meat from the other species of the *Cervidae* family has been reported to be as follows: 1.60% (Strazdiņa et al., 2011) and 1.45% (Anderson et al., 1989) in wild deer (*Cervus elaphus*), and 1.59% (Strazdiņa et al., 2011) in roe deer (*Capreolus capreolus*). Daszkiewicz et al. (2012) found a significant difference between female and male roe deer IMF percentages 1.46% and 0.83%, respectively. This indicates that meat from the other species of the *Cervidae* family also has a low IMF level. Still, in case grounded meat was used for analysis, the fat content of the meat from elk (*Cervus canadensis*) (4.5% in bulls, 7.8% in cows) was relatively high (Field et al., 2003).

In the present study the recorded fat content in moose muscles was low, ranging from 0.46–1.50%, whereas it was higher in cows and lower in bulls. Zhitenko (1984) has reported higher fat level in moose muscle (1.1–2.5%). The fat content in the muscles of adult animals was slightly (0.16%) higher than that in calves, but the difference between the age groups was not statistically significant (*P = 0.451*) (Fig. 2).

The protein content (18.5–19.0%) in the meat from wild, free-range moose does not differ significantly from that of beef (19.0–20.0%) (Rogozhin, 2009). Strazdiņa et al. (2011) showed that the protein content of the muscle from the different species of the *Cervidae* family were in deer – 22.36%, roe deer – 22.82% and moose – 22.72%. Bedilo et al. (2010) found, that the protein content of *Longissimus thoracis* of moose was 19.78%, whereas it was 4.48% higher in red meat. However, Zhitenko (1984) estimated 20.6–21.6% protein content in moose meat. The protein content of trimmed meat (21.28%) does not differ significantly from the above values (Ponamareva 1997). The highest protein level (24.94%) in the longest spinal muscles from moose was found by T. Reede (personal communication, 15. December 2013). Field et al. (2003) analysed the meat from another representative of the family *Cervidae* - the elk (*Cervus canadensis*), and determined 23.0% protein content in the meat from bulls, and 21.8% in that from cows.

Protein content in the moose muscle samples was between 21.10% and 23.30%, whereas it was 22.44 ± 0.21%, as an average, in the muscle from adult animals, i.e. significantly higher (*P = 0.045*) compared to the calf group (21.80 ± 0.18%) (Fig. 3).

Previous studies have shown that protein content does not differ much between meat cuts from different parts of moose. Hoffman & Wiklund (2006) have stated that game meat and venison fulfill the expectations and dietary requirements of the modern consumer due to low fat and high protein level.
Figure 2. Average intramuscular fat content (± standard error) of moose muscles by age group.

Figure 3. Average protein content (± standard error) of moose muscles by age group.

The highest moisture content of Longissimus thoracis from moose (78.44%) was reported by Bedilo et al. (2010). In the current study, the average moisture content found in the muscle samples from moose calves was 76.07 ± 0.21%, which was by 0.77% higher than that in adult animals (75.30 ± 0.27%). No significant difference was found between age groups, but just a tendency to it was observed (P < 0.01) (Fig. 4). The moisture content in the muscle from adult moose (74.00–76.20%) varied slightly more than that in calf group (75.50–76.80%). Similar variation (74.3–75.8%) was found by Zhitenko (1984). The lowest moisture level (69.62%) was detected by Reede (personal communication, 15 December 2013), which may have been due to the use of frozen muscle samples. Analyses of moisture content in grounded moose meat showed higher results in the works of Anderson et al. (1989) and Bedilo et al. (2010) (75.55% and 73.24%, respectively). Also, the moisture content in trimmed meat (74.85 ± 0.83%) did not differ from that of untrimmed meat (Ponamareva, 1997). Similar values (73.80% in female and 75.32% in male animals) were found in the meat from roe deer by Daszkiewicz et al. (2012).

According to literature, the total mineral content in moose meat ranged within narrow limits, from 1.05% to 1.13% (Zhitenko, 1984; Anderson et al., 1989; Bedilo et al., 2010). Ponamareva (1997) showed that trimmed meat had slightly higher ash level (1.20 ± 0.11%). The current study showed that the ash content in moose muscles was 1.03–1.32%, whereas less variability was observed in calves. Still, the average values in the calf (1.14 ± 0.03%) and adult groups (1.13 ± 0.04%) differed by only 0.01% (P = 0.816) (Fig. 5).
Technological characteristics

To determine the technological properties of muscle tissue parameters that characterize the toughness of meat, the following was studied: weight loss during heat treatment, ability to bind and retain moisture that ensures juiciness, and the pH value which is related to maturation of meat due to biochemical processes and accumulation of glycogen in muscle.

The initial average pH\(_{45\text{min}}\) values ranged from 5.06 to 6.60, whereas the highest values were determined in the muscles of female calves and in the adult moose group. The pH\(_{45\text{min}}\) value in the muscle of female animals was 5.59 which was higher than that in the muscle of males (Fig. 6a). Furthermore, the acidity of the muscles in cows dropped to the normal level (pH\(_{72\text{hr}}\) = 5.50), while that in the muscle of calves continued to increase up to pH\(_{72\text{hr}}\) 6.9 (Fig. 6b). This indicated that DFD-specific meat developed due to stress obtained during hunt. The average pH\(_{45\text{min}}\) differed by only 0.2 units (\(P = 0.549\)) between the groups, whereas it was lower in the muscles of adult moose (pH\(_{45\text{min}}\) = 5.18).

The ultimate acidity (pH\(_{72\text{hr}}\)) of moose muscles declined compared with the initial pH\(_{45\text{min}}\), and was 5.53 ± 0.03 in the adult and 5.76 ± 0.23 in the calf group (Fig. 6b). As the difference between the initial pH\(_{45\text{min}}\) values between the groups was 0.23 units, and the difference in the ultimate pH\(_{72\text{hr}}\) falls in the same range (\(P = 0.362\)), it can be concluded, that the acidity of muscles was relatively evenly changed. Bedilo et al. (2010) found that the ultimate pH of moose muscles fell in a normal range (5.47–5.71), and the calculated average value was 5.59. Field et al. (2003) determined that muscle pH in elk (Cervus canadensis) was 5.5 in both bulls and cows.

The highest initial and ultimate muscle pH was determined in calves, while calf muscle also had the highest WHC (75.2%), which indicates damage to muscle structure and emergence of excess water. (Fig. 7).
The WHC of moose muscles was considerably high (60.50–75.20%), therefore the meat can become dry and firm after ageing. Calf muscles contained 1.82% more water than those of adult animals (Fig. 7), and although this difference proved insignificant, a tendency existed ($P < 0.01$). Also, Bedilo et al. (2010) stated that the WHC of moose muscle is high (59.10–61.19%; 60.10% on average).

The highest cooking loss during thermal treatment ($29.69 \pm 1.18\%, P = 0.191$) was observed in the muscles of adult moose, while it was the lowest in calf muscle ($26.42 \pm 2.14\%$). The average cooking loss of thermally processed moose muscles ranged from $19.10\%$ to $33.90\%$, whereas there was more variation in the calf group ($19.10–33.20\%$) than in the adult group ($26.10–39.90\%$) (Fig. 8).

On the contrary, Bedilo et al. (2010) showed much higher loss during thermal treatment ($39.44\%$), which varied from $38.30\%$ to $40.58\%$. This indicates that moose meat may become dry after processing, and therefore requires specific pre-treatment.

The electroconductivity of moose muscles was in a range $4.00–13.10$ mS. The lowest average value in the calf group was observed in male calves ($5.00$ mS) and the highest in females ($10.38$ mS). The average value was quite similar in both groups, being only slightly higher in the adult group ($P = 0.885$) (Fig. 9).

Consistently uniform colour of meat attracts consumers. Bedilo et al. (2010) found that colour values of moose meat are uniform, which is in accordance with the present study, in which the colour values did not vary significantly, ranging from 85 to 90. The values indicated that moose meat is dark in colour. The average muscle colour was slightly darker (88.43) in adult moose compared to that of calves (87.6), whereas muscle colour of male animals tended to be lighter than that of females in both groups (Fig. 10).
Figure 7. Average water holding capacity (± standard error) of moose muscles by age group.

Figure 8. Average cooking loss (± standard error) of moose muscles by age group.

Figure 9. Average electroconductivity (± standard error) of moose muscles by group.

Figure 10. Average colour values (± standard error) of moose muscles by age group.

Generally, consumers prefer tender meat. Bedilo et al. (2010) concluded that elk meat was sufficiently tender (shear force 2.65 kg cm$^{-1}$ (25.99 N)), i.e. similar to the Aberdeen Angus beef (27.81 N) (Tänavots et al., 2013). Taylor et al. (2002) indicated to the reported toughness of moose meat and concluded that this is probably due to the lack of I band breaks and normal to large fibre size.

The present study showed that more force had to be used to shear adult moose muscles (32.54 N), compared to cutting veal samples (23.92 N). Although the difference between age groups was statistically insignificant, it can be concluded that the meat from adult moose was slightly tougher (Fig. 11).
Fresh or processed moose meat is an alternative to traditional meats that enables the consumer to diversify the range of exotic and low-calorie meat products. Due to its low IMF and high protein content, moose meat corresponds to the modern dietary demands. The protein and moisture content of moose muscle was significantly influenced by age. On the other hand, pH, WHC, electroconductivity and boiling loss did not differ between age groups. Although the meat from adult animals was tougher, the dry matter, protein and IMF level was higher than that in calves. Still, it can be concluded, that the meat from younger moose was more tender and of better overall quality.

Since there is small numbers of published information on moose meat, it is necessary to continue research in this field. The number of game meat handlers and their increasing production volumes also demonstrates the need for further research, which shall involve after-processing and product development issues.

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Effect of aging technologies on some qualitative characteristics of Longissimus dorsi muscle of Marchigiana beef

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Abstract. In order to determine sensory preference and value of fresh beef steak differing in aging technique, this study evaluated two aging methods: dry and wet; a quality grade on physic-chemical traits of instrumental tenderness, color, and sensory properties of Longissimus dorsi beef muscle of Marchigiana bovine, correlated to calpain proteolytic activity too. Dry-aged loins had higher weight loss than wet aged loins. Wet aged loins had higher L* values than dry aged loins. Warner-Blatzer shear force of steaks was not affected by aging method. We made a sensory panel evaluation too that showed no effect of aging method on myofibrillar tenderness, juiciness, connective tissue amount, overall tenderness or off flavor intensity.

Key words: beef, dry aging, wet aging, tenderness.

INTRODUCTION

Aging of fresh beef is essential to meet the high demands and expectations of an exceptional eating experience. Fresh meat is aged to enhance the palatability of the product, to increase the tenderness over time and to develop the flavors. Dry and wet aging are common aging techniques, dry aging is a process whereby beef carcasses, primals, and/or sub primals are stored – without protective packaging – at refrigeration temperatures for one to more weeks, while during wet aging meat is packaged in a sealed barrier film and held at a temperature above the freezing point of the product. The objective of our study was to quantify sensory differences between wet- and dry-aged strip loins and to determine the score that consumers place on their preferred product. The study was carried out on Longissimus dorsi muscles of 20 cattle of Marchigiana breed, 12 months-old, reared for 7 months in a wild state and for 5 months in a semi-wild state. Each loin was cut into half and randomly assigned to one of the two aging treatments. Loins were placed on wire racks, with the subcutaneous fat surface down. The yield, chemical composition, instrumental tenderness (Warner–Blatzer shear force) and color quality traits were determined on loin sections aged for 15 and 25 d at 2.2°C in a cooler with minimal air movement starting after 8 d post-mortem. Most important results showed that dry-aged loins had higher weight loss than wet aged loins. Moisture content of raw meat was significantly lower in dry aged group while was not different between treatment after cooking. Wet aged loins had higher L* values and lower red index (a* value) than dry aged loins. Warner–Blatzer shear force of steaks was not affected by aging method as well as sensory panel evaluation.
MATERIALS AND METHODS

The study was carried out on 20 cattle of Marchigiana breed, 12 months-old, raised for 7 months in a wild state and for 5 months in a semi-wild state.

Animals were treated according to the guidelines of the European Community on the treatment of experimental animals (Reg. CE 1/2005; directives 74/577/EEC; Law 439 2 August 1978). The slaughter house had EEC mark with reference to rules 852/853/854/2004; 2076/2005. The muscle used was Longissimus Dorsi (Ld). Each loin was cut into half and randomly assigned to one of the two aging treatments: (dry or wet). Loin sections allocated to wet aging \((n = 10)\) were vacuum-packaged \((8600-14EL, \text{Cryovac Sealed Air Corporation, Duncan})\) in vacuum bags having \(O_2\) permeability of \(3–6 \text{ ml of } O_2 \text{ m}^{-2} \text{ h}^{-1} \text{ at } 4.4\degree C\), atmospheric pressure, and 0% relative humidity; water vapor permeability of \(0.5–0.6 \text{ g } 64,516 \text{ cm}^{2} \text{ h}^{-1} \text{ at } 37.8\degree C\) and 100% relative humidity. Loin sections destined for dry aging \((n = 10)\) were aged unpackaged with direct exposure to air in the cooler. Muscle samples were withdrawn at different period \((15-\text{d and } 25-\text{d post-mortem})\) and by means of the Western Blot method, the \(\mu\)-calpain, \(p-94\) and calpastatin expressions were evaluated. Loin sections were aged from the time they were received at 8 d postmortem for 15 and 25 d at 2.2°C in a cooler with minimal air movement. Loins were placed on wire racks, with the subcutaneous fat surface down.

Steak preparation

Five 3.00 cm-thick steaks were removed from the anterior end of all loin sections and randomly assigned to cooking temperatures \((70°C)\) for Warner–Bratzler shear force determination and sensory analysis after the 15 and 25 day aging period. A sample was also taken from the most anterior end of the Ld for compositional analysis and pH. Steaks for sensory evaluation were frozen at \(-40°C\) until just before evaluations by a trained sensory panel. Each loin section was weighed before and after the assigned aging times \((15 \text{ or } 25 \text{ d})\). Weight loss percentage was calculated. After dry aging, loin sections were trimmed to remove dry and discolored portions. Wet aged loins were blotted by dry paper towels. They were calculated both percentage trim loss than combined loss.

pH, moisture, fat

Samples of Ld tissue, obtained before and after aging, were frozen in liquid nitrogen and pulverized in a warring blender. Ten grams of pulverized sample was added to 100 ml of distilled water and mixed for 30 s, and pH values were obtained with an accumet glass electrode attached to an accumet 210 pH meter (Model Hanna pH 210 microprocessor pH Meter, Italy). Moisture and fat content were determined using the CEM (CEM, Corporation; Mathews) SMART (moisture) and SMART Trac (fat) systems (AOAC PVM 1:2003; Keeton et al., 2003).

Instrumental and visual colour

Color measurements on the anterior Ld surfaces were taken after opening the sub-primal vacuum packages and allowing for 20 min of bloom time and prior to aging with a colorimeter equipped with a 2.54 cm orifice (Hunter Mini Scan XE, model 45/0-L). Color parameters were determined in triplicate with an instrument which uses the CIE \(L^* a^* b^*\) color system, by measuring lightness \((L^*)\), redness \((a^*)\), and yellowness intensities \((b^*)\). Aperture of 8 mm, illuminant D65, and 10° standard observer were used.
Calibration was performed using a white standard plate (L* = 95.26, a* = 0.89, b* = 1.18). The colorimeter was calibrated daily against black and white tiles before steak measurements. After 15 and 25 d of aging, instrumental color of Ld muscles was determined by averaging five readings on each cut steak surface after blooming. Internal visual color was evaluated to the nearest 0.5 unit on a 6-point scale: 1, raw red centre, pink border, tan edge (medium rare); 2, reddish-pink centre, pink border, tan edge; 3, pinkish red centre, pink to light brown/tan to outer surface; 4, slightly pink centre, light brown to tan edge (medium); 5, tan/brown centre and edges, no evidence of pink; and 6, dry, brown throughout (well done; AMSA, 1991).

**Shear force**

Steaks were cooked in a forced-air convection oven (DFG-102 CH3; G.S. Blodgett Co., Arlington, VT) on trays to an internal temperature of 70°C. Internal temperature was monitored by using copper-constantan thermocouples (Omega Engineering, Stamford, CT) inserted into the geometric center of each steak and connected to a Doric temperature recorder (VAS Engineering). After cooking, steaks were over wrapped in polyvinyl chloride film and stored at 2°C for 24 h. Five round cores (1.27 cm diameter) were obtained from each strip steak, parallel to the long axis of the muscle fibers (AMSA, 1995). Each core was sheared once, perpendicular to muscle-fiber orientation, with a Warner–Bratzler shear force apparatus (Vnotch blade) connected to an Instron Universal Testing Machine (Model 4201; Instron, Corp., Canton, MA) with a 50 kg compression load cell operating at a crosshead speed of 250 mm/min. Shear-force steaks also were used to determine cooking loss as: cooking loss (%) = (raw weight − cooked weight) / (aw weight) x 100.

**Sensory analysis**

Sensory evaluation was essential in the assessment of consumer products by the use of the human senses (sight, smell, taste, touch, and hearing). The sensory analysis required the use of a panel of human evaluators, wherein test results were recorded based on their responses to the products under test. Particularly they evaluated tenderness and flavor intensity for the two aging processes. Steaks were broiled at 163°C to an internal core temperature of 70°C for sensory evaluation. Sensory analysis was conducted at the Department of Agriculture. Panelists (n = 6) were highly trained in descriptive sensory principles and methods. Panelists evaluated each parameter without collaboration and recorded individual evaluations on a 14-point scale, where 1 had the lowest intensity and 14 had the greatest. The testing room was a round-table panel room and had lighting, temperature, humidity, and noise controls designed according to the guidelines of A.S.T.M. (1986). Four of the cut pieces were placed randomly into plastic cups, kept warm by placing the cups on tiles heated to 121°C, and presented to the panel within 5 min of cutting.

**Calpain activity assay**

For proteins extraction 300 mg of tissue samples from the muscle were homogenized using a Polytron (Brinkman Instruments, Westbury, NY) in 0.9 mL of post-rigor extraction buffer containing 100 mM Tris base, 10 mM EDTA, 0.05% 2-mercaptoethanol, adjusted with HCl to pH 8.3 and a cocktail of protease inhibitors (Protease Inhibitor Cocktail Tablets; Roche). After centrifugation at 8,800 g for 30 min, the supernatant (containing soluble proteins) was collected. Immunoprecipitation was
carried out as follows: 100 µl of a 1:1 slurry of protein A-Sepharose beads was incubated for 1 h at 4°C with 5 µg of anti-µ-Calpain (DOMAIN IV) (SIGMA) or anti-m-Calpain (DOMAIN III/IV) mouse IgG1 (SIGMA). The beads were washed three times with lysis buffer (NaCl 150 mM, Hepes 20 mM, glycerol 10 %, triton 1%), and incubated overnight at 4°C with 100 µg of protein extract. Immunoprecipitated proteins were washed three times with 1 ml of lysis buffer (NaCl 150mM, Hepes 20mM, glycerol 10 %, Triton 1%). Cell lysates were used to detect calpain activity by means of the Calpain-Glo protease assay (Promega) that furnishes specific calpain luminogenic substrates (O’Brien et al. 2005). The Calpain-Glo Reagent was prepared as indicated in the Promega protocol. 25 µl of Calpain-Glo Reagent and 25 µl of sample were mixed and incubated for 30 min, and the luminescence was recorded with a luminometer (GloMax™20/20 Luminometry System GloMax™ 96 Microplate Luminometer).

Western blot analysis of µ-calpain, m-calpain and calpastatin
Protein samples were subjected to 10% SDS-PAGE according to the method of Laemmli (Laemmli 1970). In briefly, muscle extracts were incubated in buffer at 37°C for 10 min and fractionated by electrophoresis. The separated protein were electrophoretically transferred to a 0.22 µm nitrocellulose membranes (Hybond-C Super or Hybond-ECL) at 4°C overnight using a Bio-Rad Transfer Blot apparatus (30m Amp). Nonspecific sites were blocked with 5% skim milk in TTBS (50 mM Tris-HCl, pH 7.5, 0.15 M NaCl, 0.05% Tween-20) for 1 h at room temperature. The membranes were incubated for 2 h at room temperature and were sequentially diluted with 1:2500 of anti-rabbit, µ-, m-calpain or calpastatin. The membranes were washed and incubated for 1 h with 1:2000 dilution of secondary (AP-conjugated goat anti-rabbit IgG). After three time washings, antibody-reactive bands were visualized by the ECL detection system (Amersham). The blots were scanned using a Leica DM 1000 (Gene Gnome syngene Bio) imaging system.

Calibration Curve
We created a calibration curve for our results. The data-the percentage of the analyte and the relative response for each standard-were obtained using linear progression analysis.

Statistical design and analysis
The experimental was designed as a split-split-plot design with the incomplete assignment of the treatment combinations to the experimental units and the treatment was replaced six times. The whole plot treatment was quality grade and the sub-plot treatment was aging method (dry aging and wet aging). Data were analyzed using the Proc Mixed procedure of SAS (2009). The degree of freedom computation method was the Kenward-Roger (ddf = kr) and the computation was included in the model statement. Means separated were significant for P > 0.05 using the PDIFF option. Least significant difference (LSD) for all significant factors were calculated and presented for ease of mean separation.

RESULTS AND DISCUSSION
The Marchigiana loins had higher weight loss during dry aging than wet 14.55% and 2.80 %, respectively (Table 1). The higher moisture content (P < 0.0001) (70.53%
for wet vs 56.64% for dry) before cooking might explain the higher eight loss due to evaporation and/or purge associated with Marchigiana bovine. In the study by Sitz, Calkins, Feuz, Umberger, and Eskridge (2006), they found that dry-aged steaks had lower moisture content than wet-aged steaks. Prime loins supporting our findings that lower intramuscular fat and higher moisture content resulted in greater weight loss during aging. Warren and Kastner (1992) also reported higher weight loss with dry aging as compared with wet aging. Similarly, Ahnströmet et al. (2006) concluded that dry aging resulted in higher weight loss than SB aging for steaks aged for 21 d. Aging method affected trim loss (Table 1), in fact it was highly significant (P < 0.0001). Dry method resulted in much higher (P < 0.0001) trim loss (23.05%) than wet aging (3.51%). This excessive trim loss required much more labour than for wet aging. Similarly Li et al., (2013) found higher ageing loss, trim loss, and total ageing and trim loss of meat aged in dry ageing bags compared to that aged in vacuum because there was a higher moisture loss when using the dry ageing bag compared to vacuum ageing. There was aging method effect for the combined loss (Table 1). Dry losses were higher than wet aging (34.98% for dry vs 6.23% for wet, Table 1). De Geer et al. (2009) reported similar combined losses for dry and SB aged strip or shell loin steaks.

Table 1. Percentage value for physical and chemical parameters in relation to aging method and end point temperature

<table>
<thead>
<tr>
<th>Trait</th>
<th>Aging method</th>
<th>End point temperature</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry</td>
<td>Wet</td>
<td>61.8</td>
</tr>
<tr>
<td>Weight loss (boneless loins)</td>
<td>14.55</td>
<td>2.80</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Trim loss % (boneless loins)</td>
<td>23.05</td>
<td>3.51</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Combined loss</td>
<td>34.98</td>
<td>6.23</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Moisture % (raw steaks)</td>
<td>56.64</td>
<td>70.53</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Moisture % (cooked steaks)</td>
<td>53.06</td>
<td>53.20</td>
<td>0.65</td>
</tr>
<tr>
<td>Fat % (raw steaks)</td>
<td>4.43</td>
<td>3.42</td>
<td>0.04</td>
</tr>
<tr>
<td>Fat % (cooked steaks)</td>
<td>5.36</td>
<td>6.41</td>
<td>0.04</td>
</tr>
<tr>
<td>WBSN (steaks)</td>
<td>29.75</td>
<td>29.80</td>
<td>0.99</td>
</tr>
<tr>
<td>Cooking loss % (steaks)</td>
<td>17.73</td>
<td>19.23</td>
<td>0.01</td>
</tr>
<tr>
<td>Visual color</td>
<td>2.80</td>
<td>2.78</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Prior to aging, Marchigiana loins had mean pH values of 5.60. The pH of dry aged loins was 5.66, which was higher (Pb < 0.01) than wet aged (5.49) loins. A faster, earlier decline in pH can cause detrimental effects on meat quality through the earlier reduction in activity in u-calpain combined with a relatively high calpastatin level resulting in tougher meat (Hwang and Thompson, 2001). Wet aged loins had higher moisture content (Table 1) than dry aging. This result was in accordance with that of Juárez et al., (2011) that in their study on pork found that dry-aged meat had lower (P < 0.001) moisture content when compared with wet aged meat. After cooking, there was no difference (P > 0.05) in moisture content due to aging method. As expected, higher end point temperature resulted in lower (P < 0.0001) moisture content (Table 1). Quality grade × aging method interaction was significant (P < 0.05) for L* values, but not for a* or b* values (Table 2). Aging method × end point temperature interaction was significant (P < 0.05) for a* and b* values. The decrease in a* values (less red) with the increased end-point temperature was not significant (P < 0.04) in dry aged steaks like it was in wet aged steaks (Table 2). The same decreasing trend in b* values with the higher end point temperature was also observed (Table 2).
In general, there was less difference in L*, a*, and b* between for dry aging and wet aging.

Table 2. Color profile of meat

<table>
<thead>
<tr>
<th>Trait</th>
<th>Marchigiana Dry</th>
<th>Marchigiana Wet</th>
<th>Aging method and end point temperature, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L*</td>
<td>a*</td>
<td>b*</td>
</tr>
<tr>
<td>L*</td>
<td>55.44</td>
<td>56.24</td>
<td>53.75</td>
</tr>
<tr>
<td>a*</td>
<td>13.00</td>
<td>12.34</td>
<td>12.87</td>
</tr>
<tr>
<td>b*</td>
<td>19.01</td>
<td>19.56</td>
<td>18.4</td>
</tr>
</tbody>
</table>

L*—lightness, a*—redness, b*— yellowness intensities.

At the endpoint temperature of 61.8°C, dry aged steaks tended to have a higher early cooking rate than wet steaks. Dry aged steaks had a mean visual color score of 3.80 (more done appearance) while wet steaks had mean visual color scores of 2.78, but the difference was not significant (P > 0.05) (Table 1). The mean visual colour scores of steaks cooked to 61.8°C and 70°C were 2.15 and 4.53 (P < 0.01), respectively. As expected higher endpoint temperature resulted in higher degree of doneness. There was no aging method effect (P > 0.05) on Warner Bratzler Shear Force (WBSF) (Table 1). In contrast George-Evins et al., 2004 found that each increase in aging period resulted in lower (P < 0.05) WBSF values. In our study, WBSF increased (P < 0.0001) as endpoint temperature increased. Similarly, George Evins et al., (2004) in their study on beef found that WBSF values increased (P < 0.05) as endpoint temperature increased. In agreement, Lorenzen et al. (2003) found that WBSF for top sirloin steaks increased when cooking to higher in internal endpoint temperatures, too. Dry aging resulted in similar cooking loss (P > 0.05) as wet aging (Table 1). Similarly, Warren and Kastner (1992) reported that wet and dry aged strip loins had similar (P > 0.05) cooking losses. However, Laster et al. (2008) reported that dry aging caused less cooking loss than wet aging. A strong (P < 0.0001) endpoint temperature effect existed for cooking loss (Table 1). Cooking loss for steaks cooked to 70 °C was about 5% higher (P < 0.0001) than that for steaks cooked to 61.8°C (Table 1). Fang Liu et al., (2013) have reported increased cooking loss with increased endpoint temperature. Cooking induces the shrinkage of myofibrillar, sarcoplasmic proteins, and shrinkage and solubilization of the connective tissue, which decrease the water holding capacity of the meat (Tornberg 2005; García-Segovia et al. 2007). According to Tornberg et al., (1997), the denaturation of sarcoplasmic proteins started at 40°C and terminated at 65°C, which might contribute to the increase in the cooking losses. The only significant effect for myofibrillar tenderness was aging method x endpoint temperature. Dry aged steaks cooked to 70°C had a mean myofibrillar tenderness score of 5.20, which was higher (P < 0.05) than that of wet aged steaks (Table 3). Quality grade and aging method did not affect (P > 0.05) juiciness but steaks cooked to 61.8°C were juicier (P < 0.05) than those cooked to 70°C (Table 3), which might be attributed to higher cooking losses with the higher endpoint temperature. Similarly, Sitz et al. (2006) reported no significant differences for flavor, juiciness, tenderness, and overall acceptability between dry-aged and wet-aged Choice strip loins. In our study, connective tissue amount, overall tenderness, flavor, and off flavor intensity were not affected (P > 0.05) by any treatment or treatment combinations (Table 3). Smith et al. (2008) similarly reported no differences in overall like scores between dry aged and wet aged steaks.
Table 3. Percentage value for physical and chemical parameters in relation to aging method and end point temperature.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Aging method</th>
<th>End point temperature (°C)</th>
<th>Aging method x endpoint temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry</td>
<td>Wet</td>
<td>P-v.</td>
</tr>
<tr>
<td>Myofibrillar tenderness</td>
<td>5.17</td>
<td>5.03</td>
<td>0.42</td>
</tr>
<tr>
<td>Beef flavor intensity</td>
<td>4.63</td>
<td>4.56</td>
<td>0.46</td>
</tr>
<tr>
<td>Juiciness</td>
<td>4.65</td>
<td>5.63</td>
<td>0.68</td>
</tr>
<tr>
<td>Connective tissue amount</td>
<td>5.86</td>
<td>5.70</td>
<td>0.31</td>
</tr>
<tr>
<td>Overall tenderness</td>
<td>5.18</td>
<td>4.99</td>
<td>0.44</td>
</tr>
<tr>
<td>Off flavor intensity</td>
<td>6.06</td>
<td>6.15</td>
<td>0.56</td>
</tr>
</tbody>
</table>

The calpain system has been extensively studied over the past few decades. Nevertheless, the mechanisms that control the calpain activity in postmortem muscle have not been fully elucidated (Lindahl et al., 2010). An important characteristic of calpains is that they autolyze once activated, ultimately leading to loss of activity (Goll et al. 2003). In bovine muscle the extractable activity of μ-calpain has been reported to decline rapidly during ageing, whereas the activity of m-calpain is more stable (Ducastaing et al., 1985 and Koohmaraie et al., 1987). Western Analysis was performed to determine μ-calpain, p-94 and calpastatin content in the Longissimus dorsi samples (Figs 1, 2). Calpain and calpastatin content were determined in the same samples that were evaluated for calpain and calpastatin activity. Our results by western blot analysis indicated that autolysis of the large subunit of μ-calpain was, already, detected in the standard sample, which was an independent at-death bovine muscle. During the period analyzed, in the muscle there was a gradual decline in the intensity of the 80 kDa band of μ-calpain while the intensity of the 60 kDa band, presumably the product of μ-calpain autolysis, increased throughout the post-mortem storage period. The expression of p94 remained the same in the muscle during the period considered, the calpastatin, at 15d post mortem, presented a slight decline.

The rate of calpastatin degradation and inactivation is related to the rate of proteolysis and tenderization observed in meat (Geesink and Koohmaraie 1999; Lonergan et al. 2001). The analysis of bioluminometer showed that the aging system significantly affected the activity on μ-calpain. Infact it was lower in dry aging system than wet aging. Extended aging from 7 to 15 days decreased μ-calpain activity. Particularly for dry aging approximatively 45% of at-death μ-calpain activity remains at mortem; 80% of at death μ-calpain activity remains after 7d of post mortem storage and approximatively 65% of at death μ-calpain activity remains after 15d of post mortem storage. For wet aging method 50% of at-death μ-calpain activity remains at mortem; 100% of at death μ-calpain activity remains after 7d of post mortem storage and approximatively 85% of at death μ-calpain activity remains after 15d of post mortem storage. Activity of p94 doesn’t change during post mortem storage in both methods (100%). Activity of calpastatin changes during post mortem storage and there wasn’t significantly differences between two methods: approximatively 100% of at death.
calpastatin remains at mortem; 70% of at death remains after 7d of post mortem storage and it was absent after 15d of post mortem storage.

Figure 1. Western blot analysis of µ-calpain, p-94 and calpastatin on Longissimus Dorsi muscle.

CONCLUSIONS

In conclusion our results showed that in the muscle object of study: µ-calpain expression was in inverse relation to the calpastatin expression; µ-calpain expression decreased in the time interval considered; µ-calpain activity increased at 7 days post mortem and decreased at 15 days post-mortem; the variability in tenderness after 15 days cannot be attributed to p-94; in particular there was much more differences for µ-calpain activity in the two aging methods while the calpastatin and p-94 followed the same trend.

Both aging methods used in our study resulted in similar palatability. However, D aging resulted in greater weight and trim loss (≥ 36%) and required extensive labor in trimming. This data are in accordance with the minor µ-calpain activity showed. A trained sensory panel revealed few, if any, differences among dry and vacuum aging. Dry aging beef process beyond the traditional aging period give to meat taste and mouth that feel optimize. Therefore, wet aging should be the preferred method of aging beef for most of the industry. We made this study in order to determine the score that consumers place on the two aging methods. A greater percentage of consumers favored wet-aged samples, indicating that high quality beef can be wet aged with desirable palatability results.

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Glyphosate attachment on aminoactivated carriers for sample stabilization and concentration

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Abstract. Glyphosate is the most widely used non-selective postemergence herbicide for weed and vegetation control. The need for monitoring glyphosate levels in environmental samples and agricultural products proceeds from its extensive use due to the unregulated application and contradictory information about its toxicity on living organisms. In order to achieve high sensitivity and reliability of glyphosate assessment, stabilization and preconcentration steps are generally required for its determination in different samples. The aim of the present study was to develop and optimize possibilities for effective glyphosate stabilization and concentration using aminoactivated nanoparticles of different materials. The results show that the usage of aminoactivated iron (II,III) oxide magnetic nanoparticles for the attachment and concentration of glyphosate is a prospective option to be integrated with in situ analytical technologies (e.g. biosensors), as the whole processes of glyphosate attachment was efficiently and reproducibly carried out within 20 minutes.

Key words: glyphosate, stabilization, concentration, analysis, amino-activated, magnetic iron oxide nanoparticles, silica.

INTRODUCTION

Glyphosate is the most widely used non-selective postemergence herbicide for weed and vegetation control. Glyphosate is commonly regarded to be safe to the environment, but several studies point out its potential toxicity on living organisms (Marc et al., 2004; Relyea, 2005a; Relyea, 2005b; Benachour & Séralini, 2008; Paganelli et al., 2010; Jones et al., 2011). Due to the unregulated application, the occurrence of glyphosate and its major metabolite aminomethylphosphonic acid (AMPA) in soil, ground and surface water has raised the need of continuous monitoring of glyphosate levels both in environment and agricultural products (Torstensson et al., 2005; Kolpin et al., 2006; Mörtl et al., 2013).

Limits have been set for glyphosate levels in drinking water and food. The maximum concentration of glyphosate in drinking water in the European Union should not exceed 0.1 μg l⁻¹ (Council Directive 98/83/EC); in US, the tolerable level is much higher, being 700 μg l⁻¹ (EPA, 2014). The maximum residue level of glyphosate in foods and feeds of plant and animal origin is currently set between 0.05–50 mg kg⁻¹, depending on the particular object (MRL Database, 2015).
The quantitative analyses of glyphosate and AMPA are commonly carried out using high performance liquid chromatography (HPLC). Although the sensitivity of HPLC is very high and the detection limits of glyphosate and AMPA are as low as 0.1 μg l⁻¹ (Lee et al., 2002), there is an urgent need for the development of analytical methods for continuous monitoring of these compounds in real-time. A promising option for rapid analyses is the application of biosensors. In order to achieve high sensitivity and reliability of detection, stabilization and preconcentration of glyphosate samples are generally required as glyphosate is highly soluble in water and is readily carried into surface water, streams and lakes (Council Directive 91/414/EC; Sanchís et al., 2012; Ding & Yang, 2013).

At present, only a limited number of glyphosate biosensors have been proposed. Sandwich-type immunosensors for glyphosate based on Au or CoB magnetic nanoparticles coupled with a fluorescence marker have been developed by Lee et al (2010; 2013). These systems are quite complex with limits of glyphosate detection (LOD) 0.01 mg l⁻¹ and 0.046 µg l⁻¹, respectively.

Glyphosate is readily degraded by microorganisms and its half-life depends on environmental conditions. The median half-life of glyphosate in water varies from a few days to 91 days (NPIC). To enhance the reliability of its quantitative analyses, preliminary glyphosate stabilization has been additionally used. Glyphosate has been covalently immobilized on amino-activated solid carriers through (N-[3-dimethylaminopropyl]-N’-ethylcarbodiimide hydrochloride) / (N-hydroxysuccinimide) or EDC/NHS chemistry. Ding & Yang (2013) used this method to immobilize glyphosate on amino-activated glass beads and developed a surface plasmon resonance biosensor with a glyphosate detection limit 98 µg/l. In this biosensor, silica beads served as a solid support to prevent the loss of glyphosate during screening procedures with oligopeptides used for glyphosate biorecognition (Ding & Yang, 2013).

In addition, glyphosate molecule acts as an anion within relevant pH ranges in water and it can be bound onto solid surfaces by sorption (Borggaard & Gimsing, 2008). Due to the high specificity of alumina towards phosphate groups, alumina-coated iron oxide nanoparticles as the affinity adsorbent for glyphosate and AMPA in aquatic solutions have been reported. This micro-scale solid phase extraction has been used in combination with electrochemical detection of glyphosate and AMPA with LODs 0.3 and 30 µg l⁻¹ respectively (Hsu & Whang, 2009). The presence of negatively charged groups of glyphosate has been also used in glyphosate analyses with the positively charged amino groups of cysteine-modified gold nanoparticles (Cs-AuNPs), where glyphosate stimulates the aggregation of Cs-AuNPs, detectable photometrically with LOD 9.9 µg l⁻¹ (Zheng et al., 2013). For a more selective adsorption of soluble glyphosate, molecularly imprinted microspheres (MIMs) have been synthesized. However, the application of MIMs in integration with the measurement of chemiluminescence resulted in higher LOD values for glyphosate: 46 μg l⁻¹ (Zhao et al., 2011). As it can be seen, the detection limits of glyphosate with biosensors are quite close to the glyphosate levels set by EU in drinking water. However, there is still a need for more sensitive analyses, which can be achieved by sample stabilization and preconcentration.

The aim of the present study was to develop rapid and reliable methods for glyphosate attachment on aminoactivated nano- and microparticles, which enable to improve the sensitivity and reliability of glyphosate analyses and can be used in further quantitative detection with biosensors or other systems (Fig. 1). Glyphosate was
immobilized onto two different types of aminoactivated solid beads – silica microparticles and magnetic iron oxide nanoparticles, which can be easily extracted after immobilization from mixtures using bead injection or magnetic separation techniques, respectively.

**Figure 1.** Steps of glyphosate analyses.

**MATERIALS AND METHODS**

Aminosilasorb (aminoactivated silica beads or ASB, $\varnothing$ 30 $\mu$m) was purchased from Chemapol (Brno, Czech Republic) and aminopropyl silane coated magnetic iron oxide nanoparticles (AFenP, $\varnothing$ 14 nm) from GreenBead OÜ (Tartu, Estonia). Both materials were fabricated with the help of 3-aminopropyltriethoxysilane and had similar chemically active groups on surface (Fig. 2).

**Figure 2.** The surface of aminoactivated beads.

The analytical standard of glyphosate (N-phosphonomethylglycine) (99.7%) was purchased from Sigma. We also used the commercial preparation of glyphosate (36% solution in water) (Monsanto Europa S.A.). EDC (N-((3-dimethylaminopropyl)-N´-ethylcarbodiimide hydrochloride (≥ 98%) and NHS (N-Hydroxysuccinimide) (98%) were purchased from Sigma; all other reagents used were of analytical grade from different producers. For preparation of all solutions the deionized water was used.

Glyphosate immobilization on the aminoactivated beads was carried out using EDC/NHS chemistry according to the reaction scheme shown on Fig. 3.

The activation of carboxyl groups of glyphosate was carried out at room temperature in 10 mM HEPES buffer (pH 7.0) at EDC and NHS concentrations 0.2 M and 0.05 M, respectively. EDC reacts with a carboxylic-acid group of glyphosate forming an amine-reactive O-acyl isourea intermediate. The addition of NHS convert it to an aminereactive NHS-ester, thus increasing the efficiency of EDC-mediated coupling reaction (Bart et al., 2009). After 10 min of incubation under constant stirring, 10–40 $\mu$l
of the solution, containing activated glyphosate was mixed with aminoactivated carrier beads. The immobilization time at room temperature (10 mM HEPES buffer, pH = 7.0) was 1–120 minutes. After immobilization, the beads were washed with 0.1 wt % SDS solution and DI water to remove unbound glyphosate. The concentration of bound glyphosate was determined photometrically measuring the light absorption of phosphorus-complexes at λ = 880 nm with spectrometer UV-1800 (Shimadzu) after treatment of glyphosate-bead complexes according to ascorbic acid method (Greenberg et al., 1985).

Figure 3. Immobilization of glyphosate onto aminoactivated beads.

RESULTS AND DISCUSSION

Glyphosate was immobilized on the aminoactivated silica beads and magnetic iron oxide nanoparticles using EDC/NHS chemistry. The optimization of immobilization process was focused on the generation of reproducible amounts of bound glyphosate within minimal time. For these studies, the concentrations of ASB and AFeNP were 200 and 20 mg l⁻¹, respectively.

As the first step, we determined the minimal number of washing procedures to remove all unbound glyphosate. Aspiration was used for ASB and magnetic extraction for AFeNPs to separate the unsoluble particles with attached glyphosate from mixtures. It was found that in case of ASB, the minimal number of washing procedures to remove all the unbound glyphosate was 10 and in the case of AFeNPs 1 (data not shown). This difference is the result of effective separation of AFeNPs with magnets, placed outside the reaction tube; at the same time, the separation of ASBs from solution was carried out with the help of vacuum filtering. The abovementioned numbers of washing procedures were applied in further studies of optimizing the immobilization time and building the calibration curves for the bound glyphosate.

The main factor of decreasing the time of the overall stabilization and concentration procedure of glyphosate is the time required for its immobilization on a carrier. The
recovery rates of glyphosate bound to ASB or AFeNP after 1–120 minutes of immobilization is shown on Fig. 4.

Figure 4. Glyphosate recovery at different immobilization times. The immobilization was carried out at room temperature in 10 mM HEPES buffer at pH 7.0. A: onto aminoactivated silica beads, [glyphosate] = 8 mg l\(^{-1}\); [ASB] = 200 mg l\(^{-1}\); B: onto AFeNP, [glyphosate] = 4 mg l\(^{-1}\); [AFeNP] = 20 mg l\(^{-1}\).

The measured maximal recovery of glyphosate was twice bigger with ASB than AFeNP, (14.9 ± 0.3) % and (7.0 ± 0.2) % on the initial amount of glyphosate in solution, respectively (Fig. 4). Based on the obtained data, we calculated the maximum specific load of immobilized glyphosate per 1 mg carrier (ML). The value of ML was (6.0 ± 0.1) µg glyphosate/mg ASB and (14.0 ± 0.4) µg glyphosate/mg AFeNP. These ML values were also used to estimate the concentration of active amino groups on the surface of the used particles. These calculated concentrations were 0.036 µM mg\(^{-1}\) for ASB and 0.083 µM mg\(^{-1}\) for AFeNP. Taking into consideration the estimated number of particles per mg and the Avogadro number, we found that both carriers used had over 10\(^3\) amino groups per particle available for glyphosate immobilization.

The maximum immobilization yield was achieved already within 1 minute incubation with ASB and there was no change in recovery during the next 119 minutes. So, using ASB, the incubation time for immobilization can be as short as 1 minute. Shorter incubation times were not studied in the present work due to technical reasons, but if ASB will be used in automated analysis system, even shorter immobilization times can be considered. For AFeNPs, the shortest immobilization time yielding maximum attachment (Fig. 4B) was 10 minutes (it was also similar for other glyphosate concentrations, data not shown).

Comparing the immobilization of glyphosate on ASBs and AFeNPs, the total time required as a sum of immobilization and washing procedures is considerably lower for AFeNPs due to the very effective separation of unbound glyphosate. For further decrease of the immobilization time, higher temperatures can be suggested.

The concentration of ASBs used for immobilization was taken as proposed by Ding & Yang (2013). As there was no data available for AFeNPs, the influence of the concentration of AFeNPs with the aim of improving the sensitivity of the system was studied. As it can be seen on Fig. 5, the detected recovery of glyphosate increases in a linear mode as a function of the concentration of AFeNPs (shown with 95% confidence band).
Figure 5. The recovery of glyphosate after immobilization as a function of the concentration of magnetic iron oxide nanoparticles. The initial concentration of glyphosate was 4 mg l\(^{-1}\). The immobilization was carried out at room temperature in 10 mM HEPES buffer at pH 7.0 for 10 min.

Increasing the concentration of AFeNPs to 80 mg l\(^{-1}\) raised the immobilization yield to over 30% of the theoretical. Higher recoveries lead to lower glyphosate detection limits, which can be further improved by combining the glyphosate attachment with more sensitive detection methods.

The glyphosate calibration curves with 95% confidence bands, using ASB or AFenp at concentrations 200 and 20 mg l\(^{-1}\) respectively, are shown on Fig. 6.

The calibration plots were linear in the studied concentration ranges. The line functions for ASB and AFenp systems were following:

\[
y = (0.022 \pm 0.001)x + (0.069 \pm 0.006) \quad \text{(ASB)}
\]
\[
y = (0.023 \pm 0.002)x + (0.085 \pm 0.005) \quad \text{(AFenp)}
\]

The high detection limits were resulting from the detection limit of phosphorus by ascorbic acid method, being 0.1–1 mg l\(^{-1}\) (Greenberg et al., 1985). The slope of calibration curve, defining the sensitivity of detection, was similar for ASB and AFenp systems, although the concentration of aminoactivated silica beads was 10 times higher. As shown on Fig. 5, the slope for AFenp system can be increased easily by increasing the concentration of AFenps. The y-intercepts of these calibration plots indicate the background signal of the used photometric detection.

Figure 6. Glyphosate calibration curves for ASB and AFenp systems. The immobilization of glyphosate was carried out at room temperature in 10 mM HEPES buffer at pH 7.0. A: with aminoactivated silica beads; [ASB] = 200 mg l\(^{-1}\); B: with AFenp; [AFenp] = 20 mg l\(^{-1}\).
CONCLUSIONS

The study indicates that the application of AFeNPs for the stabilization and concentration of glyphosate-containing probes has a high potential to be integrated with in situ analyses for continuous monitoring of glyphosate, as all necessary procedures (incl. glyphosate activation, immobilization and concentration) can be carried out with high efficiency within 20 minutes. For the development of methods for practical analyses, the detection of the bound on AFeNPs glyphosate will be further carried out with immunobiosensing system, enabling to achieve high selectivity and low detection limits, which are in line with the established glyphosate concentrations in drinking water and food.

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REFERENCES


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