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CONTENTS

The effect of cadmium and lead pollution on growth and physiological parameters of field beans (*Vicia faba*)

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Abstract. Research on the impact of soil contamination on crops is important as plants directly take up heavy metals from the soil through the roots, so heavy metals can enter the food chain. The aim of this study was to investigate the impact of cadmium (Cd) and lead (Pb) pollution on growth and physiological parameters of field beans. Plants in the vegetation experiment were grown under controlled conditions. Changes in growth and physiological parameters were studied at five levels of Cd (0–25 mg L^{-1}) and at 6 levels of Pb in substrate: from (0–$1,000$ mg L^{-1}) at the first day of the experiment, to (0–$2,000$ mg L^{-1}) at the end of the experiment after gradual Pb additions after every sample collecting day. Methods used for analysing the plant material: the content of amino acid proline and photosynthetic pigments were determined by spectrophotometry; chlorophyll *a* fluorescence parameters – using continuous excitation chlorophyll fluorimeter. The fresh weight of plant above-ground parts and roots was detected. The growth and development of field beans was slightly influenced by increasing amount of Cd and Pb in substrate only at the end of the experiment. The highest Cd treatments (Cd20 and Cd25) caused 2.5 and 1.3 times increased proline concentration in bean leaves. The chlorophyll *a* + *b* content and chlorophyll *a* fluorescence parameter F_v/F_m changed differently throughout the experiment. In general, during the experiment, there was a tendency for the content of proline in leaves for Pb treatments to be increased compared to control. At the end of the experiment the content of proline in field bean leaves of the highest Pb treatments (Pb600 + 100 + 400 + 500, Pb800 + 100 + 400 + 500 and Pb 1,000 + 100 + 400 + 500) was 1.66, 1.44 and 1.55 times higher, respectively, than that of the control plant leaves. The negative impact of exposure to Pb on chlorophyll *a* + *b*, chlorophyll *a* fluorescence parameter P_{Index} and F_v/F_m in bean leaves was less pronounced compared to Cd. The obtained results confirm that field beans until their flowering stage can grow and develop in the presence of a large amount of Cd and Pb in substrate without significant growth inhibition and detrimental impact on physiological parameters, if optimal cultivation conditions are provided.

Key words: heavy metals, *Vicia faba*, photosynthesis parameters, proline.

INTRODUCTION

With the development of agriculture and industry, research on the impact of soil contamination on crops is of vital importance not only in Europe but throughout the

world (Pourrut et al., 2011; Van Liedekerke et al., 2014, Fu et al., 2017). Plants directly take up heavy metals from the soil through the roots, so heavy metals can enter the food chain, thus affecting human health (Peralta-Videa et al., 2009). In Europe the most frequently occurring contaminant groups in soil are heavy metals and mineral oils – 35% and 24%, respectively (Van Liedekerke et al., 2014). Heavy metals (for example, Cd and Pb) can enter the soil as the result of human activities, for example, using plant fertilizers in agriculture (Grant et al., 1998; Peralta-Videa et al., 2009; Swartjes, 2011). Therefore, it is important to pay attention to the questions of phytoremediation – on how to recover the soil from heavy metal pollution. In this aspect it is relevant to find out plants with high capacity to accumulate heavy metals. Previous studies have shown that hyperaccumulants are able to accumulate up to 100 times more heavy metals in leaves than other plants (Rascio & Navari-Izzo, 2011). On the other hand, studies on tolerance/sensitivity and distribution models of heavy metals in plants are of great value also for crop plants taking into account food safety issues.

Increased amounts of heavy metals in the environment can cause detrimental changes in physiological and biochemical processes in plants, thus affecting growth and productivity (Loi et al., 2012; Loi et al., 2014). The impact of heavy metals on plant growth and development is species-specific and tissue specific, for example, in vegetables heavy metals reduce shoot – root biomass ratio (Martin et al., 2006). It is noteworthy that heavy metal transport from soil to plants are affected by different factors as soil properties (soil pH, organic matter content, granulometric composition etc.), plant species specificity, the physical and chemical properties of the elements, growing conditions, environmental biotic factors and others (Titov et al., 2007; Loi et al., 2014). It is known that under different environmental stress conditions, including heavy metal contamination, the plants may show changes both in the amount of chlorophyll $a + b$ and in the chlorophyll a fluorescence parameters (Zarco-Tejada et al., 2002; Sayed, 2003; Pourrut et al., 2011). Heavy metal induced alteration in photosynthetic pigments influenced the biosynthesis of photoassimilates which resulted in decreased biomass production and stunted plant growth (Titov et al., 2007; Sengar et al., 2008; Capelo et al., 2012).

The changes in the accumulation of amino acid proline in plants under different biotic and abiotic stresses are widely documented (Hare & Cress, 1997; Kavi Kishor et al., 2005; Verbruggen & Hermans, 2008; Szabados & Savoué, 2010). The increased proline accumulation in plants can be caused by heavy metals, drought, oxidative stress, high light and UV irradiation, high salinity, high/low temperature, atmospheric pollution and also biotic stresses (Schat et al., 1997; Trovato et al., 2008; Verbruggen & Hermans, 2008; Szabados & Savoué, 2010; Karlsons, 2011). Proline accumulation in plants can also be a stress signal that affects plant adaptive responses like as stabilizing the structure of proteins, providing a way to buffer cytosolic pH and balance cell redox status (Verbruggen & Hermans, 2008). Schat et al. (1997) have reported that the plant water balance declines under exposure to heavy metals (especially Cd). In the conditions of osmotic stress the growth of plants is inhibited, stomata closed and the rate of photosynthesis decreased (Trovato et al., 2008). Proline as essential osmolite participates in osmotic stress tolerance protecting plant cells against osmotic stress damage (Hare & Cress, 1997; Trovato et al., 2008). The initial amount of proline in plants can be species specific (Verbruggen & Hermans, 2008).

According to the regulation of the European Union starting from 2015 farmers can get a payment for agricultural practices beneficial for the climate and the environment (called: 'greening payment') (Regulation (EU) No 1307/2013, 2013; Hart et al., 2017). Diversification of crops in Latvia is a mandatory requirement from 2015. The aim of diversification of crops is to promote enhanced environmental protection, especially in the area of soil quality improvement. Field beans are one of the crops used for 'greening' to improve soil quality in Latvia (The Rural Support Service, 2018). However, so far, there is little research on field bean physiological and adaptive responses to different stress conditions, including soil contamination with heavy metals.

The aim of this study was to investigate the impact of Cd and Pb pollution on growth and physiological parameters of *Vicia faba*, to find out the changes in biomass production, photosynthesis and chlorophyll *a* fluorescence parameters as well as changes in the accumulation of amino acid proline.

MATERIALS AND METHODS

The vegetation experiment was carried out with the field bean (*Vicia faba,* cv. 'WITKIEM') as a model object. Plants were grown in 1 L polyethylene containers from seeds; quartz sand was used as a growing substrate. The following controlled conditions were provided for plant growth: photoperiod light/dark 16/8 h, moisture of substrate 60–65%, day/night temperature + 20/18 °C and a photon flux density of 160 μmol m^{-2} s^{-1} supplied by fluorescent tubes. To ensure sufficient moisture level in the substrate, it was regularly gravimetrically watered using deionized water.

Changes in growth and physiological parameters were studied at five levels of Cd in substrate: 0, 10, 15, 20, 25 mg L^{-1}. The experiment was arranged with the following 6 levels of Pb in substrate at the first day of the experiment: 0, 200, 400, 600, 800, 1,000 mg L^{-1}. To find out changes in growth and physiological parameters under conditions of variable Pb amount in substrate, the amount of Pb in the substrate was gradually added after every sample collecting day during the experiment. Thereby, after the 14th, 21st, and 28th day of the experiment 100 mg L^{-1} Pb, 400 mg L^{-1} Pb and 500 mg L^{-1} Pb, respectively, was added in substrate for every Pb treatment. Abbreviations of treatments for experiment with Pb supply in the substrate are given in the Table 1.

Table 1. Abbreviations of treatments for the experiment with Pb supply in the substrate in during the experiment

The day of sampling	14	21	28	35
Treatment 1 (control)	Pb 0	Pb 0	Pb 0	Pb 0
Treatment 2	Pb 200	Pb 200 + 100	Pb 200 + 100 + 400	Pb 200 + 100 + 400 + 500
Treatment 3	Pb 400	Pb 400 + 100	Pb 400 + 100 + 400	Pb 400 + 100 + 400 + 500
Treatment 4	Pb 600	Pb 600 + 100	Pb 600 + 100 + 400	Pb 600 + 100 + 400 + 500
Treatment 5	Pb 800	Pb 800 + 100	Pb 800 + 100 + 400	Pb 800 + 100 + 400 + 500
Treatment 6	Pb 1,000	Pb 1,000 + 100	Pb 1,000 + 100 + 400	Pb 1,000 + 100 + 400 + 500

Cd was added as $Cd(NO_3)_2$ $4H_2O$ solution in substrate and Pb was added as $Pb(CH_3COO)_2$ $3H_2O$ solution in substrate. Nutrient solution was added in substrate for control groups and for all treatment groups, it contained optimal concentrations of macronutrients and micronutrients (in mg L^{-1}: N 120, P 60, K 150, Ca 800, Mg 50, S 60, Mn 1.5, Zn 1, Cu 0.5, Mo 0.02, B 0.2, Fe 30) (Osvalde, 2011). Ca was added as two grams of $CaCO_3$ in substrate of each container at the beginning of the experiment. As the Cd standard solution contains nitrogen, adjustments were done to prepare the complete nutrient solutions for Cd treatments by reducing the content of ammonium nitrate.

The experiment lasted 35 days; plants were collected on the day of 14th, 21st, 28th and 35th of the experiment. Field bean fresh weight was determined for all sampling dates. The fresh weight of plant above-ground parts and root system was separately detected.

The content of chlorophyll $a + b$ was determined in the first fully expanded leaves of field beans. Photosynthetic pigments were determined by spectrophotometry method using JENWAY 6300 Spectrophotometer (JENWAY, UK). Pigments were extracted with 20 mL of 96% ethanol, extracts were centrifuged and absorbances were measured at 470, 649 and 664 nm. Lichtenthaler (1987) equitation was uesd to calculate amount of chlorophyll $a + b$.

Chlorophyll a fluorescence parameters such as the variable fluorescence (F_v/F_m) and Performance Index (P_{Index}) were determined with continuous excitation chlorophyll fluorimeter Handy PEA system (Hansatech, UK). *Vicia faba* leaves were dark-adapted with leaf clips for 20 minutes.

The content of amino acid proline was determined by spectrophotometry method, adjusting the method with ninhydrin (Bates et al., 1973). Fresh plant material was homogenized in sulfosalicylic acid. Two milliliters of acid ninhydrin and glacial acetic acid was added to the extract, it was heated for 1 h at 100 °C and then the mixture was extracted into a toluol (Karlsons, 2011). Absorbances were measured at 520 nm using JENWAY 6300 Spectrophotometer (JENWAY, UK).

The statistical analysis of results was done using MS Excel 2013. Standard errors (SE) were calculated in order to reflect the mean of the results.

RESULTS AND DISCUSSION

Accumulation of biomass is the key factor for evaluating plant responses to various environmental stresses. There were different changes in the fresh weight of the above-ground parts and roots of the *V. faba* under exposure to increasing Cd and Pb levels in sand substrate. Laboratory experiments showed that the fresh weight of the above-ground parts of field beans cultivated under different Cd treatments varied similarly to the control variant throughout the experiment (Fig. 1, A). In general, the effect of the Cd contamination in substrate on the fresh weight of the above-ground parts of field beans was found to be insignificant ($p < 0.1$). Thus, on the last day of the experiment, the fresh weight of bean leaves and stems at the highest contamination level (Cd 25 mg L^{-1}) was only 1.81 g lower (i.e., 9.58% lower) than that of the control plants (Cd0).

Unlike to the above-ground parts, significant effect of Cd pollution in substrate on the fresh weight of roots was found ($p < 0.05$). Field bean exposure to Cd10, Cd20 and Cd25 treatments lead to an increasing reduction in the fresh weight of roots throughout

the experiment (Fig. 1, B). The treatment of Cd 25 mg L^{-1} resulted in the 1.51 g lower (i.e., 14.5% lower) fresh weight of roots than that of the control level at the last sampling time (the 35th day).

More pronounced inhibition on the fresh weight of roots under heavy metal pollution could be mainly explained by root direct contact with the contaminated substrate (Pourrut et al., 2011). Thus, the negative effects of Cd could first affect plant roots.
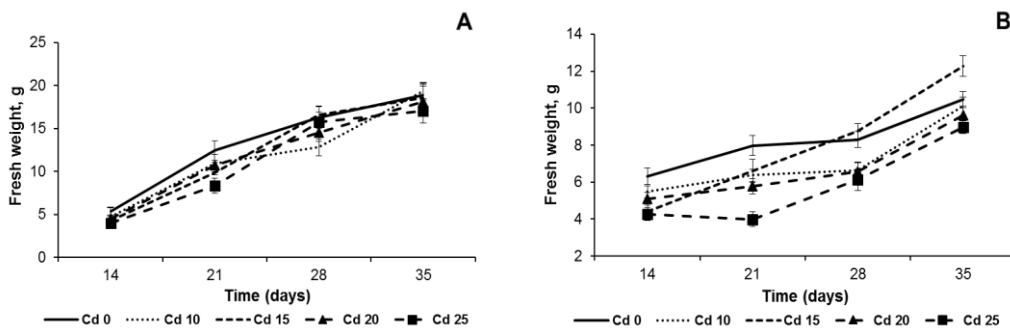
Figure 1. Fresh weight of the above-ground parts (A) and roots (B) of *V. faba* at five levels of Cd added in substrate, ±SE.

There was similar pattern of Pb impact on the fresh weight of above-ground parts and roots of *V. faba* as described for Cd treatments (Fig. 2, A, B) despite the fact that the level of Pb in the substrate during the experiment was gradually increased for all treatment variants.

Overall, a significant effect of Pb in substrate on fresh weight of field bean roots ($p < 0.05$) was observed.

On the last day of the experiment, fresh weight of the above-ground parts was 10.44% lower than that of the control plants at the highest contamination level (Pb 1,000 + 100 + 400 + 500 mg L^{-1}) in the substrate (Fig. 2, A). For roots this reduction reached 20% (Fig. 2, B).

Thus, no significant inhibitory effect of Cd and Pb on the fresh weight of the above-ground parts of *V. faba* was found until flowering stage. Only a slight trend of reduction was stated. In general, there is contradictory evidence about the effect of Cd on field bean weight. Sajwani et al. (1996) have found that Cd, Ni and Se did not affect the biomass on *Phaseolus vulgaris*. Likewise, Loi et al. (2014) have reported no significant Cd impact on the *Faba vulgaris* dry mass for treatments with Cd 0, 25, 50 and 75 mg L^{-1} in substrate. Similar conclusion is made by Jin at al. (2017) in the study on effect of Cd stress on broad bean. In contrast, Simek & Tuma (2016) reported about stimulating impact of Cd (Cd 2 and 20 mg kg^{-1}) on the production of *Phaseolus vulgaris* biomass.

The content of chlorophyll *a* + *b* in *V. faba* leaves varied throughout the experiment, both in treatment with Cd and Pb (Fig. 3, A, B). The negative impact of Cd on chlorophyll *a* + *b* in bean leaves was more pronounced compared to Pb.

Figure 2. Fresh weight of the above-ground parts (A) and roots (B) of *V. faba* at 6 levels of Pb added in substrate, ±SE.

In general, a significant effect of Cd pollution in the substrate on chlorophyll *a* + *b* content in leaves of *V. faba* ($p < 0.05$) was observed. During the experiment, the chlorophyll *a* + *b* content in plant leaves for all Cd treatments was lower than that in control plants (Fig. 3, A).

Unlike to Cd, the content of the chlorophyll *a* + *b* in bean leaves for Pb treatments was lower than that of the control plants mainly in the second half of the experiment, that is, from the 28th to 35th day of the experiment (Fig. 3, B). It should be noted that, the chlorophyll *a* + *b* content in plants in the variants Pb400 + 100 and Pb600 + 100 changed uniformly from day 21 to day 35 of the experiment.

On the 21st day of the experiment, a decrease in the content of chlorophyll *a* + *b* was observed for almost all treatments (except only: Cd20, Pb800 + 100), including control. This may be due to a certain stage of plant development or probably due to the impact of environmental factors. After this point, the decrease in chlorophyll content was mainly found in the conditions of higher pollution doses.

According to Masarovičová et al. (2010), heavy metal toxicity resulted in reduction of total chlorophyll content and, consequently, in photosynthesis inhibition in plants. Pb and Cd was reported to disrupt photosynthesis by changed chloroplast ultrastructure, reduced synthesis of chlorophyll, plastoquinone, carotenoids, disturbed electron transport (Seregin & Ivanov, 2001; Cheng, 2003; Titov et al., 2007). In general, various stress conditions can also cause chlorophyll hydrolysis to chlorophyllides and phytol (Pshibytko et al., 2004). Loi et al. (2012) studies have shown that Cd in the substrate has a negligible effect on the photosynthesis process in the *Faba vulgaris,* indicating on the tolerance of beans to heavy metal pollution.

Figure 3. Chlorophyll $a + b$ content (mg g^{-1}) in *V. faba* leaves at five levels of Cd (A) and at 6 levels of Pb (B) added in substrate, ±SE.

Chlorophyll *a* fluorescence parameters are sensitive to changes in photosynthesis process depending on the environmental impact, so they are used to describe photosynthesis process of plants (Sayed, 2003; Kalaji et al., 2014). Chlorophyll *a* fluorescence parameter P_{Index} and F_v/F_m during the experiment changed differently depending on Cd level in the substrate (Fig. 4, A, B). The relative values of the parameter P_{Index} for all treatments (Cd10, Cd15, Cd20 and Cd25) were lower than that of the control variant. A similar situation was found for the relative values of the parameter F_v/F_m. On the 35th day of the experiment, there was a significant increase of the relative values of P_{Index} for all variants compared to the 28th day of the experiment: P_{Index} values increased 1.9 times for Cd0 plants, 2.0 times for Cd10, 2.2 times for Cd15 and Cd20, and 2.3 times for Cd25 (Fig. 4, A). Conversely, on the last day of the experiment, rapidly reduced values of F_v/F_m for plants of all variants were found: for control variant plants the F_v/F_m value decreased to 0.827, while for Cd treatments they ranged from 0.817 to 0.823 (Fig. 4, B). It is assumed that 0.8 is the limit of F_v/F_m value between optimal and stress conditions (Gailite, 2012). Since none of the treatments had a F_v/F_m value lower than 0.8, Cd as a stress factor probably did not cause photo-inhibition for field beans.

In general, a significant effect of Cd on P_{Index} values was observed during the experiment ($p < 0.05$). As P_{Index} represents vitality of plants, our results showed that at the flowering phase (end of the experiment) vitality of the field beans increased for all Cd treatments, indicating possible adaptation to stress conditions.
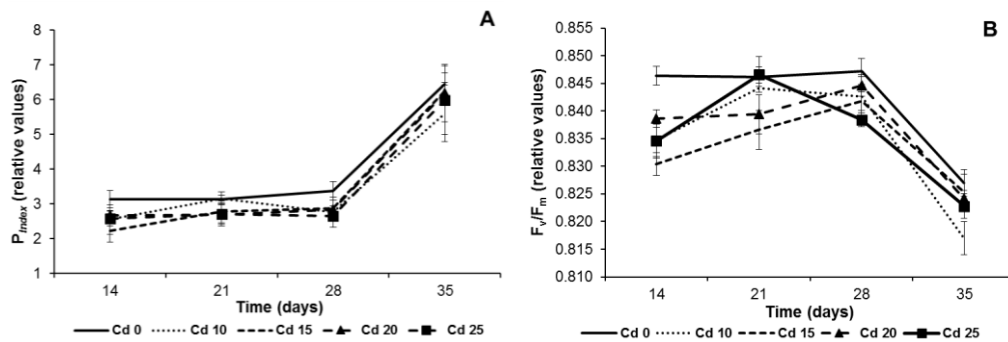
Figure 4. Chlorophyll *a* fluorescence parameter P*Index* (relative values) (A) and F$_v$/F$_m$ (relative values) (B) of *V. faba* at five levels of Cd added in substrate, ±SE.

It was found that the relative values of P*Index* and F$_v$/F$_m$ in the experiment with Pb pollution in the substrate changed similarly to those stated in the Cd experiment (Fig. 5, A, B). In general, the effect of Pb on changes in chlorophyll *a* fluorescence parameters were less pronounced than they were in the Cd variants even under conditions when Pb level of pollution in the substrate, gradually increased during the experiment, was very high reaching 1,200, 1,400, 1,600, 1,800, 2,000 mg L^{-1} at the end of the experiment.

Thereby, field bean as an experimental model object showed high adaptation potential to stress conditions in the case of Cd and Pb contamination in the growing medium.

* Abbreviations;

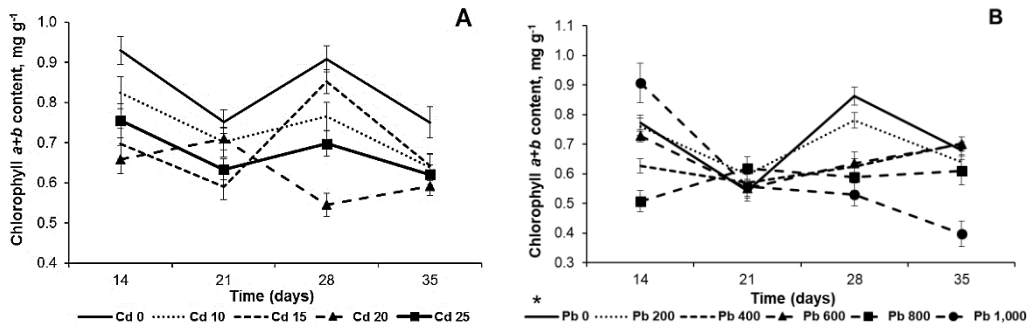| Days			
14	21	28	35
Pb 0	Pb 0	Pb 0	Pb 0
Pb 200	Pb 200 + 100	Pb 200 + 100 + 400	Pb 200 + 100 + 400 + 500
Pb 400	Pb 400 + 100	Pb 400 + 100 + 400	Pb 400 + 100 + 400 + 500
Pb 600	Pb 600 + 100	Pb 600 + 100 + 400	Pb 600 + 100 + 400 + 500
Pb 800	Pb 800 + 100	Pb 800 + 100 + 400	Pb 800 + 100 + 400 + 500
Pb 1,000	Pb 1,000 + 100	Pb 1,000 + 100 + 400	Pb 1,000 + 100 + 400 + 500

Figure 5. Chlorophyll *a* fluorescence parameter P*Index* (relative values) (A) and F$_v$/F$_m$ (relative values) (B) of *V. faba* at 6 levels of Pb added in substrate, ±SE.

The research revealed a significant increase in the content of amino acid proline in the field bean leaves with increase of Cd in the substrate till the 21st day of the experiment (Fig. 6, A).

The highest Cd treatments Cd20 and Cd25 caused 2.5 and 1.3 times, respectively, increased proline concentration in *V. faba* leaves. Further in the course of the experiment, this plant biochemical response significantly decreased. Nevertheless, on the last day of the experiment, the content of proline in the treatments Cd20 and Cd25 was 22% higher than that in the control variant plants.

There are evidences in the literature (Loi et al., 2014) that the content of proline in field beans in Cd treatments (Cd 2, 5, 10, 20 and 50 mg kg^{-1} in soil) is similar to the content of proline in the control variant plants on the 21st day of the experiment.

In experiment with Pb pollution, only one treatment (Pb600) induced massive accumulation of free proline in field bean leaves on the 14th day of the experiment. It is notable that in the conditions of Pb800 this impact was lost and the content of proline in the leaves was even 9.68% lower than that of the control plants (Fig. 6, B).

* Abbreviations;

Days			
14	21	28	35
Pb 0	Pb 0	Pb 0	Pb 0
Pb 200	Pb 200 + 100	Pb 200 + 100 + 400	Pb 200 + 100 + 400 + 500
Pb 400	Pb 400 + 100	Pb 400 + 100 + 400	Pb 400 + 100 + 400 + 500
Pb 600	Pb 600 + 100	Pb 600 + 100 + 400	Pb 600 + 100 + 400 + 500
Pb 800	Pb 800 + 100	Pb 800 + 100 + 400	Pb 800 + 100 + 400 + 500
Pb 1,000	Pb 1,000 + 100	Pb 1,000 + 100 + 400	Pb 1,000 + 100 + 400 + 500

Figure 6. Proline content (µmol g^{-1}) in *V. faba* leaves at five levels of Cd (A) and at 6 levels of Pb (B) added in substrate, ±SE.

On the 35th day of the experiment the content of proline in the field bean leaves of the highest treatments: Pb600 + 100 + 400 + 500, Pb800 + 100 + 400 + 500 and Pb 1,000 + 100 + 400 + 500 was 66.67%, 44.44% and 55.56%, respectively, higher than that of the control plant leaves. In general, during the experiment, there was a tendency for the content of proline in the leaves for Pb treatment variants to be increased compared to control. Thus, Cd and Pb showed impact on proline biosynthesis in *V. faba* leaves. Increased proline accumulation could be regarded as *V. faba* adaptive response to Cd and Pb pollution in growing medium. Pb induced increase in proline content was also reported for cowpea (*Vigna unguiculata*) (Krishnaveni et al., 2015).

CONCLUSIONS

Summarizing the results, it can be concluded that in laboratory experiment in sand substrate cultivated *V. faba* showed different changes in the fresh weight of the above-ground parts and roots under increasing Cd and Pb levels in substrate. It is evident that both Cd and Pb significantly decreased root fresh weight, while Cd pollution had negative effect also on the fresh weight of above-ground parts. Increase of Cd and Pb in the substrate showed slight impact on the photosynthetic performance of *V. faba*. The negative impact of exposure to Cd on chlorophyll *a + b*, chlorophyll *a* fluorescence parameter P$_{Index}$ and F$_v$/F$_m$ in bean leaves was more pronounced compared to Pb. In general, chlorophyll *a* fluorescence parameter P$_{Index}$ and F$_v$/F$_m$ during the experiment changed differently depending on Cd and Pb level in the substrate and plant development stage. Since none of the treatments had a F$_v$/F$_m$ value lower than 0.8, Cd and Pb as a stress factor probably did not cause photo-inhibition for field beans. According to the results, Cd and Pb showed stimulative impact on proline biosynthesis in *V. faba* leaves. Increased proline accumulation could be regarded as *V. faba* adaptive response to Cd and Pb pollution in growing medium. Thereby, field beans showed high adaptation potential to stress conditions in the case of Cd and Pb contamination in the growing medium.

As the results on the content of Cd and Pb in the above-ground parts and roots of the field beans is still in the process of determining, discussion about possible use of field beans for forage or in phytoremediation from/of polluted territories is the subject of next paper.

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Effect of concentrate supplementation on fattening performance and carcass composition of finished meat-goat kids

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Abstract. A study on the effective usage of the concentrated feed supplement to Boer meat goat kids was carried out within the framework of the project 'Zootechnical and economic efficiency of feeding of fodder pulses to ruminant's meat production'. Since the Boer goat breed in Latvia is still very rare, there is a lack of experience in the feeding and production of meat goat for better carcass traits and meat quality. This study was arranged in autumn period, using the Boer cross breed male kids born in 2018. In control group (OG) four kids were kept together with mothers till finishing and were fed by oats as concentrated feed supplement. In research group (BG) four goat kids were weaned from mothers and placed in shed to explain influence of mix of 85% of oats and 15% of fodder beans as protein supplement to the fattening outcomes. In the trial kids were weighed at the starting and ending of the trial. Carcass quality is assessed according to the European standard for the classification of carcasses of sheep, where EUROP letter designations have been used to denote musculature development, and the fat deposition level is indicated by numbers 1–5. The length of the carcass and the circumference of the hips were measured using the tape measure. The carcasses were analyzed by type of tissue: muscle, bone, fat. The fastest growing rate during the fattening period of 72 days was given to BG kids with a mean daily live weight gain of 72 g, while the OG kids achieved 69 g. After slaughter, the carcass yield of OG kids was from 42.5% to 51.4%, but for BG kids was 38.5% to 42.5%. The quality of the obtained carcasses was an average, and score for musculature was from R to P class, but the average score for fat deposition was from 2.25 to 2.75 points and higher fat cover was observed in BG kids. Higher proportion of lean meat (59.1%) and fat tissues (16.3%) were observed to OG kids, but higher proportion of bone (25.9%) was in BG kid's carcasses. The consumption of concentrated feed for 1 kg of live weight gain indicates the conversion of feed nutrients. The consumption of oats per kilogram of live weight gain was 3.21 kg for OG kids, but the consumption of the feed mix of oats and beans for BG kids was 2.83 kg. Based on physical parameters of goat kid carcass and high level of lean meat, fattening of Boer cross breed goat kids by using of oats or feed mix can be an ideal choice for farmers, which try to find new products and free market for consumers.

Key words: kids, carcass characteristics, meat quality.

INTRODUCTION

In the Baltic States goat farming focuses mainly on milk production, however, recently there has been increased interest and demand for goat meat. Boer goats were imported into Latvia in 2005 and used in cross breeding for the improvement of goat

meat quality and quantity. At present, the Latvian goat breeding industry is in early stage of its development. Although there is a demand for goat meat in Latvia, the number of meat goats is decreasing. In order to successfully develop goat meat production, it is necessary to increase the number of animals and continue to promote goat meat production in Latvia (Lauksaimniecības zinojums par 2014. gadu ...).

If you breed meat goats, then you will not get milk because goats give milk only for kids. Meanwhile, there will be a lot of meat production and goat meat is very valuable. The favorite is 6–8-week old goat kid meat, which is very similar to lamb (Piliena & Spruzs, 2007).

Small ruminants are the most efficient transformers of low quality forage into high quality animal products with distinguished chemical composition and organoleptic characteristics (Zervas & Tsiplakou, 2011).

The feeding system effects on meat quality are more difficult to be identified because kids of different breed, weaned at different age and live weight or raised on different types of pastures have different growth rate and carcass characteristics like level of fatness, flavor, tenderness, taste, etc. It has been demonstrated that kids raised under a grazing system without any supplementation, present an inferior fatness degree and a higher meat fat concentration of n−3 PUFA and CLA (Zervas & Tsiplakou, 2011). Goat meat has different distribution of fat compared to that in sheep meat. At similar carcass weight goats may be expected to produce carcass with lower proportion of subcutaneous and intramuscular carcass fat and a higher proportion of non-carcass fat in the abdominal cavity, compared to sheep. This lower proportion of carcass fat is reflected in a higher proportion of bone in the carcass of goats (Morand-Fehr et al., 1991). Consumers are interested in goat meat as a source of relatively lean meat, especially in developed countries with a high incidence of cardiovascular diseases (Zervas & Tsiplakou, 2011).

The nutritional requirements of goats managed primarily for milk production and those managed primarily for meat production are quite similar with perhaps two notable differences. First, meat goats need only achieve a 4–7 month lactation with high initial milk flow, persistency beyond 4 months being of lesser concern. Secondly, lactating meat goats are not usually fed concentrates in addition to their forage diet because the extra kid growth achieved from the extra milk may well not repay the added costs. In those situations in which the plants are too low in protein, additional protein must be offered to maintain acceptable goat performance. High protein supplemental feedstuffs, used only occasionally by meat goat owners, are soybean meal, peas and field beans meal, urea and others. Choosing between alternative high protein feedstuffs is largely an economic decision (Pinkerton & Pinkerton, 2015).

Confinement feeding of diets with concentrate can affect various carcass characteristics as well as internal and carcass fat levels (Goetch et al., 2011). In addition to effects of dietary inclusion of concentrate, the level feed can affect mass of non-carcass organs and tissues as well as carcass weight and composition. Intake and average daily gain (ADG) by crossbred goats in a 90-day experiment increased as *ad libitum* access to concentrate, which was accompanied by increasing liver (% of empty BW) and internal (primarily omental) and total body fat mass (Mushi et al., 2009b). According the study of Wuliji et al. (2003) growing Spanish kids consumed more dehydrated alfalfa pellets than a 70% concentrate diet, resulting in similar ADG. Nonetheless, fat accreted in non-carcass tissues was greater for the concentrate diet. Goats appear to differ from

cattle and sheep in performance benefits from diets very high in concentrate (Mushi et al., 2009a). For example, ADG and carcass weight of growing Angora wethers were similar between confinement feeding of diets consisting of 80% concentrate or 24% concentrate and 76% forage (20% cottonseed hulls and 56% alfalfa hay. Although above it was generalized that growth performance of goats is not improved by high dietary concentrate levels. Level of concentrate had few effects on meat quality, but the total level of fat in dissected rack tissue was greater for the highest vs. intermediate dietary concentrate level and the level of subcutaneous fat was greatest among diets for the diet highest in concentrate. But the research of Lee et al. (2008) found that there were considerably greater ADG for continuous feeding of the concentrate vs. hay diet (134 vs. 41 g) and much greater carcass weight for the concentrate diet. High internal fat deposition by goats has heightened interest in feeding practices to maintain high lean tissue gain and meat quality with little fat accumulation. One such method is limited intake of concentrate-containing diets, which in beef cattle offers a means of achieving efficient feed utilization perhaps by minimizing the maintenance energy requirement at least in part because of limiting energy use by splanchnic tissues (Galyean, 1999), as would be expected in goats as well (Tovar-Luna et al., 2007).

The objective of our study was to assess the efficiency of feeding a compound feed containing fodder beans to Boer kid meat productivity and to study its impact on meat quality.

MATERIALS AND METHODS

The study was carried out on a farm whose main activity is the production of goat meat. This study included 8 Boer cross breed goat kids born in this farm in spring 2018. The kids were divided into two groups of 4 animals each similar in age and live weight. After 7 days adaption period at beginning of fattening the control group kids average age was 209 days and the average live weight was 30.0 kg, but the research group kids average age was 215 days and the average live weight was 28.1 kg. Dietary treatment was conducted according to research scheme (Table 1).

In the summer, goats of all ages and genders were grazed in the farm, and 1st control group kids together with their mothers were separated from herd and fed with pasture grass, hay (*ad libitum)* and oats as additive of concentrated feed. For the purposes of the study, the 2nd research group kids

Table 1. The research scheme

Groups	Number of kids	Concentrated feed
1st control group	4	Oats
2nd research group	4	85% oats + 15% fodder beans

at the age of 7 months were separated from their mothers and placed in a barn and fed with hay (*ad libitum*) and self-made grain mixture made of whole oats and bean flour.At the beginning of the study for 32 days the proportion of beans in the concentrated feed mixture was 10%, from the mid-term to the end of fattening – 20%, so the average proportion of fodder beans in the concentrated feed mixture was 15%. During the study there were counted the amount of consumed concentrated feed, but the amount of grass feed was *ad libitum*. All goat kids were slaughtered after 72 days of experimental period.

The following feed nutrient biochemical parameters were established before the start of the trial according with generally accepted methods of analysis: dry matter (DM)

according ISO 6496:1999 method; neutral detergent fiber (NDF) according LVS EN ISO 16472:2006; acid detergent fiber (ADF) according LVS EN ISO 13906:2008; crude protein (CP) according LVS EN ISO 5983-2:2009; ether extract (EE) according ISO 6492:1999; starch according LVS EN ISO 10520:2001, calcium (Ca) according LVS EN ISO 6869:2002; phosphorus (P) according ISO 6491:1998; but the net energy for lactation (NEL) were calculated based on the results of the analysis performed. The quality indicators for nutrients were determined by the accredited laboratory of Agronomic analysis of the Latvia University of Life Sciences and Technologies.

In trial period goat kids were weighed at birth and at the age of 50 days and also at the beginning and at the end of fattening with an electronic scale (accuracy of 0.01 kg). Absolute live weight gain per day per period for analyzed kids was calculated by formula (1):

$$a = (W_t - W_0)/t \tag{1}$$

where a – live weight gain per day, g; W_t – live weight at the end of period, g; W_0 – live weight at the beginning of period, g; t – period, days.

At the end of the feeding period kids were held overnight without feed before slaughter. Each goat kid was weighed before slaughter to determine the slaughter live weight. Hot carcass weights were taken immediately after slaughter and removal of non-carcass components. Weight of non-carcass components recorded included heart, kidney and liver. Carcasses were chilled for 24 h at 4°C. In 24 h *post mortem*, the carcasses were reweighed. Dressing percentage was computed as a proportion of the cold carcass weight to the slaughter live weight by formula (2).

$$K = \frac{Km}{Wk} \cdot 100 \tag{2}$$

where K – dressing, %; Wk – live weight before slaughter, kg; Km – carcass weight, kg.

Each carcass was evaluated for conformation and fatness according to the SEUROP carcass classification system for lambs (CEE No. 1249/2008). Carcass was classified for conformation (scale from S = Superior (0) to P = poor (5) and fatness (scale from 1 = traces of or no fat visible to 5 = very thick fat cover) according to the visual scores in the SEUROP system. After evaluation of conformation and fatness, the length of the carcass and the circumference of the hips are measured using a tape measure. The neck was then separated and carcass was split longitudinally on a saw. The left side of carcass was also separated into five prime cuts: shoulder blade and shoulder, hip with leg, ribs, flank, sirloin (Fig. 1).
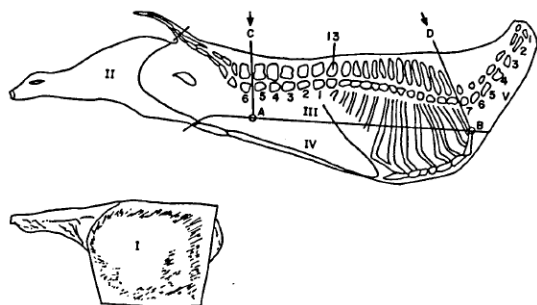
where,
I – Shoulder blade and shoulder; II – Hip with leg (cut between 5/6 lumbar vertebrae); III – Ribs+ chest vertebrae + back vertebrae (from D to 13 vertebrae); IV – Flank; V – Neck; Sirloin (1–5 lumbar vertebrae with fillets).

Figure 1. Goat kid carcass parts (Colomer-Rocher et.al., 1987).

The cuts were weighed and expressed as a percentage of the total weight of the carcass. Fat depth (body fat) over the midpoint of *longissimus do*rsi muscle at the 13th rib was determined 24 h postmortem. The *M. longissimus dorsi* (MLD) were carefully dissected from the left side sampled, weighted and the ribeye area (REA) was measured on a transparent plastic grid (Fig. 2). The guidance for grid assessment of REA was based on a mathematical theorem for determining the area of a polygonal object located on a grid of equally spaced points, Pick's Theorem (Pick, 1899).

Figure 2. Grid with a contour of MLD.

For calculations of REA we used Pick (1899) theorem (3).

$$A = ID + (BD/2) - 1 \qquad (3)$$

where A – area, cm^2; ID – interior dots; BD – boundary dots.

Each cut was dissected into components of meat, bone and fat. The dissection technique used for measuring muscle, bone, and fat composition was described by Colomer-Rocher et al. (1987). Firstly, the muscle was individually removed from their attachment and then the external fat was removed. Muscle, fat, and bone were separated and weighed individually.

After boning the carcasses, the tissue ratios were calculated: meat (muscle tissue + adipose tissue) and bone tissue, muscle tissue and bone tissue, muscle tissue and adipose tissue. Kidney and pericardial obesity was evaluated on a 1–3 point scale according to the standard method for estimating goat carcass (Colomer-Rocher et al., 1987), where 1 – minimal obesity, 2 – moderate obesity; 3 – very large obesity.

Using the data on consumed concentrated feed and the obtained live weight gain, the economic efficiency of the use of concentrated feed was calculated. Average daily gain was calculated from weight change within a given duration. Concentrate efficiency was calculated as the amount of concentrate consumed per unit of live weight gain.

Fattening performance and carcass parameters were analyzed with mathematical data processing methods. In all analyses, statistical significance was declared at $P < 0.05$. The physical data were subjected to analysis of variance (ANOVA). The results are reported as least square means (LSM) and the standard error of the mean (SEM).

RESULTS AND DISCUSSION

The evaluation of the results of the research was started with the analysis of the composition of the concentrated feed.

Since the study did not limit the feeding of grass – pasture grass and hay, and was not calculated the consumed amount, it is not possible to analyze the effect of the grass feed on the kid's growth rates. During the study, whole oats were fed as a source of

energy for the control group's kids, while mix of grains and beans was additionally fed to the kids in the 2nd research group after a 7-day period of adaptation. At the beginning of the study for 32 days the proportion of beans in the concentrated feed mixture was 10%, from the mid-term to the end of fattening – 20%.

Table 2. Chemical composition of concentrated feed

Nutrient	Oat	Fodder bean	10% bean + 90% oat	20% bean + 80% oat
Dry matter (DM), %	91.77	86.90	91.28	87.78
Crude protein (CP), % DM	10.37	30.39	12.37	14.37
NDF, % DM	32.61	18.24	31.17	29.74
ADF, % DM	18.01	15.36	17.75	17.48
NEL, MJ kg^{-1} DM	7.17	7.38	7.19	7.21
Ether extract (EE), % DM	5.80	1.25	5.35	4.89
Starch, % DM	44.77	40.94	44.39	44.00
Ca, % DM	0.11	0.14	0.11	0.12
P, % DM	0.36	0.73	0.40	0.43

According to Table 2, the feed mix with higher proportion of fodder bean has higher energy (NEL) and protein content, while lower NDF, ADF, EE and starch content. Taking into account the climatic conditions of 2018, when a very hot and dry summer was observed, we assume that the quality of grass feed in the second half of the summer fell sharply, and that in order to provide the goat kids with the nutrients they need, it was necessary to include concentrated feed in feed ration, which provides additional nutrients during the fattening of the kid (Table 3).

Table 3. Feed nutrient intake per head per day from concentrated feed

Nutrient	1st control group	2nd research group		
		At the beginning	In mid term	At the end
DM, g	203.3	190.2	182.9	186.5
CP, g	21.1	23.5	26.3	24.9
NDF, g	66.3	59.3	54.4	56.8
ADF, g	36.6	33.7	32.0	32.9
NEL, MJ	1.5	1.4	1.3	1.3
EE, g	11.8	10.2	8.9	9.6
Starch, g	91.0	84.4	80.5	82.4
Ca, g	0.2	0.2	0.2	0.2
P, g	0.7	0.8	0.8	0.8

By adding fodder beans to the compound feed in 2nd research group, the daily intake of crude protein increases by 18.0%, but decreases the intake of starch (-9.5%), fat (-18.7%) and energy (-13.4%) compared to feeding of only oats as concentrated feed. Dietary protein requirements of growing goats vary with energy intake. Interactions between dietary energy and protein levels are common but may not always be observed due to method of diet formulation. In this regard, Kannan et al. (2006) did not influence average daily gain (ADG) or meat characteristics of dairy goats fed concentrate diets differing in CP and digestible energy (DE) concentrations.

The analysis of the results of the study was continued with growth rates, i.e. changes in the kids' live weight during the fattening.

To carry out the research, 8 kids were separated from the goat herd. The control group 4 kids average age at beginning of fattening was 209 days and the average weight was 30.0 kg, but the control group 4 kids were on average 5.7 days older and 1.9 kg lighter (Table 4).

Table 4. The age and live weight of kids at the beginning of fattening

Group	Age at beginning of fattening, days				Live weihgt at beginning of fattening, kg			
	$\bar{x} \pm s_{\bar{x}}$	min	max	V,%	$\bar{x} \pm s_{\bar{x}}$	min	max	V,%
1st control	209.0 ± 9.71	184	228	9.3	30.0 ± 2.92	24.0	35.5	19.5
2nd research	214.7 ± 6.29	204	228	5.8	28.1 ± 2.68	23.0	33.0	19.0

Where V, % – coefficient of variation; s_x – standard error of the mean.

According Table 4, kids of both groups were adjusted by age at the beginning of fattening, but relatively unadjusted by the live weight – the coefficient of variation 19.0–19.5%, however, no significant differences ($P > 0.05$) were found between the groups.

The age of the kids and their live weight before slaughter are summarized in Table 5. The 2nd research group's kids were 1 day younger and 2.16 kg lighter ($P > 0.05$) than the control group kids. When the fattening was started, the kids in the 2nd reasearch group were by 6.4% lighter, but at the end of the fattening, they were by 6.1% lighter, indicating that after including of feed bean mixture to the feed ration, there was no increase in live weight as expected. In the control and research groups during the fattening period the live weight of the kids increased by 5.46–5.20 kg, respectively. The fattening period of the control group kids was 79 days, and of the research group kids – 72 days old, reaching a daily live weight gain of 69 and 72 g, respectively.

Table 5. Live weight and daily live weigt gain of goat kids

Indices	1st control group ($n = 4$)			2nd research group ($n = 4$)		
	Average	V,%	SEM	Average	V,%	SEM
Birth live weight, kg	3.3	15.3	0.26	3.65	13.7	0.25
Live weight at age of 50 days, kg	12.43	4.6	0.28	11.53	11.5	0.66
Daily live weight gain till age of 50 days, g	183	3.3	3.5	158	19.1	15.4
Age at the end of fattening, days	288	6.7	9.71	287	4.4	6.29
Live weight at the end of fattening, kg	35.46	14.6	2.59	33.30	17.9	2.99
Daily live weight gain till end of fattening, g	112	16.1	9.3	103	17.4	9.1
Daily live weight gain in fattening period, g	69	34.8	11.8	72	36.2	12.8

Where V, % – coefficient of variation; SEM – standard error of the mean.

According to the data in Table 5, the birth weight of 2nd research groups kids were by 0.35 kg higher than that of the control group kids, but the difference are not significant ($P > 0.05$). The all control group kids were born as twins, but the research group's 2 kids were single at birth. Birth weight of goat's decreases with increasing litter size (Todaro et al., 2006). Pre-weaning growth rate is greater for single kid litters compared with kids of multiple births depending on factors influencing milk production. Concentrate supplementation should increase pre-weaning growth when milk yield is low regardless of litter size but not with moderate-high milk yield when concentrate substitutes for milk (Goetsch et al., 2011, Todaro et al., 2006). Asizua et al. (2014) found that the growth rates of both the grazing goats and supplemented goats were similar, and average daily gain (ADG) for Boer crossbreed kids on pastures were 60–80 g. According to Uganda scientists investigation the increase in ADG resulting from concentrate intake was considerable especially in the last 34 (56–90) days of the trial where growth rate of the supplemented goats was more than double that of the control goats (Asizua et.al., 2014).

For six Boer goat kids crossing level for both the control group and the study group kids (3 in each group) is 50.00–82.03%, and for one kid in each group this is only 16.80%. The average daily live weight gain up to age of 50 days reaches only 160–183 g. According to breeding program of Boer goats the fertility should be 180–200%, and the ADG up to 50 days should be 180–230g (Ciltsdarba programma..., 2016). According to the data in Table 5 the feeding of goat mothers should also be reviewed on the farm to achieve the growth rates of kids indicated in the Boer goat breeding program.

After the slaughtering of the kids the result of the carcass yield (dressing) was calculated (Table 6).

Table 6. Means (± S.E.) of carcass measurements

Group	Dressing, %	Carcass length, cm	Hip circumference, cm	Body fat thickness, cm	Ribeye area, cm^2
1st control	45.1 ± 2.12[a]	71.0 ± 3.72	56.7 ± 4.64	0.27 ± 0.05	14.5 ± 0.73
2nd research	39.9 ± 0.94[b]	63.0 ± 2.79	52.5 ± 2.54	0.35 ± 0.09	11.6 ± 1.40

In the control group the cold carcass yield (dressing) was between 42.5% and 51.4%, but in the research group between 38.5% and 42.5%. According to several researchers (Solaimana et al., 2011; Asizua et al., 2014; Turner at al., 2014) investigations pure and cross breads Boer goats dressing percentage were from 43.0% to 56.4% and feeding has significant effect on dressing percentage. In Solaimana et al. (2011) investigation *M. longissimus dorsi* ribeye area (REA) for pure Boer goats was 9.8 cm2, where in our research REA wary from 11.6 to 14.5 cm^2. Estimates of REA have been reported to indicate relative muscling in meat goats (Browning et al., 2012). According Turner et al. (2014), the REA:body weight (BW) ratio was 0.24–0.31 and was not different among different feeding groups, but in our research ratio among REA:BW was 0.41 in control group and 0.35 in trial group ($P > 0.05$), which mean the higher meatiness of our kids carcasses. Mourad et al. (2001) found that dressing percentage, carcass conformation, carcass compactness, and leg conformation of West African dwarf goats improved with increasing BW. Substantially lower dressing percentage in various goat genotypes in Kenya occurred with slaughter at 7.2 vs. 14.7 and 23.9 months of age (Ruvuna et al., 1992).

According to the results, the carcass yield of 2nd research group kids was by 5.2% less ($P < 0.05$) than in the control group. After slaughter were obtained carcasses weighing on average 13.38 kg (2nd research group) to 16.08 kg (1st control group).

The carcasses of the 2nd research group were by 8 cm shorter, 4.2 cm smaller hip circumference, 0.8 mm more fat layer at the last rib (no significant difference), but in overall with weaker muscle development according to the SEUROP carcass evaluation system (Table 7).

Table 7. Carcass conformation and evaluation of body organs

Group	Conformation, number			Carcass fatness, points	Kidney fat, g	Kidney fatness, points,	Heart fat, g	Heart fatness, points
	R	O	P					
1st control	2	2	0	2.75	5.00 ± 2.9	1.25	17.50 ± 4.8^a	1.25
2nd research	0	2	2	2.25	9.50 ± 4.9	1.50	50.50 ± 13.4^b	2.25

A very important indicator for goat kid carcasses is the stratification of fat because of the amount of subcutaneous fat depends the quality of the meat during storage. If there is a small layer of fat, the storage and freezing of the carcasses may be difficult because the carcasses are damaged by dehydration. When visually evaluating the entire carcass, higher fat deposits are presented in the 1st control group carcasses – 2.75 points, compared to the 2nd research group – 2.25 points. The amount of fat in the carcass is clearly related to the dietary energy availability, with diets of higher energy density producing fatter carcasses (Chestnutt, 1994). In our investigation kids from 1st control group received concentrated feed higher in energy content which lead to fatter carcasses. When the diets are isoenergetic, the protein level produces only slight and frequently non significant modifications in fatness (Jason & Mantecon, 1993). A number of studies have demonstrated that lambs raised under a grazing system without any supplementation presented a slightly inferior fatness degree, as a consequence of the lower energy intake from pasture, combined with the higher energy expenditure of the grazing animals in relation to those supplemented with concentrate, since the amount of carcass fat depots is related positively with the energy intake (Chestnutt, 1994). When grazing lambs were supplemented with concentrate, the total body fat increased from 21.8% to 32% (Murphy et al., 1994; Carrasco et al., 2009).

The evaluation of carcasses also includes evaluation of pericardial and kidney obesity. Lower kidney and pericardial obesity ($P < 0.05$) has been found in the control group of the kids (Table 7), which can be explained by increased exercise possibilities and physical activity in pasture, possibly with better kid health. Asizuza et al. (2014) found that the considerable increase in non-carcass fat with supplementation was probably partly responsible for the relatively low live weight gain per kg of concentrate. Supplementation compared to sole grazing increased non-carcass fat (pericardial, kidney, omental, mesenteric, scrotal) with 2.9–4.7 as percent-units of empty body weight. Increase in internal and carcass fat in goats due to increasing intake of energy in diets has been reported by various authors (Goetsch et al., 2011; Zervas & Tsiplakou, 2011). Effects of feeding regime on the distribution of non-carcass components as percentage of empty body weight is studied by Ugandan researchers, where kidney fat were from 0.5 (pasture feeding) to 1.8% (supplemented feeding) (Asizua, 2014), but in our investigation kidney fat were only from 0.01 (1st control group) to 0.03% (2nd

research group) of final body weight. According Asizua et al. (2014) investigation, pericardial fat content is not affected by feeding regime and is 0.2% of empty body weight, but our study shows that it is affected by feed supplementation where content of pericardial fat were 0.05 (1st control group) to 0.15% (2nd research group) of final body weight.

In the marketplace, in the interests of consumers, most carcasses are jointed and offered at a different price, which can be explained by the different proportion of bones in cuts. As the most valuable carcass cuts are the hip with leg, the sirloin and the ribs that meat consumers have classified in the Extra category; the less valuable part is included in 1st category – shoulder blade and shoulder, while 2nd category includes the flank and the neck (Colomer-Rocher et.al. 1987) (Table 8).

Table 8. Carcass parts

Carcass parts	1st control group (n = 4)			2nd research group (n = 4)		
	Average	V,%	SEM	Average	V,%	SEM
Half-carcass weight, kg	7.63	17.7	0.68	5.98	21.2	0.64
Hip + leg, kg	2.31	21.5	0.25	1.90	22.6	0.22
Flank, kg	0.59	8.5	0.03	0.57	49.6	0.14
Sirloin, kg	0.63[a]	12.5	0.04	0.34[b]	30.0	0.05
Ribs+ chest vertebrae + back vertebrae, kg	2.59[a]	22.4	0.29	1.67[b]	21.2	0.18
Shoulder blade + shoulder, kg	1.51	16.4	0.12	1.49	16.1	0.12
Proportion of half-carcass parts:						
Hip + leg, %	30.09	8.1	1.22	31.79	9.4	1.51
Flank, %	7.92	20.8	0.83	9.21	31.2	1.44
Sirloin, %	8.41	15.3	0.65	5.74	19.3	0.56
Ribs+ chest vertebrae + back vertebrae, %	33.71	5.6	0.94	28.10	12.2	1.72
Shoulder blade + shoulder, %	19.87	3.6	0.36	25.12	8.5	1.08

Where V, % – coefficient of variation; SEM – standard error of the mean.

Higher proportion of the most valuable and the most expensive parts (extra category) has from 1st control group carcasses, where the sirloin reaches 8.41% ($P < 0.05$) and the rib cuts reaches 33.71% ($P < 0.05$) of the half carcass, while the hip and leg cuts are larger in the 2nd research group carcasses, reaching 31.79% of the half carcass weight. In general, the cuts included in the Extra category for the carcasses of the 1st control group is 72.21%, but for the carcasses of the 2nd research group by 6.58% less – 65.63% of the half carcass.

The results of the carcass tissue parts and the ratio between muscles and fat tissue and also between meat (Muscles + fat tissues) and bones are shown in Fig. 3.

For the most demanded tissue parts of the consumer – the muscle tissue, the highest proportion in the carcass was obtained from the control group carcasses, on average – 59.1%, which indicates a tendency of increased formation of muscle mass to the kids fed oats. Oats have an increased starch and fat content, which provide kids with a higher amount of easy-to-use energy during the fattening period, which has a positive effect on kid's growth and fattening. The highest proportions of inferior parts – bone tissue derived from the carcasses of the 2nd research group – 25.9%, but these differences are not significant ($P > 0.05$). According to Solaimana et al. (2011) research proportion of bone,

muscle and fat in Boer goat carcass was 25.9%, 54.1% and 19.9% respectively, which is quite close to our investigation. As with other ruminant livestock species, fat is the most variable component of goat carcasses (Mahgoub et al., 2004). Castration generally increases carcass fatness depending largely on plane of nutrition (Ruvuna et al., 1992; Abdullah and Musallam, 2007). Fat is deposited relatively more rapidly in carcass and non-carcass tissue pools by females than intact males, with wethers being intermediate (Mahgoub et al., 2004). Such differences are most likely with diets relatively high in energy and concentrate feedstuffs. That is, limited nutrient intake maximizes lean tissue accretion and minimizes fat deposition regardless of gender.
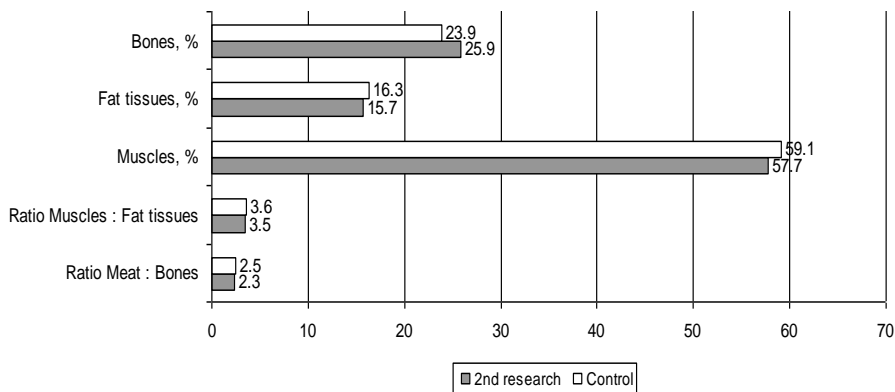
Figure 3. Carcass tissue parts and the ratio.

The highest meat-to-bone ratio is derived from the 1st control group carcasses, where to 1 kg of bones we got 2.5 kg of meat. However, ratio between muscle tissue and fat tissue in both groups of carcasses had practically similar, with 3.5 kg to 3.6 kg of lean muscle per 1 kg of fat tissue. The carcass fat content is highly variable and can be influenced by breed, age, sex, nutrition, BW, physiological condition and physical activities (Owen et al., 1978; Kirton, 1988). As fat is nearly water free and high in energy density, this is enormous energy storage, with low slaughter value but very important as energy storage for the goat (Asizua et al., 2014).

During the fattening of the goat kids, consumed concentrated feed was recorded. The amount and economical effect of concentrated feed during the fattening is summarized in Table 9.

Table 9. Economical effect of feeding of concentrated feed for goat kids

Group	Daily concentrated feed for 1 kid		Concentrated feed to 1 kg of live weight gain	
	Amount, kg	Cost, EUR	Amount, kg	Cost, EUR
1st control	0.222	0.036	3.21	0.51
2nd research	0.208	0.038	2.83	0.52

According the results the control group's kids used more concentrates per day for 1 kid than the 2nd research group's kids, but the cost per day for one kid was by 5.5% lower than for the kids group, where as protein feed was included fodder beans. In turn,

the amount of feed consumed per 1 kg of live weight gain was lower for the 2nd research group kids, and in this case the cost of consumed concentrated feed was only by 1 euro cents or by 1.9% higher than the control group. It means, that feed effectiveness in both groups is rather hight and in fattening of goat kids is acceptable using of both feeding systems.

CONCLUSIONS

Results of this study provide new knowledge about the influence of different concentrated feeds on Boer goat kid's productivity. Usually in farms is used only one grain source for concentrated feed for fattening of kids, but our experimental activities had shown that by inclusion of fodder beans as protein source in feed ration of goats kids, it provided moderate fast growing (average daily gain was 72 g per kid during 72 days of fattening) by using a 2.83 kg concentrated feed mix for 1 kg of live weight gain. Feeding the grain mixture resulted in a 39.9% carcass yield and in a 65.47% Extra category cuts of carcass weight. Cost of oatmeal and fodder bean consumed per day per animal was 3.8 cents; concentrated feed costs per 1 kg live weight gain – 0.52 EUR. Feed effectiveness in both groups is rather hight and in fattening of goat kids is acceptable using of both feeding systems. Overall, meat goat kids finished on grass feed compounded with oats or oat-bean mix produced desirable final live weights (> 33 kg) for niche markets in the Latvia. Chevon could be a low-fat, red meat option for human diets in the Latvia and other countries.

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Use of lipids of Chlorella microalgae in poultry meat marinades and sauces recipes

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Abstract. The aim of this study is to develop formulations and technologies for fermented poultry meat products with the addition of whey and lipid extracts obtained from Chlorella microalgae. Lyophilized microalgal biomass was obtained from cell suspensions of *Chlorella sorokiniana* (strain 211-8k) cultivated in a closed photobioreactor under laboratory conditions. For the cell wall disintegration, the biomass samples were homogenized using a high-speed homogenizer at 10,000 vol min^{-1} for 5 minutes. The lipid extraction was performed on a Sohxlet apparatus Buchi E-812 SOX with the solvent extraction system ethanol: n-hexane (1: 9). The higher fatty acids composition of the obtained microalgal lipid extracts was determined by gas chromatography with flame-ionization detection using nitrogen as a carrier gas. The ω-3 and ω-6 content represented 26.59% and 19.05% respectively, which indicates that these lipid extracts have high nutritional values. The curd whey was obtained from cow's milk of summer and winter production from 2017 to 2018 (Lomonosov district auxiliary farm, Leningrad region); and lyophilized Direct Vat Set (DVS) cultures (Ch. Hansen, Denmark). The organic acids and carbohydrate content in the serum was determined by ion-exclusion HPLC. The FD-DVS CHN-19 culture was selected to produce a serum with improved organoleptic characteristics and a lower propionic acid content (0.01 g L^{-1}). To obtain an optimal ω-3 / ω-6 ratio, a phyto-additive mixture based on sunflower oil and lipid extracts from *C. sorokiniana* microalgae at a ratio of 5–10: 1 is proposed to be used in recipes and technologies of sauces and marinades. It is established that the use of curd whey marinades allow to increase the water-holding capacity (WHC) by 6−8% and to reduce losses during heat treatment of poultry meat from 2 to 11%.

Key words: microalgal biomass, *Chlorella*, microalgal lipid extract, curd whey, DVS-cultures, marinades and sauces, poultry meat.

INTRODUCTION

Building a national system for the production of healthy food using innovative food ingredients is one of the priorities of the Russian technological platform BIOTECH 2030.

Analysis of the current nutrition and assessment of the nutritional status of the Russian population in various regions shows, that for most population categories, diet is significantly deficient in omega-3 and omega-6 polyunsaturated fatty acids and a wide range of vitamin-like substances of natural origin (Hu et al., 2008). In this regard, it is

highly important to develop and improve Eco-friendly production technologies of biologically active compounds.

Microalgae are rich sources of valuable nutrients, antioxidants and cancer-preventive compounds (Reyna-Martinez et al., 2018). Chlorella miroalga is a promising genus as its biomass is characterized by a high specific growth rate and a rich protein, carbohydrate, essential fats and pigment content.

Microalga *Chlorella sorokiniana* has a high growth with a maximum specific growth rate that can reach 0.12 h^{-1} and a productivity of 0.66 g L^{-1} (Lizzul et al., 2018). The biomass contains in average 40%, carbohydrates - 30–38%, lipids - 18–22% by dry weight (Belkoura et al., 1997; Lizzul et al., 2018). Microalgae biomass is a source of antioxidants such as carotenoids (Damerg et al., 2017) that can reach 0.69% of the dry weight (Lizzul et al., 2018). A previous study compared four microalgae species encluding Chlorella pyrosenoida. The average PUFA content reached 61.17% and chlorella biomass was found to have a high palmitic acid (59.12%) and oleic acid (29.69%) content (Silva Gorgônio et al., 2013).

It is necessary to adapt and improve the cultivation and deep processing of microalgae biomass with a high added value before reaching the stage of a large-scale production (Matos, 2017).

The issue of whey processing in northwest Russia is relevant and has been long discussed. 50% of the dry matter of milk passes to the whey. This includes 20% of the total proteins, 95% of the lactose, 80% of the mineral substances and 10% of milk fat by dry weight. Also, whey has the ability to loosen muscle proteins and emulsify fat.

The development of food formulations such as emulsions stabilized by whey with the addition of microalgae lipids is fairly relevant. The serum obtained after the acid coagulation of milk contains a wide range of Lactobacillus. These bacteria secrete lactic acid, use carbohydrates as an energy source (Hansen, 2002; Leroy & De Vuyst, 2004; Seskin & Bazarnova, 2016) and are widely used in the meat fermentation technology.

However, the specific flavor and short shelf life of whey create problems for its widespread use in food formulations. The organoleptic characteristics of dairy products largely depend on the hydrolysis of milk protein and fat resulting in the accumulation of nitrogenous compounds, free amino acids and fatty acids, which are precursors of many taste and aroma-forming substances (Korenman et al., 2006; Boeva, 2007). The formation of these compounds depends highly on the enzymatic activity of lactic acid bacteria (Evans et al., 2009; Croissant et al., 2011). The components responsible for most of the peculiar smell of cottage cheese whey are volatile fatty acids: acetic, propionic, butyric and formic acids. Reducing the specific taste and smell of whey is possible by selecting the appropriate cultures of microorganisms for milk fermentation and by masking the taste with flavoring additives. Actually, the use starter culture so-called DVS culture - Direct Vat Set meaning 'direct introduction of pure cultures into processed milk', is quite popular for the production of fermented milk products. The DVS cultures include strains of Lactococcus lactis subspecies cremoris, Lactococcus lactis subspecies lactis, Leuconostoc mesenteroides subspecies cremoris and Lactococcus lactis subspecies diacetylactis (Varnam & Sutherland, 1994; Kuznetsova, 2005). To obtain dairy products and whey with predictable properties, it is advisable to preliminarily study the activity of the fermentation enzymes, composition of which differs in the ratio of strains that the manufacturer does not report.

Broiler chicken meat includes high-grade, highly digestible animal proteins but has a low content of essential lipids (Donskova et al., 2018). It would be valuable to develop recipes for seasoning chicken meat semi-finished products and other food products that will be enriched with essential fatty acids.

The peculiar development of the sauces market is related to higher incomes and an increase in travelling opportunities. Consumers are increasingly inclined to experiment with food and get more interested in world cuisine. A promising direction for the development of sauces is the creation of seasoning dishes based on natural food additives, without the use of synthetic preservatives, flavors, antioxidants and dyes (Böhm et al., 1998). The seasoning sauces category is the most promising niche for innovation, packaging experiments and flavors.

The aim of this study is to develop formulations and technologies for marinades and sauces for poultry meat using whey additioned with lipid extracts from *Chlorella* microalgae.

MATERIALS AND METHODS

This study was carried out in the Graduate School of Biotechnology and Food Science and in the LTC 'Analytics. Materials. Technologies 'SPbPU.''

To obtain microalgae biomass lyophilisates, crude cell suspensions of *C. sorokiniana* (strain 211-8k) from the collection of algae at the University of Göttingen (international Acronym SAG) were cultivated under laboratory conditions. The biomass cultivation took place in a pilot bioreactor (Fig. 1).
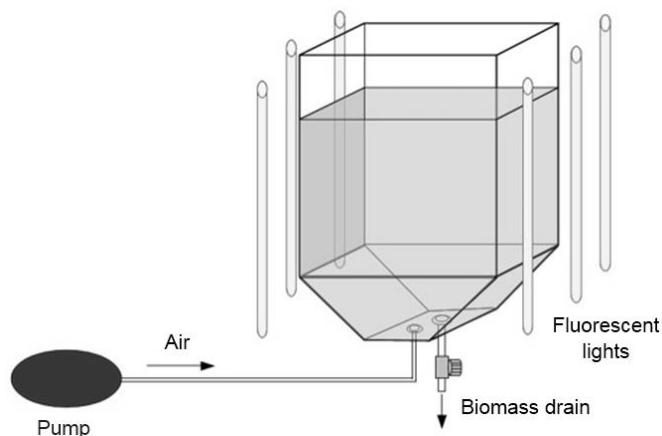
Figure 1. Cultivation of *Chlorella sorokiniana* microalgae in a pilot bioreactor.

For a surface illumination regulated to a day-night mode, a fluorescent lamp was used at luminous flux of $2,500 \pm 300$ Lx, T (K) 400. The temperature of cultivation was 23 ± 1 °C; the intensity of aeration of the mixture - 1.5 L min^{-1}; the mixing mode - periodic (15 min once a day) and the mixing speed - 500 rpm. The used cultivation medium is described as balanced in macro - and microelements (Crofcheck et al., 2012). The maximum concentration 41×10^6 cells mL^{-1} was reached on the 9th day of

cultivation. The specific growth rate of the linear phase of biomass growth was $\mu = 0.26$ day^{-1} (Politaeva et al., 2017).

The concentration of the biomass was performed by auto-flocculation. In the depletion phase, a 0.1 N NaOH solution was added during cultivation while constantly stirring. At pH 11, the flocculation efficiency reached 95.4% and the flocculation took no more than 30 minutes (Bazarnova et al., 2018; Kuznetsova et al., 2018).

The microalgae suspensions concentrates were lyophilized under 1 mbar at a temperature of -55 °C in an Alpha 1–2 LD plus freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH).

For a mechanic cell wall disintegration, a 3 g of freeze-dried biomass sample was mixed with 10 mL of mixture of n-hexane: ethanol (9:1) and subjected to homogenization using a Silent Crusher M high-speed homogenizer (IKA® Werke, T25 Basic) at 10,000 vol min^{-1} for 5 minutes. The lipid extractions were performed on a Sohxlet apparatus Buchi E-812 SOX with the solvent extraction system ethanol: n-hexane (1: 9) for 15 cycles and the extraction lasted 2 hours in total. These parameters were shown to be optimal for a maximum lipid yield in a previous study (Toumi et al., 2018). The extraction temperature corresponded to the boiling point of the solvent mixture, which was 69–70 °C.

The higher fatty acids composition in the lipid extract of microalgae *C. sorokiniana* was determined by gas chromatography with flame-ionization detection Agilent Technologies Sales & Services GmbH & Co.KG, on a BPX70 column (60 m × 0.25 mm × 0.25 micron), SGE Analytical Science, VWR International GmbH; using nitrogen as a carrier gas. For their identification, the Supelco 37 Component FAME Mix standard containing 37 fatty acid methyl esters was used.

Curd whey was obtained from cow's milk of summer and winter production from 2017 to 2018 (Lomonosov district auxiliary farm, Leningrad region). This serum was produced by acid coagulation of cow's milk with a 3.5% fat by mass using lyophilized DVS cultures for cottage cheese and cheese making (Ch. Hansen, Denmark). To establish the compliance of the DVS starters culture with the specified microflora composition, they were pre-microscoped after activation by adding them to pasteurized skimmed milk and thermostating at 38 °C for 24 hours.

The proteolytic activity of the starter's microflora was determined by the method of Anson and Mirsky (Srichanun, 2012). The organic acids and carbohydrate content in the serum was determined by ion-exclusion HPLC according to (Kruchina-Bogdanov & Anosov, 2003). The chromatographic system included a Water 510 pump, a Rheodyne sample injector (volume 20 µl) and a 9 × 600 mm glass column with resin Hitachi-2614. A column with a water jacket allowed the separation of a series of organic acids contained in the samples. A 90 mM sulfuric acid in 2% by volume of aqueous acetonitrile as used as a mobile phase; with an eluent feed rate of 1.4 mL min^{-1} at a column temperature of 50 °C. All reagents were analytical grade.

The fatty acid composition of whey was determined by Gas Chromatography (GC) according to on a CBP 5-25 column (25 m × 0.22 mm × 0.2 µm), carrier gas – nitrogen at a rate of 20 cm s^{-1}; flame ionization detector; temperature gradient - from 70 to 300 at a rate of 4 °C min^{-1} (Deeth et al., 1983; Liguori et al., 2015). The temperatures of the injector and the detector were 280 °C and 311 °C in this order. Tridecanoic acid (REAHIM AO LLC) was used as an internal standard.

Marinades and sauces were prepared from pasteurized whey (95 °CC, 20–30 min) and refined sunflower oil with the addition of lipid extracts of *C. sorokiniana* microalgae.

Marinated semi-finished products from poultry meat were produced from chilled broiler chicken carcasses produced by the Udarnik poultry farm (Leningrad Region). Marinating broiler chicken meat was carried out at a temperature of 2–4 °C for 12 hours. The functional and technological parameters of meat were examined before and after marinating: the moisture content and water-holding capacity (WHC) of meat, as well as losses during heat treatment (roasting).

RESULTS AND DISCUSSION

The main technological steps for microalgae lipid extraction included the preparation of cell suspension, dehydration of biomass, mechanical disintegration of the cell wall, extraction in Sohxlet apparatus and obtaining lipid extract after evaporating the extraction solvents (Fig. 2).
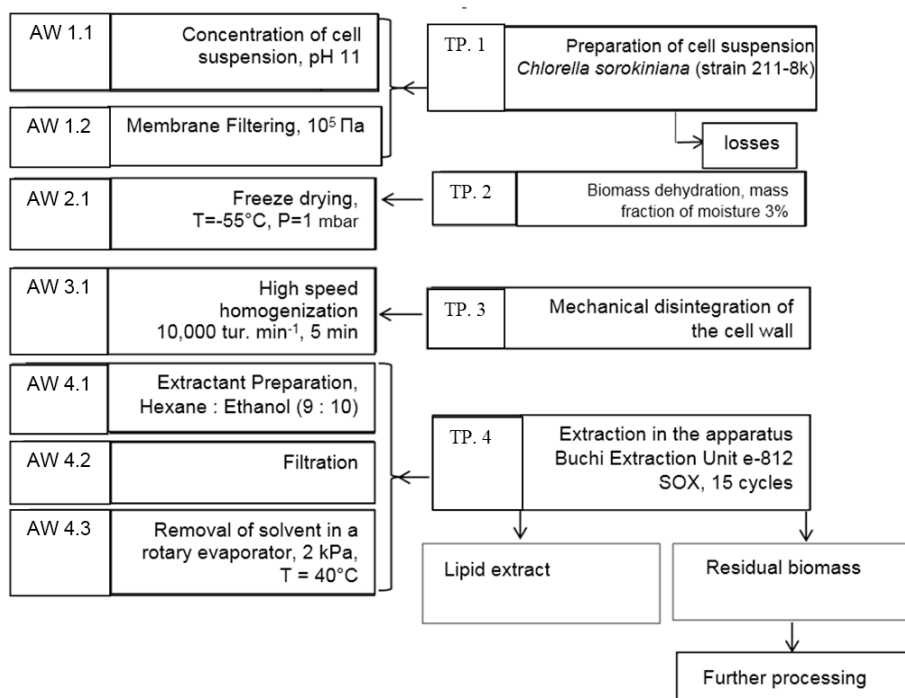
Figure 2. Scheme of the leading steps to obtaining lipid extracts from *C. sorokiniana* biomass. TP - technological process, AW - auxiliary work.

The lipid yield at the selected extraction mode represented about 20% by dry weight of microalgal biomass, which corresponds to the previously obtained results (Silva Gorgônio et al., 2013; Lizzul et al., 2018). The obtained lipid extracts are characterized by a dark brown color and a pronounced fishy taste and smell, therefore it was proposed to include these extracts to edible vegetable oils for further use in oil-based spreads, sauces and marinades.

The fatty acid composition of the lipid extract obtained from lyophilized biomass samples of *C. sorokiniana* microalgae is shown in Table 1.

The ω-3 and ω-6 fatty acid content in the lipid extracts as well as the FAO / WHO recommendations on the optimal content of saturated, monounsaturated and polyunsaturated fatty acids are listed in Table 2.

Among all the identified fatty acids, palmitic acid, trans and cis-linoleic acids, as well as α-linolenic acid, have the highest yield, which corresponds to the data on the study of high-fatty acids in several species freshwater algae. An increase in the

Table 1. Fatty acids composition of the lipid extracts of *C. sorokiniana*

Yields, %	Cn:m	Fatty acids
17.80	16:0	palmitic acid
0.80	16:1	palmitoleic acid
1.88	17:1	cis-10-Heptadecenoic acid
1.72	18:0	stearic acid
18.35	18:1, 9	oleic acid
6.10	18:1, 7	vaccenic acid
3.32	18:2, 6	trans-linoleic acid
14.80	18:2, 6	cis-linoleic acid
7.54	18:3, 6	octadecatriene acid
26.06	18:3, 3	α-linolenic acid
0.93	20:4, 6	α-linolenic acid
0.53	20:5, 3	eicosapentaenoic acid
0.17	23:0	tricosanoic acid

oleic acid content (3,670 mg per 100 g of dry biomass according to our results) is associated with adaptive effects in response to stress factors or an increased duration of cultivation (Molino et al., 2018; Van Wagenen et al., 2012).

Table 2. Characterization of the biological value of *C. sorokiniana* lipid extracts

Lipid fractions	Content in microalgal lipids, %	FAO/WHO recommendations, g 100 g^{-1} of lipids*
Unsaturated Fatty Acids (UFA)	19.7	30.0
Monounsaturated Fatty Acids (MUFA)	27.1	60.0
Polyunsaturated Fatty Acids (PUFA)	53.2	10.0
total *ω-3*	26.6	
total *ω-6*	19.1	

*– FAO. 2010. Fats and fatty acids in human nutrition. Report of an expert consultation. FAO 'Food and Nutrition Organisation'. Geneva, 91. Rome.

The ratio of saturated to unsaturated fatty acids in the lipid extract represented 3:10, and the fatty acids: PUFA ratio constituted 8:10. The high ω-3 and ω-6 content indicates a high nutritional value of the selected lipids (Meyer et al., 2003, Silva Gorgônio, Gomes Aranda & Couri, 2013). However, a fat with high content of essential unsaturated fatty acids does not guarantee its usefulness and indicates a need to assess their balance and redundancy.

For the use of microalgae lipids in the formulation of emulsion products, it is advisable to prepare concentrates in edible vegetable oils, which can be mixed with the fatty basis of spreads, sauces, marinades. As previously stated, the lipid extract is brown and has a pronounced fish like smell, so, it is advisable to select its dilution in such a way as to exclude foreign flavor in food products.

The World Health Organization and a number of national medical organizations have recommended a daily intake of 500–1,000 mg of ω-3 for the prevention of cardiovascular diseases (Simopoulos, 2002; Kris-Etherton et al., 2009). At the same

time, the ratio of the consumed ω-6 and ω-3 polyunsaturated fatty acids should be no higher than 2: 1-3:1 according to the recommendations of the US National Institutes of Health (Davis, Kris-Etherton, 2003).

The microscopy of the starter cultures used for fermentation of milk showed no sign of extraneous microflora (yeast, mycelium of microscopic fungi). In the CHN-11 sample, short chains of streptococci predominated (4–5 pieces in the chain). Diplococci and cocci were present as well. Sample CHN-22 showed similar results. The CHN-19 sample showed a distinctive feature: predominance of long chains of cocci in the form of beads with 8–10 cocci per chain in average. The most intensive development of cocci was observed after 6 hours of fermentation. During the depletion phase, 24 hours after the start of ripening, their proportion decreases, which is associated with the adverse effect of acid accumulated as a result of fermentation. The study of the dynamics of acid accumulation during milk fermentation indicated that, in all three samples of the activated starter cultures, the process proceeded almost equally.

The results of the study of the proteolytic activity of the starter microflora under different acidity values of the medium showed that, at pH 5, the proteolytic activity of the CHN-19 starter was significantly higher than the in CHN-11 and CHN-22 samples.

The results of organic acids and carbohydrate composition of milk whey are presented in Table 3 and Fig. 3 shows their chromatographic profiles.

Table 3. The organic acids and carbohydrate composition of milk whey

| Organic acids | Content, g L^{-1} | | |
	CHN-11	CHN-22	CHN-19
citric acid ($C_6H_8O_7$)	0.07 ± 0.01	0.11 ± 0.01	0.09 ± 0.01
malic acid ($C_4H_6O_5$)	1.04 ± 0.05	1.01 ± 0.05	0.71 ± 0.04
pyruvic acid ($C_3H_4O_3$)	0.25 ± 0.01	0.45 ± 0.02	0.04 ± 0.01
succinic acid ($C_4H_6O_4$)	0.24 ± 0.01	0.32 ± 0.02	0.24 ± 0.01
lactic acid ($C_3H_6O_3$)	5.07 ± 0.25	5.24 ± 0.26	5.57 ± 0.29
acetic acid (CH_3COOH)	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
propionic acid ($C_3H_6O_2$)	0.06 ± 0.01	0.09 ± 0.01	0.010 ± 0.002
butyric acid ($C_4H_8O_2$)	0.050 ± 0.003	0.051 ± 0.003	0.042 ± 0.003
lactose ($C_{12}H_{22}O_{11}$)	16.08 ± 1.10	17.07 ± 0.85	17.51 ± 0.87
galactose ($C_6H_{12}O_6$)	6.86 ± 0.35	6.67 ± 0.33	5.07 ± 0.25

The highest lactic acid content in whey was reached using the CHN-19 starter culture. This serum distinguished itself from others as it had a higher proteolytic activity and better organoleptic indicators due to a decrease in propionic and butyric acid content, giving it a 'whey' flavor (Korenman, 2006). It was established that the best organoleptic indicators characterized the serum obtained by fermenting milk with CHN-19 cultures.

Marinade formulations based on curd whey and refined sunflower oil additioned with *C. sorokiniana* lipids were developed. Serum content in the marinades ranged from 40 to 50%; sunflower oil with phytoadditives - from 15 to 30%; dry mixes of spices - from 2.5 to 2.7% by dry weight of marinades.

To get the necessary consistency for the marinade, a mixture of guar and xanthan gums was used at a ratio of 1: 7, the content of which represented 1.5% by weight of marinade.
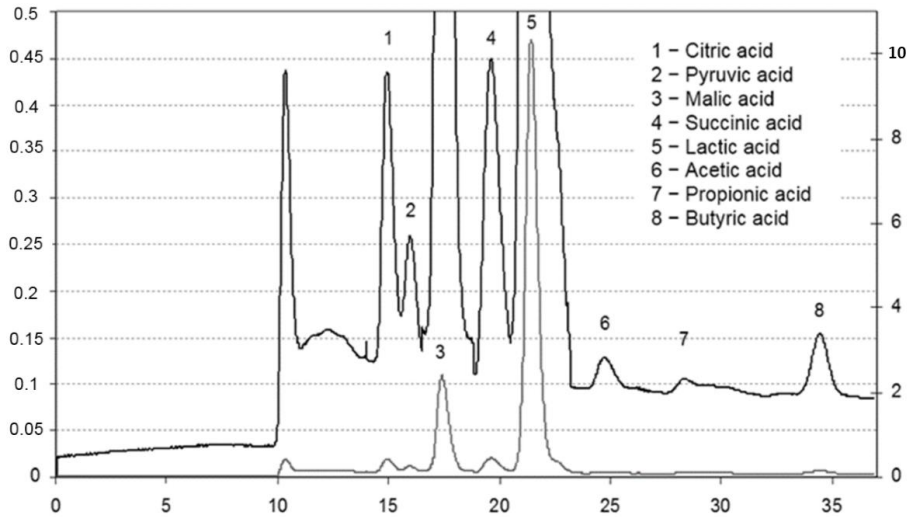
Figure 3. Chromatographic profile of organic acid in milk whey. X axis – time (h); Y axis – optical density at 210 nm.

The preparation of the marinade included the preparation of an emulsion base, for which the required amount of refined sunflower oil with the addition of microalgae lipids was loaded into the mixer at a temperature of 3–5 °C, water and curd serum at a temperature of 3–5 °C and mixed for 1 minute until an emulsion was formed, then the remaining water was added and the dry components of the marinade were evenly spread over the entire surface and mixed for 7–10 minutes until a homogeneous mass was obtained.

Ground spices, spicy greens, and whole spice seeds were used to compile the aromatic composition of marinades. Spice mixtures were added to the finished emulsion.

Table 4. Functional and technological indicators of marinated chicken meat

	Functional and technological indicators of chicken meat			
Whey content in marinades, %	Humidity content, %	pH	WHC, %	Losses after heat treatment, %
Breast fillet raw material				
-	76.9 ± 0.5	6.3	47.6 ± 0.3	30.1 ± 0.2
Marinated breast fillet				
Control	75.1 ± 0.5	6.5	53.3 ± 0.3	38.3 ± 0.2
30	77.0 ± 0.5	6.4	54.9 ± 0.3	36.1 ± 0.2
50	78.5 ± 0.5	6.2	55.3 ± 0.3	27.3 ± 0.2
Chicken legs raw material				
-	76.7 ± 0.08	6.3	49.2 ± 0.3	25.1 ± 0.2
Marinated chicken legs				
Control	78.0 ± 0.5	6.5	53.6 ± 0.3	27.3 ± 0.2
30	79.6 ± 0.5	6.4	54.7 ± 0.3	24.6 ± 0.2
50	80.8 ± 0.5	6.3	55.4 ± 0.3	22.8 ± 0.2

The results of the studies of the effect of marinades on the functional and technological indicators of broiler chicken meat (Table 4) indicate that the use of marinades based on milk whey allows to increase the WHC of chicken breasts fillets by about 8% and the chicken legs by 6% relative to the control sample.

Heat treatment loss of marinated meat decreased from 2 to 11% (breast fillets) and from 3 to 5% (leg meat) relative to the control sample.

Table 5 shows the formulations of sauces for poultry meat with the addition of lipids of microalgae.

The technology for making seasoning sauces hown in Fig. 4 is similar It is similar to the technology of cold Italian vegetable oil based sauces.

Table 5. Sauces recipes for poultry meat with the addition of microalgae lipids

Raw material and ingredient names	Mass ratio of the ingredients, kg per kg of sauce	
	'Siberian'	'Baltic'
Crushed pine nuts	0.080	-
Olive oil	0.200	0.100
Sunflower oil additioned with microalgal lipids	0.300	0.130
Parmesan cheese	0.100	-
Crushed basil	0.300	0.250
Crushed garlic	0.020	0.020
Sour cream 15% fat	-	0.500
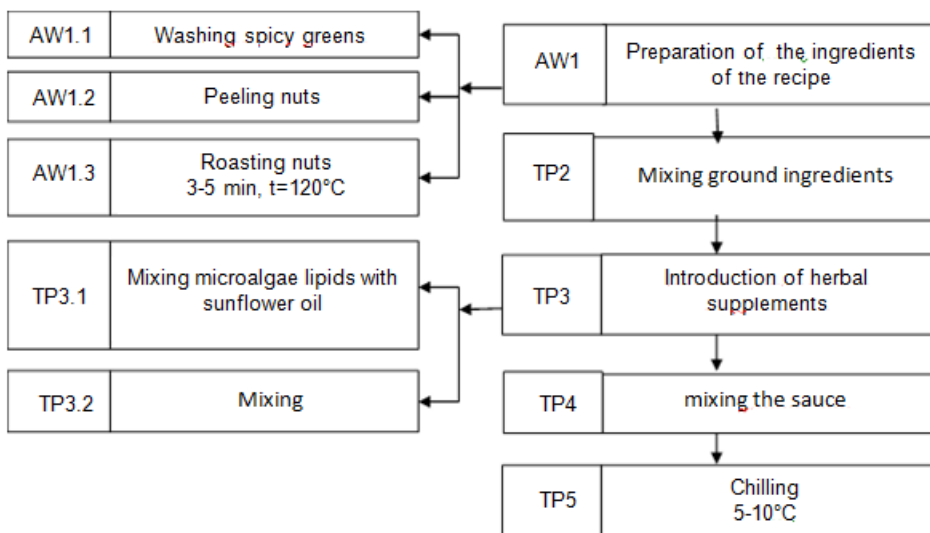
Figure 4. Technological operations for the preparation of seasoning sauces with phytoadditives of *C. sorokiniana* lipid extract. TP – technological process, AW – auxiliary work.

CONCLUSIONS

Aquatic ecosystems are the main birthplace of long-chain ω-3 PUFAs synthesized by microalgae (Gladyshev et al., 2012). Omega-3 fatty acids - eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), synthesized by microalgae via trophic chains, are transmitted to aquatic invertebrates, fish and then, to humans. The use of lipid extracts of microalgae rich in omega-3 is important for achieving a balance of PUFAs in food products, which is recommended by the FAO.

Chlorella sorokiniana has a high growth rate (Belkoura, Benider & Dauta, 1997; Politaeva et al., 2017.). Biomass for research was obtained during the cultivation of microalgae in a laboratory bioreactor when illuminated with a fluorescent lamp ($2,500 \pm 300$ Lx), while the specific growth rate during the linear phase of biomass growth was $\mu = 0.26$ day^{-1}. From 1 liter of biomass, 0.60 ± 0.05 g of air-dry biomass was obtained.

The proposed technology for the extraction of lipids from lyophilized biomass of C. sorokiniana microalgae allowed obtaining samples of lipid extracts with lipid yield from 13.5 to 20% of dry biomass, which indicates the high efficiency of lipid extraction regimes (Belkoura et al., 1997; Lizzul et al., 2018).

The fatty acid composition of the isolated lipids of C. sorokiniana microalgae indicates their high nutritional value, however, the ratio of the sum of ω-3 and ω-6 acids in the obtained lipid extract determines the expediency of mixing it with edible oils, which are dominated by linoleic acid, for example, sunflower oil Simopoulos, 2002), which will allow to achieve the desired balance (FAO. 2010. Report of an expert consultation. FAO 'Food and Nutrition'. Geneva, 91. Rome).

To obtain the optimal ratio of ω-3 / ω-6, a mixture of sunflower oil and lipid extract of *C. sorokiniana* microalgae at a ratio of 5–10: 1 by weight was proposed for further use for emulsion sauces and marinades making.

A comparative analysis of the volatile aroma-forming acids and carbohydrates content of milk whey samples obtained by acid coagulation of cow's milk with the use of starter cultures was carried out. The effect of propionic acid on the formation of whey flavor has been established. The use of DVS-starter CHN-19 for to produce a serum with improved organoleptic characteristics and a reduced propionic acid content (0.01 g L^{-1}) was aproved.

Studies on the content of volatile aromatic acids and carbohydrates of curd whey samples allowed to determine the effect of propionic acid content on the formation of whey flavor, which is consistent with the data found in literature (Korenman et al., 2006; Boyeva et al., 2007).

Formulations of marinades and seasonings 'Baltic' and 'Siberian' sauces based on curd whey and a supplement of the extract of the lipid extract of the microalga C. sorokiniana were developed and introduced in Gastroman LLC (St. Petersburg).

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Accelerated technology of rye bread with improved quality and increased nutritional value

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Abstract. Accelerated bakery technologies do not always ensure high bread quality. The taste and smell of bread is less pronounced when compared with the traditionally prepared bread and it is quickly subjected to microbial spoilage. The aim of the research was to develop an improved composite mixture for the accelerated technology of rye bread, which would improve its quality, nutritional value, extend shelf life and microbiological stability. Rowan powder (botanical species *Sorbus aucuparia*) as unconventional raw ingredients of high nutritional and biological value was used. Rowan powder has high acidity (40 degrees or 5.7% in terms of malic acid) and contains a wide range of organic acids, including volatile acids (2–3%) and preservative acids (such as sorbic acid), as well as other micro- and macronutrients. New acidifying additive with rowan powder was created. The optimal dosage of rowan powder in the new acidifying additive by 13% per 100 kg of flour allows bread making with higher specific volume, acidity and porosity of the crumb compared with the control sample. The research proves that rowan powder usage in the accelerated bread technology improves its organoleptic and physico-chemical indicators and also increases the content of dietary fiber, vitamins and minerals. The content of fibers in custard bread with rowan powder was 1.85 times higher than in the control sample. The rowan powder usage has a positive effect on the preservation of bread freshness during its storage. The rowan powder usage slows down the custard bread mould disease.

Key words: bread, rye, rowan powder.

INTRODUCTION

Bread made from rye flour or its mixture with wheat flour, including custard bread with scalded flour (like Swedish bread Sillabröd), is traditionally one of the main food products of the Russian population living in the North-West, Central and North-East regions, Belarus, Ukraine, Lithuania, Latvia, Estonia, as well as in Germany, Poland, Finland, Austria and other countries (Fuckerer et al., 2016). It is well-known that bread with rye flour, including custard bread, is technologically impossible to prepare without acidifying dough (Birch, 2013; Fuckerer et al., 2016; Gagiu et al., 2017). This is due to the peculiarities of rye flour (Tatham & Shewry, 1991; Wrigley & Bushuk, 2010; Ficco

et al., 2018). The protein content of rye grain is similar to that of wheat. But rye flour proteins do not form a gluten skeleton like bread wheat (Shewry et al., 1997; Shewry et al., 2002; Tosi et al., 2011) and durum wheat proteins (Carrillo et al., 1990; Pogna et al., 1990; Palumbo et al., 2002).

The monomeric gluten proteins of rye are called secalins (equivalent to wheat gliadins) and the polymeric gluten proteins (polypeptides or subunits) take the generic name of glutelin (equivalent to wheat glutenin). The secalins of rye comprise four major groups of proteins, three of which are closely related to groups of wheat and barley prolamins (Tatham & Shewry, 1991; Wrigley & Bushuk, 2010; Ficco et al., 2018). Wheat flour protein is an important component having an influence on the quality of pasta and bread. The glutenin and gliadin proteins, the types present and their ratio influence dough properties. The disulfide bonds presented in the wheat gluten structure contribute to the process of dough formation through the process of disulfide-sulfhydryl exchange. Tyrosine bonds also form in wheat doughs during the processes of mixing and baking, contributing to the structure of the gluten network (Tilley et al., 2001; Sissons, 2008). Increasing of the number of high molecular weight glutenin subunits to obtain more varied dough properties improve the breadmaking properties of wheat and durum flour (Žilić et al., 2011). The rheological properties of gluten are needed not only for bread production, but also in the wider range of foods that can only be made from wheat, viz., noodles, pasta, pocket breads, pastries, cookies, and other products.

Rye have slightly less than wheat of the nitrogen-rich amino acids, such as glutamine and proline. Rye flour have a similar or higher content of gluten than wheat flour and the rye protein is forming gluten on wetting, but its resistance to stretching of the resulting dough is less than wheat gluten. Machine processing of rye dough is more difficult than for wheat dough due to the stickiness of rye dough. The resulting baked rye loaf is poorer in volume, with coarser crumb structure, but the distinctive flavour of rye makes it especially attractive to many customers (Cauvain et al., 2005; Wrigley & Bushuk, 2010).

That is why the starch, pentosans and dextrins of rye flour play a major role in the formation of crumb structure. An increase in acidity in the dough contributes to their swelling, which leads to the increase in the viscosity of the rye dough, its gas-holding capacity, the decrease in stickiness and hardenability of the crumb due to inactivation of the α-amylase. Biological or chemical methods are used for acidification. The most common ways are the usage of sourdough or of organic acids (Kosovan, 2008; Wrigley & Bushuk, 2010).

Traditional sourdough rye custard bread technology consists of several stages such as flour scalding and saccharification, sourdough and dough (Kosovan, 2008). So it is laborious and economically unprofitable, especially in the conditions of small enterprises. Artisan bakeries and bakeries working in discrete conditions are forced to move to an accelerated production method. Accelerated production is possible if complex acidifying additives and improvers are used (Kosovan, 2008; Lambert-Merete et al., 2010; Gagiu et al., 2017; Gioia et al., 2017). The quality of the rye custard bread produced in the accelerated way is worse than the one of the traditional bread. It does not have harmonious and traditional taste and smell, typical for rye bread made with scalded flour (like Swedish bread Sillabröd), because the substances involved in the formation of taste and smell accumulate in the sourdough fermentation process (Jensen

et al., 2011; Onishi et al., 2011; Plessas et al., 2011; Demin et al., 2013). Such bread lacks aromatic substances and thus has weak, empty smell and taste.

The disadvantages of accelerated bread production technology include many other factors. It includes low content of vitamins, minerals, dietary fiber in finished products; the lack of guarantees in obtaining stable quality products, lower quality of bread. Crumb bread can be crumbling. This bread has low resistance to microbiological spoilage and short shelf-life. Consequently, accelerated technology bread does not meet the food security doctrine. That is why the main task for scientists and bakers is to improve the quality of bread prepared in an accelerated way and to make it useful. Therefore, introducing new biologically valuable components into the bread recipe is an important task (Corsetti et al., 2000; Dubrovskaya et al., 2017).

Due to the inconvenience of using biological starters in discrete conditions, it is necessary to use acidifying additives containing citric acid, as a rule, which may have a negative effect on health. For example, citric acid in an acidifying additive may cause an allergic reaction in people who are sensitive to acetylsalicylic acid or have asthma (Sweis & Cressey, 2018).

Accelerated technology of rye bread using acidifying additives and extruded flour was created at Saint-Petersburg branch of State Research Institute of Baking Industry (Kosovan, 2008). Flour eliminates the stage of flour scalding and saccharification. But bread prepared acceleratedly with this additive has a faint smell and taste and is quickly subjected to molding. To acceleratedly produced custard bread with a more pronounced smell and taste, it is advisable to use raw materials that not only participate in the formation of a harmonious taste and smell but also lead to an increase in the quality and nutritional value of the bread (Wieser, 2007; Dubrovskaya, 2012).

The aim of the research was to develop an improved composite mixture for the accelerated technology of rye bread, which would improve its quality, nutritional value, extend shelf-life and microbiological stability.

The powder from the fruit of the rowan (botanical species *Sorbus aucuparia)* was used as an enriching additive. Rowan powder has high acidity (40 degrees or 5.7% in terms of malic acid), has rich biochemical composition, as well as a significant amount of dietary fiber (56.3–59.9%) and volatile acids (2–3%) (Dubrovskaya, 2012).

MATERIALS AND METHODS

Characteristic of ingredients

The powder from the fruit of the ordinary rowan (botanical species *Sorbus aucuparia)* was used.

Rowan powder was obtained from the fruits of rowan *Sorbus aucuparia*. The rowan fruits were dried in a vacuum drier (SVK-1/4) at a temperature 58 ± 2 °C to a moisture content of 8–10%. The dried rowan fruits were ground into powder by knife mills. Particle size was 560–1,000 μm.

The biochemical composition of rowan powder used as an acidifying additive was investigated.

A new acidifying additive with extruded rye flour and rowan powder was developed. When developing a new additive, the following components were varied: rowan powder, extruded rye flour, fermented and unfermented rye malt, coriander. Formulation of acidifying additive is presented in Table 1.

Table 1. Formulation used to prepare different acidifying additive types

Ingredients, g	Control	Samples						
		No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7
Extruded rye flour	60.0	65.0	56.0	47.0	34.0	25.0	17.0	9.0
Fermented malt	25.0	20.0	20.0	20.0	20.0	25.0	20.0	20.0
Unfermented malt	9.0	-	-	-	4.0	-	4.0	4.0
Coriander	2.5	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Citric acid	3.5	-	-	-	-	-	-	-
Rowan powder	-	13.0	22.0	31.0	40.0	48.0	57.0	65.0
Total	100							

Bread making procedure

The optimal amount of rowan powder in the recipe of dry complex brewing was determined as a result of test laboratory baking of custard rye-wheat bread. The dough was made from following ingredients (g per 100 g total amount of flour): rye flour (45.0), wheat flour baking first grade (35.0), new acidifying additive (20.0) salt (1.5), white sugar (4.0) and yeast (1.2). Water was added in an amount to ensure the humidity of the dough 48.5%–49.0%. All the components were mixed in a kneading machine Ankarsrum Original Assistant (Sweden) at a speed of 200 rpm for 15 minutes. After mixing, dough was fermented at a temperature of 30 ± 2 °C for 90 minutes. Then, dough pieces shaped into 310 g loaves, placed in baking forms, and leavened at a 35–40 °C until the volume was twice that of the initial volume. The leavened dough samples were cooked in an oven SvebaDahlen (Sweden) at the temperature at 180 °C for 28 minutes with steam for 6 seconds. The control was bread on a well-known acidifying additive with extruded flour, developed by the St. Petersburg branch of State Research Institute of Baking Industry, which was used in the same amount.

Analysis of the biochemical composition of rowan powder

The analysis of the biochemical composition of the powder of mountain ash was performed using gas-liquid chromatography with mass spectrometry (GC–MS) on an Agilent 6850 chromatograph (USA). Acidity was determined according to State Standard of the Russian Federation (GOST R 52061–2003). The content of vitamins was determined in accordance with different State Standards of the Russian Federation and by special methods. Ascorbic acid was determined by photometric method (State Standard of the Russian Federation GOST 24556–89 P). The method is based on the extraction of vitamin C with metaphosphoric acid or acetic acid mixture and metaphosphoric acids, the reduction of ascorbic sodium 2.6-dichlorophenolindophenolate sodium acid, followed by extraction with an organic solvent (amyl acetate, butyl acetate or xylene) an excess of 2.6-dichlorophenolindophenol sodium and photometric measurement of organic extract at a wavelength of 500 nm.

ß-carotene (provitamin A) was determined by Murri's colorimetric method, based on photometric determination of the mass concentration of carotene in a solution obtained after extraction of carotene from products with an organic solvent and purified from related dyes using column chromatography. Tocopherol was determined by photoelectric colorimetric method (Skurikhin, 1991).

Vitamin B_1 (thiamine chloride hydrochloride) and vitamin B_2 (riboflavin) in food products, food raw materials and dietary supplements were determined by the method based on acid and enzymatic hydrolysis of the sample, as a result of which the release of related forms of vitamins occurs (Method M 04–56–2009). To determine the mass fraction of vitamin B_1, the resulting hydrolyzate is purified with isobutanol. Under the action of potassium-synergistic potassium in an alkaline medium, vitamin B_1 is oxidized to thiochrome, which is extracted with isobutanoll, and the fluorescence intensity of the resulting extract is measured using a FLUORAT®–02 fluid analyzer. To determine the mass fraction of vitamin B_2, the fluorescence intensity of the hydrolyzate is measured using a FLUORAT®–02 fluid analyzer. Then, to assess the influence of fluorescent impurities, riboflavin thiourea luminescence is quenched. The concentration of vitamin B_2 is calculated by the difference in values before and after extinguishing.

The dough assessment

Mass proportion of moisture of the dry microbial composition and of the sourdough was determined by drying at a temperature of 130 °C during 40 minutes in drier SHS-1M. The lifting capacity was determined by the rate of floating up of the 10 g of dough shaped in the ball with humidity of 45% in a glass of water with a temperature of 32 °C. The increase in volume was calculated by the ratio of the final volume to the initial volume multiplied by 100% (Puchkova, 2004). Acidity was determined by titration, using 0.1 N solution of NaOH (State Standard of the Russian Federation GOST 5670–96, 1996).

The gas-forming and gas-holding capacity of the dough was determined using a F3 Chopin Reofermentometer. Samples of the test weighing 315 g were placed on the bottom of the drum, preheated to 28.5 °C. A piston with a load of 2,000 g (4 plates of 500 g each) was installed on the dough and the system was tightly closed with a lid. The duration of the experiment was 90 minutes. The essence of the method is that the pressure generated by the dough in the fermentation process is alternately released into the atmosphere through a soda lime cartridge that retains carbon dioxide, the gas holding capacity of the test is estimated from the volume of which, expressed in cm^3. The rise of the dough in the fermentation process is estimated by the movement of the piston, which is mounted directly on the dough. During the analysis, two coordinate systems are displayed on the instrument display. On one (top) the dough rise dynamics are drawn in mm, on the second (bottom) – the dynamics of change in gas-forming ability and gas-holding capacity of dough in mm of water column.

Assessment of baked bread
Assessment of quality

The assessment of bread quality levels was carried out in relation to the following properties: organoleptic appearance (shape, surface, crumb color), condition of crumb (porosity and texture), taste and smell; physic-chemical and physical – the mass proportion of moisture was determined by drying at a temperature of 130 °C during 45 minutes in drier (SHS-1M, Russia), acidity was determined by titration, using a 0.1 N solution of NaOH (State Standard of the Russian Federation, 1996), 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 bread, compressibility was determined on the automatic penetrometer Labor (Hungary). The swelling of the crumb was determined by the amount of water absorbed by the crumb of bakery products for a certain period of time (Goryacheva, 1983).

The chemical composition of the bread was obtained by calculation (Kosovan, 2008), taking into account the content of nutrients in the used raw materials accordingly (Skurikhin & Tutelyan, 2002).

Sensory evaluation

The panel of 10 non-specialists was used to evaluate the sensory characteristics of the bread. Then, they were asked to evaluate separately appearance (shape, surface, crumb color) and the crumb (color, smell, taste, chewiness and porosity). The ranking scale ranged from 1 to 5 (5-like extremely, 4.5-like very much, 4-like moderately, 3.5-like slightly, 3-neither like not dislike, 2.5-dislike slightly, 2-dislike moderately, 1.5-dislike very much, 1-dislike extremely).

Mould spoilage assessment

The impact of the sourdough and rowan powder on mould disease of custard bread was investigated. Sterile bread slices were contaminated by a pure culture of the mould *Penicillium chrysogenum.* Immediately after baking in the oven opening, the loaves were packed into sterile paper, placed in a sterile room, and cooled to a temperature of between 25–28 °C. After cooling the bread was cut in a sterile environment, with slices being taken at a size of 3.5×6.5 cm and at a thickness of 0.3–0.4 cm. The slices were placed in sterile Petri dishes. An aqueous suspension of a pure culture of the mould, *Penicillium chrysogenum*, was prepared for the infection of slices of bread. The biomaterial of *Penicillium chrysogenum* was transferred from a tube containing a pure culture of mould grown on malt agar to 1 mL of sterile water using 'Tween-80' and was thoroughly suspended. The suspension was inoculated into each slice of bread in three shots using a microbiological needle. Petri dishes with infected slices were incubated at a temperature of 25 ± 1 °C until the first signs appeared of a growth of mould colonies Dubrovskaya, 2018).

Statistical analysis of the data

When analyzing the results of experiments, standard approaches of probability theory and mathematical statistics were used: One-way ANOVA to test the hypothesis of equality of averages of several independent samples, Tukey tests (for a posteriori quality control of conclusions) and the Dunnett test for assessing the relationship with the test sample, paired t- test for samples with different variances to test the hypothesis of the difference between the two means.

RESULTS AND DISCUSSION

It was established that rowan powder contains vitamins, minerals, as well as a significant amount of dietary fiber, carbohydrates and volatile acids (Table 2). This allows suggesting that rowan powder will increase the nutritional value of bread.

Rowan powder had a high acidity (40 degrees or 5.7% in terms of malic acid), acidifying additives. The effect of the quantity of rowan powder in the composition of new acidifying additive with extruded flour on the quality of custard bread was investigated (Table 3). With increasing dosage of rowan powder, the acidity of dough and bread increases. The specific volume of bread with rowan powder was slightly higher than that of the control bread. Increasing the dosage of the rowan powder to 15% (Sample 7) leads to a decrease in specific volume compared with other samples, but it is comparable with the control. It was established that the optimal amount of rowan powder in the composition of new acidifying additive is 11 and 13% of rowan powder per 100 kg of flour in the dough (Samples 5 and 6). With such a content of rowan powder, the analyzed parameters increase in comparison with the control sample.

Table 2. Rowan powder nutrient content

Vitamins:	Range
Ascorbic acid, mg 100 g^{-1}	40–65
Vitamin A, mg·100 g^{-1}	0.12–0.16
Vitamin E, mg kg^{-1}	7.9–9.2
Vitamin B_1, mg 100 g^{-1}	0.028–0.029
Vitamin B_2, mg 100 g^{-1}	0.37–0.434
Mineral substances:	
Potassium, mg 100 g^{-1}	780–980
Calcium, mg 100 g^{-1}	260–300
Iron, mg·100 g^{-1}	4.8–25.4
Manganese, mg 100 g^{-1}	5.9–6.7
Copper, mg 100 g^{-1}	0.4–0.5
Zinc, mg 100 g^{-1}	1.02–1.07
Selenium, µg kg^{-1}	16.8–17.3
Dietary fiber, g 100 g^{-1}	56.3–59.9
Carbohydrates, g 100 g^{-1}	26–30
Volatile acids, g 100 g^{-1}	2–3

Table 3. Physical and chemical indicators of dough and bread*

Indicators Dough:	Samples* control	No.1	No.2	No.3	No.4	No.5	No.6	No.7	F
Acidity, degrees N	10.5 ± 0.5	5.5 ± 0.3	6.7 ± 0.3	7.3 ± 0.4	8.1 ± 0.4	9.1 ± 0.5	10.5 ± 0.5	10.5 ± 0.5	33.3
Lifting capacity, min.	10.0 ± 0.5	8.0 ± 0.4	8.0 ± 0.4	8.0 ± 0.4	9.0 ± 0.4	9.0 ± 0.4	10.0 ± 0.4	11.0 ± 0.6	12.3
Bread:									
Acidity, degrees N	5.0 ± 0.3	3.6 ± 0.2	3.8 ± 0.2	4.4 ± 0.2	5.0 ± 0.5	6.2 ± 0.3	6.8 ± 0.3	7.0 ± 0.4	25.2
Porosity, %	60 ± 3.0	60 ± 3.0	62 ± 3.0	62 ± 3.0	63 ± 3.0	64 ± 3.0	65 ± 3.0	65 ± 3.0	0.72[a]
Specific volume, cm^3 g^{-1}	1.7 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.5[a]
Compressibility, units of the device	19 ± 1.0	20 ± 1.0	20 ± 1.0	19 ± 1.0	19 ± 1.0	20 ± 1.0	20 ± 1.0	19 ± 1.0	0.41[a]

*The letter (a) means acceptance of the hypothesis (a slight difference in the results at the level of 0.05); calculated value of the F-criterion does not exceed the tabular value of the F-criterion - 2.66.

The acid-forming and gas-holding capacity of the dough with an acidifying additive containing 13% rowan powder (Sample No.7) was studied (Table 4).

It was found that the amount of gas released in the test sample on a new acidifying additive with rowan powder is reduced by 10.8% relative to the control sample (Fig. 1). The decrease in the total volume of gas released in the sample being analyzed is directly related to the high acidity of the rowan powder used. It is known that high acidity inhibits the activity of yeast.

Table 4. Dough characteristics*

Indicators	Control	Sample No.7	t*
Total volume of released CO_2, cm^3	962 ± 48	858 ± 43	2.03^a
Volume of CO_2 retained, cm^3	864 ± 43	776 ± 39	2.01^a
Gas retention coefficient, %	89.8 ± 4.5	90.4 ± 4.5	0.11^a

*The letter (a) means acceptance of the hypothesis (a slight difference in the results at the level of 0.05), the calculated value of the t-test does not exceed the tabular value of the Student's t-test (from 2.77 to 4.3).
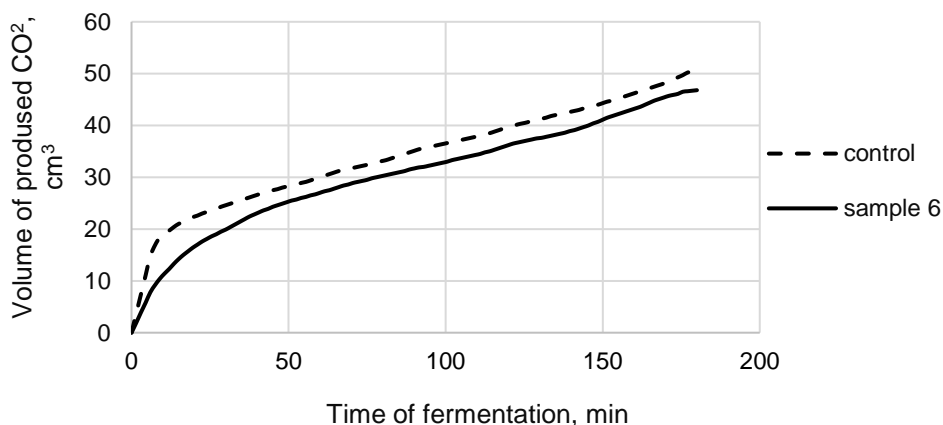
Figure 1. Dynamics of gas formation in the dough.

It was established that the gas holding coefficient in the dough samples was higher than in the control one (Fig. 2). This indicates a small amount of lost CO_2, which is also explained by the presence of rowan powder. The gas is retained in the dough due to the presence of pectic substances with a high degree of esterification in the rowan powder. Pectic substances of the powder make the dough stronger and more elastic, increasing its water-absorbing ability. Consequently, the specific volume of the experimental samples should be higher than that of the control, which is confirmed by physico-chemical and organoleptic characteristics (Tables 4, 5).
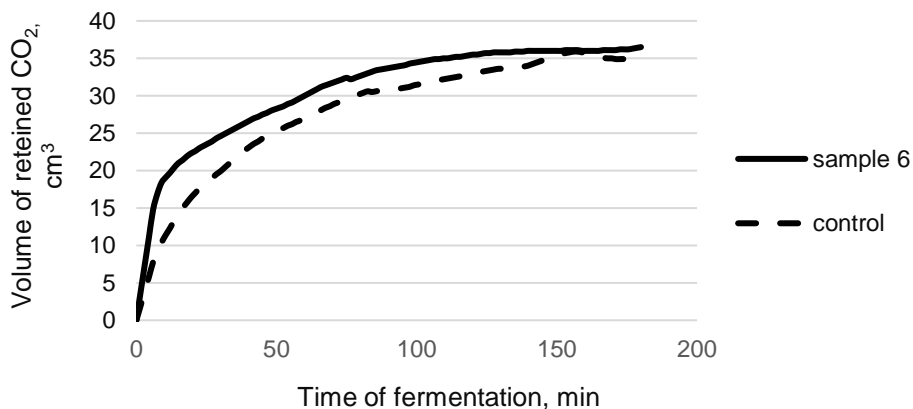
Figure 2. Dynamics of gas containment in the dough.

The sensory characteristics of custard rye-wheat bread were investigated (Table 5). The positive effect of rowan powder on sensory characteristics was found. It is established that increasing dosage (from 7 to 15%) in the composition of the acidifying additive leads to the improvement in the elasticity and color of the crumb, as well as the smell and taste of custard bread. It has been established that increasing the amount of rowan powder in a mixture is impractical, since the taste of the bread becomes sour and the smell is pronounced fruity. At the same time, we found out that the optimum content of a mountain rowan powder had samples 5 and 6.

It was established that the rowan powder in the acidifying additive improves the organoleptic and physic-chemical characteristics of the custard bread and increases the nutritional value.

Table 5. Sensory characteristics of custard bread

Indicators	Samples								
	control	No.1	No.2	No.3	No.4	No.5	No.6	No.7	F*
Crust:									
Shape	4.80 ± 0.18	4.83 ± 0.23	4.77 ± 0.22	4.77 ± 0.22	4.80 ± 0.21	4.82 ± 0.32	4.82 ± 0.32	4.82 ± 0.32	0.05[a]
Surface	4.80 ± 0.18	4.83 ± 0.23	4.77 ± 0.22	4.77 ± 0.22	4.80 ± 0.21	4.82 ± 0.32	4.82 ± 0.32	4.82 ± 0.32	0.01[a]
Color	3.20 ± 0.16	3.19 ± 0.19	3.89 ± 0.04	4.09 ± 0.12	4.73 ± 0.11	4.90 ± 0.15	4.90 ± 0.15	4.79 ± 0.12	9.8
Crumb:									
Color	3.49 ± 0.38	3.5 ± 0.10	3.64 ± 0.14	4.80 ± 0.21	4.83 ± 0.27	4.72 ± 0.32	4.72 ± 0.82	3.24 ± 0.12	6.06
Odour	2.08 ± 0.18	2.87 ± 0.28	2.92 ± 0.23	3.91 ± 0.06	4.11 ± 0.12	4.79 ± 0.12	4.90 ± 0.15	2.88 ± 0.28	45.4
Taste	2.22 ± 0.11	2.75 ± 0.18	2.95 ± 0.18	3.19 ± 0.19	3.43 ± 0.23	4.01 ± 0.04	4.09 ± 0.04	4.01 ± 0.18	38.2
Chewiness	3.19 ± 0.19	3.34 ± 0.29	3.25 ± 0.15	3.89 ± 0.14	4.01 ± 0.10	3.92 ± 0.05	4.05 ± 0.09	4.01 ± 0.10	9.94
Porosity	4.59 ± 0.23	4.65 ± 0.31	4.64 ± 0.25	4.69 ± 0.38	4.49 ± 0.22	4.57 ± 0.28	4.48 ± 0.38	4.39 ± 0.32	0.17[a]

*The letter (a) means acceptance of the hypothesis (a slight difference in the results at the level of 0.05); calculated value of the F-criterion does not exceed the tabular value of the F-criterion - 2.66.

Table 6 shows the effect of a rowan powder on increasing the nutritional value of custard bread and on meeting the daily need for nutritional components necessary for the normal development of the body.

The greatest impact of rowan powder was on the content of dietary fiber. Its content increased in 1.85 times relative to the control. Also, there is a decrease in the total content of digestible carbohydrates by reducing the digestible carbohydrates by 11.6%, which will have a positive impact on human health. When using rowan powder the content of vitamins and minerals, increased. Vitamins A, E and ascorbic acid were also found in the bread with rowan powder (Table 4). At the same time, the maximum degree of satisfaction of daily need was observed for vitamins A and E (11% and 13%, respectively).

Table 6. Content of basic nutrients in bread

Substances	Daily needs	Control	Sample No.6	t*
Proteins, g	75	6.6	6.4	3.57
Fat, g	83	1.1	1.0	0.6[a]
Digestible carbohydrates, g	365	43.0	38.0	4.05[a]
Dietary fiber, g	30	5.7	10.6	20.2
Ash, g		1.6	2.0	2.18[a]
Vitamins, mg 100 g^{-1}:				
vitamin C	70	-	1.4	
vitamin A	0.1	-	0.011	
vitamin E	10	-	1.26	
vitamin B$_1$	1.5	0.127	0.129	0.68[a]
vitamin B$_2$	1.8	0.06	0.095	0.18[a]
vitamin PP	20	0.76	0.79	0.5[a]
Mineral substances, mg 100 g^{-1}:				
Sodium	5,000	389	400.18	1.13[a]
Magnesium	400	35.5	42.47	12.24
Potassium	3,500	175	236.62	10.1
Calcium	1,000	23	48.55	45.8
Phosphorus	1,000	107.5	137.13	30.83
Iron	14	2.5	3.0	1.81[a]
Selenium	0.07	-	0.00015	
Manganese	7.5	-	0.52	
Zinc	15	-	0.09	

*The letter (a) means acceptance of the hypothesis (a slight difference in the results at the level of 0.05); calculated value of the t-test does not exceed the tabular value of the Student's t-test (from 2.77 to 4.3).

The total ash content also increased, respectively, by 17% and amounted to 1.6 g 100 g^{-1} in the control sample, and 2.0 g 100g^{-1} in the experimental sample. The most significant impact rowan powder had on the enrichment of custard bread with phosphorus, magnesium, satisfying the daily requirement of 21%, 13% and 11%, respectively. In addition, manganese and selenium were not found in the control bread, but they were found in sample with rowan powder.

The use of rowan powder in the recipe of custard bread leads to an increase in its consumer advantages and quality.

However, during storage, the quality of bread begins to decline due to the processes of staling and drying. The process of staling is irreversible, but it can be slowed down. Therefore, the next stage of research was to study the effect of new additive on changes in moisture, structural and mechanical properties and swelling of the crumb of custard rye-wheat bread during its storage.

It was established that the loss of moisture during storage was less in the sample with rowan powder. Moisture content in control bread decreased by 1.2% compared to its initial value, and in the experimental one only by 0.6% during storage period (Fig. 3).
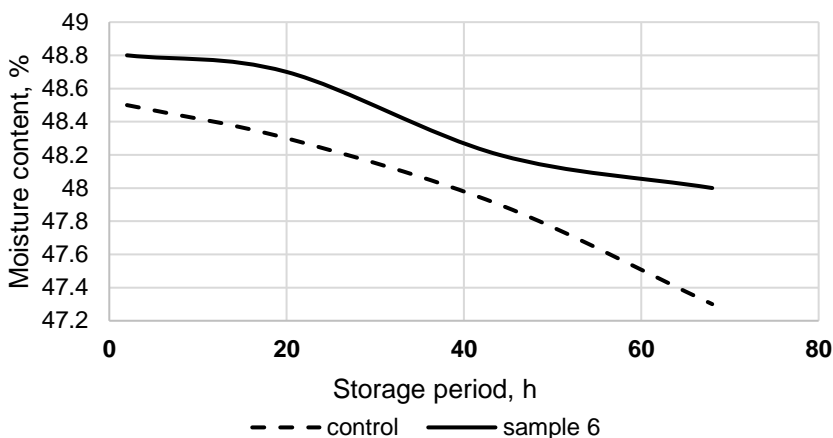
Figure 3. The change in moisture content of the crumb during the storage.

The compressibility of the bread crumb was examined (Fig. 4). It was found that the sample with rowan powder had the best compressibility throughout the entire storage period for 68 hours. The compressibility of the crumb of the control sample, compared to the sample with rowan powder, decreases with greater speed, especially during 20 hours of storage. By the end of storage, the compressibility of the control bread decreased by 57%, and that of bread with rowan powder decreased only by 37%. Such a pattern is associated with an increase in the mechanical strength of the pore walls, slowing down the process of drying and staling.
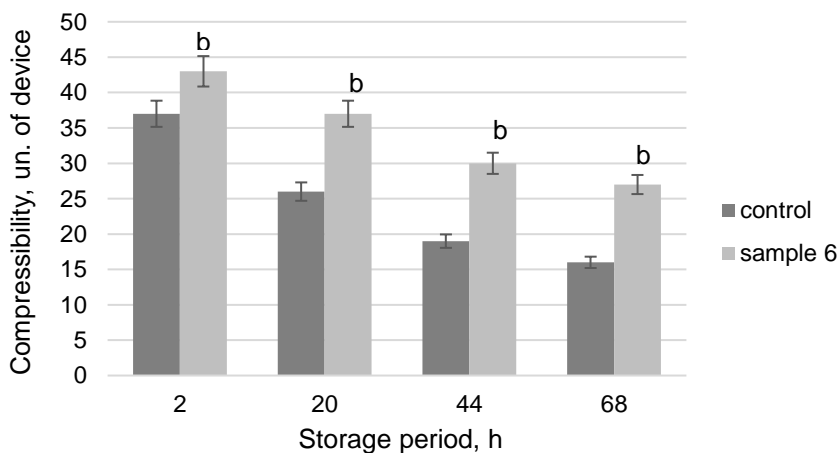
Figure 4. Crush compressibility during bread storage*.

* The letter (b) means the rejection of the hypothesis (a significant difference in the results at the level of 0.05), the calculated value of the t-value criterion is the tabular value of the t-student criterion.

When studying swelling, its decrease during storage was established (Fig. 5). Changes in the swelling index of the samples of custard bread under study occur most intensively also during 20 hours of storage. This is probably due to a decrease in the ability of colloidal substances to absorb water by compacting the structure of starch and

proteins during their aging. The swelling capacity of the control bread was less than that of the samples with rowan powder during the storage period. At the same time, the swelling of the control decreased by 1.5% from the initial value (from 4.1%), and the swelling of bread with rowan powder decreased only by 1.2% (from 4.8%). That may be due to the high fiber content in bread with rowan powder, which has a high swelling index. Rowan powder has high water absorption capacity due to the high content of pectin with the high degree of esterification - 85%.

Thus, this studies have shown that the use of rowan powder in an acidifying additive has a positive effect on the preservation of freshness during storage of custard rye-wheat bread prepared in an accelerated way. The use of rowan powder leads to an improvement in the structural-mechanical properties (compressibility, swelling) of the bread prototype. The result can be explained by the fact that it is difficult to release free moisture during the storage of bread due to the influence of the rowan powder, which has a high water absorption capacity, caused by the content of pectin with a high degree of esterification (above 85%).
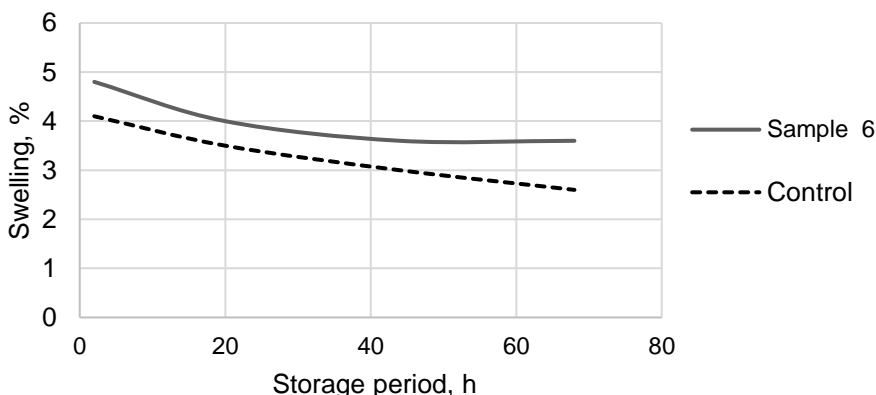
Figure 5. Change in the swelling of the crumb during the storage of bread.

The effect of the rowan powder on the custard bread resistance to the moulds was established. It was found out that in the control bread slices, contaminated by *Penicillium chrysogenum*, the growth of mould colonies was observed in 48 hours, and in samples 7 was not observed during 7 days storage until total slice staling. The usage of the rowan powder allowed slowing down the custard bread mould disease.

CONCLUSIONS

To improve the quality and nutritional value of custard rye-wheat bread produced in an accelerated technology, new acidifying additive with rowan powder was created. The value of rowan powder is that it contains biologically active substances. The optimal dosage of rowan powder in the new acidifying additive was revealed (13% per 100 kg of flour in the dough). It was established experimentally that the use of rowan powder in the accelerated bread technology improves its organoleptic and physico-chemical indicators, and also increases the content of dietary fiber (in 1.85 times), total ash content (by 17%), vitamins and minerals. The compressibility of the control bread decreased by

57%, and that of bread with rowan powder decreased only by 37% during storage, so it confirmed that the rowan powder usage had a positive effect on the preservation of bread freshness during its storage. Therefore, the use of rowan powder allows delaying the release of free moisture and slowing down the staling. The rowan powder usage (13% per 100 kg of flour in the dough) also allowed slowing down the custard bread mould disease.

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Development of formulation and technology of yogurt with prolonged shelf life enriched with biologically active substances from fennel seed extract

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Abstract. Spray drying is most common drying technology in food industry and can be used as an alternative to freeze drying method for the production of extracts in powder form. Fennel seeds are used to treat diabetes, bronchitis and chronic cough. They possess antibacterial, antifungal, antithrombotic, anti-inflammatory, hepatoprotective and antidiabetic activities. The aim of this study was to obtain fennel extract in dry form and investigate the influence of dry fennel extract incorporation on the possibility of yogurt production. The effect of inlet temperatures on wettability, solubility, moisture content and water activity of spray-dried fennel seed extract obtained by decoction technique was investigated. The inlet temperature 165 °C was preferred. Lactic acid accumulation during fermentation occurs faster in the sample with fennel powder. Based on the results of rheological, organoleptic, physico-chemical properties, water-holding capacity and shelf life of the finished product, the recommended doses of dried fennel powder for yogurt manufacture is not more than 1%.

Key words: decoction, fennel extract, natural food preservation, spray drying.

INTRUDUCTION

Natural foods that promote well-being and reduce the risk of various diseases cause an increased interest among consumers. The consumers are more attracted by products containing natural antioxidants, rather than synthetic ones, whose use is limited due to their toxicity and carcinogenic effect. Dairy products themselves are among the most interesting and promising products in terms of antioxidant activity, due to the presence of caseins and whey proteins, as well as traces of various antioxidants (Niero et al., 2016). Medicinal plants, rich in natural antioxidants and phenols, are increasingly used in the manufacture of dairy products to improve their nutritional and therapeutic properties. Fermented milk products are among them (Alenisan, M.A. et al., 2017).

There are lots of studies proving the undeniable benefits of yogurt. Yogurt is considered as a nutrient-dense food that contains essential nutrients such as protein, vitamins and minerals necessary for growth. Yogurt consumption reduces the risk of

diseases that cause an increase in systolic and diastolic arterial (Dong et al., 2013), reduces the concentration of certain cariogenic bacteria in the mouth (mainly streptococci and lactobacilli), and also increases the concentration of calcium and phosphorus in plaque (Ravishankar et al., 2012). Studies conducted by Dougkas et al. (2012) have shown that consuming yogurt reduces hunger and calorie intake by the next meal by 11%. The results of seven studies involving 254,892 people showed that consuming 200 grams of yogurt per day reduces the risk of diabetes by 22% (Aune et al., 2013). According to the research of Burgain et al. (2012), yogurt consumption is recommended for people suffering from lactose intolerance, which reduces the risk of calcium and vitamin D deficiency in the diet. In view of the above mentioned consumption of dairy products such as yogurt helps to improve the overall quality of the diet.

Spray drying is the most widely used technology in the food industry and the pharmaceutical industry. This process is continuous, ensuring a constant drying temperature and product flow rate (Cosmin et al., 2013). It allows to turn a liquid into a powder with the exact characteristics (particle size, morphology, physical and chemical stability) and it is a relatively economically viable process. The spray drying process makes it possible to preserve the content of polyphenols in the extracts to the greatest extent possible and can be used as an alternative to freeze drying for the production of extracts in the form of powder (Michalska et al., 2018).

There is a growing interest, both in industry and in research to aromatic and medicinal plants due to their antioxidant properties and content of many active substances, such as tocopherols, flavonoids, terpenoids, etc. ((El-Belghiti). Herbal extracts are widely used in the dairy products as a source of antioxidants (Shori & Baba, 2011a; Shori & Baba, 2011b; O'Sullivan et al., 2014; El-Said, 2014).

Fennel (*Foenicnicum vulgare*) is a plant belonging to the Apiaceae family, and thanks to its edible and very fragrant leaves and seeds has a long history of use.

Fennel seeds contain polyphenols and flavanoids and are used for medicinal purposes and as a flavor component. Fennel is used for diabetes, bronchitis, chronic cough, and kidney stone treatments (Angelov & Boyadzhieva, 2016).

The results obtained by Oktay et al. (2003) indicate that the fennel (*F. vulgare*) seed is a potential source of natural antioxidant. Fennel decoction, also employed as a carminative. In the Indian subcontinent, fennel seeds are eaten raw, sometimes with some sweetener to improve eyesight. Extracts of fennel seeds have been shown in animal studies to have a potential use in the treatment of glaucoma and a potential drug for the treatment of hypertension. It has been used as a galactagogue improving the milk supply of a breast feeding mother (Manzoor et al., 2016). Fennel is known in folk medicine for its diuretic and digestive aid. Phenolic compounds of fennel contribute to the prevention of chronic diseases such as cancer, cardiovascular diseases. Fennel components have antibacterial, antifungal, antithrombotic, anti-inflammatory, hepatoprotective effects (Ferioli et al., 2017).

The aim of this study is to create an optimal formulation and technology for yogurt manufacture with the addition of fennel extract, which would have not only satisfactory organoleptic properties, but could also contribute to maintaining high quality in the lack of milk fat.

MATERIALS AND METHODS

Fennel decoction preparation

Fennel seeds were purchased from the drugstore in St. Petersburg, Russia. The samples were reduced to powder (328 ± 8 µm) and added (120 g) to 1 L of distilled water. The mixture was boiled for 4 min, left to stand for 15 min and then filtered using 3 layers of cheese cloth to avoid the presence of any suspended particles. The fennel extracts were concentrated in a rotary evaporator (BUCHI, B-491) until 14% total solids and kept at 4 °C until spray drying.

Spray drying process

In the present study, the fennel decoction was spray dried using a lab–scale spray dryer Eyela SD-1000 (Tokyo Rikakikai Co. Ltd, Japan) using two nozzles of 0.4 mm in diameter. The emulsion was fed into the main chamber with the aid of peristaltic pump. The fennel decoction was mixed continuously by a magnetic stirrer during the entire process of spray drying. The inlet temperature varied from 120 °C to 165 °C with the increment of 15 °C. The atomization pressure was fixed as 90 kPa, while the drying air flow rate varied from 0.45–0.50 m^3 min^{-3}. The outlet temperature was kept constant and controlled by the flow rate of the feed product. For each spray-drying experiment, 1,000 mL of feed was prepared. The powder obtained from spray drying, both in the chamber and the cyclone, was stored in airtight containers before analysis.

Yogurt production

For the yogurt production skimmed milk powder (commercial brand Central Lechera Asturiana, Corporación Alimentaria Peñasanta, S.A., Spain) was reconstituted at 11% w/v solids-non-fat to be further completed with fennel powder. The skimmed milk powder reconstitution was carried out using distilled water at a temperature 45 °C. Milk was fortified with dry fennel powder in the amount ranged from 1% to 2% of milk weight with the increment of 1% and pasteurized at 85–87 °C for 10 min. Control sample was made without fennel powder. Samples were inoculated with 5% of reactivated commercial yogurt starter culture (containing *Lactobacillus* subsp. *bulgaricus* and *Streptococcus thermophilus*) at 43 °C. Fermentations were stopped by rapid cooling and the samples were placed in a cold storage at 5 ± 1 °C. All the yogurts were prepared in triplicate.

The samples were analyzed immediately after cooling and during the shelf life at 5 ± 1 °C. Solids-non-fat contents were measured using milk analyzer 'Klever-2M' (LLC Scientific and production Enterprise 'BIOMER', Russia). pH values were measured using pH-meter pH-410 with combined glass electrode (Scientific Production Association 'TECHNOKOM', Russia). Titratable acidity was measured according to AOAC method 947.05 (AOAC, 2007), using NaOH 0.1 N and phenolphthalein solution as an indicator. Acidity was expressed as % lactic acid.

Antioxidant activity was determined according to the method of Najgebauer-Lejko et al. (2011) using DPPH reagent (DPPH) 1, 1-diphenyl-2-picrylhydrazyl. First, an aqueous extract of yogurt was obtained according to Shori & Baba (2011a). Samples of yogurt with fennel extract or without fennel extract (10 g) were mixed with 2.5 mL of distilled water and acidified with 0.1 HCl to pH 4.0. Acidified yogurts were incubated for 10 min in a water bath at a temperature of 45 °C, followed by centrifugation

(5,000 rpm, 10 minutes, laboratory centrifuge Sigma). The pH value of the resulting supernatant was adjusted to 7.0 by the addition of 0.1 M NaOH and centrifuged (5,000 rpm, 10 min., Sigma laboratory centrifuge). To determine the antioxidant activity, samples of yogurt extracts were mixed with 3.0 mL 0.1 mM DPPH solution (39.4 mg DPPH in 1 liter of methanol) and incubated for 2 hours at room temperature in a dark place. Absorbency (Abs) was determined using a Shimadzu UV-1800 spectrophotometer at a wavelength of 515 nm. Control was a mixture of methanol and DPPH reagent. Antioxidant activity was calculated using the formula as follows:

$$Antioxidant\ activity, \% = 1 - \left(\frac{Abs\ treatment\ sample}{Abs\ control\ sample}\right) \cdot 100$$

Wetting time was measured according to Pabari & Ramtoola (2012) with some modifications. A Whatman No. 1 filter paper disk folded once diametrically was placed in a Petri dish of 8.5 cm in diameter. A small volume (8 mL) of distillated water was added to the filter. Then, the compact was carefully placed on the wetted tissue paper at $t = 0$ (initial time) and the time for complete wetting of the sample was measured.

Solubility of fennel powder was determined by Eastman & Moore's (1984) method with some modifications. Sample of fennel powder (1 g) was mixed thoroughly with 100 mL of distilled water. This solution was transferred to some experimental tubes and centrifuged at 3,000 rpm for 5 min and allowed to settle for 30 min. An aliquot of 25 mL of the supernatant was transferred to preweighed porcelain cup and immediately oven-dried at 105 °C for 5 h. The solubility (%) was calculated as the weight difference.

The moisture content was determined gravimetrically by estimating the powder's weight loss after oven drying at 105 °C until a constant weight (AOAC, 2007).

The water activity of the powders was measured using a water activity meter Aqualab CX-2 (Aqualab Series 4TE, Decagon Devices, Inc., Pullman, Washington, USA).

The forced syneresis was estimated by the amount of separated whey during the centrifugation (1,500 rpm) of 10 g of control yogurt samples or samples with different amount of fennel powder. Each sample was weight into centrifuge tubes. The tubes were centrifuged and the amount of separated whey was measured every 5 min during 30 min. The liquid expelled from the yogurt gel was denoted as expressible whey.

Lactic acid bacteria were determined according to GOST 10444.11-2103. Moulds and yeasts were determined according to GOST 10444.12-12013.

The suitable level of fennel powder determined using a 5-point Just About Right (JAR) scale (Rothman & Parker, 2009), where 1 = not enough attribute, 3 = just right and 5 = too much of the attribute. Each consumer testing consisted of 67 panelists who were mainly 18 to 45 years of age (> 70% of total populace) with > 70% being frequent yogurt consumers, including university staff and students. Samples were presented in 3 digit-coded 2-oz soufflé cups with lids.

Rheological measurements were carried out in triplicate with the aid of Rheotest 2 type rotating viscometer (VEB-MEDINGEN, Germany). For this study, the coaxial cylinder device S2 was used. The shear rate varied from 0.3333 to 437.4 s^{-1}. The rheological measurements were performed at controlled temperature of 20.0 ± 0.5 °C. The shear stress was calculated at increasing shear rates (upward flow curve) followed by decreasing shear rates (downward flow curve). The areas under the upward and

downward flow curves were estimates. Apparent viscosity η, Pa s, was calculated using the following formula:

$$\eta = \frac{\tau}{\gamma} \qquad (1)$$

where γ – shear rate, s^{-1}; τ – shear stress, Pa.

The storage time was estimated according to MUK 4.2.1847-04. According to recommendations the product research dates should be longer than the expected shelf life for the time determined by the so-called reserve ratio. The reserve ratio for perishable products with a shelf life of up to 30 days is in 1.3. MUK 4.2.1847-04 also establishes the frequency of microbiological analyzes. To determine the shelf life of the finished product, the control and treatment samples of yogurt were packed in glass containers and stored at $4 \pm 2\ °C$.

The experiments were replicated three times. Significant differences were determined at $\alpha = 0.05$. Statistical processing of data was carried out using Microsoft Office Excel 2010 and Mathcad 15.0

RESULTS AND DISCUSSIONS

The data in Table 1 show the influence of inlet temperature on quality parameters of fennel powder. It can be seen from the Table the moisture content of the powders increased with increasing inlet temperature. Apparently, an increase in the inlet air temperature results in a rapid formation of dried layer on the droplet surface and causes the formation of impermeable films on the particle surface, followed by the formation of a crust on the drop surface. Moisture content of fennel powders varied from 3.34 to 4.33% and this amount of moisture is sufficient to assure microbiological safety of food powder.

Table 1. The influence of inlet temperature on quality parameters of fennel powder

No.	Inlet temperature, °C	Outlet temperature, °C	Wettability, s	Solubility, %	Moisture content, %	Water activity, a_w (25 °C)
1	120	79–82	430 ± 7	93.94 ± 0.98	3.34 ± 0.06	0.28 ± 0.01
2	135	79–82	349 ± 9	94.23 ± 0.96	3.69 ± 0.05	0.26 ± 0.01
3	150	79–82	280 ± 8	96.12 ± 1.01	4.14 ± 0.05	0.22 ± 0.01
4	165	79–82	117 ± 9	98.56 ± 0.99	4.33 ± 0.06	0.19 ± 0.01

Wettability can be defined as the ability of a powder bulk to be penetrated by a liquid caused by capillary forces (Hogekamp & Schubert, 2003). Powders produced with an inlet temperature of 165 °C showed the lowest wetting time (117 s), whereas the highest value (430 s) was shown by sample with an inlet temperature of 120 °C. Therefore, the presented data show that an increase in the inlet temperature decreased the wetting time and can reduce the incidence of powder clumping upon contact with water.

The results also showed that the solubility of the powders increased with the inlet temperature increase. At the highest temperature (165 °C) fennel powder showed the highest solubility (98.56%), whereas at the lowest temperature (120 °C), fennel powder showed the lowest solubility.

All powders obtained showed water activity values below 0.3. The average value of water activity varied from 0.19 to 0.28, therefore, the obtained dry extracts are biochemically and microbiologically stable.

As a result of the analysis of the data presented in Table 1, it was concluded that, based on the combination of the studied parameters, the most suitable for further use and research is fennel powder obtained at a drying temperature of 165 °C.

The inclusion of fennel powder into milk prior to bacterial fermentation influence on titratable activity, pH and lactic acid content compared to milk alone (Table 2). Yogurt fermentation was monitored every hour up to 6 h. The pH of yogurt after 6 h fermentation was in the range of 4.82 to 4.61. Titratable acidity, lactic acid content varied from 80 to 104 °T and 0.720 to 0.936% LA, respectively and was found to vary with the fennel powder concentrations.

The presented data showed that with an increase of the concentration of the applied powder, the acidity during the fermentation process increases faster and the lactic acid accumulates to higher level.

The forced syneresis values of yogurts were affected fennel powder concentration and the changes are shown in (Fig. 1). As seen in Fig. 1, the addition of fennel powder caused a decrease of the amount of whey expelled. All yogurts with fennel showed a lower syneresis percentage compared to plain yogurt. Yogurt with 2% (w/v) fennel powder showed the highest water holding capacity, while the control sample had the lowest water holding capacity.
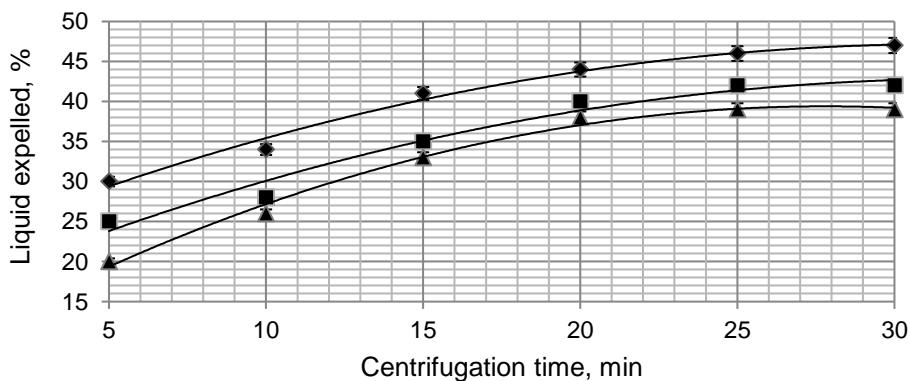
Figure 1. Estimation of the water holding capacities of yogurt samples made using: ◆ – control sample; ■ – treatment sample (1% of fennel powder); ▲ – treatment sample (2% of fennel powder).

Table 2. The effect of the fennel extract concentration on the dynamic of acid accumulation

Fermentation time, h	Control sample			Sample with 1% of fennel powder			Sample with 2% of fennel powder		
	°T	%LA	pH	°T	%LA	pH	°T	%LA	pH
0 (before fermentation)	23 ± 2	0.207 ± 0.01	6.25 ± 0.02	21 ± 1	0.189 ± 0.01	6.27 ± 0.05	22 ± 1	0.198 ± 0.01	6.29 ± 0.04
1	24 ± 2	0.216 ± 0.01	6.15 ± 0.04	23 ± 1	0.207 ± 0.01	6.13 ± 0.04	26 ± 2	0.234 ± 0.01	6.09 ± 0.03
2	33 ± 3	0.297 ± 0.01	5.96 ± 0.02	37 ± 1	0.333 ± 0.01	5.83 ± 0.06	39 ± 2	0.351 ± 0.01	5.76 ± 0.06
3	48 ± 2	0.432 ± 0.01	5.61 ± 0.04	54 ± 2	0.486 ± 0.01	5.46 ± 0.05	56 ± 3	0.504 ± 0.01	5.37 ± 0.06
4	62 ± 2	0.558 ± 0.01	5.35 ± 0.05	69 ± 2	0.621 ± 0.01	5.15 ± 0.04	76 ± 3	0.684 ± 0.01	4.82 ± 0.03
5	69 ± 2	0.621 ± 0.01	5.05 ± 0.04	78 ± 1	0.702 ± 0.01	4.95 ± 0.04	94 ± 2	0.846 ± 0.01	4.72 ± 0.04
6	80 ± 1	0.720 ± 0.01	4.82 ± 0.04	94 ± 2	0.846 ± 0.01	4.72 ± 0.05	104 ± 3	0.936 ± 0.01	4.61 ± 0.04

Table 3. Quality indicators of control and treatment samples of yogurt

Indicators	Storage time, days								
	Control sample				Treatment sample with 1% of fennel powder				
	0	5	10	13	0	5	10	15	20
Titratable acidity, °T	80 ± 1	92 ± 1	103 ± 2	116 ± 2	104 ± 2	112 ± 3	124 ± 2	134 ± 2	144 ± 2
DPPH radical scavenging activity, %	32.8 ± 1.1	30.5 ± 1.2	27.9 ± 0.9	25.6 ± 1.0	51.4 ± 2.1	48.3 ± 1.6	45.6 ± 1.4	43.2 ± 1.7	41.4 ± 1.2
Lactic acid bacteria CFU mL^{-1}	4.2×10^9	4.9×10^9	6.2×10^8	1.1×10^7	4.9×10^9	5.3×10^9	6.8×10^8	4.1×10^7	1.2×10^7
Yeasts, CFU mL^{-1}	<10	<10	16	28	<10	<10	<10	16	26
Molds, CFU mL^{-1}	<10	<10	<10	31	<10	<10	<10	15	30

Fig. 2 shows the flow curves for yogurt of different fennel powder concentration. Data presented showed that the degree of thixotropy in yogurt samples decreased with the fennel powder content.
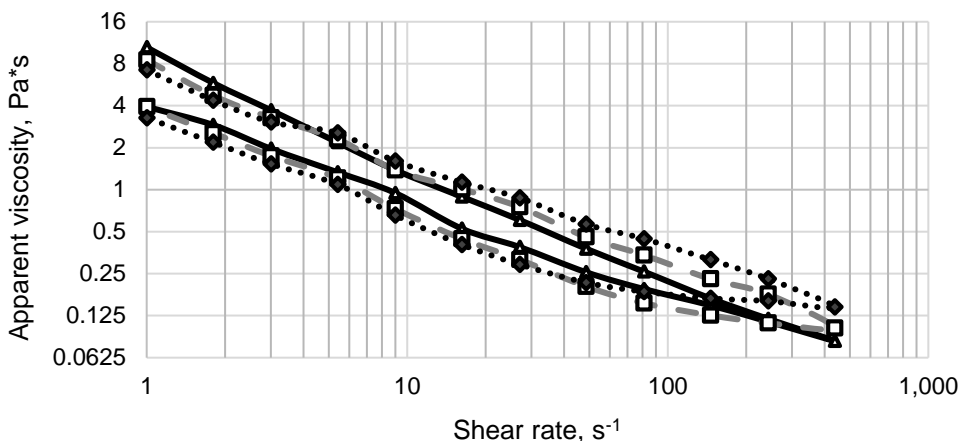
Figure 2. Hysteresis loop of the yogurt samples of different fennel powder content (—△— – 0%; —□— – 1% (w/v); ••◆•• – 2% (w/v)).

Control sample without fennel powder showed more resistant to action of shear forces. This can be attributed to the increased strength of interparticle interactions in the plain yogurt, mainly caused by self-aggregation of proteins. Analyzing the area between the upward and downward flow curves of each analysis sample, it can be seen that the rheological properties of the sample with 1% of fennel powder is the closest in degree of thixotropy to the control sample, whereas the hysteresis loop area of the sample with 2% of the extract was the largest. The presence of hysteresis loops, i.e., the difference existing between the forward and backward measurements, reveals that all yogurt samples would exhibit a thixotropic behavior, but sample with 2% of fennel powder has decreased the ability for rebuilding. The sample assessment, considering the attribute of fennel concentration is shown in Fig. 3.
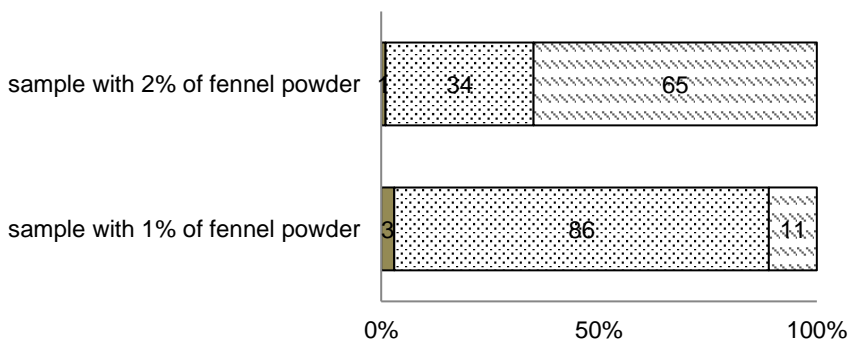
Figure 3. 'Just Right' ratings: ■ – 'Not enough'; ▒ – 'Just right'; ▨ – 'Too much'.

From the graphics above, the yogurt sample with 2% of fennel powder was considered as having too much fennel (65%). Overall, 86% of the assessors scored 1% of fennel powder 'Just right,' whereas only 34% scored 2% of fennel powder 'Just right'. Based on the obtained data, the most optimal concentration of fennel powder is 1% (w/v).

Based on the results of preliminary organoleptic evaluation, titratable acidity and pH the expected shelf life of control and treatment sample was 10 and 15 days, respectively. According to MUK 4.2.1847-04 the frequency of analysis was established (Table 3). Changes in titratable acidity, microbiological indicators and antioxidant activity during estimated shelf life are presented in Table 3. Analyzing the data presented, it can be concluded that quality indicators during the shelf life of the samples do not exceed the established parameters for yogurt. The results of the present study showed that the antioxidant activity of yogurts was enhanced by the presence of natural extracts, such fennel powder. The results presented in Table 3 revealed that the recommended period of storage for fortified yogurt is 15 days at 4 ± 2 °C.

CONCLUSIONS

The incorporation of fennel extract into yogurt allowed to create a product with unique organoleptic characteristics and health benefits. The present investigation concludes that the inlet temperature have significant effects on physicochemical properties. The spray-dried powders produced using higher inlet air temperatures showed higher moisture contents, solubility, lower water activity and requires less time for wetting. The results obtained indicate that good quality powders can be produced by spray drying if the inlet temperature of 165 °C is used. The addition of fennel powder accelerates acid accumulation and increases water holding capacity of yogurt gel in comparison with plain yogurt.

The incorporation of fennel powder appeared to extend the shelf life of yogurt from 10 to 15 days and guarantees the quality preservation of the product at a storage temperature of 4 ± 2 °C. The recommended dose of the fennel powder is 1% of mixture weight. This fennel powder positively affected the antioxidant activity of yogurt during the refrigerated storage. Since fennel powder is a natural product with therapeutic and antioxidant properties it is recommended as a novel ingredient to enhance yogurt's properties.

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Development of formulation and technology of fermented dairy beverage for musculoskeletal disease prevention

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Abstract. According to the data of World Health Organization, 20–33% of people across the world suffer from painful musculoskeletal conditions, which lead to restricted mobility, dexterity and functional mobility. The aim of the research was to develop formulation and technology of yogurt for prevention of musculoskeletal disease. The results of sensory characteristics, physico-chemical parameters, rheological characteristics, fatty acid composition have shown that it is possible to create the new product with curcumin, grape seed oil, hyaluronic acid and chondroitin sulfate, which are recommended to use for improving various symptoms of musculoskeletal disease. However, the chosen components increase the manufacturing process. It was observed that fermentation time increase was caused by addition of curcumin, which inhibits the lactic acid bacteria growth within 2.5–3 h. The combination Tween 80 and lecithin allows to obtain stable product during the storage period.

Key words: musculoskeletal disease prevention, curcumin, grape seed oil, emulsion.

INTRUDUCTION

Musculoskeletal health was included as a noncommunicable disease target since 2016 in the *Action plan for the prevention and control of noncommunicable diseases in the WHO (2016) European Region*. The Global Burden of Disease study reported that the 20-33% of people around the world suffers from a painful musculoskeletal condition (GBD, 2017).

Musculoskeletal conditions can be caused or made worse by many factors. They can be divided into three categories. The first is professional and work-related factors.

Studies have shown that long-term repetitive operations, incorrect postures, long-term repeated lifting, pushing, pulling, and transporting heavy objects increase the incidence of musculoskeletal injuries (Bernard, 1997; Podniece & Taylor, 2008; Okunribido & Wynn, 2010; Ge et al., 2018).

The second is the individual factor. Factors such as age, gender, genetics, smoking, alcohol, poor nutrition, low phisical activity, hormones, inadequate dietary intake of calcium and vitamin D, overweight and obesity, ethnicity (Karponis, 2004; Guh et al., 2009; Arthritis research UK, 2017; Ge et al., 2018). The third is social and psychological factors, such as job satisfaction, high workload, work monotony, job control, and social support, depression (Arthritis research UK, 2017; Ge et al., 2018).

Musculoskeletal conditions are one of the leading causes of morbidity and disability, giving rise to huge economic burdens to the country and enterprises. The pain and disability caused by musculoskeletal diseases result in a substantial loss in quality of life. Musculoskeletal conditions are a leading cause of work limitations and working days lost (Woolf & Pfleger, 2003; Buckle, 2005; Miller et al., 2005, Arthritis research UK, 2017; Briggs et al., 2018; Ge et al., 2018).

In this study yogurt was chosen as a vehicle for the oral administration of these natural components. Yoghurt is one of the highly-consumed dairy foods in the world and it also demonstrates unique health benefits. It contains *Lactobacillus* subsp. *bulgaricus, which* is one of the first *probiotic* strains ever studied. It also helps reduce the risk of caries, calcium and vitamin D deficiency, reduce body mass index, blood pressure, sugar levels (Burgain, 2012; Kim, 2013; Wu et al., 2013). For this investigation yogurt and grape seed oil were also selected based on the recommendation to consume curcumin with milk and with any edible oil (Gouda & Bhandary, 2018).

Curcumin (diferuloylmethane) is a yellow pigment isolated from *Curcuma longa* L. (turmeric) (Gutierres et al., 2015). Curcumin is stable during thermal treatment. Results of numerous research have showed that curcumin can reduce severity of pain in adults suffer from arthritis in comparison with placebo or analgesic medications and improve, functional outcomes (Madhu et al., 2012; Lakhan et al., 2015; Daily et al., 2016; Ross; 2016). Besides,curcumin shows significant antioxidant, anticancer, anti-flammatory, antimutagenic, antimicrobial, gastroprotective properties and play an in the prevention and treatment of diabetes, and its associated disorders (Zhang et al., 2013; Jovičić et al., 2017). Research suggests that curcumin can help in the management of oxidative and inflammatory conditions, metabolic syndrome, arthritis, anxiety, and hyperlipidemia. It helps to protect liver, brain, age-related diseases and cancer (Ferrari, 2013; Pulido-Moran et al., 2016). Dietary intake of isolated curcumin could be more effective compared to whole turmeric powder. It is recommended to consume curcumin with milk, pepper, honey or with any edible oil (Gouda & Bhandary, 2018).

Hyaluronic acid is a glycosaminoglycan, which is a substance that attaches to collagen and elastin to form cartilage and also helps to increase supplies of joint-lubricating synovial fluid, thus protecting the articular cartilage. Due to its viscoelasticity, it absorbs mechanical impacts and avoids friction between the bone-ends (Fallacara et al., 2018). The functions of hyaluronic acid include preventing cartilage denaturation, protecting the outer layer of cartilage, blocking synovial inflammation, increasing chondrocyte density, promoting synovium metabolism, normalizing synovial fluid, and treating sharp pain (Oe et al., 2016).

Therefore, without adequate amounts of hyaluronic acid, disorders such as rheumatoid arthritis and osteoarthritis occur, the joints will become brittle and deteriorate (Manasa et al., 2012; Tamer, 2013; Fallacara et al., 2018). Additionally, hyaluronic acid was found to be beneficial also for the treatment of joint (Fallacara et al., 2018). A 70 kg human contains around 15 g total of hyaluronic acid of which about 2–4 g L^{-1} in the synovial fluid. Hyaluronic acid is also present in skin, blood vessels, serum, brain, heart valves, and the umbilical cord (Frasher et al., 2003; Volpi et al., 2009; Oe et al., 2016).

Chondroitin sulfate is a glycosaminoglycan formed naturally by the body for the synthesis and maintenance of connective tissue. It is also can be extracted from animal cartilage (cows, pigs, birds, and fish) (Jerosch, 2011; Pelletier et al., 2016).

Due to the negative charge of chondroitin sulfate, it is responsible for the water retention of the cartilage, which is important for pressure resistance. Chondroitin sulfate is a symptomatic slow-acting drug. The first effects become noticeable after 2–3 weeks of regular intake and remain for up to several months (Jerosch, 2011). Chondroitin sulfate supports and protects structure and function of cartilage as well as other connective tissue in numerous ways.

Results of many studies demonstarted that chondroitin sulfate has a beneficial effect on pain and joint space narrowing in patients with knee osteoarthritis, decreased cartilage volume loss, improves spinal function and allows to slow down musculoskeletal disease. Chondroitin sulfate inhibits the enzymes leukocyte elastase and hyaluronidase, which are found in high concentration in the synovial fluid of patients with rheumatic diseases (Oliviero et al., 1991; Pepitone, 1991; Bucci & Poor, 1998; Uebelhart, 1998; Shostak et al., 2002, Mazurov & Belyaeva, 2004; Kahan et al., 2009; Jerosch, 2011; Wildi et al., 2011).

Grape seed oil is recognized as beneficial wine industry by-product (Pardo et al., 2009). Besides pleasant sensory characteristics grape seed oil has a high linoleic acid content, high vitamin E and F contents, polyphenols, flavonoids, carotenoids, minerals like zinc potassium, copper, calcium, phosphorus, magnesium, iron and selenium and low values of cholesterol. Grape seed oil is rich in proanthocyanidins which antioxidant properties are 50 times more effective than Vitamin E and 20 times stronger than Vitamin C (Herting et al., 1963; Nash, 2004; Pardo et al., 2009; Erlich, 2012). Grape seed oil has also high concentration of tannins, oligomeric proanthocyanosides at 1,000 times higher than the other oils and that is the reason why it has high stability and resistant to oxidation reaction (Sotiropoulou et al., 2015).

The highlight health benefits that offers the consumption of grape seed oil including antitumor, antioxidant, anti-inflammatory, cardioprotective, neuroprotective, antimicrobial effects which has been proven by preclinical tests and studies in humans (Shinagawa et al., 2015; Garavaglia et.al., 2016).

The aim of this study is to investigate the possibility of using the combination of hyaluronic acid, curcumin and chondroitin sulfate in the manufacture of beneficial product, which would help prevent musculoskeletal disease.

MATERIALS AND METHODS

Skimmed milk powder (commercial brand Central Lechera Asturiana, Corporación Alimentaria Peñasanta, S.A., Spain, 34 g protein and 52 g carbohydrates per 100 g) reconstituted with distilled water at a temperature 45 °C and stirred for enough time to assure solubilisation and hydration of solids.

Control sample preparation
Reconstituted skimmed milk was heated to 90 ± 2 °C for 5–7 min, and then cooled immediately to 42 ± 1 °C. Milk was inoculated with the starter culture at a rate of 3% (*Lactobacillus delbrueckii subsp. Bulgaricus* and *Streptococcus thermophilus*) and fermented at 42 ± 1 °C until the complete coagulation of yoghurt. The yoghurt samples were stored at about 5 °C at refrigeration until used.

Treatment samples preparation

Chondroitine sulfate (0.6% (w/v), Now Foods, USA) and hyaluronic acid (0.05% (w/v), Jarrow Formulas, USA) were dissolved in distilled water at a temperature 50 ± 2 ºC with holding time 10–15 min. These solutions were added to the reconstituted skimmed milk and mixed for 15 min (1,050 rpm, RZR 2020, Heidolph Instruments GmbH). The amount of water for skimmed milk reconstitution was reduced by the amount required for chondroitin sulfate and hyaluronic acid dissolution. Curcumin (0.3% (w/v), Thompson, USA) was dissolved in grape seed oil (Fratelli Mantova, Italia) at 50 ± 2 °C with holding time 10 min. For the preparation of oil in water (O/W) emulsions, method of two-step mechanical homogenization was used. Based on preliminary studies (data not shown) grape seed oil 7% (w/w) was added gradually and the most suitable homogenization conditions were 6,500 rpm for 5 min (1 step) and 21,500 for 3 minutes (2 step). The emulsification process was carried out using Ultra-Turrax® (IKA T 25, Germany). The homogenization temperature was kept at 50–55 °C. Based on the results of another part of our study concerning lecithin and Tween 80 concentration selection, in order to improve emulsion final stability, lecithin (Cargill Inc., Germany) and Tween 80 (Polyethylene glycol sorbitan monooleate, Sigma-Aldrich) in the ratio 8:2 (1.2% v/v) were added to the formula. Further step were made according to the same process of control sample manufacture. The amount of starter culture varied from 3% to 5%, with the increment of 1%.

Methods

pH values were measured using pH–meter pH–410 with combined glass electrode (Scientific Production Association 'TECHNOKOM', Russia).

Titratable acidity was measured according to AOAC method 947.05 (AOAC, 2007).

Centrifugally separated whey was estimated visually and by the amount of separated whey during the centrifugation of 10 g of yogurt. Each sample was weight into centrifuge tubes, centrifuged and the amount of separated whey was measured every 5 min during 30 min.

The rheological measurements were performed at controlled temperature of 20.0 ± 0.2 °C and 4 ± 2 °C using rotational rheometer Rheotest RN 4.1 (RHEOTEST Medingen GmbH, Germany). Since the structure of yoghurt gel is sensitive to its shear deformation history, samples were carefully loaded to the rheometer. The measurement were taken using spindle (H), sample were subjected to shear rates ranging from 0.11 to 20.43 s^{-1}. Rheological analysis was carried out after 24 hours of yogurt production. Before measurements, yogurt samples were gently stirred with a spatula for 30 seconds to ensure homogeneity.

Vitamin E concentration was determined according to M 04-10-2007 using HPLC 'Lumachrom'. The method based on sample alkaline hydrolysis. As a result, in conjunction with saponification vitamin essential form lipid transformation in alcohol forms occurs; hexane extraction followed by separation and determination of vitamin E using HPLC method with fluorimetric detection.

Fatty acid composition of yogurt was analysed was determined by gas chomatograph (GC-2010, Shimadzu, Tokyo, Japan) equipped with a flame ionization detector and a capillary column DB-23 (60 m x 0.25 mm x 0.25 µm) (Agilent Technologies, Santa Clara,CA, USA). Injector and detector temperatures were set as 250 °C and 280 °C, respectively.

Emulsion stability of control and treatment samples of yogurt was determined experimentally by the measurement of the creaming index (CI) (Eq. 1) in 24 h after yogurt manufacture and during the storage period every 5 days. An emulsion (35 mL) was placed into a 50 mL centrifugal plastic tube and centrifuged at 2,500 rpm for 15 min at 25 °C. An emulsion (30 mL) was placed into a 50 mL centrifugal plastic tube and centrifuged at 7,500 g for 15 min at 25 °C. The CI values were obtained from the ratio between the total height of cream layer (CC) and the total height of emulsion layer (TE).

$$CI(\%) = \frac{CC}{TE} \cdot 100 \qquad (1)$$

Lactobacillus delbrueckii subsp. Bulgaricus and *Streptococcus thermophilus* were counted according to GOST 33951-2016.

Organoleptic characteristics were assessed using 9–point hedonic scale (Clark et al., 2009) with four positive categories in the upper pole, a centered neutral category and four negative categories in the lower pole (Table 1). Yogurt sample were assessed by a 36-member panel (50% of participants were female from 20 to 70 years old and 50% of participants were male from 19 to 65 years old) selected from students and staff members of our Faculty and were unsalted cracker for palate cleansing between the samples.

Table 1. Numerical and verbal representations of the 9–point hedonic scale

Score	Categories
9	Like extremely
8	Like very much
7	Like moderately
6	Like slightly
5	Neither like nor dislike
4	Dislike slightly
3	Dislike moderately
2	Dislike very much
1	Dislike extremely

Statistical analysis

All measurements were replicated at least 3 times for each sample.

All experiments were performed at least in triplicate. 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 DISCUSSIONS

Values presented in Figs 1, 2 showed the pH and titratable acidity changes during fermentation process.

During the fermemtation GSO, HA slightly affect the changes in titrated acidity compared to the control sample. HS decrease the fermentation by about 30 minutes. The combination of all additives (sample with GSO, HA, CS and curcumin) significantly decrease the process of fermentation.

In the samples with GSO and HA, the mean pH value changes were close to the control sample. In the sample with HS, the pH drops slower. In general, the pH values of the treatment sample with GSO, HA, CS and curcumin decreased significantly slower during fermentation in comparison with other treatment and control samples.
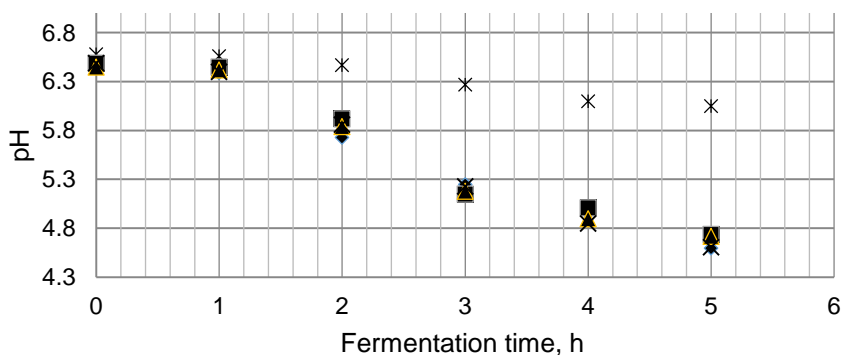
Figure 1. Changes in pH during fermentation: ◆ – control sample; ■ – sample with HA; ▲ – sample with CS; ✖ – sample with CS, GSO, HA, curcumin; ✕ – sample with GSO.
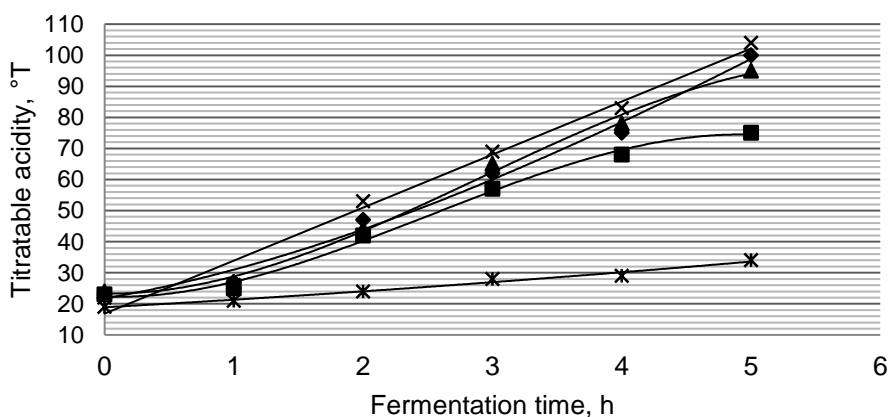
Figure 2. Changes in titratable acidity during fermentation: ◆ – control sample; ■ – sample with HA; ▲ – sample with CS; ✖ – sample with CS, GSO, HA, curcumin; ✕ – sample with GSO.

Increasing the amount of starter affects the time of fermentation (Figs 3, 4). However, during the first 3 hours, the titratable acidity and pH, regardless of the amount of the starter, change in the same way. In further, the time of fermentation varies depending on the amount of the added starter: an increase in the amount of starter by 1% reduces the time of fermentation by 1 hour. However, the fermentation time is equal at least 6 hours.

This increasing in fermentation time is probably caused by the addition of curcumin and lactic acid bacteria need more time for adaptation (approximately 2.5–3 h) compared to the plain yogurt. In other words, curcumin does not allow bacteria to develop immediately, as occurs in the control sample, they begin to increase population only after adaptation to the environment.
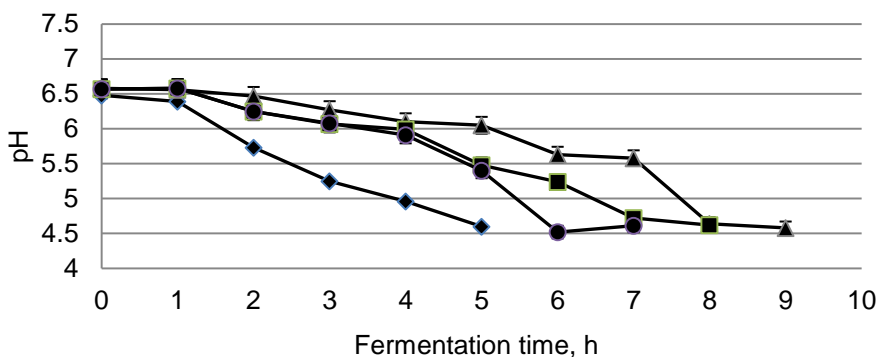
Figure 3. Effect of starter culture amount on pH-values of yogurt during fermentation: ◆ – control sample; ▲ – treatment sample with 3% of starter culture; ■ – treatment sample with 4% of starter culture; ● – treatment sample with 5% of starter culture.
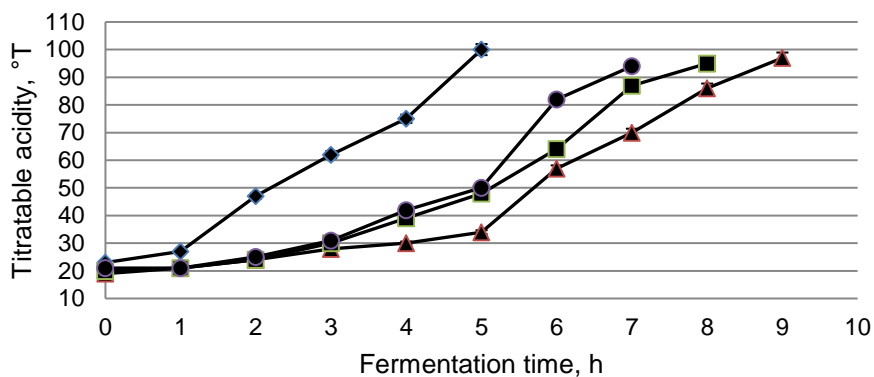
Figure 4. Effect of starter culture amount on titratable acidity changes during fermentation: ◆ – control sample; ▲ – treatment sample with 3% of starter culture; ■ – treatment sample with 4% of starter culture; ● – treatment sample with 5% of starter culture.

In 5 hours of fermentation (time is needed for the complete fermentation of the control sample with 1% of starter culture) the lowest numbers of *Lactobacillus delbrueckii subsp. bulgaricus* were observed for the treatment sample (1% of starter culture). The numbers of *Lactobacillus bulgaricus* for the control and treatment samples were $5.0\ 10^8$ CFU mL^{-1} and $5.0\ 10^6$ CFU mL^{-1}, respectively. The numbers of *Streptococcus thermophilus* for the control and treatment samples were 1.1×10^8 CFU mL^{-1}.

The results of water holding capacity of the control and treatment samples are shown in Fig. 5.

The experimental results can verify the water holding capacity and, therefore, the porosity of the yogurt samples as well. The amount of liquid separated from the samples affirmed that the yogurt with addition of CS, HA or GSO are more resistant against whey explusion. The results of centrifugally separated whey are presented in Fig. 5.

Based on the results (Fig. 5), the sample with GSO had the smallest amount of whey expelled indicating that this sample had the highest water holding capacity, while the

control sample had the smallest water holding capacity. Yogurt samples with addition of CS showed a water holding capacity close to that of the control sample. The decrease in the amount of whey expelled in yogurt samples with addition of CS can be due to the negative charge of CS (Jerosch, 2011). Concerning the sample with HA, the less amount of whey can be caused by hydrogen bond formation between water molecules and adjacent carboxyl and N-acetyl groups of HA (Manasa et al., 2012).
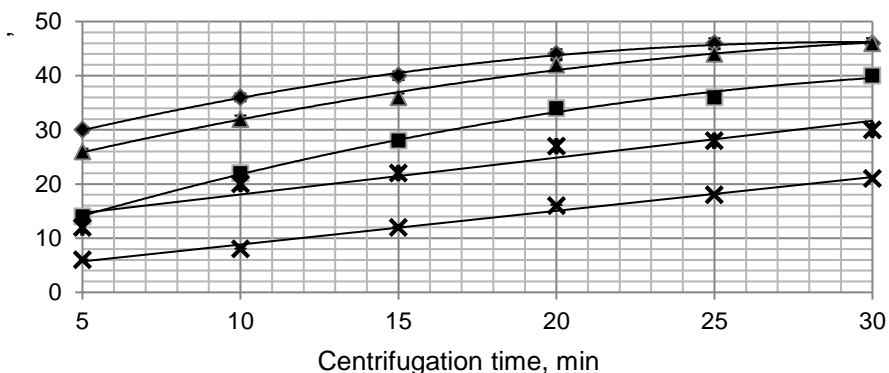
Figure 5. Water holding capacities of yogurt samples: ◆ – control sample; ■ – sample with HA; ▲ – sample with CS; ✖ – sample with CS, GSO, HA, curcumin; ✖ – sample with GSO.

Yogurt apparent viscosity has been evaluated 4 °C and 20 °C. The data are shown in Fig. 6 and Fig. 7. The apparent viscosity of the control and treatment samples decreased as the shear rate increased due to shear thinning behaviour and implied that the fluid does not have a true viscosity. Since apparent viscosity is affected by different factors such as milk composition, heat treatment of milk and additives, the apparent viscosity of the of treatment sample showed significant lower values than those of control sample. These results could be due to the intermolecular bonds reduction in treatment sample. The results obtained for the treatment sample found the same tendency for changes in the rheological properties of the control sample in both cases understudy, despite the component introduction.
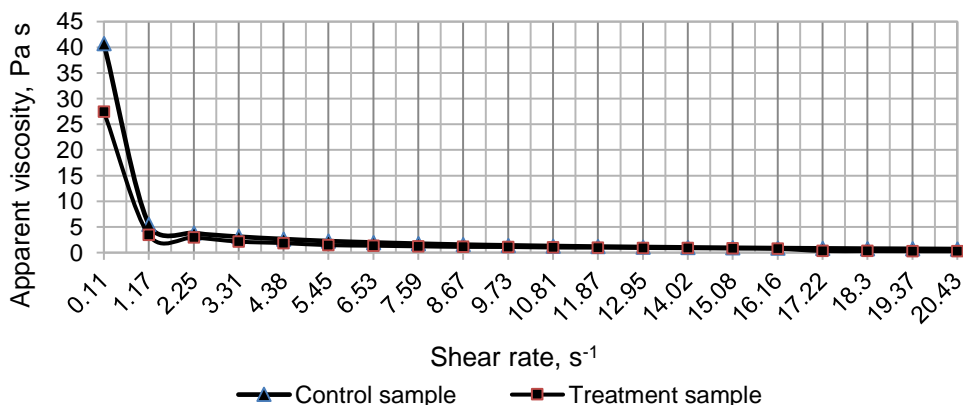
Figure 6. Dependence of the apparent viscosity of yogurt samples on the shear rate at 4 °C.
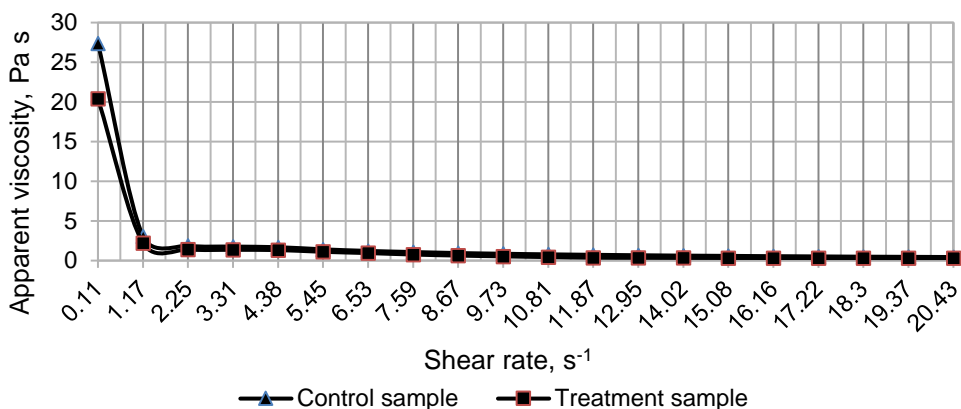
Figure 7. Dependence of the apparent viscosity of yogurt samples on the shear rate at 20 °C.

By increasing shear rate from 0.11 to 1.17 s^{-1}, the enriched yogurt showed a greater decrease in apparent viscosity as compared to the control (Figs 6, 7), indicating faster disruption of protein aggregates in the enriched yogurt.

The addition of GSO (Table 2) can achieve fortified yogurt containing omega-3 polyunsaturated fatty acid. The contents of most important fatty acids for human health – C18:1, C18:2 were significantly higher in the treatment yogurt than in skimmed yogurt. The incorporation of GSO also allows to fortify yogurt with vitamin E. As the recommended daily intake of vitamin E (Institute of Medicine. Food and Nutrition Board, 2000) is 15 mg for an adult the developed product (100 g) satisfies the daily intake for vitamin E up to 26.7%.

Table 2. Fatty acid composition of the treatment yogurt

Fatty acid profile/Vitamin E	Fatty acid content, %
Palmitic	0.5
Palmitoleic (16:1)	-
Stearic (18:0)	0.27
Oleic (18:1)	1.44
linoleic acid (18:2) (w-6 PUFA)	4.62
Linolenic (18:3)	-
Arachidic (20:0)	-
Gadoleic (20:1)	-
Vitamin E	4.0 ± 2.3 mg per 100 g

The results of the short term stability study are shown in Fig. 8. According to data, the emulsions showed different behavior to creaming during the storage time The higher the creaming index value is, the more destabilized the emulsion is. Regarding the creaming index data, the lowest CI rates were observed at time 1 day for the treatment sample with emulsifiers, where CI was 1.01%. After 25 days, lower CI was also observed for the treatment sample with selected emulsifiers (2.63%). It can be concluded that yogurt samples made with the mixture of Tween 80 and lecithin (2:8) are more stable than those made without emulsifiers.
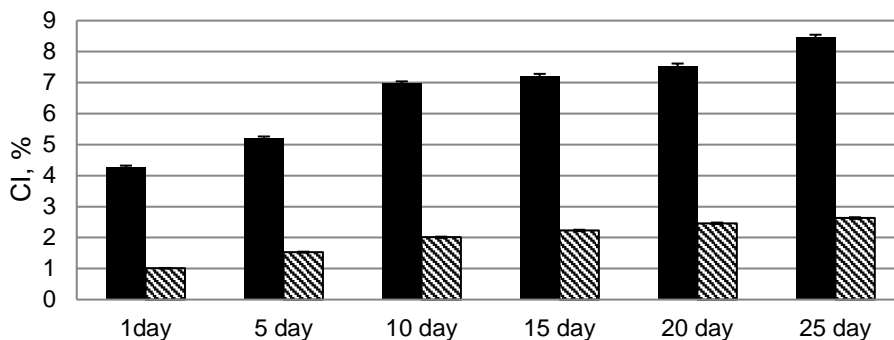
Figure 8. Creaming index versus days of storage: ■ – treatment sample without emulsifiers; ▨ – treatment sample with emulsifiers.

Data from the Fig. 9 present the sensory evaluation of yogurt samples. It can be seen, that the mean scores for overall acceptance obtained for treatment sample was higher than for the control sample. In terms of appearance, treatment sample presented slightly lower degree of liking by the panelists, reflecting differences in outcomes by age or sex among judges. The bright yellow color of the treatment sample gained higher scores among people at the age between 19–49. In terms of taste and flavor all yogurt samples were well accepted by the panelists, and the treatment sample was the product of greater acceptance.
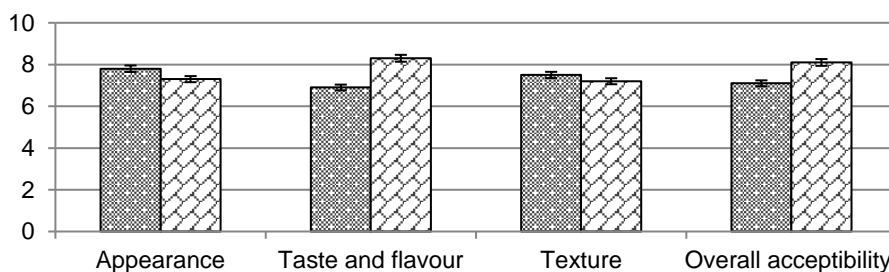
Figure 9. Hedonic scale for appearance and overall acceptability of the samples: ▨ – control sample; ▨ – treatment sample with emulsifiers.

Consequently, sensory analysis indicated that chosen sensory attributes and overall acceptability were very satisfactory and graded with the highest score treatment sample.

CONCLUSIONS

The present work demonstrated the feasibility of yogurt with incorporated curcumin, hyaluronic acid, chondroitine sulfate and grape seed oil. The results indicate that it is possible to produce product of good sensory quality for the prevention of musculoskeletal disease prevention. The fortification of yogurt with chosen components

allows to increase the storage period of yogurt in comparison to the plain yogurt, 10 and 21 days, respectively.

The selected components improve the properties of yogurt, however, affect the time of its manufacture. The developed technology and formulation can be an excellent outlet for the use of valuable skimmed milk solids. The developed product is a good source of vitamin E and w-6 PUFA. The combination of emulsifiers could be recommended for creation stable product during the storage.

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Surface wax composition of wild and cultivated Northern berries

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Abstract. Surface wax of plants is the outer layer, which protects the plant from dehydration, extreme temperatures, UV radiation and changes in the environment, as well as attacks from moulds and bacteria. Studies of berry surface wax are of importance to understand metabolism character (factors affecting wax layer composition in different berry species) as well as to increase the shelf life of berries and increase the microbial resistance. The aim of this study was analysis of surface wax composition of commercially grown 8 blueberry (*Vaccinium corymbosum*) varieties, wild bilberry (*Vaccinium myrtillus* L.) and bog bilberry (*Vaccinium uliginosum* L.). More than 80 different compounds were identified and quantified belonging to 9 groups of compounds, namely, alkanes, phytosterols, alcohols, fatty acids, phenolic acids, ketones, aldehydes, esters and tocopherols. Significant differences were found between blueberry (*Vaccinium corymbosum*) and bog bilberry (*Vaccinium uliginosum* L.) surface wax composition. Amongst studied berries differences were found in concentrations of triterpenes (up to 62% in blueberries), and fatty acids (up to 26% in bilberries) identifying species related differences influencing associated functional properties of berry wax (antimicrobial activity, stress caused by environmental changes). Blueberry variety 'Polaris' had the highest amount of ursolic acid (9.30 g 100 g^{-1}), alpha-amyrin (11.07 g 100 g^{-1}) and lupeol (10.2 g 100 g^{-1}). Research on berry surface wax composition could help reduce loss of commercially produced berries due to environmental impacts or microbial attacks, prolonging shelf life and overall quality of fruits and vegetables post-harvest.

Key words: blueberry, bilberry, bog bilberry, cuticular, cuticle, surface wax, chemical composition, lipids.

INTRODUCTION

Blueberries (*Vaccinium corymbosum* L.) are rich source of polyphenolic substances, carbohydrates, vitamins as well as lipids (Kim et al., 2013) and they are one of the commercially important berry species. Blueberries have large fruits, long shelf life, excellent taste properties and they are suggested as part of a healthy diet (Nile & Park, 2014). Blueberry cultivation takes place worldwide and the demand is steadily increasing (Venskutonis et al., 2016). Blueberries can be considered as functional food and many cultivars (varieties) are developed to differ in taste, size and colour of berries, improving productivity of blueberry bushes and prolonging growing season (Burdulis et al., 2007). Blueberries contain many phenolics responsible for their colour

(anthocyanins) and high radical scavenging capacity, which is considered beneficial for human consumption (Cho et al., 2004). Another important group of substances in the composition of *Vaccinium corymbosum* berries are their lipids and waxes. Berry lipids are berry skin waxes, seed lipids and cytoplasm lipids. By the chemical composition *Vaccinium* berry lipids include triglycerides, fatty acids, alcohols, alkanes as well as sterols, terpenes and other groups of substances with low polarity (Dulf et al., 2012).

An important group of berry lipids are their cuticular wax, which have glossy or glaucous appearance; it acts as an interface between plant and the environment (Yeats & Rose, 2013). The outermost layer of plant organs- the cuticle- protect the plant from abiotic stresses such as dehydration, extreme temperatures (frost, heat) and other factors presented by the environmental changes in the growth area. Cuticular wax is a complex mixture of various aliphatic and aromatic compounds. Plant waxes consist of low- to intermediate-polarity compounds, they are hydrophobic, non reactive, long-chain (chain length from C_{12} up to C_{70}) chemical compounds (Jetter et al., 2006). The main compound classes found in the wax are n-alkanes, fatty acids, primary alcohols, aldehydes, secondary alcohols, ketones, phytosterols and esters. To obtain waxes care should be taken to avoid co-extraction of cytoplasmic and membrane lipids and it is suggested to do it by treatment of fresh berries with hydrophobic solvents such as chloroform, hexane or petroleum ether (Sharma et al., 2018).

The importance of cuticular wax studies of blueberries is related to the need to understand the natural protection mechanisms of berries as well as to consider possibilities to increase the quality of produce by increasing the shelf-life during berry storage, fruit quality and reduce possible microbial infections (Lara et al., 2014). Functional foods, nutraceuticals and healthcare products containing plant lipids (waxes) are being developed as innovative, consumer friendly products (Weingartner et al., 2014). The hydrophobic properties of plant cuticular waxes are being investigated for implementation as part of antimicrobial paints, windshield coatings, stain resistant textiles and biodegradable plastics (Li et al., 2007; Yadav et al., 2014).

The composition of berry waxes depend on the species, their growth location and thus it is important to study berries composition in each specific site. Lipids of *Vaccinium corymbosum* have been studied by other authors, however, no information is present for the wax composition of Northern blueberry varieties. The aim of the present article is to study and to compare the composition of waxes of 8 bilberry *Vaccinium corymbosum* cultivars as well as comparison with related species bilberry *Vaccinium myrtillus* L. and bog bilberry *Vaccinium uliginosum* L. cuticular wax.

MATERIALS AND METHODS

Plant material
In this study, three berry species were examined for their cuticular wax composition. Examined berries were- bog bilberry (*Vaccinium uliginosum* L.), bilberry (*Vaccinium myrtillus* L.) and eight varieties of blueberry (*Vaccinium corymbosum* L.), namely, 'Blue crop', 'Blue gold', 'Chandler', 'Chippewa', 'Duke', 'North blue', 'Patriot', 'Polaris'. The different blueberry varieties were harvested at a commercial blueberry farm Z/S 'Strelnieki' located on the outskirts of town Jurmala, Latvia. Bog bilberries and bilberries were harvested from the forests belonging to Kemeru National Park. To avoid contamination and possible damage to the outer layer of berries they were

harvested into glass containers previously washed with chloroform (≥ 99%, Sigma Aldrich) using metal forceps. In total approximately 700 berries were harvested for each sample, all berries were harvested in the summer/autumn of 2018. After the harvest berries were placed into a refrigerated sample box and delivered to the laboratory for immediate extraction of waxes.

Extraction of cuticular wax

Extraction of cuticular wax was done using two extraction solvents, chloroform and a mixture of hexane/ethyl acetate (1:1) (≥ 99%, Sigma Aldrich). Each extraction solvent was used three times for the extraction of respective berry species. For extraction, 150 mL of extraction solvent was poured into three separate glass beakers that were previously cleaned with the same solvent. A hundred berries were picked from the harvested sample and sequentially dipped one by one into the extraction solvent for 30 seconds in each of the three beakers containing the solvent. Clean metal forceps were used for the berry dipping. After the berry dipping all of the contents of the three used beakers were filtered and combined into an evaporation flask. Each beaker was further washed twice with extraction solvent and added to the combined extract. Samples were evaporated under reduced pressure using Hi-Vap Advantage (Heidolph, Switzerland) evaporator. Samples were evaporated to approximately 5 mL and transferred to clean glass tubes. The remaining solvent was evaporated in a water bath (40 °C) (Cole Parmer) under a gentle stream of nitrogen until dry. The dried berry cuticular wax samples were stored into a freezer (-20 °C) until analysis.

Analysis using Gas Chromatography-Mass Spectrometry (GC-MS)

The cuticular berry wax chloroform extracts were evaporated under a flow of nitrogen. Silylation was done using N,O-bis (trimethylsilyl) trifluoroacetamide, BSTFA (200 µl, Sigma-Aldrich) in pyridine (1,300 µl, Sigma-Aldrich), for 1 hour at 60 °C. Quantification was performed using three external standard curves: ergosterol (Sigma-Aldrich), stearic acid (Sigma-Aldrich) and tetracosane. GC- MS analysis was performed using GC-2010 plus coupled with GC/MS QP-2010 Ultra mass detector (Shimadzu, Japan). The column used was Restek Rxi®-5MS (30 m × 0.25 mm × 0.25 µm; Crossbond ® 5% diphenyl + 95% dimethyl polysiloxane) with working temperature range 40 to 350 °C. He (Helium) was used as carrier gas with a total flow rate of 10.8 mL min^{-1} and column flow rate of 0.71 mL min^{-1} flow rate. The split ratio was 1:10 and injection temperature 290 °C. The temperature programme used was: oven temperature 200 °C (2 min) increased to 250 °C at the rate of 30 °C min^{-1} and held for 7 min then increased to 310 °C at the rate of 10 °C min^{-1} and kept for 14 min. Injection of 1.0 µL sample was performed using an autosampler. Mass selective detector with quadrupole mass analyser was used with electron impact (EI) ionisation, ionization voltage of 70eV. The ion source temperature was 230 °C and interface temperature 290 °C. Identification of the compounds separated in the GC was performed using Shimadzu LabSolutions 4.30 software, coupled with NIST'14 spectral library.

Data analysis

Quantitative data of cuticular wax composition was subjected to two-way analysis of variance (ANOVA) to evaluate the differences between the analysed berries. Principal component analysis (PCA) on correlation matrix and hierarchical cluster analysis using

Ward's method with standardized data was performed to evaluate relationship among various tested berries. Statistical analysis and data visualisation was done using SAS JMP®, Version 13 (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Composition of berry cuticular wax was investigated for three species, both grown commercially and found in the wild forests and bogs of Latvia. Studied berries were chosen in order to cover *Vaccinium* genus berries and compare wild and cultivated berries (Table 1). Substances found as part of the cuticular wax were identified and quantified to evaluate variations of composition and contents among berries.

The amount of extracted cuticular wax ranged from 0.65 to 0.90 mg berry^{-1} for the investigated blueberry varieties (Table 1). The amount of extracted wax is similar in the blueberry varieties, bilberries and bog bilberries, these berries have white, textured cuticular wax layer (crystal forming) (Jeffree, 2006). The glaucous appearance in analysed berries could be attributed to the presence of high triterpenoid contents (Fig. 1, B), however, in this study the morphology of cuticular wax layer was not investigated. In terms of morphology, wax layer of blueberries

Table 1. Studied berries and the extracted cuticular wax amount per berry

Studied berry	Variety	Wax, mg berry^{-1}
Bog bilberry		0.95[a] ± 0.09
Bilberry		0.63[b] ± 0.05
Blueberry	Blue crop	0.74[c] ± 0.04
	Blue gold	0.67[b] ± 0.03
	Chandler	0.83[c] ± 0.05
	Chippewa	0.90[c] ± 0.07
	Duke	0.57[c] ± 0.02
	North blue	0.65[b] ± 0.02
	Patriot	0.84[c] ± 0.03
	Polaris	0.87[c] ± 0.03

[a,b,c] – represent significant differences (α = 0.05) between different groups (ANOVA, Tukeys HSD).

is considered to belong to β-diketone tubes (tubule shaped wax crystals (Barthlott et al., 1998)), where diketones and triterpenoids were found to be the major compounds (Chu et al., 2017). The results reported by Chu et al. (2017) are in agreement with our findings - blueberry cultivars have high triterpenoid contents and, among other studied berries, contained high amounts of hentriacontane-10,12-dione (up to 6.0 g 100 g^{-1} extract in 'North Blue').

As part of the berry waxes 9 groups of compounds were found, namely, alkanes, phytosterols (triterpenoids), alcohols, fatty acids, phenolic acids, ketones, tocopherols and aldehydes (Fig. 1, B). Obtained cuticular wax extracts from different berries show a similar pattern of plant wax constituents, where triterpenes (up to 62% of total wax content in 'Blue Gold' and 'Blue Crop') and alcohols (up to 28% of total wax content in 'North Blue') are the major groups of cuticular wax components (Fig. 1). Aldehydes were found in all of the berries, however, their concentrations were low (Fig. 1, A). As minor groups of compounds found in the berries, phenolic acids and tocopherols were identified, despite the low concentration of these substances, they have a vital role in the plant - pathogen interaction (Fig. 1, B) (Kolattukudy et al., 1995). Phenolic acids and tocopherols have been reported to have protective abilities against UV radiation and antimicrobial activity, respectively (Kolb et al., 2003; Ahmed et al., 2014; Andrade et al., 2014; Ghimire et al., 2017).

Triterpenes were the most abundant components in the cuticular wax of the studied 3 species of berries, varying from 32% ('Chippewa') to 62% ('Blue Gold') of total wax contents (Fig. 1). Eleven different triterpenes were identified as part of the cuticular wax. The triterpene acid ursolic acid was found in all of the studied berries in varying amounts. In the blueberry variety 'Chippewa' 0.46 g 100 g^{-1} ursolic acid was found. The analysed blueberry varieties show different triterpene composition patterns. Variety 'Polaris' has the highest amount of ursolic acid (9.30 g 100 g^{-1}), alpha-amyrin (11.07 g 100 g^{-1}) and lupeol (10.2 g 100 g^{-1}) among all of the studied berries. Alpha – amyrin, beta – amyrin and lupeol are triterpene alcohols that are dominant in both, the cultivated and wild *Vaccinium* berries. Lanosterol was found only in, blueberry variety 'Chandler' (0.34 g 100 g^{-1}).
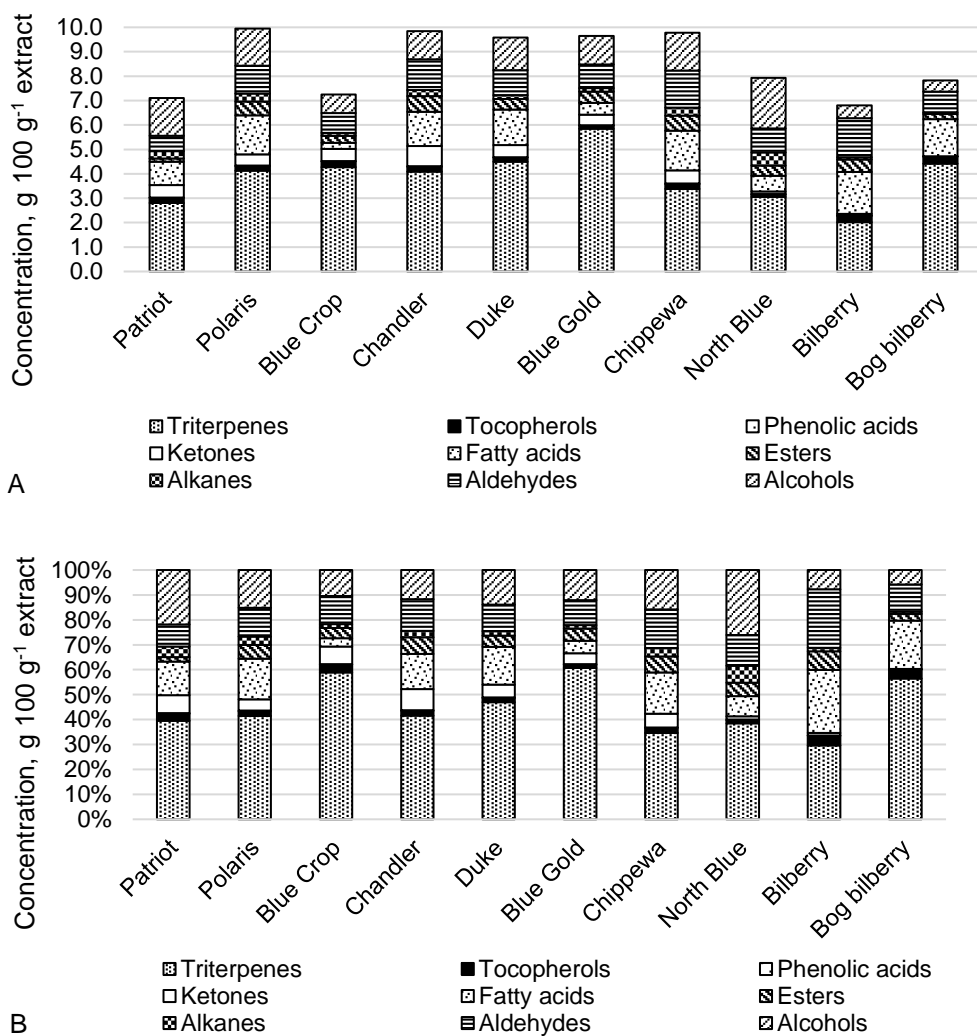
Figure 1. Absolute (A) and relative (B) amounts of identified compound groups in studied berries.

Triterpenoids as part of cuticular wax have been reported previously in a wide variety of plants, for example, grapes, apples cherries, tomatoes, blueberries and plums (Szakiel et al., 2012; Lara et al., 2014; Pensec et al., 2014; Chu et al., 2017). Considering the knowledge about the function of cuticular wax, where, for example, alkanes are believed to be responsible for the prevention of water loss, triterpenoids, on the other hand, might play a role in plant-pathogen interaction/recognition. Various biological activities and health promoting effects are attributed to triterpenoid molecules as part of human diet. Triterpenoids were reported to be the main group of compounds in blueberry cuticular wax (Chu et al., 2017), it was found that triterpenoids composed 64.2% of total wax, which is in agreement with the reported total wax amounts of specific varieties of blueberries analysed in this study (up to 62%, Fig. 1), also, the reported dominant triterpenoids are the same in both studies. During the GC/MS analysis, compounds in minor concentrations with unidentified MS spectra were recorded, possibly belonging to ursane- or oleanane type triterpenes. The unidentified compounds could contribute to species specificity and cuticular wax protective properties.

Alkanes with chain length from C20 to C33 were found in all of the studied berries. Blueberry varieties and the rest of the studied berries contained from 1.5–7% alkanes of total wax contents (Fig. 1). Differences among the relative alkane distribution in berries could be explained by the morphology of the cuticular wax- alkanes are possibly related to the glossiness of the berry, as glaucous berries have lower alkane concentration (Barthlott et al., 1998). The main alkanes found in the cuticular wax of studied berries were the C29 (nonacosane) and C31 (hentriacontane) alkanes. The dominant alkanes found on the surface of the berries are odd-numbered chain length.

Analysed blueberry varieties contained from 1.5 to 7% alkanes of total wax, which is higher than that reported by Chu et al., 2017, however, the previously reported alkane composition seems to be similar among the tested varieties, where the odd-numbered alkanes, specifically, C29 and C31were the dominant alkanes (Chu et al., 2017). Alkanes have been found to be part of outermost layer (cuticle) of many fruits, the dominant C29 alkane has been found on the surfaces of plums, apples and cherries (Lara et al., 2014). Main function of alkanes as part of the cuticular wax is to control transpirational water loss of the plant (Parsons et al., 2012).

Saturated fatty acids contribute to 26% in bilberry and 20% of total wax in bog bilberry. Blueberry varieties 'Chippewa' and 'Chandler' contained 24% and 20% fatty acids of total wax content (Fig. 1). Overall, the fatty acid distribution in studied berries was higher in the blueberries and bilberries. Also, the total amount fatty acids was higher in bluberries with up to 15.6 g 100 g^{-1} in 'Chandler'. As the most abundant fatty acid in the blueberry varieties triacontanoic acid (C30:0) was found, however, this fatty acid was not found in variety 'Chippewa', while variety 'North Blue' contained 9.6 g 100 g^{-1} of this fatty acid. Bilberry and bog bilberry present hexacosanoic acid (C26:0) as the major fatty acid with 7.6 and 5.2 g 100 g^{-1} extract, respectively. Identified fatty acids ranged from C16:0 to C30:0 in different berry species. In blueberry varieties grown in China the same chain length fatty acids were identified as part of the cuticular wax with C30:0 beeing the most abundant, followed by C28:0 (Chu et al., 2017).
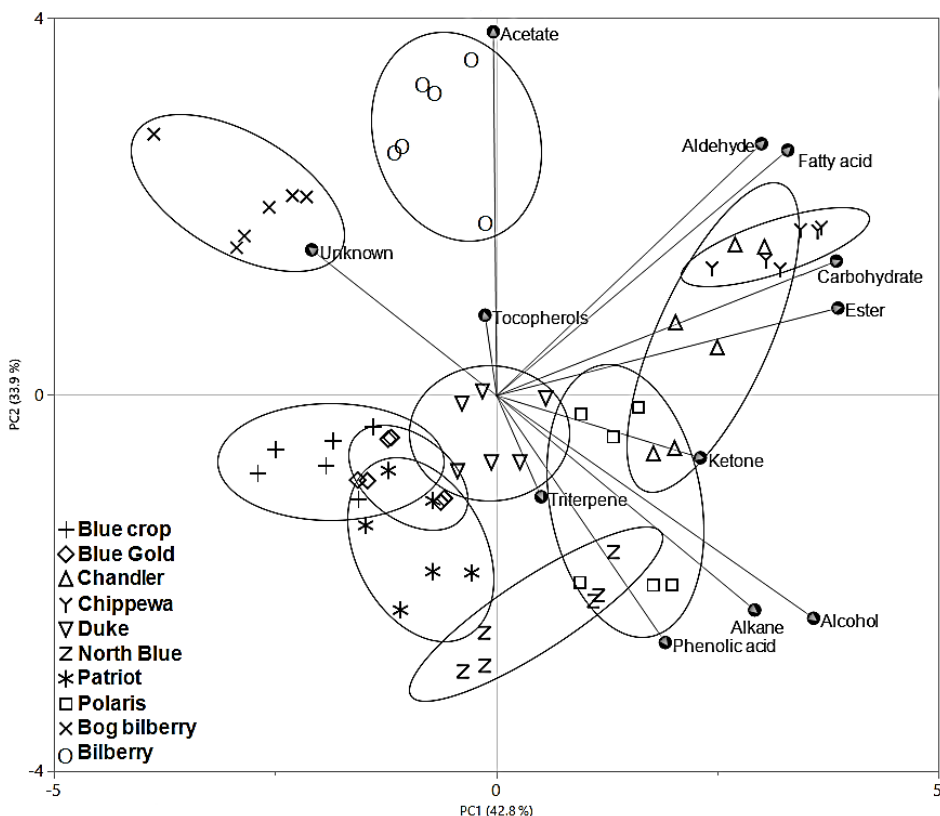
Figure 2. Principal components analysis of different berry species and blueberry varieties based on the identified compound classes of berry waxes.

Principal component analysis of the studied berry species and blueberry varieties based on the identified compound classes (Fig. 2) demonstrates significant differences amongst bluberry varieties on one hand and bilberies and bog bilberries on the other hand, thus supporting relationship between these berries based on chemical composition. However, also among varieties of blueberries, which belong to the same species, significant differences can be found. Also, main classes of compounds forming waxes can be grouped in the PCA plot, indicating similarities in their metabolic functions: long chain aldehydes, fatty acids, esters in one gorup, ketones, fatty alcohols, alkanes in another group (Fig. 2). Acyl- coenzymeA is directly responsible for the synthesis of esters, fatty acids and primary alchols which are later transformed to alkanes, seccondary alcohols and ketones by different oxidases (Goodwin et al., 2005). Grouped separately are tocopherols and terpenes indicating their different biological functions probably related to berry protective functions against microrganisms.

CONCLUSIONS

Study of berry waxes are of importance for commercially cultivated species as waxes influence berry appearance and storage quality. By means of gas chromatography-mass spectrometry more than 80 different compounds were identified and quantified in

8 varieties of commercially cultivated blueberry (*Vaccinium corymbosum*), bilberry (*Vaccinium myrtillus* L.) and bog bilberry (*Vaccinium uliginosum* L.). Identified berry wax components belong to 9 groups of compounds, namely, alkanes, triterpenes, alcohols, fatty acids, phenolic acids, ketones, aldehydes, esters and tocopherols. Significant differences in the wax composition was found amongst berries from different species, as well as differences among varieties within the same species. Presented results suggest that analysis of cuticular wax can be used to distinguish between berries within the same species and among different species, implicating use in berry authenticity testing, however, more testing should be done to avoid the interference of varying environmental factors.

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Investigation of extruded cereals enriched with plant by-products and their use in fermented beverage production

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Abstract. The aim of the study was to analyse the quality of extruded cereals enriched with plant by-products and to obtain fermented drinks from production rejects. Extrusion was performed with co-rotating twin-screw extruder (compression ratio 8:1) at MILZU Ltd. from rye and oat flour (80:20, control samples) with addition of apple (ABF), carrot (CBF) and pumpkin (PBF) by-product flour in various amounts (10%, 15% and 20%). Naturally fermented kvass production process was used for non-alcoholic fermented beverage production. Total dietary fibre (TDF), textural properties and sensory features of extruded products after addition of by-products (BP) were determined. Dry matter, active acidity and sensory properties were analysed in fermented beverages. The obtained results showed a 12-55% increase in TDF of extruded cereals (11.8 g 100 g^{-1}) after addition of plant by-products. All extruded samples with BP showed lower hardness levels than control (35.55 ± 2.95 N); samples with PBF were the least hard ($P < 0.05$). Samples with the lowest bulk density were obtained by the addition of 10% and 15% PBF, and 15% CBF, whereas addition of apple by-product flour in all tested concentrations gave the samples a higher bulk density compared to control. Highest taste and aftertaste scores using 5-point hedonic scale were given to samples with addition of 15% and 20% ABF, which also showed high consumer acceptance. With regards to fermented drinks, the highest dry matter content was found in PBF and ABF drink, 8.1 ± 0.1 and 7.0 ± 0.1, respectively. Sensory evaluation of fermented beverages showed that the intensity of flavour, acidity and aroma was most pronounced in sample with ABF, whereas colour was most pronounced in sample with PBF. In order to reduce production costs, it is possible to use production rejects of extruded cereals enriched with plant by-products to obtain new products.

Key words: by–products, extrusion, dietary fibre, fermented beverage, sensory properties.

INTRODUCTION

Fruits and vegetables have the highest wastage rates of any food (Vaqué, 2015). Apple, carrot and pumpkin processing industry produces such by-products as pomace, peel, and seeds. They are used, possibly as animal feed, for biogas production, or not used at all and utilized. About 100 million tons of food are destroyed in the European Union annually (Vaqué, 2015). Disposal of food is not only an ethical or economic issue, but it also has important consequences for the exhaustion of natural resources. By-products of vegetable and fruit production still contain such biologically valuable

substances as dietary fibre, vitamins, carotenes, antioxidants, organic acids that play an important role in human health (Gupta et al., 2015; Sharma et al., 2016; Lucera et al., 2018). Nowadays, the interest in products that are not only healthy, sensory acceptable and price-responsive to a wide range of consumers, but also contain nutrients that can provide the consumer the required daily intake is increasing.

Several researchers have used such fruit and vegetable by-products as apples, pears, oranges, peaches, black currants, cherries, artichokes, asparagus, carrot, legumes and tomatoes (Černiauskiene et al., 2014; Zargar et al., 2014) as dietary supplements in functional foods to contribute to optimal health (Arscott & Tanumihardjo, 2010; Karthika et al., 2016; Sharma et al., 2016).

By-products of apple (*Malus domestico*), carrot (*Daucus carota*) and pumpkin (*Cucurbita pepo*) processing are peel, pomace and seeds that contain a significant amount of fibre, vitamins, enzymes, sugars (Wolfe et al., 2003) which can be used for functional foods and beverages (Henríquez et al., 2013). One of such products is extruded cereals.

Extruded products are relatively new in the grocery segment, but can compete with other snack products. High-temperature, short-time extrusion technology (HTST) has become popular in preparing snacks and breakfast cereals using starchy base products (Nikmaram et al., 2017). Extrusion could be a viable and new method of adding fruit and vegetable by-products to convert them into innovative and functional foods in order to offer a healthy range of products for consumers.

However, similar to by-products which are considered a food waste, such production rejects as deformed product pieces are made during extrusion cooking, which do not have a demand in market. According to production data of Milzu Ltd., production rejects composed 1,500 kg monthly in 2018, which accounts to 10% of total production. At the moment, production rejects are donated as feed for forest animals.

In order to promote non-waste technology and reduce production costs, several possibilities to utilise production rejects could be considered, e.g., alternative to breadcrumbs for oven baked or fried products, compressed crispbreads, crackers or cookies, or fermented beverage production in order to transform this type of food waste into value products. Naturally fermented beverages are considered healthier alternatives to water compared to soft drinks (Wilburn & Ryan, 2016), therefore, given that consumers often prefer soft beverages to water, testing of alternative raw materials for fermentation is necessary.

The aim of the study was to analyse the quality of extruded cereals enriched with plant by-products and to obtain a fermented beverage from production rejects.

MATERIALS AND METHODS

The study was carried out at the scientific laboratories of the Faculty of Food Technology, Latvia University of Life Sciences and Technologies and production facility of Milzu Ltd.

Production of extruded cereals enriched with plant by-products
Control samples were prepared from rye and oat flour (80:20) and 17% water. Enriched experimental samples were prepared by replacing 10%, 15% and 20% of the

dry ingredients with apple (ABF), carrot (CBF) and pumpkin (PBF) by-product flour (Table 1).

Table 1. Composition of experimental samples.

Sample	Composition
Control crispbread	80% - rye, 20% - oats
A10	90% - control crispbread, 10% - apple by-product flour (ABF)
A15	85% - control crispbread, 15% - apple by-product flour (ABF)
A20	80% - control crispbread, 20% - apple by-product flour (ABF)
C10	90% - control crispbread, 10% - carrot by-product flour (CBF)
C15	85% - control crispbread, 15% - carrot by-product flour (CBF)
C20	80% - control crispbread, 20% - carrot by-product flour (CBF)
P10	90% - control crispbread, 10% - pumpkin by-product flour (PBF)
P15	85% - control crispbread, 15% - pumpkin by-product flour (PBF)
P20	80% - control crispbread, 20% - pumpkin by-product flour (PBF)

Extruded cereals (crispbreads) were prepared using a co-rotating twin-screw extruder SLG65 – III (Datong Machinery, China) (compression ratio 8:1) at Milzu Ltd. The main drive of extruder was provided with a 7.5 HP motor (400 V, 3 HP, 50 cycles). Temperatures for extrusion zones were 125 °C /135 °C /145 °C according to developed process at Milzu Ltd.

Quality analysis of extruded cereals enriched with plant by-products
The content of total dietary fibre was assessed according to AOAC 985.29 using FOSS Fibertec™ 1023 Dietary Fibre analyser and Megazyme enzymes.

Textural properties and hardness of extruded cereal samples were determined instrumentally using TA.XTplus Texture Analyser (Stable Micro Systems, UK), data processing was completed with Texture Exponent 32. Samples were compressed with a 35 mm compression plate with test speed 2 mm s^{-1}, compressive force was measured.

Bulk density of the extrudates was calculated according to the equation (Varsha & Mohan, 2016):

$$Bulk\ density = \frac{Weight\ of\ the\ smaple, g}{Volume\ of\ the\ sample, mL}$$

5-point hedonic scale (ISO 4121:2003) and ranking test (ISO 8587:2006) were applied to determine sensory features of products. Overall liking (hedonic scale) was used to define acceptance by trained panellists. Extruded cereals enriched with plant by-products were evaluated by 35 trained panellists (20% men and 80% women), average age 32 years. Samples with different by-product flour addition were assessed on separate days. Such sensory parameters as taste, aftertaste, colour intensity, volume and crispness were determined. The product was considered acceptable if the it scored 3 or more points on the scale by 50% of evaluators. Ranking test (1 – most appealing, 4 – least appealing) was used to define consumer acceptance during the international food exhibition RigaFood in 2017; a total of 200 consumers participated (40% men and 60% women, average age 28 years).

Production of non-alcoholic fermented beverages from extruded cereals enriched with plant by-products

Naturally fermented kvass production process (Lidums et al., 2014) was modified to produce fermented beverages from extrudates. The following materials were used: extruded cereal production rejects with 15% apple, carrot and pumpkin addition, baker's yeast *Saccharomyces cerevisiae* (JSC Rigas Raugs), lactic acid bacteria *Leuconostoc mesentericus* (Chr. Hansen Ltd), beet sugar (Dansukker Ltd) and dark malt extract (Coopers Ltd).

One litre beverage mash was produced by soaking 200 g of extruded cereal rejects and 2 g dark malt in 2 litres of hot water (78 ± 2 °C) for 3 hours. Then the suspension was filtered (300 µm) and liquid fraction was cooled. 1 g baker's yeast, 2 units of lactic acid starter and 10 g of sugar were added to 1 litre extruded cereal mash, fermentation time – 9 hours at 27 ± 1 °C. Then fermented beverages were cooled to 3 ± 1 °C, filtered (5 µm) to remove yeasts and the remaining sugar (20 g) was added. Fermented beverages were matured in 0.5 litre PET bottles for 12 hours at 6 ± 1 °C.

Quality analysis of fermented beverages from extruded cereals enriched with plant by-products

Active acidity (pH) was assessed according to AACC 02-31 method, dry matter (ISO 6496) was determined using hand-held refractometer HR32B (Schmidt + Haensch, Germany).

Sensory evaluation of fermented drinks was carried out by 25 trained panellists (40% men and 60% women, average age 37 years). Four samples of fermented drinks were served to each panellist in a randomised sequence. Line scale (ISO 4121:2003) was used to evaluate such parameters as the intensity of aroma, acidity, colour and flavour of fermented drinks.

Data processing

Microsoft Excel v16.0 for Windows was used to process the obtained data; mean ± standard deviation was calculated. Cross-comparison of data was performed using ANOVA, Tukey's test and correlation analysis. Friedmann test is used to evaluate the results of ranking test. For the interpretation of the results it is assumed that $\alpha=0.05$ with 95% confidence.

RESULTS AND DISCUSSION

Dietary fibre in extruded cereals enriched with plant by-products

The addition of plant by-products influenced the amount of TDF in samples (Fig. 1). TDF content in control sample was 11.8 g 100 g^{-1}, partial replacement of rye-oat base with by-product flour (BPF) increased total dietary fibre content in all samples. In samples with the highest proportion of BPF (20%) total dietary fibre content increase was 18.29 g 100 g^{-1} for apple, 14.82 g 100 g^{-1} for carrot and 15.49 g 100 g^{-1} for pumpkin by-product flour. A total of 12–55% increase in TDF of extruded cereals with BPF was observed, depending on the replacement of BPF in base recipe. The most noticeable increase in TDF was obtained when using apple by-product flour, especially at 20%. A similar trend has been observed by several researchers (Karthika et al., 2016; Sharma et al., 2016, Lucera et al., 2018).

Incorporation of fibre-rich ingredients influences physical properties of the extrudates such as oil and water binding ability, gel formation, texture and crispness (Elleuch et al., 2011). The quality of snacks depends upon the solubility of fibres (Henríquez et al., 2010). Soluble fibre enhances bubble formation and the crispness of extrudate (Varsha & Mohan, 2016).
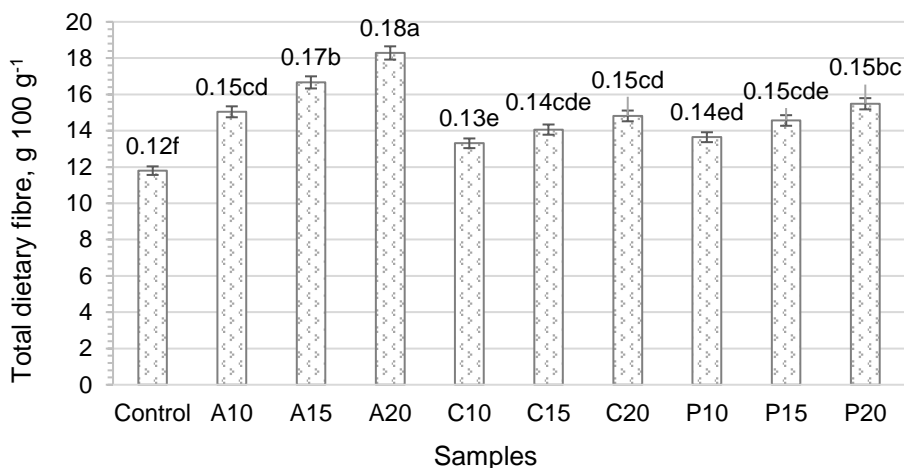
Figure 1. Content of dietary fibre in extruded samples. Values sharing the same letters are not significantly different ($P > 0.05$). Sample abbreviations are summarised in Table 1.

Bulk density and hardness of extruded cereals enriched with plant by-products

Textural properties of extruded products are perceived by the final consumer as prime criteria of acceptance.

Hardness (Table 2) of extruded products is the surface property, which is essential to obtain the shape, and it is expressed as the maximum force applied by teeth to compress the food (Varsha & Mohan, 2016).

Extruded product having minimum hardness is highly acceptable among the consumers, as it is easier to chew. The minimum force to break the product was found for samples with 10% carrot (16.28 ± 2.80 N) and pumpkin (16.70 ± 1.20 N) addition, and 15% apple (22.42 ± 4.28 N) addition. The hardness of the extruded products is highly dependent on their moisture content and density.

Table 2. Bulk density and hardness of extrudates

Sample	Bulk density, g mL^{-1}	Hardness, N
Control	10.85 ± 0.85 cd[*]	35.55 ± 2.95 a
A10	11.82 ± 0.18 bc	33.59 ± 2.78 a
A15	14.62 ± 0.35 b	22.42 ± 4.28 b
A20	17.41 ± 0.44 a	18.80 ± 0.42 cd
B10	9.71 ± 0.07 de	16.28 ± 2.80 d
B15	8.80 ± 0.19 e	17.69 ± 3.84 cd
B20	13.70 ± 0.67 b	19.30 ± 3.86 c
P10	8.56 ± 0.47 e	16.70 ± 1.20 cd
P15	8.13 ± 0.04 e	19.29 ± 0.22 c
P20	12.64 ± 0.65 bc	18.95 ± 2.35 c

[*] Values within the same column sharing the same letters are not significantly different ($P > 0.05$).

Bulk density (Table 2) of extruded products is an important factor in quality acceptance, storage conditions, as well as desired form. Minimum bulk density is desired for an acceptable product. Bulk density is the parameter that shows how much space is between cells of the product and the volume of it; lower bulk density gives a better porosity and extrudate aeration of the structure, which is desirable mainly for cereal snacks (Liu & Yao, 2007). A significantly lower bulk density was found for extruded cereals with 15% pumpkin (8.13 ± 0.04 g mL^{-1}) or carrot (8.80 ± 0.19 g mL^{-1}) and 10% pumpkin (8.56 ± 0.47 g mL^{-1}) addition, whereas addition of apple by-product flour in all tested concentrations gave the samples a higher bulk density compared to control.

Correlation analysis of several parameters (Table 3) reviled that in all samples with added BPF correlation between TDF and bulk density was very strong, i.e., 0.78 ($P < 0.05$), whereas correlation between TDF and hardness showed the negative correlation of -0.28, therefore it can be concluded that addition of dietary fibre-rich ingredients aids in decreasing the hardness of extrudates. Correlation between bulk density and hardness of samples was very week (0.08, $P < 0.05$).

Table 3. Correlation (Pearson's) coefficients between the TDF, bulk density and hardness of samples

Correlation between groups	
TDF and bulk density	0.78 ($P < 0.05$)
TDF and hardness	-0.28 ($P < 0.05$)
bulk density and hardness	0.08 ($P < 0.05$)

Sensory evaluation of extruded cereals enriched with plant by-products

Sensory parameters of foods are important when developing new products or improving the recipes as negative sensory experiences can reduce consumer adherence to purchasing specific food products (Olsen et al., 2012). As BPF contain sugar, they can alter the taste of products (Konrade et al., 2017). Within each group of base recipe replacement with the same fruit/vegetable BPF, the highest taste scores (Table 4) were given to extruded cereal samples with addition of 20% apple by-product flour – 4.2 (A20), 15% carrot BPF – 3.7 (C15) and 15% pumpkin BPF – 3.8 (P15); the taste was significantly more pleasant than that of control sample ($P < 0.05$). Out of samples with 20% addition of BPF, only sample with apple showed potential in terms of taste.

With regards to aftertaste, control sample (3.9) and samples A15 (3.9) and C10 (3.7) scored higher numbers. The lowest preference of taste and aftertaste was found in extruded cereals with 20% addition of carrot and pumpkin BPF.

The addition of BPF caused significant colour changes for all samples. Panellists liked the colour of samples with 20% BPF the most, namely A20 (3.8), C20 (4.5), P20 (3.0), the colour was significantly better than that of control sample ($P < 0.05$). Colour intensity has been previously associated with biologically active carotenoids the composition of which is different in BPF (Delgado-Nieblas et al., 2015). A previous study showed that it is possible to increase the content of carotenes in extruded cereals when adding plant by-product flour from 0.77 ± 0.01 mg 100 g^{-1} (control sample) up to 6.51 ± 0.02 mg 100 g^{-1} in samples with 20% pumpkin BPF (Konrade et al., 2018).

The acceptability of crispiness decreased for all samples with BPF compared to control, which was also observed instrumentally (Table 2). Higher crispiness level was detected in samples with 15% and 20% apple BPF, 4.3 and 4.3, respectively. These sensory data coincide with instrumentally obtained data.

Table 4 ensory properties of extruded cereal samples enriched with by-product flour. Sample abbreviations are summarised in Table 1.

Sample	Colour intensity	Volume	Crispness	Taste	Aftertaste
Control	2.1 d[*]	3.6 ab	4.1 ab	2.9 cd	3.9 a
A10	3.1 bc	3.8 a	3.7 b	2.8 d	3.5 b
A15	3.6 ab	3.0 bc	4.3 a	3.7 b	3.9 a
A20	3.8 a	2.9 c	4.3 a	4.2 a	3.4 bc
C10	2.2 d	3.6 ab	3.9 ab	2.8 d	3.7 ab
C15	2.9 c	2.9 c	3.6 b	3.7 b	3.1 c
C20	4.5 a	1.9 d	2.6 cd	2.2 e	2.5 d
P10	2.8 c	3.8 a	3.4 bc	3.2 c	3.4 b
P15	2.9 c	3.0 bc	3.2 bc	3.8 ab	3.2 c
P20	3.0 c	2.0 d	3.0 c	2.2 e	1.6 e

[*] Values within the same column sharing the same letters are not significantly different ($P > 0.05$).

The results of ranking test (1 – most appealing, 4 – least appealing) of products showed the consumers' acceptance for all examples with 15% BPF addition ($P < 0.05$). Consumers ranged sample A15 as the most appealing and samples C20 and P20 as the least appealing.

Based on sensory evaluation results, samples with 15% BPF showed the highest consumer acceptance, therefore, the next step of the research, namely development of fermented beverages, was carried out using production rejects with 15% apple, pumpkin and carrot by-product flour addition.

Quality evaluation of non-alcoholic fermented beverages

Fermentation, being a quite inexpensive technology, plays an important role in food processing of many countries (Misihairabgwi & Cheikhyoussef, 2017). The fermented non-alcoholic beverage best known to Eastern European countries is kvass which has been traditionally made from bread; nowadays, however, such modifications as fruits and vegetables as the main ingredient exist (Lidums et al., 2016). Therefore, the possibility of using product rejects of extruded cereals for kvass-like fermented beverage production was considered.

The obtained pH levels of apple, carrot and pumpkin enriched extruded cereal beverages were within the range of 4.24 to 4.35; ABF beverage had the highest acidity. Dry matter content in ABF, PBF and CBF beverages was 7.0 ± 0.1, 8.1 ± 0.1 and 5.3 ± 0.1, respectively. pH level in experimental fermented beverages was higher compared to traditional kvass (pH 3.88 ± 0.02), whereas dry matter content was similar (7.0 to 8.6 for kvass) (Lidums et al., 2016). Rye bread, which is traditionally used for naturally fermented kvass production in the form of bread rusks, contains higher amounts of sugar, thus explaining the lower pH levels in traditional kvass as more sugar is available for fermentation and acid production by yeasts and lactic acid bacteria (Salovaara & Gänzle, 2011). Other researchers have also shown similar results on pH and dry matter levels of fermented beverages from plant-based ingredients (pearl millet, chickpea, soy) (Misihairabgwi & Cheikhyoussef, 2017; Wang et al., 2018).

The evaluation of the intensity of sensory properties of fermented beverages from extruded cereals enriched with plant by-product flour showed differences within all tested sensory properties (Fig. 3). The most intense colour was found in sample made from cereals with pumpkin by-product flour addition ($P < 0.05$). The intensity of flavour,

acidity and aroma was significantly more pronounced in sample with ABF ($P < 0.05$), compared to other samples. The lowest intensity for all sensory properties was found in sample with CBF.
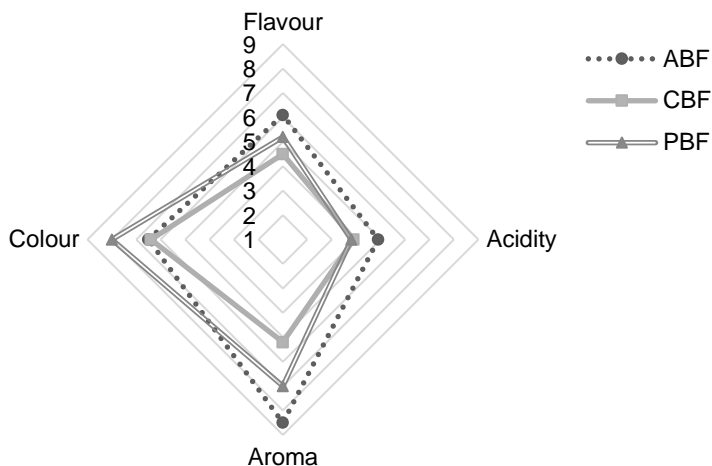
Figure 3. Intensity of sensory properties of fermented beverages from extruded cereals enriched with plant by-product flour. Sample abbreviations are summarised in Table 1.

All sensory properties of the experimental fermented beverage samples were lower than in traditional kvass, especially acidity. Kvass has a slightly more pronounced colour (9.0), flavour (8.9) and aroma (10.4) (Lidums et al., 2016). The mouthfeel and flavour of the experimental fermented beverage samples was more similar to kombucha which is not a traditional beverage in Latvia. A recent research on chickpea as an alternative to soy in fermented plant-based beverages associated lower scores with the cultural background of panellists (Wang et al., 2018); thus, suggesting that consumer habits have a significant effect on acceptance of new products.

CONCLUSIONS

The addition of fruit and vegetable by-product flour can be used to create added value to rye-oat extruded cereals. Increasing the amount of fruit and vegetable by-product flour in the extrusion blend increases total dietary fibre content and bulk density, while decreasing hardness of the extruded cereals enriched with plant by-product flour. Sensory evaluation of extruded cereals enriched with plant by-product flour showed that addition of BPF decreased crispiness and increased colour intensity of products compared to control. The most acceptable products by consumers were extruded cereals enriched with 15% apple, carrot and pumpkin by-product flour, which would form the majority of production rejects.

The results of preliminary research on the production of fermented beverages from production rejects of extruded cereals enriched with plant by-product flour on a pilot scale established that visually defective products could be used to transform this type of food waste into value products, promoting non-waste technology and reducing

production costs. However, research on a production scale is necessary, in addition to recipe modification according to sensory properties more accepted by consumers.

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The effect of humic acids on the natural resistance of the body of broiler chickens and the quality of their meat

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Abstract. The aim of the work is to study the effectiveness of the concentration of humic acids of *Reasil Humic Vet* feed additive on the increase of the feed bioavailability for poultry and the probability of its negative impact on the safety and marketable characteristics of the final product. Studies were conducted on the basis of the Saratov State Agrarian University in two similar groups of broiler chickens 'Cobb 500', 100 heads each. Poultry feeding consisted of the same complete feed, but the drinking water for the broilers of the experimental group was enriched by humates in the amount of 0.5 ml L^{-1}. Based on the data obtained by daily weighing of the poultry and considering the feed intake, a positive trend of the influence of the feed additive on the average daily weight gain and feed conversion per unit of production was noted. The results of slaughter and anatomical cutting of broiler carcasses revealed that metabolic processes were more active in the body of an experimental poultry, reflected in the intensive growth of muscle tissue and fat deposition, which contributed to an increase in the yield of edible parts from carcasses by 9.9%. Studies of composition of broiler blood indicate non-toxicity of the recommended concentration of humates in the feed additive, its stimulation of non-specific resistance of the organism, contributing to the functioning of the immune system and the development of internal organs and, as a consequence, ensuring the safety of the products obtained from them.

Key words: body resistance, humic acid, *Reasil Humic* additive, live weight, muscle and adipose tissue, safety.

INTRODUCTION

The poultry industry occupies a significant place in the maintenance of the meat balance of Russia. This industry is capable to significantly increase its resources in the shortest time provided a reliable forage basis and, in particular, the biological availability of nutrients in the diet. However, the natural potential of meat productivity of agricultural poultry and the improvement of the composition and environmental friendliness of its meat is hampered by the use of mixed feeds. The problem is that these feeds are based on local crops, which have a large microbial contamination. They are supersaturated with chemical toxins that have a mutagenic and carcinogenic effect, and thus, a negative

impact on adaptive and immune properties of the poultry organism (Gamko et al., 2015). At the same time, changes in the proteins and protein fractions, the dynamics of blood hematological parameters, the fluctuations in the activity of aminotransferases may indicate a lack of effectiveness of the body's protective properties on external stimuli, and may be accompanied by metabolic disturbances, lagging in poultry growth, development of internal organs, and the emergence of various diseases. To solve this problem, scientists and specialists of the agro-industrial complex are engaged in the development of feed additives and supplements based on natural components of various biochemical composition (Hayirli et al., 2005; Dolgopolov, 2006; Ghahri et al., 2009; Ghahri et al., 2010; Arif et al., 2016; Arafat et al., 2017; Vasiliev et al., 2018). World experience has shown that humic acids or humates are of practical interest for agriculture due to their composition and properties and the wide distribution of such raw materials as leonardite, brown coal, peat and saprofel (Schnitzer & Khan, 1972; Orlov, 1974; Orlov et al., 1996).

Scientific studies of humic acids have shown that in addition to their common properties there are a number of differences resulting from the nature of the original substance and the method of isolation. A common feature for humates is the presence of an aromatic substance, hydroxyl of alcohol or phenolic character, carbonyl, carboxyl and methoxyl groups. It is established that humic acids perform transportational, regulatory and protective functions in the body; they are harmless to animals, birds and humans, having no allergic, anaphylactogenic, teratogenic, embryotoxic and carcinogenic effects when used in recommended doses (Bollag & Mayers, 1992; Ziechmann, 1996; Anisimov & Likhatskaya, 2001; Potapova et al., 2012).

Humates, being biologically active compounds, can be sources of new various biologically active substances depending on the method of their processing in each specific case (Platonov et al., 2010; Nebbioso & Piccolo, 2011). In this regard, there is a great need for the development and implementation of additives based on humic acids of various origins in the production and processing of poultry meat.

The aim of our research was to study the effect of the humate-based product with the commercial name *Reasil Humic Vet* in combination with the main ration of feeding broiler chickens on the natural resistance of the organism and, as a consequence, the quality of meat.

MATERIALS AND METHODS

Preparation of the drug and bioavailability

The product under study is a dark-colored liquid obtained by alkaline extraction of natural leonardite and containing humates. The structural part of humate molecule consists of polysaccharides, peptides, amino acids, vitamins, minerals, sterols, hormones, fatty acids, polyphenol and ketone with subgroups, including flavonoids, flavones, flavins, catechins, tannins, quinones, isoflavones and tocopherols. At the same time, *Reasil Humic Vet* has a high bioavailability and according to the results of testing at the Testing Center of the FGBU 'Leningradskaya MVL', the maximum sorption capacity of the additive in relation to T-2 toxin, aflotoxin B1, zearalenone, and orahtraxin of feed is 84%; 100%; 100% and 97.7% respectively.

Experimental design and feeding program

An experiment on poultry 'Cobb 500' lasted for 42 days. It was conducted at the department of veterinary medicine and biotechnology of Saratov State Agrarian University, Russia. The research implied the analog principle, where two groups of 100 animals each were formed out of one hatch of broiler chickens: I – control, II – experimental. The chickens were kept in the same microclimatic conditions (Table 1).

Table 1. Experiment scheme

Group	Flock, heads	Duration, days	Feeding ration
I-C	100	42	BR (Basic ration)
II-E	100	42	BR + 0.5 mL *Reasil Humic Vet* per 1 liter water

I-C – control group; II-E – experimental group.

As a basic ration, experimental broiler chickens received mixed fodder (Table 2) prepared on the basis of individual entrepreneur farm 'Korostin' in the Markovsky district of the Saratov region.

Table 2. Composition and nutritional value of mixed feed for experimental poultry

Ingridients	Universal feed mixture from 7th to 42nd day of fattening, %
Wheat	40.61
Corn	15.00
Full-fat soya bean (FFS)(31.5% crude fat, 17% crude protein)	24.62
Soybean meal (45% crude protein)	1.00
Sunflower cake (34% crude protein, 18% crude fiber)	3.00
Fish flour (64% crude protein)	1.49
Monocalcium phosphate	0.53
Lime dust	0.75
Premix 2% Chicken premix 5 agrostimulus	2.00
100 g containing (nutrient composition, %):	
Metabolic energy, MJ	1.30
Crude protein	22.00
Crude fat	6.14
Linoleic acid	3.10
Crude fiber	4.50
Lysin	1.43
Methionine	0.62
Methionine+cystine	0.92
Threonine	0.87
Tryptophane	0.25
Ca	1.00
P	0.84
P digestible	0.50
Na	0.19
Cl	0.17
NaCl	0.10

The differences were that the drinking water for livestock of broiler chickens of the II E group was supplied with the feed additive based on humates at a dose of 0.5 mL L^{-1}, recommended by the manufacturer based on the literature data analysis. To achieve our goal, we studied a set of indicators of experimental poultry.

Measurement of growth performance

Changes in the live weight of broiler chickens were measured by their individual weekly weighing on electronic scales, each group starting from the 7th day of life. Based on the results of weighing, we calculated the absolute and average daily growth of broilers. Conversion of feed (kg kg^{-1} of body weight) was determined by the ratio of the amount of feed spent during the entire period of experiment to the number of products obtained.

Sample collection and measurements

To assess the physiological state of the broiler body when consuming humic acids, a clinical blood analysis was performed using the instrumental method on hematology analyzers *PSE 90 Vet*, *Biochem SA* (US-made). The safety of poultry was determined by daily accounting of dead birds.

Upon completion of fattening, a control slaughter (3 heads from each group with a live weight close to the average value) and the total slaughter of broilers in groups were conducted to study both the indicators of their meat productivity and anatomical and morphological composition of carcasses. When cutting carcasses, the following indicators were taken into account: a pre-slaughter mass, a mass of non-gutted (without blood, feathers, down) carcass, gutted carcasses (without internal organs, head, neck and legs), mass of internal organs. The slaughter yield was calculated as the ratio of the slaughter mass to the pre-slaughter live weight, expressed as a percentage. Subsequently, the skin and subcutaneous fat were removed from the carcass, the muscles were separated from the bones.

In the meat samples, the following parameters were determined: moisture – by drying the substance to constant weight; protein (total) – by Kendall's method; fat – using the Soxhlet extractor, ashes – by burning the sample in a muffle furnace to constant weight; mineral substances – by atomic absorption spectrometry.

To determine the taste qualities of broiler meat, a tasting was conducted on the basis of the research and production laboratory of the Food Technology Department at the Saratov State Agrarian University; the taste was measured within 5-scale system. Firstly, poultry carcasses were evaluated in its raw form (uncut), and then on the cut product for such indicators as appearance, color, smell, texture. After the meat was subjected to heat treatment (cooking, frying) the smell (aroma), texture (hardness, tenderness), taste, weight loss, the yield of the finished product were estimated.

Statistical analysis

The obtained data was processed on a personal computer using software *Stat Plus* and *Microsoft Excel* (Glantz, 1998; Dolgopolov, 2006).

RESULTS AND DISCUSSION

Growth Performance

One of the main indicators determining the effectiveness of growing broiler chickens is their live weight. It was established that with almost the same starting weight of experimental chickens at 7 days of age (Table 3), broilers of group II, consuming the additives with humic acids, already surpassed their peers from group I by this indicator by 15.56 g (4.36%) at 14 days of age; at the end of the fattening period (42 days) they reached a live weight of 2,826 g, which was 228 g or 11.36% more than the control group broilers' weight. The advantage of poultry in the group with humates was also manifested in the intensity of the growth energy. Thus, the absolute and average daily gain in live weight of broilers of the experimental group for the reporting period was equal to 2,734.33 g and 71.95 g against 2,444.67 g and 64.33 g in the control group respectively.

Table 3. Live broiler weight dynamics, g ($n = 12$)

Age of poultry, days	I-C	II-E
7	93.33 ± 0.56	$91.67 \pm 0.32^{*}$
14	357.17 ± 1.11	$372.73 \pm 1.17^{**}$
21	685.75 ± 1.82	$781.82 \pm 2.22^{**}$
28	$1,237.42 \pm 1.44$	$1,336.64 \pm 1.61^{**}$
35	$1,857.75 \pm 1.23$	$2,009.09 \pm 2.02^{**}$
42	$2,538.00 \pm 1.44$	$2,826.00 \pm 2.05^{**}$

$* - p \leq 0.05$; $** - p \leq 0.001$, experimental group (II-E) compared to the control group (I-C).

Feed intake

The best feed conversion in the compared groups was observed in broiler chickens of group II and amounted to 1.93 kg kg^{-1} increase in live weight, which is 0.3 kg or 13.5% less than that of the control poultry. This was due to the fact that humic acids in the composition of the studied feed additive help stimulate the development of intestinal villi, increase the amount of enzymes secreted, enhance absorption processes and improve feed digestibility by suppressing the growth of pathogenic bacteria.

Blood parameters

Blood in the body of a bird is the most important biological fluid that provides almost all metabolic, protective and adaptive functions. When there are changes in the external environment, compensatory mechanisms, that restore disturbed properties, are activated. This sets a new level of homeostatic indicators. Therefore, a more accurate picture of the effect of the studied additive on the biochemical processes in the body of broiler chickens can be obtained by analyzing their blood. It was established that the use of humates in the composition of the studied feed additive did not have a significant effect on the number of red blood cells in the blood of broiler chickens (Table 4). The level of red blood cells in the control poultry at the age of 42 days was 2.54 million μl^{-1} (normal 3–4 million μl^{-1}). The broilers of the experimental group showed a slight tendency to increase to 2.57 million mL^{-1}. A similar trend, which does not go beyond the criteria of the physiological norm, is traced in relation to the amount of hemoglobin as the main component of red blood cells, the main function of which is to transfer oxygen from the lungs to the tissues, and carbon dioxide, the main function of which is to transfer oxygen from the tissues to the lungs and participate in maintaining acid-base balance in the body, thus possessing buffer properties. In our studies, the control group hemoglobin

level was 115.67 g L^{-1}, *Reasil Humic Vet* group hemoglobin level was 117 g L^{-1} or 1% more, with the norm of 115–128 g L^{-1}.

Table 4. Morphological and biochemical blood parameters of broiler chickens

Indicator	Measuring Unit	Group	
		I-C	II-E
Morphological parameters			
Erythrocytes	mln pcs mL^{-1}	2.54 ± 0.13	2.57 ± 0.04
Leucocytes	thousand µL^{-1}	22.38 ± 0.74	20.41 ± 0.66
Hemoglobin	g L^{-1}	115.67 ± 2.60	117.00 ± 7.23
Thrombocytes	thousand pcs	18.00 ± 2.08	20.67 ± 0.33
Hematocrit	%	33.46 ± 1.04	32.70 ± 0.73
Biochemical parameters			
Total bilirubin	µmol L^{-1}	11.43 ± 1.15	11.20 ± 1.09
Aspartate – transaminase *(AST)*	u L^{-1}	87.83 ± 6.61	55.17 ± 9.85*
Glutamic-pyruvic transaminase *(GPT)*	u L^{-1}	62.43 ± 10.71	49.73 ± 7.34
Total protein	g L^{-1}	82.57 ± 4.86	61.90 ± 3.44
Total creatinine	µmol L^{-1}	152.00 ± 5.07	112.67 ± 5.74*
Blood urea	mmol L^{-1}	5.50 ± 0.29	7.03 ± 0.23*
Glucose	mmol L^{-1}	6.00 ± 1.62	7.47 ± 0.58
Cholesterol	mmol L^{-1}	5.10 ± 0.29	4.17 ± 0.43
Ca	mmol L^{-1}	4.20 ± 0.21	4.80 ± 0.21
P	mmol L^{-1}	1.73 ± 0.12	2.33 ± 0.13
Mg	mmol L^{-1}	1.13 ± 0.09	1.40 ± 0.06
Na	mmol L^{-1}	126.77 ± 2.66	124.33 ± 0.62
K	mmol L^{-1}	22.36 ± 0.99	23.50 ± 0.60
Fe	µmol L^{-1}	24.73 ± 0.93	24.57 ± 0.52

* – $p \leq 0.01$; experimental group (II-E) compared to the control group (I-C).

In the poultry blood, leukocytes serve as an important link in the mechanism of immunological protection, interacting with lymphoid cells in certain phases of immunological reactions. Due to their phagocytic activity and participation in cellular and humoral immunity, the exchange of histamine, heparin, antimicrobial, antitoxic, antibody-forming and other important components of immunological reactions are performed.

The number of leukocytes in the blood of experimental broilers was within the physiological norm (20–24 thousand µl^{-1}) and amounted to 22.38 thousand µl^{-1} in group I-K against 20.41 thousand µl^{-1} in group II-E. Due to the fact that leukocytes play a major role in the specific protective reactions of the body, these results indicate the harmlessness of the feed additive based on humic acids.

Hematocrit is the ratio between the plasma volume and blood cells, expressed as a percentage by volume. It is essential to calculate hematocrit for an objective assessment of laboratory blood parameters. An increase in hematocrit value is noted in case of anemia, while a decrease points out to the thickening of blood and dehydration of the body. With an average hematocrit rate of 39–40% in poultry, this indicator was evenly lower than the norm in the blood of broilers of all groups by 6–7%.

Blood biochemical parameters are necessary for assessing the physiological status of the body of poultry and for timely diagnosis of pathological conditions, allowing to

evaluate the functional state of the body, the liver, kidneys, pancreas and other organs, as well as the state of protein, carbohydrate, fat and mineral metabolism and correct the diet in time.

According to our research, the content of bilirubin in the blood of broilers was within the physiological norms (8.5–20 µmol L^{-1}) and amounted to 11.43–11.2 µmol L^{-1}.

The total protein content in the blood characterizes the protein metabolism in the body. A decrease in its level in blood serum is observed with a low protein content in the diet, with a disturbance of the digestive system. In our experiment, the level of protein in the blood of control group before slaughter deviated from the average norm (53–59 g L^{-1}) by 39% and amounted to 82.57 g L^{-1} with a significantly low rate of the same indicator in the experimental group, which was much closer to physiological norms.

Along with proteins, blood serum contains various nitrogen-containing non-protein substances, which are called residual nitrogen, the main component of which in poultry is urea.

The amount of uric acid in the blood depends not only on the kidney function, but also on the amount of protein consumed or the rate of its breakdown in the body. In particular, the concentration of uric acid in the blood increases with bleeding, gout, leukemia, or pneumonia. The lack of protein in the diet, on the contrary, leads to a decrease in the concentration of uric acid in the blood serum.

The normal concentration of this indicator in poultry ranges from 2.5 to 8.32 mmol L^{-1}. It was established that the urea content in the blood of all experimental chickens was within the physiological norm. However, in the blood of broilers consuming the feed additive, this figure was 7.03 mmol L^{-1}, which exceeds the same indicator in control group broilers by 1.52 mmol L^{-1}.

Along with the calculation of uric acid concentration in blood, it is recommended to determine – as an additional factor – the concentration of creatinine synthesized in the liver and transported to skeletal muscle. The concentration of creatinine in the blood is fairly constant, reflecting muscle mass and not dependent on feeding and other factors.

Normal serum creatinine concentrations are 44–100 µmol L^{-1}. In our experiment, elevated creatinine levels were noted in all groups. At the same time, in the blood of chickens of the control group, 152 µmol L^{-1} creatinine was found, and in the blood of experimental chickens, the creatinine levels were much closer to the physiological norm (112.67 µmol L^{-1}) and significantly lower than in the control group ($p \leq 0.05$). This indicates that in the poultry consuming humates more amino acids worked for the anabolic processes and a higher supply of potential energy was accumulating in glycogen of the muscle tissue.

Blood glucose is the main indicator of carbohydrate metabolism. It reflects the ratio between the processes of its formation and use in tissues. Depending on the type of feeding, the concentration of glucose in the blood usually varies within the physiological norm. The blood glucose concentration rate is the result of the hormones balance regulating it.

In our studies, the blood glucose level in experimental chickens did not significantly differ from the norms (6–9 µmol L^{-1}) and was 6 mol L^{-1} in the control group with a tendency to increase to 7.47 mmol L^{-1} in the experimental group ($p \leq 0.05$), treated with humates added to drinking water (0.5 mL L^{-1}).

Cholesterol is found in all tissues of the body, being a component of cell membranes. It can be synthesized in small quantities by the intestinal wall and supplied with food. Based on it, bile acids, sex hormones, adrenal hormones are synthesized, the product of cholesterol oxidation in the skin is converted into vitamin D3. According to the research it was found that the level of cholesterol in the blood of broiler chickens in the experimental group was 4.17 mmol L^{-1} (at a rate of 3.6) versus 5.1 mmol L^{-1} in the control group. Such elevated cholesterol in the blood of broiler chickens can be observed when feeding with rations enriched with solid feed fats.

The basis of many pathological and pre-pathological states of the body in poultry is the disruption of the enzyme systems. The pathological process is accompanied by an increase in the permeability of cell membranes or the death of cells. Thus, the enzymes from the cells enter the blood, where the corresponding enzymatic activity increases dramatically, since the content of enzymes in the cell is much higher than in the blood. An increase in the activity of blood enzymes can be the result of accelerating the processes of synthesis, lowering the rate of elimination of enzymes, increasing the permeability of cell membranes, the action of activators and cell necrosis. A decrease in the activity of blood enzymes on the contrary indicates an increase in the rate of elimination of the enzymes, the action of inhibitors and inhibition of synthesis.

Aspartate – transaminase *(AST)* is not specific for the liver, but its level in poultry can indirectly show the function of this organ. Normal values are up to 330 u L^{-1} for most bird species. Elevation usually occurs with muscle damage or liver cell damage. In our experiment, the *AST* data in the control group amounted to 87.83 u L^{-1} with significantly low values in the experimental group of 55.17 u L^{-1}, which indicates a more intensive biosynthetic process.

Of the blood mineral substances, the important role of the constant concentration of calcium and inorganic phosphorus should be noted. The level of serum calcium and phosphorus is regulated by derivatives of vitamin C, calcitonin and parathyroid hormone. The calcium content in the blood depends on the species, age, constitution of the bird, the quality of the water taken in, and the amount of calcium in the diet.

Phosphorus is found in biological compounds and tissues in the form of phosphoric acid. It takes an active part in the metabolism of carbohydrates, proteins, fats and minerals also regulating acid-base balance. A low content of phosphorus in the blood is noted in cases of vitamin D avitaminosis, hyperparathyroidism, impaired intestinal absorption, an incomplete diet and kidney disease. The normal level of phosphorus is 0.64–1.45 mmol L^{-1}. The phosphorus content in the blood of chickens from the control and experimental group exceeded the physiological norm by 19.3% and 60.7%, respectively.

Calcium provides mechanical strength of bones, takes an active part in the metabolism of proteins, fats, carbohydrates and mineral and in the process of blood coagulation, activation of enzymes and hormones. The normal level of calcium in the blood is from 2 to 4.5 mmol L^{-1}. Increased calcium content may occur when the amount of vitamin D3 is exceeded or as a result of normal physiological changes. Reduced calcium levels occur in diets that consist only of grain mixtures, or may be caused by kidney disorders. It was found that the level of calcium in the blood of broiler chickens of all groups was also within the physiological limits from 4.2 mmol L^{-1} in the control group to 4.8 mmol L^{-1} in the experimental group.

The physiological norm of magnesium usually does not exceed more than 1.2 mmol L^{-1}. In our experiment, the control group (1.13 mmol L^{-1}) fits into this norm, and the experimental group shows minor deviations in magnesium. The difference constitues 0.27 mmol L^{-1}.

In the blood of experimental broilers, the sodium level was almost within the normal range (138–146 mmol L^{-1}) and amounted to 126.77–124.33 mmol L^{-1}. However, there was a tendency to a decrease in this indicator by 2.44 mmol L^{-1} in chickens consuming humates.

At the same time, the safety of the flock of the experimental broiler chickens was within 98% and did not depend on the factor studied; the loss of the population was the result of technological trauma or asphyxiation.

Development of the internal organs

The work of the internal organs has a great importance for the growth and development of the skeleton, muscles and other tissues of the body. According to the results of the control slaughter of the experimental poultry population at the age of 42 days, the greatest weight of the heart was observed in chickens of group II – 16.86 g, which is 20.7% more than the control counterparts (Table 5).

A similar pattern is noted for the mass of the liver, spleen, and gallbladder.

An important role in the formation of the gastrointestinal tract is played by the pancreas, the mass of which in the chickens of the control group turned out to be less than the experimental group data by 13.6%. At the same time, the poultry of the experimental group was inferior in weight of the stomach by 4.0% of the counterparts from the control group.

Table 5. Humic acid impact on the development of the internal organs of broilers, g ($n = 3$)

Indicator	I-C	II-E
Heart weight	13.96 ± 0.25	$16.86 \pm 0.17^{**}$
Liver without gall	49.90 ± 0.15	$61.20 \pm 0.21^{**}$
Spleen	2.23 ± 0.08	$3.03 \pm 0.02^{**}$
Pancreas gland	3.96 ± 0.05	$4.50 \pm 0.19^{*}$
Gallbladder	2.60 ± 0.23	4.00 ± 0.03
Gizzard stomach	27.76 ± 0.14	$26.66 \pm 0.25^{*}$

$* - p \leq 0.05$; $** - p \leq 0.001$, experimental group (II-E) compared to the control group (I-C).

Humic acid impact of the slaughter quality of broilers

The results of slaughter showed that the use of humic acids with drinking water did not significantly affect the slaughter yield of broiler chickens (Table 6). This indicator in group II was 74.89%, which is 0.55% more than in control group chickens.

Muscle, connective and adipose tissue play the largest role in the tissue composition of muscles. The ratio between these components characterizes the multifunctional state of muscle, in addition to many morphological and chemical indicators that determine the quality of meat.

The results of the anatomical cutting of poultry carcasses indicate that the inclusion of *Reasil Humic Vet* in a dose of 0.5 mL per 1 liter of water to the basic ration of broilers had a positive effect on the synthesis of muscle and fat tissue. Thus, the carcasses of broilers of group II showed muscle mass equal to 1,251.2 g, fat – 31 g, which is more than the benchmark by 111.3 g and 6.7 g, respectively. In terms of the content of edible parts, the chickens of the experimental group turned out to be the best and surpassed their counterparts from the control group by 9.9%.

Table 6. Slaughter and meat qualities of broilers, g ($n = 3$)

Indicator	Group	
	I-C	II-E
Pre-slaughter mass	$2{,}511.70 \pm 1.09$	$2{,}788.40 \pm 1.15^{**}$
Mass of gutted carcass	$1{,}867.20 \pm 2.45$	$2{,}088.30 \pm 6.64^{**}$
Slaughter yield, %	74.34	74.89
Muscle mass with skin	$1{,}337.00 \pm 2.01$	$1{,}465.00 \pm 2.75^{**}$
Skin mass with subcutaneous fat	197.10 ± 2.47	$213.80 \pm 1.96^{*}$
Muscle mass	$1{,}139.90 \pm 1.34$	$1{,}251.20 \pm 0.77^{**}$
% by weight of the gutted carcass	61.0	65.2
Subcutaneous fat	24.30 ± 0.56	$31.00 \pm 0.95^{*}$
Edible parts (muscle + skin + fat)	$1{,}361.30 \pm 0.89$	$1{,}496.00 \pm 2.13^{**}$

$* - p \leq 0.01$; $** - p \leq 0.001$, experimental group (II-E) compared to the control group (I-C).

Meat guality

The data of the chemical composition of broiler meat presented in Table 7 show that the protein content in chickens of the control and experimental groups was almost at the same level and was respectively 22.03–22.31%. At the same time, the amount of fat in samples of meat of broilers of group II, which received humic acids with drinking water, was less by 1.5% than in the meat of their control counterparts.

The appearance assessment of the broiler carcasses showed that the smell was mild, and in the experimental groups the presence of light acidity was noted. The skin of all carcasses was thick, elastic, and the color ranged from white to yellow. Fat deposition was observed in the neck and cloaca area in the same amount in all groups.

Table 7. Chemical composition of broiler chickens, %

Indicator	I-C	II-E
Water	66.90 ± 0.1	$67.90 \pm 0.1^{*}$
Dry matter	32.85 ± 0.1	$31.44 \pm 0.1^{*}$
Protein	22.03 ± 0.1	22.31 ± 0.1
Fat	9.40 ± 0.1	$7.90 \pm 0.1^{*}$
Ash	1.42 ± 0.1	1.23 ± 0.1

$* - p \leq 0.01$; experimental group (II-E) compared to the control group (I-C).

The results of the organoleptic assessment indicate that the use of the examined feed additive in the drinking water of broiler chickens did not lead to a decrease in the taste of meat. It should be noted that the samples in the control group scored the maximum number – 19 points on organoleptic indicators, and samples of group II were 1.7 points less when boiled and 2 points less when fried.

The maximum yield of the finished product when cooked is set in group II (81.7%, which is 16.6% more than that of the control broilers). A similar trend is observed when frying carcasses. In the control group the yield of the finished product was 66.8%, while in the experimental group this indicator was 2.5% higher.

CONCLUSIONS

Thus, the introduction of the dietary supplement *Reasil Humic Vet* based on humic acids of natural origin at the rate of 0.5 ml L^{-1} of drinking water to the diet of broiler chickens has a positive effect on the body's natural resistance and feed digestibility. At the same time, protein metabolism as well as the utilization of protein substances in biosynthetic processes in the poultry muscle tissue become more intensive, which makes it possible to obtain larger body weight gains and yields of edible carcass parts.

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Phenolic and volatile compound composition influence to specialty coffee cup quality

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Abstract. With increasing specialty coffee consumption, more attention is focused not only on the cup quality (sensory quality) of the coffee beverage but also about the impact of coffee on health. The beneficial effects of coffee on human health are mainly based on a wide range of biologically active components, including phenolic compounds. The aim of the study was to evaluate the influence of phenolic and volatile compound composition to specialty coffee cup quality. Seven specialty coffees from two Latvian roasteries were selected and analysed. Total phenolic and flavanoid content and radical scavenging activity by DPPH and ABTS assay were determined spectrophotometrically. Sensory evaluation (cup quality) was performed by trained panellist team using the SCAA protocols cupping specialty coffee. Volatile compounds were extracted by SPME and analysed by Gas Chromatography-Mass Spectrometry (GC–MS). Coffee final cup quality score ranged in amplitude of 83–90.25 points. HON_2 with dry fruits and melon characteristics has shown the highest final cup quality score. Almost detected volatile compounds in KEN_1 is associated with positive specialty coffee characteristics. In ETH_1 coffee with the final cup quality score 88.25 was detected highest floral, fruity compounds and highest coffee–like roasted notes. The highest total phenolic content and DPPH, $ABTS^{+\cdot}$ value showed Roastery_1 coffee samples (HON_1; KEN_1; COL_1) and the lowest values Roastery_2 coffee samples (HON_2; ETH_1; HON_3; SAL_1).The results indicate that the roastery specific roasting process parameters could influence not only volatile compounds profile and cup quality but also the total and individual phenolic compound content.

Key words: phenolic compounds, coffee aroma, cup quality, volatile compounds.

INTRODUCTION

Product quality is the key factor for consumer choice of product purchasing today. The quality of coffee is based on its flavour and aroma characteristics, and specialty coffee is a coffee which has been standardized from the coffee harvesting until its delivery to the consumer, in compliance with all quality standards, to highlight the best flavour of the coffee beverage (Hendon et al., 2014; Kwak et al., 2017).With increasing coffee consumption, more attention is focused not only for the flavour and texture of the coffee brew but also about the impact of coffee on health. The beneficial effects of coffee on human health are mainly based on a wide range of biologically active compounds. From the medical point of view, the positive effects of coffee on human health are even more prominent (Carman et al., 2014). Moderate and regular coffee consumption has been more positively associated with the wide range of bioactive compounds in it.

Research studies have shown that both caffeinated and non–caffeinated coffee consumption is proven to gain health benefits. This statement set, that the phenolic compounds in coffee have equally beneficial characteristics as caffeine (Ludwig et al., 2012; Martínez–Lopez et al., 2018).

Therefore, it is important to evaluate the best way to balance specialty coffee as the high–quality coffee drink not only with great aroma and flavour but also with positive biologically active compound value for human health. The desired aroma profile for specialty coffees has sweet, fruity and floral notes, which are formed in light–medium roast level. For the phenolic compounds, their concentration in coffee is rapidly decreasing during roasting. With the phenolic compounds, like chlorogenic acid, degradation, there are formed compounds with strong bitterness, which is not tolerated in good cup quality (Fischer et al., 2001; Fuller & Rao, 2017). During the roasting process chlorogenic acid converts to chlorogenic acid lactones and at this point having pleasant bitterness. But further roasting process from light to medium transforms the chlorogenic acid lactones into phenylindanes. Phenylindanes shows harsh bitterness which is not related to coffee–like bitterness (Frank et al., 2007).

To avoid in the roasting process formed phenolic compounds with the harsh bitter taste, it is important to know phenolic and volatile compound profile composition in coffee and their influence to cup quality. The aim of the study was to evaluate the influence of phenolic and volatile compound composition to specialty coffee cup quality.

MATERIALS AND METHODS

Samples

Seven samples of coffee *(Coffea arabica L.)* beans were collected from two different coffee roasteries in Latvia roasted at light–medium roast level (roasted at a maximum temperature of 214 °C; 11 min.). All coffee bean sample packages were sealed and stored in dry, cool place till coffee beverage preparation and analysed in one-month interval from coffee bean roasting. The main characteristics of coffee samples are summarized in Table 1.

Table 1. Main characteristics of the samples

Coffee sample	Roastery(roaster)	Origin	Roasting level
HON_1	Roastery 1 (Besca BSC-01, Turkey)	Honduras	Light–Medium (max. temperature of 193 °C; 11 min.)
KEN_1	Roastery 1 (Besca BSC-01, Turkey)	Kenya	Light–Medium (max. temperature of 193 °C; 11 min.)
COL_1	Roastery 1 (Besca BSC-01, Turkey)	Columbia	Light–Medium (max. temperature of 193 °C; 11 min.)
HON_2	Roastery 2 (Loring Smart Roast Kestrel35, USA)	Honduras	Light–Medium (max. temperature of 214 °C; 11 min.)
ETH_1	Roastery 2 (Loring Smart Roast Kestrel35, USA)	Ethiopia	Light–Medium (max. temperature of 214 °C; 11 min.)
HON_3	Roastery 2 (Loring Smart Roast Kestrel35, USA)	Honduras	Light–Medium (max. temperature of 214 °C; 11 min.)
SAL_1	Roastery 2 (Loring Smart Roast Kestrel35, USA)	El Salvador	Light–Medium (max. temperature of 214 °C; 11 min.)

Brewing method

After coffee bean sample package opening the coffee beans were (16 g per sample) and immediately were grind (coarse particle size 1.00–2.0 mm) (DeLonghi KG79 Coffee grinder/ Italy). The grind coffee was prepared by French Press brewing technique (SCAA Best Practice, 2016): 16 g of coarse grind coffee to 150 mL of 93ºC water (Neptunas/ Lithuania) and the extraction time was 4 min. All brewed coffee samples were prepared by triplicates and immediately was analysed.

Determination of total phenolic content

Total phenolic content (TPC) was determined by spectrophotometric method using Folin–Ciocalteu reagent by Singleton et al. (1999) using a gallic acid as standard. A 2.5 mL of Folin–Ciocalteu reagent (Sigma–Aldrich Chemie, Steinheim, Germany) (diluted in proportion 1:10 with distilled water) was added to 0.5 mL diluted coffee extract (diluted in proportion 1:33 with distilled water). After 5 minutes, 2.0 mL of 7.5% Na_2CO_3 solution was added. After 30 minutes incubation at room temperature the absorbance of samples was measured at 765 nm using a Spectophotometer (Jenway 6300). The total phenolic content was expressed as gallic acid equivalent (GAE)100 g^{-1} using standard curve of gallic acid (y = 0.1069x–0.0107; R^2 = 0.9991).

Determination of total flavanoid content

Total flavonoid content (TF) was determined by spectrophotometric method reported by Zhishen et al. (1999)with some modifications. To 2.0 mL of distilled water and 0.5 mL diluted coffee extract (diluted in proportion 1:33 with distilled water) was added 0.15 mL 5% $NaNO_2$ solution. After 5 minutes, 0.15 mL of 10% $AlCl_3*6H_2O$ solution was added. After 5 minutes, 1.0 mL of 1M NaOH solution was added. Each coffee sample flask was mixed and after 15 min incubation at room temperature the absorbance of samples was measured at 415 nm with a Spectophotometer (Jenway 6300). Total flavonoid content was expressed as catechin equivalent (CE) 100 g^{-1} using standard curve of catechin (y = 2.7592x+0.0244; R^2 = 0.9982).

Determination of ABTS radical scavenging activity

ABTS radical scavenging activity was determined by Re et al. (1999) method with some modifications. To prepare ABTS radical, 2,2–azinobis(3–ethylbenzothiazoline–6–sulfonic acid (Sigma–Aldrich Chemie, Steinheim, Germany) was dissolved in phosphate buffer (PBS) solution and oxidized with potassium persulfate. The solution was kept in the dark at room temperature for 16h before further use. The ABTS$^{•+}$solution was diluted with PBS solution to an absorbance of0.70ABS (± 0.02) at 734 nm. To 0.05 mL of diluted coffee extract (diluted in proportion 1:33 with distilled water) 5 mL of diluted ABTS$^{•+}$solution was added. After 30 minutes incubation in dark, the absorbance of samples was measured at 734 nm using a Spectophotometer (Jenway 6300). Trolox (6–hydroxy–2,5,7,8–tetramethylchromane–2–carboxylic acid) (Sigma–Aldrich Chemie, Steinheim, Germany) was used as standard and the ABTS radical scavenging activity was expressed as µmol Trolox equivalent 100 g^{-1}using standard curve of Trolox (y = –0.9755x + 0.7604; R^2 = 0.9948).

Determination of DPPH radical scavenging activity

DPPH radical scavenging activity was assessed by Brand–Williams et al. (1995) with some modifications. The 2,2–diphenyl–1–picrylhydrazyl (DPPH) was dissolved in ethanol and the DPPH solution was diluted to an absorbance of 1.00 ABS (± 0.02) at 517 nm. To 0.5 mL of diluted coffee extract (diluted in proportion 1:33 with distilled water) 3.5 mL of diluted DPPH$^{•+}$solution was added. After 30 minutes incubation in dark, the absorbance of samples was measured at 517 nm using a Spectophotometer (Jenway 6300). Trolox (6–hydroxy–2,5,7,8–tetramethylchromane–2–carboxylic acid) (Sigma–Aldrich Chemie, Steinheim, Germany) was used as standard and the DPPH radical scavenging activity was expressed as µmol Trolox equivalent 100 g^{-1} using standard curve of Trolox (y = –11,579x + 0.8931; R^2 = 0.9985).

Volatile compounds profile analysis

For volatile compounds extraction by solid phase micro–extraction (SPME) thedivinylbenzene/carboxen/poly (dimethylsiloxane) (DVB/CAR/PDMS) fibre was used. Akiyama et al. (2007) and Mestdagh et al. (2014) have also reported the DVB/CAR/PDMS fibre as suitable and efficient for coffee brew volatile compound detection. Coffee extract (5 mL) was placed in 20 mL glass container. The SPME parameters were chosen according to Gloess et al. (2013) with some modifications: extraction temperature: +50 ± 2 °C; incubation time: 4 minutes; extraction time: 7 minutes. Injection parameters: desorption time: 15 minutes; temperature: +250 °C.

For gas chromatography method 'Perkin Elmer Clarus 500' chromatograph with mass spectrometer and 'Elite–Waw ETR'(60 mx 0.25 mminternal diameter; DF 0.25 column were used. The column initial flow rate of 1 mL min^{-1} was held using helium as carrier gas. The outlet split 1:2 and between 40 and 300 mass–to–charge ratios were scanned. Oven temperature start – 40 °C, hold 7 min, programmed from 40 to 160 °C at 6 °C min^{-1}, and from 160 to 210 °C at 10 °C min^{-1}, hold 5 min; carrier gas (He) – 1 mL min^{-1}; split ratio – 2:1; ionization – EI+ mode; acquisition parameters in full scan mode – scanned m/z 40–400.

The compounds were tentatively identified using mass spectral database 'Nist98' (Gloess et al., 2013; Steen et al., 2017).

Sensory analysis

Sensory analysis was performed by six trained panellists according to the SCAA protocols cupping specialty coffee (SCAA, 2015). Each coffee sample was made with five replicates. The coffee evaluation process is stated in three steps: 1) evaluating the aroma of dry ground samples (15 minutes after the coffee sample was ground); 2) evaluating aroma of coffee brew after 3 minutes from extraction (coffee brew temperature ±93 °C); 3) evaluation of coffee brew flavour after 8–10 minutes from extraction (flavour, aftertaste at ± 71 °C; acidity, body and balance at 71–60 °C). Coffees were measured by ten specialty cup quality attributes: cup cleanness, acidity, body, flavour, aroma, after taste, uniformity, sweetness, balance and overall cup preference. The panellists also gave a description of specific flavour, aroma perceived according to The World Coffee Research Sensory Lexicon (2017) statements. Each attribute was

evaluated in scale from 1 to 10, with the final cup quality score of 100 points. The specialty coffee grade only applies if the total specialty cup quality score is 80 points or above (Figueiredo et al., 2013; Tolessa et al., 2016; Bressanello et al., 2017).

Statistical Analysis

One–way ANOVA analysis was used to statistically evaluate the differences between total phenolic, flavanoid content, antiradical activity and final cup quality score. The sensory analysis measures were carried out in five replicates and all chemical analysis measures were carried out in triplicate. The data were express as means. The significant differences were stated if $p \leq 0.05$. The data was analysed with Microsoft Office Excel 2013. A linear correlation analysis was performed in order to determine relationship between TPC, TF, antioxidant activity such as DPPH$^\cdot$ and ABTS$^\cdot$+, volatile compounds and final cup quality score.

RESULTS AND DISCUSSION

Sensory analysis

The final cup quality score and panellists sensory flavour and aroma description is shown in Table 2. All coffees scored in range 83–90.25. The lowest score (83 points) had SAL_1 coffee sample, it can be associated with the high acidity, which can disbalance the overall cup preference. From other point of view HON_2 with highest score (90.25 points) shown balance between the acidity and sweetness of the coffee brew.

Table 2. Final cup quality scores with sensory description

Roastery	Coffee sample	Final cup quality score	Sensory description
Roastery_1	HON_1	86.50	dark plum, grapes, red pepper, toffee (caramel)
	KEN_1	88.75	blackberry, red pepper, roses, dark chocolate
	COL_1	89.00	pineapple, dried apricot, elderflower
Roastery_2	HON_2	90.25	dried fruits, passion fruit, melon, kombucha
	ETH_1	88.25	lime, jasmine, chocolate cream, cherry brandy
	HON_3	85.00	cacao, red apples, dried fruits
	SAL_1	83.00	nutty and creamy notes

The balance between acidity and sweetness is correlated with higher final cup quality score as was showed in study Alex et al. (2016) that analysed relationship between different coffee plantation regions in Brazil and cup quality attributes, and in results the fruit, caramel flavour notes and pleasant acidity positively correlated with coffees whose quality standards was the most in accordance with the SCAA standards (SCAA, 2105). Final cup quality score has also shown strong correlation with sucrose and pleasant acidity in Borém et al. (2016) research. Pleasant acidity mostly is associated with dry fruit taste (Poltronieri & Rossi, 2016)and this could be the reason why HON_2 with dry fruits and melon characteristics shown the highest final cup quality score.

The major volatile compounds in coffee brew samples were furans, pyrazines, aldehydes and ketones (Table 3).Furans set the highest concentration, but the pyrazines, ketones and aldehydes contain some of the most important volatile compounds which are associated with a pleasant aroma and flavour notes in specialty coffee (Moon & Shibamoto, 2009; Parenti et al., 2014; Piccino et al., 2014; Steen et al., 2017; Yang et al., 2016). In Caporaso et al. (2018) study grouping volatile compounds by their chemical classes positive correlation was detected between aldehydes and ketones, but negative correlation between aldehydes and pyrazines. Ketones and aldehydes are also associated with floral, fruity aroma and pleasant acidity in coffee. More studies are approving the positive correlation between coffee cup quality and volatile compound concentration with floral, fruity aroma notes (Piccino et al., 2014; Poltronieri & Rossi, 2016). Ribeiro et al. (2009) study reports that higher concentration of 5–methyl–2–furancarboxaldehyde and furfural increased the overall quality of Brazilian *Coffea arabica L.* coffee samples.

If the fermentation process is not controlled at coffee bean harvesting moment, and also in roasting process, then the desired aldehyde and ketone compounds can easily transform in spirits, which can imbalance the coffee volatile compound composition (Preedy, 2015). Isoamyl acetate which was detected in HON_2 coffee have specific fermented aroma and flavour, with potential brandy, over ripe fruit notes (Toledo et al., 2016), and in sensory analyses panellists detected kambucha (non–alcoholic fermented fruit beverage) notes. In this situation fermented flavour notes are associated with positive cup quality characteristics and the high final cup quality score is in the line with trend in specialty market – exploring the fermented and specific aroma notes (Sepúlveda et al., 2016).

In previous studies about volatile compound composition and final cup score, coffee furanone (dihydro–2–methyl–3–furanone) concentration is positively associated with higher final cup quality scores (Toledo et al., 2016). Only dihydro–2–methyl–3–furanone was only detected in KEN_1 coffee samples. All detected volatile compounds in KEN_1 is associated with positive specialty coffee characteristics (Steen et al., 2017).In ETH_1 coffee with the final cup quality score 88.25 was detected highest floral, fruity compounds, like furfuryl acetate, 2–furanmethanol, and highest coffee–like roasted notes (2–methyl butyraldehyde,2–methyl–propanal).

None of the coffee samples were detected compounds with strong association with defected coffee quality. This approves that the specialty coffee high standards for green coffee beans limits the risk of damaged or unripe beans. By limiting coffee defects it also excludes possible defective/unpleasant volatile phenolic compound presents in coffee brew, for an example, 4-ethyl-2-methoxyphenol, 2-methylphenol (Giacalone et al., 2019; Steen et al., 2017).

Table 3. In the headspaceof coffee brew samples identified volatile compounds

Compounds	GC–MS peak area (x10^6)							Compound sensory description
	HON_1	KEN_1	COL_1	HON_2	ETH_1	HON_3	SAL_1	
1–(2–Furanylmethyl)–1H–pyrrole				7.025a	7.847b	8.539c	7.597b	savory, vegetables**
2.3 Pentanedione	17.453b	20.989c	23.374d	12.765a	26.013e	30.189f	26.266e	buttery***
2.6–Dimethyl–4–thiopyrone				5.006				caramel**
5–Methyl–2–furancarboxaldehyde	48.053b	40.476a	76.429e	51.623c	63.474d	97.233e	76.848f	red pepper *
2–Furanmethanol	39.029c	37.860b	55.160e	32.837a	49.475d	72.414e	70.254f	floral***
Furfuryl acetate	38.760b	31.600a	93.105e		78.378c	98.544f	83.259d	floral, fruity****
Dihydro–2–methyl–3(2H)–furanone		17.567						coffee–like, caramel****
2–Methyl–butanal	14.937a	21.138b	36.067c	93.308e	36.939c	93.311e	39.428d	cacao, coffee–like*****
1–(2–Furanyl), ethanone			22.425					nutty ***
2–(Methoxymethyl)furan					4.591			roasted almond, coffee***
2–Methyl–furan					24.119	18.563		caramel***
Furfural		10.800a	102.107e	64.532b	95.721c	133.029f	99.558d	roasted sweet almond**
2–Methyl–propanal				19.239	9.889			chocolate*****
Ethyl–pyrazine				8.465	7.456	11.145		chocolate–peanut, nutty **
4–Methyl–pyrimidine	14.156		26.268	1.433	24.662	32.687	2.707	popcorn*
2–Methoxy–4–vinylphenol		1,923						spicy, floral*****

* – Toledo et al., 2016;** – Bressanello et al., 2017; *** – Lee et al., 2017; **** – Steen et al., 2017; ***** – Piccino et al., 2014; Different letters indicate statistically significant ($p < 0.05$) differences between samples.

Total phenolic and flavonoid content

Significant differences were detected between coffee roasteries and total phenolic content in coffee brews. It can be associated with different technical conditions in the roasting process and with the specific characteristics of each raw materials have.
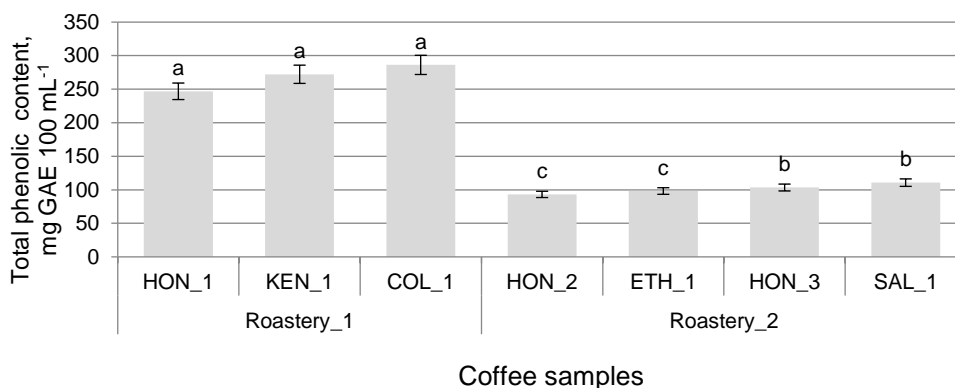
Figure 1. Total phenolic content in coffee brews.

Similar results showed also total flavonoid content in coffee brews (Fig. 2). The highest phenolic and flavonoid content were detected in COL_1 and the lowest in HON_2. From volatile compounds profile COL_1showed more balanced composition in comparison with HON_2. COL_1 volatile compounds profile has fruity and floral aroma, flavour notes from organic acids and also phenolic acids. But HON_2 dominated in higher nutty, chocolate flavour notes from furans.
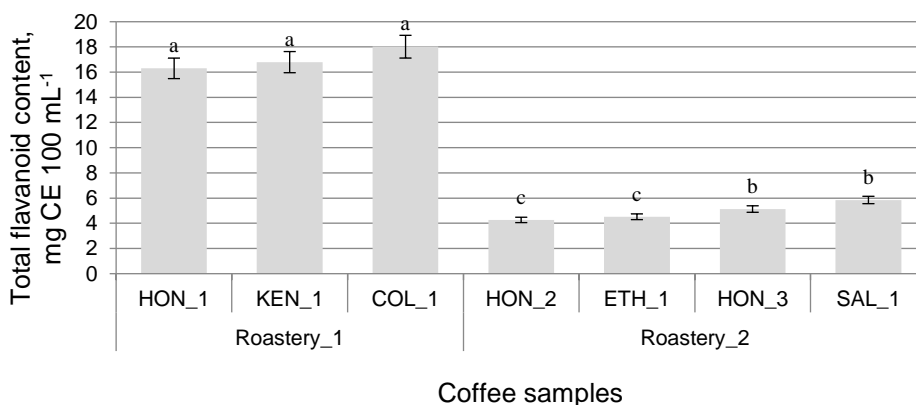
Figure 2. Total flavanoid content in coffee brews.

It has been proven that between green coffee samples the compositions of phenolic compounds are similar, but between roasted coffee samples phenolic compound composition can change significantly for various reasons, for example, roasting temperature, time, storage etc. (Somporn et al., 2011; Cheong et al., 2013), this can be

one of the main reasons why results for high quality coffees have significant differences between roasteries.

ABTS and DPPH radical scavenging activity
Similar to total phenolic and flavonoid content COL_1 showed the highest DPPH value (832.441 µmol trolox equivalent g^{-1}) and ABTS+˙value (34.127 µmol trolox equivalent g^{-1}) and HON_2 showed the lowest DPPH value (505.20 µmol trolox equivalent g^{-1}) and ABTS+˙value (34.127 µmol trolox equivalent g^{-1}).

There was high correlation between DPPH and ABTS+˙ assay with total phenolic content in coffee brews. DPPH had higher correlation ($r = 0.996$) than ABTS+˙($r = 0.9345$) but both assays showed similar results with other research studies (Somporn et al., 2011; Daniel & Workneh, 2017). Also, the radical scavenging activity showed significant differences between coffee roasteries.

Correlation between total phenolic content and cup quality
There was no correlation detected between final cup quality scores and total phenolic content in coffee brews overall. But by grouping coffee brews by roasteries, there was positive correlation ($r = 0.971$) between final cup quality scores and total phenolic content for Roastery_1 coffee brews (Fig. 4, B.) and negative correlation ($r = -0.957$) between final cup quality scores and total phenolic content for Roastery_2 coffee brews (Fig. 4, A).
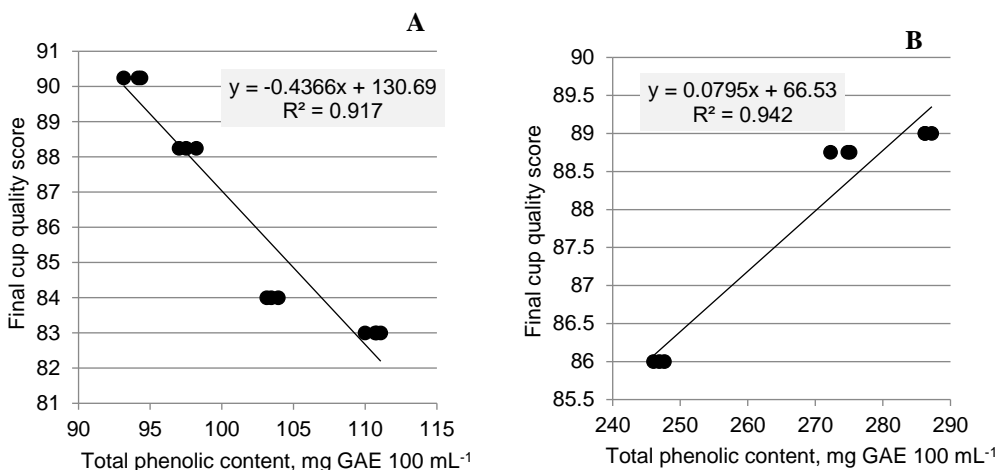
Figure 4. Correlation between final cup quality scores and total phenolic content in coffee brews. (A) Roastery_2 coffee brews; (B) Roastery_1 coffee brews.

The opposite correlations between two roasteries could suggest that roasting process parameter influence important chemical compound content in coffee brews differently. Roastery_1 coffees volatile compounds profile is more balanced and focus to fruity and floral compounds, like furfuryl acetate, 2–furanmethanol, while Roastery_2 coffee volatile compounds profiles have a higher number of volatile compounds (2–methyl–propanal – chocolate notes; 1–(2–Furanylmethyl)–1H–pyrrole – savory notes; 4–methyl–pyrimidine– popcorn, roasted bread notes). The sensory results for

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Roastery_1 coffee samples showed minimal final cup quality score differences, while Roastery_2 had wider amplitude from 83 point to 90.25 points. These two results could suggest that it is possible to maintain high phenolic compound content in coffee brew if the volatile compounds composition is focused on specific aroma attributes like sweet and pleasant acidity of fruity, floral aroma, flavour notes.

Analysing other studies about phenolic content correlation with sensory results in coffee, it also shows opposite results about cup quality and phenolic compound composition. In some research studies phenolic compounds like 5–caffeoylquinic acid (5–CQA) and feruloylquinic acid (5–FQA) is associated with lower cup quality, because of the bitterness(Fujioka & Shibamoto, 2008). Fank et al. (2007) analysing the bitter–tasting compounds in roasted coffee, states that in sensory analysis 5–CQA is associated with coffee–like bitterness, caffeic acid with strong roasted coffee bitterness and only ferulic acid and trigonelline has association with harshly strong bitterness. Phenolic compound as2–methoxy–4–vinylphenol brings pleasant spicy, floral notes to coffee brew in low concentrations (Piccino et al., 2014). In low concentrations 2–methoxy–4–vinylphenol was detected in Roastery _1 Kenya_1 (II) coffee brew and its volatile compounds sensory descriptions match with the panellists compound sensory description. Moon & Shibamoto (2009) research states that phenolic compounds with pleasant and fresh aroma, flavour notes as 2–methoxyphenol, chlorogenic acids and 2–methoxy–4–vinylphenol rapidly decreases after light roasting level but caffeic acid, catechol increases with the roasting level bringing harsh bitterness to coffee brew. Zanin et al. (2016) in his research proved that it is possible to contain good cup quality without losing the valuable chlorogenic acid content. These studies suggest that individual phenolic compounds could affect differently the overall sensory characteristics of the coffee. This is one of the reasons why it is important to determine individual phenolic content and analyse its correlation with the sensory analysis results.

CONCLUSIONS

Final cup quality score in sensory analysis varied in the range of 83–90.25 points with the highest score had HON_2 and the lowest score had SAL_1. Roastery_1 coffees volatile compounds profile was more balanced with fruity and floral compounds, like furfuryl acetate, 2–furan methanol, while Roastery_2 coffee volatile compounds profile had chocolate, nutty, roasted aroma notes. The difference between the roastery coffee sample total phenol, flavonoid content and antiradical scavenging activity also showed significant differences. A positive correlation was found between final cup quality scores and total phenolic content for Roastery_1 coffee brews and a negative correlation between final cup quality scores and total phenolic content for Roastery_2 coffee brews. The different correlations could be associated with specific phenolic compound presents in the coffee brew. These results indicate that the roastery specific roasting process parameters could influence not only volatile compounds profile but also the total and individual phenolic compound content. To better predict roasting process influence to phenolic compound composition it is important to further analyse the specific roasting parameters and individual phenolic compounds with volatile compounds profile correlation.

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Winter rye grain quality of hybrid and population cultivars

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Abstract. Rye (*Secale cereale* L.) is an important European crop used for food that is grown primarily in Eastern, Central and Northern Europe. Consuming rye grain products provides a rich source of dietary fibre as well as several bioactive compounds with potentially positive health implications. The goal of the research was to compare the rye grain quality of hybrid and population cultivars. A field trial was carried out in Priekuli Research Centre, Institute of Agricultural Resourses and Economic (in Latvia) during a three-year period: 2014/2015, 2015/2016, and 2016/2017. The trial included population winter rye cultivars 'Kaupo', 'Amilo', 'Dankowskie Amber' and hybrid rye cultivars 'Brasetto', 'Su Drive', 'Su Mephisto'. Rye grain quality indices were analysed at Latvia University of Life Sciences and Technologies, in Grain and Seed Research laboratory. Average data in our investigation (three years) show that cultivar, crop-year (weather conditions) and cultivar×crop-year interaction significantly ($P < 0.05$) affected rye grain protein content, starch content and Hagberg falling number. A significant negative correlation was found between protein content and starch content $r = -0.937$ (population cultivars grain), $r = -0.944$ (hybrid cultivars grain), medium strong negative correlation was found between protein content and falling number, respective $r = -0.549$ and $r = -0.573$. Differences between hybrid cultivar grain protein content, falling number and starch content comparing with population cultivar grains were not observed. The results of the current research show that the quality of all the studied cultivars meets the requirements for high-grade rye grains for food consumption.

Key words: winter rye, protein content, starch content, Hagberg falling number.

INTRODUCTION

Winter rye (*Secale cereale* L.) is an important grain crop in Latvia, where it is mainly used for baking rye bread, which is a popular staple. The chemical composition of rye grain promises health benefits and it contributes to higher intake of dietary fibre. The chemical composition of rye grain differs from wheat, rye contains less starch and protein but more dietary fibre than wheat (Linina & Ruza, 2012; Alijošius et al., 2016). In rye flours proteins do not form a gluten network but they seem to be important during the dough mixing step, since they have some aggregation abilities and are surface active

(Banu et al., 2006). Protein composition and contents play a critical role in bread quality and are governed by a combination of genetic and environmental factors (Hansen et al., 2004). Rola et al. (2008) stated that protein content among the different population cultivars of rye ranged between 9.4% ('Palazzo') and 11.3% ('Virgiai'), while Vidmantiene & Juodeikiene (2010) reported that protein content was from 7.5% to 11.2%, similarly also Stępniewska et al. (2018): 8.0–11.1%.

Starch is an important ingredient of rye flour. Its properties and quality determine the usefulness of flour for bread baking (Laiding et al., 2017).

Rye quality depends on weather conditions in the growing years (Chmielewski & Kohn, 2000; Nowotna et al., 2006; Kučerová, 2009; Blecharczyk et al., 2016; Kottman et al., 2016). Rye is sensitive to the prevailing weather conditions, such as precipitation. Rainy periods in the ripening stage sometimes cause the pre-harvest sprouting of rye grains. Low falling number (under 100 s) of flour made from sprouted grains is caused by the increased content of α-amylase (Dvorakova et al., 2012). The falling number is of significant meaning as it describes the α-amylase activity in flour. Excessive α-amylase activity has an adverse effect on the quality of baked products. Falling number depend on agrotechnical and weather conditions as well as on genetically determined plant properties (Dubis et al., 2008, Linina & Ruza, 2015).

Hybrid rye breeding started in 1970 at the University of Hohenheim in Germany and the first hybrid cultivars were reased in Germany at 1984 (Wang et al., 2014). Economically important traits in hybrid rye are grain yield and plant height in context of productivity as well as starch content and total pentosan content with regard to end user quality. Hybrids are grown on about 60–70% of the total rye acreage owing to their yield superiority and better uniformity as compared to population cultivars. German commercial hybrid cultivars are also realised and grown in Denmark, Austria, Poland (Miedaner & Hübner, 2011) and Latvia.

Grain yield and quality significantly varied depending on the cultivars as previously observed (Peltonen-Sainio et al., 2007; Wang et al., 2014; Alijošius et al., 2016; Linina & Ruza, 2018). Rola et al. (2009) reported that falling number was from 160 to 325 s in population rye cultivar 'Fernando' and from 113 s to 297 s in hybrid cultivars 'Stach' grown in Poland, while protein content, respectively: from 7.9% to 12%, and from 8.4% to 11.6%.

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

The goal of the research was to compare the rye grain quality of hybrid and population cultivars.

MATERIALS AND METHODS

Field experiments. A field trial was carried out in Priekuli Research Centre, Institute of Agricultural Resources and Economics in 2014/2015, 2015/2016 and 2016/2017. The soil type was sod-podzoloic loam, with close to neutral acidity (pH_{KCl} 5.6–6.0), medium high phosphorus and potassium, organic mater 1.7–2.5 g kg^{-1}. In trial traditional soil treatment was used, which involves soil ploughing. Winter rye population cultivars (POP) 'Kaupo' (Latvia), 'Amilo', 'Dankowskie Amber' (both Poland) and

hybrid cultivars (HYB) 'Brasetto', 'Su Drive', 'Su Mephisto' (all Germany) was sown (17 September in 2014, 24 September in 2015 and 19 September in 2016) after spring barley. Seeding rate was 500 seeds per 1 m². Trials were arranged using split plot design in four replications. Before sowing in autumn complex mineral fertilizers ensuring following amounts of pure elements were applied: N12, P_2O_5 52, K_2O 60 kg ha^{-1}. Nitrogen (N) was applied N68 kg ha^{-1} in spring after resumption of vegetative growth and N31 kg ha^{-1} at the shooting stage (BBCH-scale). All the necessary plant protection measures (herbicides, plant growth regulators and fungicides) were performed. Rye grain was harvested at full ripening (BBCH-scale: 90–92) on 4 August in 2015, on 5 August in 2016 and on 3 August in 2017. Harvested grain of each cultivar and replication was put into separate bags. The grain with a moisture content exceeding 14% was dried.

Weather conditions. During three investigation years weather conditions were different. Winter rye sown in 2014, 2015 and 2016 overwintered successfully. The winter of 2014/2015, 2015/2016, 2016/2017 were generally favourable for rye wintering. The air temperature in investigation years (Fig. 1) in April was close to long-term mean (LTM) observations. May in 2015 and 2017 was colder, while in 2016 it was 2.7 °C warmer, which promoted plant growth and development. Average daily temperature in June 2015 and 2017 was lower compared to long-term average data, in 2016 air temperature was warmer by 1.5 °C which contributed to the accumulation of protein. Temperature in the grain filling period (July), which is the most decisive for grain quality formation, was colder in 2015 and 2017, while in 2016 it was by 1 °C higher than the long-term average mean data.
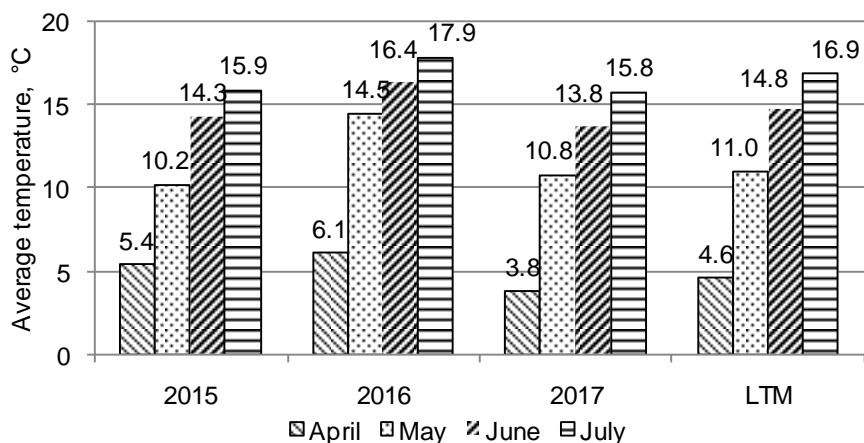
Figure 1. Average air temperature in vegetation period, and long-term mean (LTM) °C.

Water availability has effect on rye grain quality. Precipitation in April 2015, 2016 and 2017 was more than long-term means data. May in 2016 was dry. Precipitation in June 2015 was less than to long-term mean, while in 2016 and 2017 was more than long-term means data. Precipitation in all investigation years was close to long-term average observations (Fig. 2).
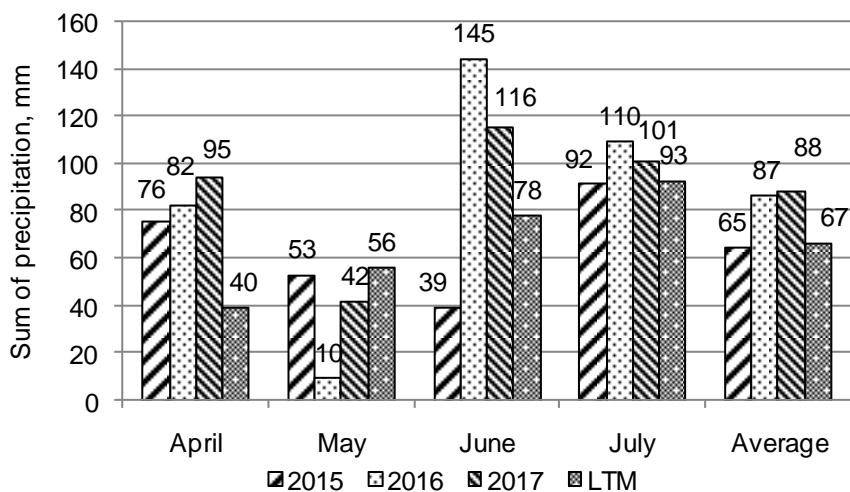
Figure 2. Average sum of precipitation, and long-term mean, mm.

Sample analysis. The rye grains were analysed at Latvia University of Life Sciences and Technologies in Grain and Seed Research laboratory. Quality indices: protein content (%) and starch content (%) were analysed by grain analyser Infratec 1241 (FOSS, Denmark), which employs the near-infrared analysis within the wavelength range 570–1,110 nm. The Hagberg falling number – α-amylase activity – was measured by the Hagberg-Perten method using a Perten Instruments (Sweden) Falling number 1500 assessed to LVS EN ISO 3093 using 7 g of flour adjusted for moisture content to 15%.

Statistical analysis. Experimental data evaluation was done using two factor analysis of variance by Fisher's criteria, which were applied to estimate the effects of year (meteorological conditions) and cultivars. Component of variance ANOVA for each quality characteristic was expressed as percentage to illustrate the relative impact of each source to the total variance. Differences of the grain quality indices between population and hybrid rye cultivars were determined by t-test: Two Sample Assuming Unequal variance. Correlation analysis between protein content and other grain quality indices was carried out.

RESULTS AND DISCUSSION

The content of protein, starch and falling number are two important criteria for the quality of cereals (Miedaner et al., 2012; Stępień et al., 2016). Rye quality is subject to large year-to-year fluctuation (Laiding et al., 2017). In our investigation grain protein content, starch content and falling number significantly ($P > 0.05$) varied depending on the cultivars and meteorological conditions. The least variation of the grain starch content (average 61.4%) was noticed in the hybrid cultivars, with coefficient of variation of 3.1%. The greatest variations of the falling number (average 230%) were observed in population cultivars: V = 21.4% (Table 1).

Table 1. Winter rye quality indices

Indicators	Population cultivars (POP)			Hybrid cultivars (HYB)		
	PC, %	SC, %	FN, s	PC, %	SC, %	FN, s
Mean ± standard error	10.3 ± 0.7	61.4 ± 0.8	230 ± 16.4	9.8 ± 0.6	61.4 ± 0.6	230 ± 13.0
min	7.7	58.8	130	7.7	58.8	158
max	13.1	64.8	305	12.2	64.1	275
V %	19.4	3.9	21.4	18.4	3.1	18.5

PC – protein content; SC – starch content; FN – falling number.

The **protein content (PC)** in population cultivars ranged from 7.7% ('Kaupo') to 13.1% ('Amilo'), similarly, it was also for hybrid cultivars: from 7.7% ('Su Drive') to 12.2% ('Brasetto') (Fig. 3). The investigation with winter rye in the western region of Lithuania indicates small changes of protein content 7 to 8% (Skuodienė & Nekrošienė, 2009), while in Estonia (seven years study of winter rye cultivar 'Elvi') it was 8.4 to 11.9% (Järvan et al., 2018) and 9 to 13% in Serbia (Žilic et al., 2011). The content of protein rye grain was differentiated by weather conditions in the years. In harvest year 2016 the content of protein in rye grains was higher as compared with other years. In 2016 a higher mean air temperature in summer favoured a greater concentration of protein. Similar dependences of protein accumulation on weather conditions were confirmed by the study of Stępień et al. (2016). In rye baking the amount and quality of protein are not as important as in wheat baking (Salmenkallio-Marttila & Hovinen 2005); also grain processing companies in Latvia do not take this into account.
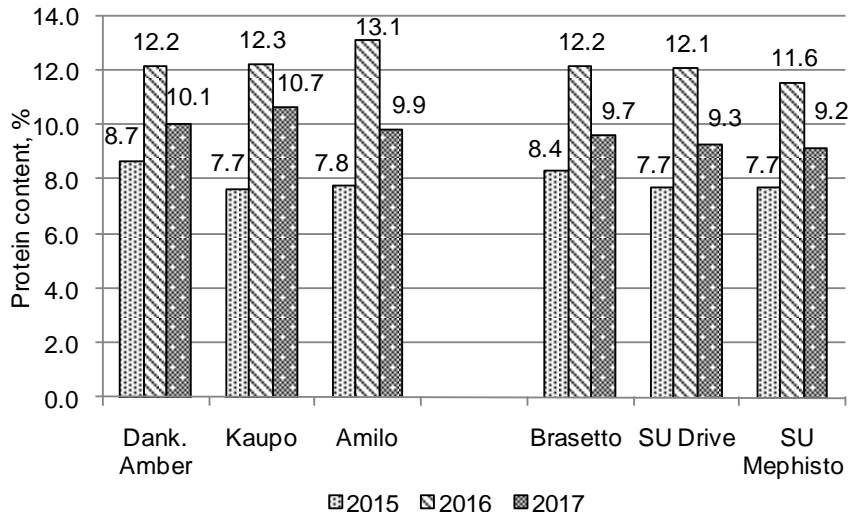
Figure 3. Winter rye grain protein content, %.

Influence of each factor (crop-year, cultivar and crop-year×cultivar interaction of them) was calculated of two-factor analysis of variance for winter rye protein, starch content and falling number. It was expressed as percentage to the total variance. Data in our experiment (3 years) suggest that protein content was significantly ($P < 0.05$) influenced by harvest year (POP 95.0%, HYB 97.2%), while cultivars (POP 0.1%,

HYB 2.0%) and cultivar×crop-year influence were small (POP 4.8%, HYB 0.4%) (Table 2). However, in experiments performed in Denmark (Hansen et al., 2004) protein content in rye grain dependency on cultivar complete to 67%, the influence of year – 17%, but cultivar×crop-year influence was small – 4%.

Table 2. Impact factors of rye grain quality indices, %

Source of variation	Population cultivars (POP)			Hybrid cultivars (HYB)		
	PC, %	SC, %	FN, s	PC, %	SC, %	FN, s
Year (Y)	95.0	96.6	46.5	97.2	92.8	80.5
Cultivar (C)	0.1	0.9	29.5	2.0	4.9	11.2
Y × C interraction	4.8	1.6	22.0	0.4	1.3	7.4

PC – protein content, SC – starch content, FN – falling number.

Starch is an important ingredient of rye flour. Its properties and quality determine the usefulness of flour for bread baking. Rye starches exhibit greater values of solubility in comparison to wheat (Banu et al., 2006).

The average winter rye **starch content (SC)** (Fig. 4) in three investigation years was from 58.8% to 64.8% (POP), which was very similar to the HYB: 58.8 to 64.1%. The content of starch measured in this study is in accordance with the findings by other authors (Marczewski et al., 2012; Miedaner et al., 2012; Wang et al., 2014; Järvan et al., 2018). The starch content was significantly ($P < 0.05$) influenced by year (POP 96.6%, HYB 92.8), while cultivar influence (POP 0.9%, HYB 4.9%) and cultivar×crop-year influence (POP 1.6%, HYB 1.3%) were small (Table 2).
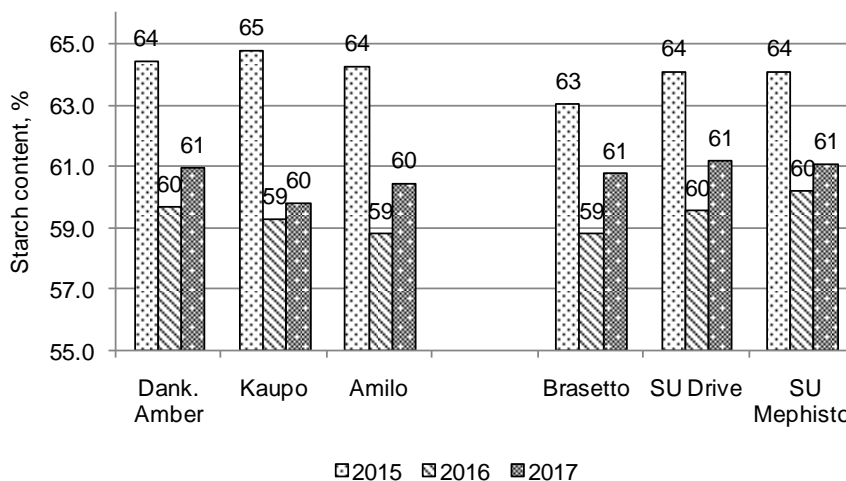
Figure 4. Winter rye grain starch content, %.

Falling number (FN) is the most commonly used method for rye quality control. Falling number is an indication of degree of soundness of rye in terms of freedom from sprouting which causes the production and activation of α-amylase inside the rye kernel which, in turn, has a very drastic effect on the dough and bread making process. To obtain rye flour with the falling number level appropriate for the baking process one

should use grain with the falling number within the interval 110–190 s (Salmenkallio-Marttila & Hovinen, 2005). In Latvia the falling number of rye flour used from bread baking is 120 s.

In our investigation the falling number of grain was significantly ($P < 0.05$) different for cultivars. Falling number in population cultivars ranged from 130 s ('Kaupo') to 305 s ('Amilo'), for hybrid cultivars: from 158 s ('Su Drive') to 275 s ('Brasetto') (Fig. 5).
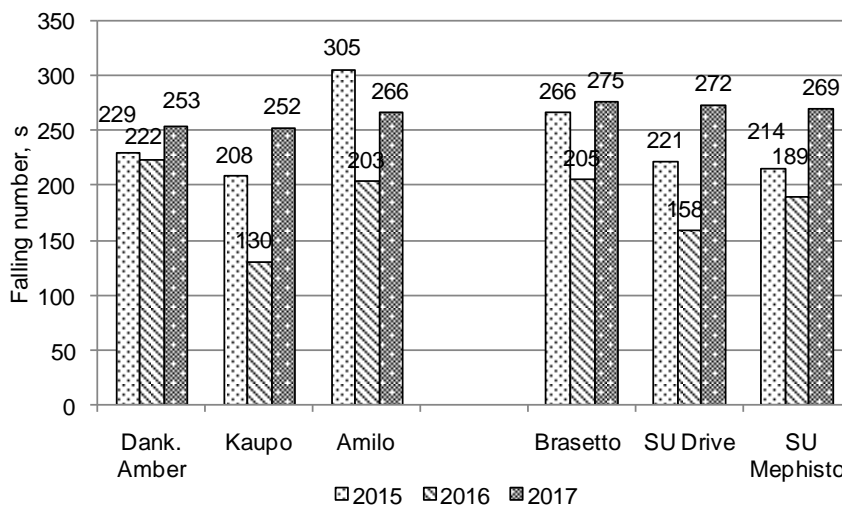
Figure 5. Winter rye grain falling number, s.

Salmenkallio-Marttila & Hovinen (2005) in Finland determined that the falling number from rye population cultivars was lower (111–204 s), compared to hybrid cultivars (256–261 s). Zdubel et al. (2009), Vidmantiene & Joudeikiene, (2010), Marczewski et al. (2012) and Ismagilov et al. (2018) also confirmed that the falling number of different cultivars may vary in the same growing conditions. In the present trial, differences between hybrid cultivar grain protein, starch content and falling number in comparison with the population of cultivar grains were not observed (*t-test, $P < 0.05$*). All properties of winter rye demonstrate that the quality of the studied cultivars corresponds to the requirements for high-grade rye for food consumption.

Weather conditions in investigation years influenced grain α–amylase activity. The falling number is affected by precipitation during grain maturation. High rainfall in grain maturation period results in higher α-amylase activity and lower falling number (Salmenkallio-Marttila & Hovinen, 2005). In Järvan et al. (2018) trial, the falling number for rye grain (seven crop-years) was from 62 to 289 s. In our investigation rainfall in 2016 during rye grain maturation was exceeded the long-term mean data, therefore falling number for rye grain was lower.

The falling number was influenced by crop-year (POP 46.5%, HYB 80.5%) and cultivar (POP 29.5%, HYB 11.2%) whereas the effect of cultivar×crop-year accounted: POP 22.0% and HYB 7.4%) (Table 2), similar results have also been demonstrated by Hansen et al. (2004).

A significant negative correlation was found between rye grain protein content and starch content $r = -0.937$, $R^2 = 0.878$ (POP), $r = -0.944$ (HYB) $R^2 = 0.891$ ($n = 12$, $\alpha_{001} = 0.708$, $\alpha_{005} = 0.576$) (Fig. 6). Similar results were obtained in previous research in Germany (Miedaner & Hübner, 2011; Miedaner et al., 2012) and also in Estonia (Järvan et al., 2018).
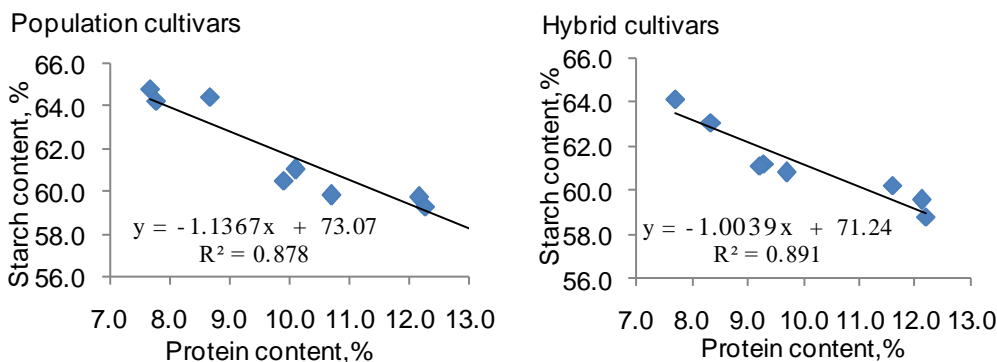
Figure 6. Winter rye grain starch and protein content relationships.

Medium negative correlation was found between protein content and falling number, respective $r = -0.549$ (POP) and $r = -0.573$ (HYB).

CONCLUSIONS

In the present research it was found, that rye cultivar, differences in their genetic as well as weather conditions significantly influenced protein formation in grains. Higher protein content was found in the analysed rye samples in the period when the growing conditions were warmer (2016). Lower falling number was obtained in winter rye grain, in 2016 because there was more precipitation in June and July. Differences between hybrid cultivar grain protein and starch content, falling number in comparison with population cultivar grains were not observed. Winter rye properties demonstrate that the quality of the studied cultivars correspond to the requirements for high-grade rye for food consumption. The strong negative correlation was found between protein content and starch content.

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Investigation of various factors on the germination of chia seeds sprouts *(Salvia hispanica L.)*

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Abstract. *Salvia hispanica L.* is capable to produce a large amount of green matter, which can be used as a source of biologically active substances. The purpose of this research was to select the optimal factors for the chia seed sprouts *(Salvia hispanica L.)* germination. Dark variety chia seeds (100 grains/sample) were investigated. The most significant factors for the process of sprouting were selected as the study factors, such as the water mass fraction, the temperature and the light exposure for seed germination. The output parameters of the experiment were seed germination energy, germination of seeds, speed of germination and seedling vigor. It was revealed that the mass fraction of added water had the greatest influence on the growing process of chia seed sprouts. The optimal amount of water for producing the chia seed sprouts was in the average of 4 mL/sample. As a result, it was noted that an insufficient or excessive amount of water had a negative effect on the chia seed sprouts germination. The optimum temperature for germination of chia seed sprouts was 25 °C. The optimal light factor was also determined; in particular light exposure peaks occur in the red spectrum with a wavelength of 660 nm and a blue spectrum with a wavelength of 450 nm.

Key words: chia seed sprouts, *Salvia hispanica L.*, germination factors, microgreens.

INTRODUCTION

Salvia hispanica L. (Spanish sage, chia) is an annual herb that adapts to arid and semi-arid climates, particularly in the United States, Mexico, Chile, Peru, Colombia, Guatemala, Bolivia, Argentina and Australia, as well as in India, China and some countries in Europe and Africa (Ayerza, 2013; Busilacchi et al., 2013; Sreedhar et al., 2015; Zayova et al., 2016; Elshafie et al., 2018; Win et al., 2018).

This plant has received wide popularity since the chia seeds are a rich source of polyunsaturated fatty acids with a high nutritional value. The chemical composition of plant and chia seeds, in particular, the oil fraction, is critically dependent on the height

of the plant and on the temperature at which they are grown (Ahmed et al., 1994; Baginsky et al., 2016).

In addition, the plant *Salvia hispanica L.* is able to produce a large amount of leaves that can be used as a source of biologically active substances, especially in cases when the plant does not enter the reproductive phase in some regions (Zayova et al., 2016) due to its photosensitivity. In contrast to the seeds, the chemical composition, pharmacological and nutritional value of the leaves and sprouts of this Spanish sage have not been well studied (Ahmed et al., 1994; Ouzounidou et al., 2015; Zayova et al., 2016; Elshafie et al., 2018; Pająk et al., 2019).

Currently, the relevance of this study on chia seeds is to obtain microgreens for the food industry. It is promising to study the application of plant sprouts as part of functional products that can have a therapeutic effect on the state of the gastrointestinal tract and on humans, in general. According to some reports, the inclusion of sprouts in the diet can enrich it with enzymes, antioxidants, polysaccharides, etc. (Gómez-Favela et al., 2017).

In the germination process, plant resources are activated and the nutritive value of seeds is increased, including phenolic compounds and mineral composition, besides resulting in an increase in their bioavailability (Gómez-Favela et al., 2017; Pająk et al., 2019). According to studies, in the analysis of the vitamins and carotenoids content in 25 plant species, it was revealed that harvesting the microgreens contain 10 times more antioxidant compounds as compared with the traditional harvesting in the commercial ripeness phase (Ivanova et al., 2016). There is also a possible difference in chemical composition of seedlings during each day of growth due to the high intensity of bioprocesses (Pająk et al., 2019).

Chia seeds revealed a significant increase in the total content of phenolic compounds in the process of germination, including the content of γ-aminobutyric acid (GABA) (Gómez-Favela et al., 2017; Pająk et al., 2019). In the oil obtained from the leaves of *Salvia hispanica L.* about 80 compounds have been identified, most of which belong to the class of sesquiterpenes, apart from monoterpenes, phenolic compounds, and flavonoids (Ahmed et al., 1994; Amato et al., 2015; Zayova et al., 2016; Elshafie et al., 2018). It is important to note that the antioxidant activity of chia seeds is widely studied, however, data on the antioxidant effect of seedlings and of chia green part are extremely limited (Zayova et al., 2016).

Gómez-Favela et al. (2017) report that the amount of protein and insoluble dietary fibers increase in germinated chia seeds, while the content of soluble dietary fibers and the lipid component decreases. During the growth cycle of *Salvia hispanica L.*, the solids content increases rapidly (from 84 to 224 g kg^{-1} of the mass of the fresh plant) (Peiretti, 2010). The content of polyunsaturated fatty acids in plant biomass also varies depending on the growth stage. Thus, a decrease in the content of alpha-linolenic acid from 649 g kg^{-1} at an early stage of vegetation to 499 g kg^{-1} at the budding stage was noted, while the remaining fatty acids showed a reverse trend. The optimal composition of plants for harvesting is determined during the period of shoots before budding (Peiretti & Gai, 2009).

Germinated chia seeds are of great interest for the food industry, and the process of seed germination is of interest for agricultural production. In addition, the determination of optimal parameters could be used in the further cultivation of the plant *Salvia hispanica L.* in case of using green matter as a biologically active component.

The seed germination depends on a number of internal (dormant, genotype, maturity) and external factors, including temperature, salinity, light and moisture conditions (Jafarinia & Yazdanbakhsh, 2016; Pająk et al., 2019).

The sensitivity of various seeds to light regime depends on their species, the light factor is decisive in the process of photosynthesis. Some seeds germinate better in the dark, while in other seeds, there is also a dependence on the light spectrum (Jafarinia & Yazdanbakhsh, 2016).

Growth temperature affects both the percentage and the speed of germination, impeding water absorption and the flow of biochemical reactions (de Souza & Chaves, 2016).

The dormant dry seed must be fed with water to activate the germination process (Jafarinia & Yazdanbakhsh, 2016). In addition, it has been proven that seed germination speed decreases with decreasing external water potential, there is a critical value of water potential, below which seed germination does not occur (Hadas, 1976). One of the characteristics of chia seeds is the ability to produce a significant amount of mucilage during hydration. This gel completely envelops the seed, which also needs to be considered in the germination process (Geneve et al., 2017).

In the natural conditions, *Salvia hispanica L.* grows in tropical and subtropical regions; the minimum and maximum temperature of plant growth is 11 °C and 36 °C, respectively, with an optimal growth range from 16 °C to 26 °C (Ayerza & Coates, 2009). During the growing season, Spanish sage needs a uniform amount of rainfall and a dry climate during ripening (Win et al., 2018).

A number of studies have been conducted in the global community on the chia seed germination depending on the temperature factor and light factor (Stefanello et al., 2015b; Paiva et al., 2016; Possenti et al., 2016; Gómez-Favela et al., 2017), and the effects of salt stress (Raimondi et al., 2015; Stefanello et al., 2015a; de Souza & Chaves, 2016) and the mucilage formation factor (Geneve et al., 2017).

The optimum temperatures for germinating chia seeds are different. It is noted that the light factor does not have a significant effect on the seeds, but seedlings accumulate dry matter better in the presence of light (Stefanello et al., 2015b; Paiva et al., 2016; Possenti et al., 2016). Chia seeds are moderately tolerant to certain levels of salinity, but higher or lower values can be disastrous, especially in the early stages of seedling development (Raimondi et al., 2015; Stefanello et al., 2015a; de Souza & Chaves, 2016). Under optimal conditions, mucilage from chia seeds does not affect germination; however it slows down the germination process while increasing the survival rate of seeds under stressful environmental conditions (Geneve et al., 2017).

In this study, the experiment taking into account three significant parameters – the water mass fraction, the temperature and the light exposure was staged for the first time. These factors would determine the optimal conditions for the chia seed germination on an industrial scale.

MATERIALS AND METHODS

Materials

The chia seeds *(Salvia hispanica L.)* of dark varieties (the brand 'Era Green') with the following composition were used as an object of research: proteins 24%, fats 31%, carbohydrates 34%.

Conducting germination tests
Sample preparation
The research was conducted according to GOST 12038-84 'Agricultural seeds. Methods for determination of germination'. Sample preparation for germination of each experimental part was carried out by counting 100 grains of chia seeds, each sample was studied in quadruplicate.

Germination of seeds was carried out in ethanol-disinfected Petri dishes, on two layers of filter paper. Pre-prepared chia seed samples were evenly distributed in Petri dishes on paper layers and were moistened with distilled water.

Every day, Petri dishes were opened in a sterile environment for several seconds for air ventilation.

Method of counting germinated seeds
Counting germinated seeds was carried out every day for 7 days. Each Petri dish was numbered and counted accordingly, each experiment (moisture, light, temperature) was independent, and for each sample there were 4 replications. The seed germination energy of the studied samples was determined on day 3, the germination of seeds - on day 7 of the experiment.

Seeds that had formed sprouts were attributed as the 'normally germinated' seeds while the 'not germinated' seeds were hard seeds, which at the time of germination were not swollen and did not change in their appearance.

Moisture test
To assess the effect of the moisture regime on the dynamics of the chia seed germination, samples were prepared with the addition of distilled water in a different volume: sample 1–1 mL of water; sample 2 – 2 mL of water; sample 3 – 3 mL of water; sample 4 – 4 mL of water; sample 5 – 5 mL of water; sample 6 – 6 mL of water. The samples investigated for the effect of the moisture factor were kept at room temperature (25 °C) in a laboratory under natural daylight.

Light test
To analyze the effect of the light factor on the chia seed germination, the test samples were placed as follows: sample 1' – in the absence of light (in a dark place); sample 2' – under natural daylight; sample 3'– under artificial light at a distance of 30 cm from the lamp.

An LED luminaire with a specific wavelength was used to study sample 3'. The line emission spectrum of the installed LED lamps was the maximum value at 440 nm in the blue region of the spectrum and 660 nm in the red region. The test was carried out with the best previously detected values of moisture regime and at room temperature (25 °C).

Temperature test
A study to determine the optimal temperature for chia seed germination was carried out with given temperatures: sample 1' – 20 °C, sample 2' – 25 °C, sample 3'– 30 °C with optimum water mass fraction and values of light exposure detected in previous stages.

Evaluation methods

The arithmetic mean value (average) of the results in the above experiments for four replicates was taken as the analysis result.

The speed of germination and the seedling vigor were determined.

The speed of germination characterizes the weighted average number of days for one seed to grow. This indicator (in days) was calculated by the formula (1):

$$\text{The speed of germination} = \frac{(A1 \cdot 1) + (A2 \cdot 2) + \cdots + (An \cdot n)}{(A1 + A2 + \cdots + An)} \qquad (1)$$

where A(n) is the number of seeds germinated in 1, 2, ... n day of germination; 1, 2,… n is the day of seeds germination.

The seedling vigor determines the average number of seeds germinated in one day. This indicator (in grains) was calculated by the formula (2):

$$\text{The seedling vigor} = \frac{A}{N} \qquad (2)$$

where A is the number of germinated seeds (in terms of 100 seeds) for the entire period of experiment (7th day) or full germination; N is the number of days of seeds germination (7 days).

Statistical analysis

The experiments were performed in quadruplicate. The data were processed by the method of mathematical statistics with using MS Excel. The value of the results is represented by the average and the standard deviation obtained using Student's criterion under the condition of 0.95 confidence intervals.

RESULTS AND DISCUSSION

In this study, for the first time, a comprehensive assessment of the influence of some of the major external factors on the process of obtaining chia seedlings (sprouts) was carried out. The optimal regimes for the production of Salvia hispanica L. sprouts were revealed.

The following parameters were studied as input factors of the experiment: the effect of the added water amount, the effect of light, and the effect of temperature on the process of chia seed sprouting.

At the first stage of the study, the influence of the moisture factor was evaluated. Samples under the numbers corresponding to the amount of water added, mL, were used as the test samples.

Table 1 presents the results of counting chia seeds within 7 days. According to the data obtained, all samples began to germinate on day 3. The amount of introduced moisture practically did not affect the seed germination energy of chia at the initial stage. However, the dependence on the specified factor was noted during subsequent observation, in particular, from 4 to 7 day of the experiment.

Samples 1 and 2 dried up on the fourth day due to insufficient volume of applied water, providing on the third day of observation 40 and 42% of germinated seeds, respectively. This volume, like the volume of 2 mL of water, was insufficient for the germination of 100 chia seeds. Further growth dynamics of chia seeds in these samples was not observed.

Table 1. The dynamics of chia seed germination depending on the moisture regime, %

Sample No.	Day 1a	Day 2a	Day 3a	Day 4a	Day 5a	Day 6a	Day 7a
1	0.0	0.0	40.0 ± 1.6	40.0 ± 1.6	40.0 ± 1.6	40.0 ± 1.6	40.0 ± 1.6
2	0.0	0.0	42.0 ± 1.3	42.0 ± 1.3	42.0 ± 1.3	42.0 ± 1.3	42.0 ± 1.3
3	0.0	0.0	42.0 ± 2.1	71.0 ± 1.9	85.0 ± 0.8	88.0 ± 0.6	90.0 ± 1.2
4	0.0	0.0	44.0 ± 1.4	78.0 ± 0.5	86.0 ± 1.3	88.0 ± 2.1	92.0 ± 1.0
5	0.0	0.0	40.0 ± 2.8	78.0 ± 0.8	85.0 ± 1.3	86.0 ± 1.8	90.0 ± 0.8
6	0.0	0.0	39.0 ± 0.8	70.0 ± 1.4	73.0 ± 1.4	72.0 ± 1.3	73.0 ± 1.4

[a]Average ± standart deviation ($n = 4$).

In sample 4 on the third day of the experiment, it was 44% germinated seeds (the seed germination energy), by the end of the experiment, it was 92% (the germination of seeds). Samples 3 and 5 showed similar dynamics. In sample 6, there was an excess of introduced moisture, which negatively affected the process of seed germination and, by the end of the experiment, this set showed the least amount of germinated seeds in comparison with samples 3, 4 and 5.

Fig. 1 shows the results in the most representative samples with the addition of 1, 4, 6 mL of water, which confirms the above-described results of the study.

Figure 1. The dynamics of chia seed germination depending on the moisture regime (from left to right 1, 4, 6 mL, respectively) on the 7th day of observation.

The seed germination energy, the germination of seeds, the speed of germination and the seedling vigor were calculated for the most viable samples of the experiment and are shown in Table 2.

Table 2. The influence of the moisture regime on the ability of chia seeds to germinate for samples 3, 4 and 5

Indicators	Sample 3[a]	Sample 4[a]	Sample 5[a]
The speed of germination, days	4.0 ± 0.1	5.2 ± 0.1	3.8 ± 0.1
The seedling vigor, grains	12.9 ± 0.2	13.1 ± 0.1	12.9 ± 0.1
The seed germination energy, %	42.0 ± 2.1	44.0 ± 1.4	40.0 ± 2.8
The germination of seeds, %	90.0 ± 1.2	92.0 ± 1.0	90.0 ± 0.8

[a]Average ± standart deviation ($n = 4$).

Thus, in terms of the seed germination energy, the germination of seeds, the speed of germination and the seedling vigor, the optimal values were obtained in sample 4. It was noted that insufficiency or excess moisture had a negative effect on the chia seed germination, but does not affect the seed germination energy, which on an average for chia seeds was about 40%.

At the second stage of the experiment, the effect of light on the chia seed germination was determined. The effect of the intensity and spectral composition of light on the efficiency of photosynthesis and productivity of various plants was studied by Protasova, 1987; Protasova et al., 1990. According to the research, it was found that the most favorable condition for growing light-loving plants were light intensities within 150–220 W m^{-2}. The optimal composition of radiation had the following ratio of energy over the spectrum: 30% in the blue region (380–490 nm), 20% in the green (490–590 nm) and 50% in the red region (600–700 nm). In general, light of the visible radiation spectrum or photosynthetically active radiation 390–710 nm has a favorable effect on the development of the plants, in particular, in the processes of chlorophyll formation, cell division and stretching, plant growth, etc. (Massa et al., 2008). The blue spectrum with a wavelength in the range of 430–470 nm contributes to the synthesis of chlorophyll, inhibits the growth of the stem, and red with a wavelength in the range of 640–660 nm promotes flowering and growth of the stems (Massa et al., 2008; Olle & Viršile, 2013). There is also evidence that the optical spectra of the red and blue regions with wavelengths of 640–660 nm and 430–460 nm, respectively, are most effective for plant growth, in particular, the 660 + 450 nm mode (Kondratyeva et al., 2018).

Table 3 presents the results of counting chia seeds within 7 days. As at the previous stage of the study, all samples began to germinate on day 3. Obviously, light exposure did not significantly affect the number of germinated seeds, which correlates with previous data (Stefanello et al., 2015b; Paiva et al., 2016).

Table 3. The dynamics of chia seed germination depending on the light, %

Sample No.	Day 1 [a]	Day 2 [a]	Day 3 [a]	Day 4 [a]	Day 5 [a]	Day 6 [a]	Day 7 [a]
1'	0.0	0.0	46.0 ± 0.8	73.0 ± 1.6	83.0 ± 1.4	86.0 ± 1.3	91.0 ± 1.3
2'	0.0	0.0	46.0 ± 1.7	72.0 ± 1.3	83.0 ± 1.0	85.0 ± 0.5	89.0 ± 0.5
3'	0.0	0.0	47.0 ± 2.6	71.0 ± 0.5	85.0 ± 1.3	88.0 ± 0.5	91.0 ± 0.5

[a]Average ± standart deviation ($n = 4$).

However, the study results showed that the line emission spectrum of the LED lamps with peaks at 440 nm in the blue region and 660 nm in the red region of the spectrum was optimal for the process of chia seed germination.

The seed germination energy, the germination of seeds, the speed of germination and the seedling vigor were calculated by samples of the experiment and are shown in Table 4.

Table 4. The influence of the light factor on the ability of chia seeds to germinate

Indicators	Sample 1'[a]	Sample 2'[a]	Sample 3'[a]
The speed of germination, days	5.2 ± 0.2	5.2 ± 0.2	5.3 ± 0.2
The seedling vigor, grains	13.0 ± 0.2	12.7 ± 0.1	13.0 ± 0.1
The seed germination energy, %	46.0 ± 0.8	46.0 ± 1.7	47.0 ± 2.6
The germination of seeds, %	91.0 ± 1.3	89.0 ± 0.5	90.0 ± 0.5

[a]Average ± standart deviation ($n = 4$).

According to the above indicators, it could not be defined the leading sample, since all samples had relatively identical values.

Fig. 2 shows the dynamics of chia seeds germination in the samples on the 7th day of the experiment.

Figure 2. The dynamics of chia seed germination depending on the light factor (sample 1', 2', 3' from left to right, respectively) on the 7th day of observation.

Fig. 2 demonstrates the formation of various pigments in the studied samples. The germination of chia seeds in the dark ensures the formation of pale yellow etiolated cotyledons, with an elongated stem and undeveloped leaves, which indicates that chlorophyll synthesis was absent in this sample.

The germination of chia seeds in natural daylight allowed obtaining sprouts of a pale green color, which also indicated an insufficient synthesis of chlorophyll in the sample.

Only the impact of the LED lamp ensured the growth of saturated green color due to the formation of a sufficient amount of chlorophyll in the chia seeds sprouts.

Thus, the optimal conditions for the germination of chia seeds were detected with light exposure with specific wavelengths, in particular 440 nm in the blue region and 660 nm in the red region of the spectrum, since the greatest synthesis of chlorophyll was observed (green, not elongated stem and opened leaves).

The presented data confirmed the feasibility of using LED lamps to obtain a qualitative composition of chia seedlings. Another advantage of LED lamps was low heat generation, so they could be placed in the immediate vicinity of the plants without the risk of damaging them, which was used in this experiment.

At the third stage, the effect of temperature on the process of obtaining chia seeds sprouts was studied by varying the temperature factor within 20–30 °C with a step of 5 °C.

Table 5 presents the results of counting chia seeds within 7 days. According to the data obtained, all samples began to germinate on day 3, which did not contradict the studies at the previous stages. Sample 3'' dried on the day 5 of the experiment due to the high temperature and insufficient moisture.

Table 5. The dynamics of chia seed germination depending on the temperature, %

Sample No.	Day 1[a]	Day 2[a]	Day 3[a]	Day 4[a]	Day 5[a]	Day 6[a]	Day 7[a]
1''	0.0	0.0	42.0 ± 0.8	75.0 ± 1.7	83.0 ± 2.5	86.0 ± 1.3	88.0 ± 0.8
2''	0.0	0.0	45.0 ± 1.3	80.0 ± 1.0	86.0 ± 1.5	92.0 ± 1.7	92.0 ± 0.6
3''	0.0	0.0	35.0 ± 1.0	61.0 ± 1.7	68.0 ± 0.8	68.0 ± 0.6	68.0 ± 0.5

[a]Average±standart deviation ($n = 4$).

The seed germination energy, the germination of seeds, the speed of germination and the seedling vigor were calculated by samples of the experiment and are shown in Table 6.

Table 6. The influence of the temperature factor on the chia seeds ability to germinate

Indicators	Sample 1" [a]	Sample 2" [a]	Sample 3" [a]
The speed of germination, days	5.2 ± 0.1	5.3 ± 0.1	5.2 ± 0.1
The seedling vigor, grains	12.6 ± 0.1	13.1 ± 0.1	9.7 ± 0.1
The seed germination energy, %	42.0 ± 0.8	45.0 ± 1.3	35.0 ± 1.0
The germination of seeds, %	88.0 ± 0.8	92.0 ± 0.6	75.0 ± 0.5

[a]Average ± standart deviation ($n = 4$).

Fig. 3 shows the dynamics of chia seeds germination depending on the temperature factor on the 7th day of the experiment.

Figure 3. The dynamics of chia seed germination depending on the temperature (from left to right 20 °C, 25 °C, 30 °C, respectively) on the 7th day of observation.

Therefore, the temperature of 25 °C was determined as the optimum for chia seed germination based on the results of the seed germination energy, the germination of seeds, the speed of germination and the seedling vigor. Previous studies confirm (Stefanello et al., 2015b; Paiva et al., 2016; Possenti et al., 2016; Gómez-Favela et al., 2017) that the influence of temperature factor was an important indicator in relation to chia seed germination. In comparative experiments, it was shown that the germination of chia seeds with low temperature (below 20 °C) and high temperature (above 30 °C) limits plant growth. The optimum range for chia seed germination is 20–25 °C, which correlates with results of this research.

Thus, according to the results of the experiment, the necessary conditions for the chia seed germination were identified for the first time; in particular, the moisture content was 4 mL of water per 100 seeds at a temperature of 25 °C with light exposure at a wavelength of 440 nm and 660 nm. These technological characteristics are recommended for utilization in obtaining chia seedlings using the soil and the hydroponic method of chia seed germination.

CONCLUSIONS

In the framework of this study, the simultaneous influence of moisture, temperature and light factors on the germination of chia seeds (Salvia hispanica L.) was studied for the first time. The optimal amount of water was 4 mL per 100 seeds, based on the seed

germination energy, the germination of seeds, the speed of germination and the seedling vigor. An insufficient or excessive amount of water had a negative effect on the germination of chia seeds.

It was revealed that the chia seed germination with an LED lamp with peaks at 440 nm and 660 nm promoted the accumulation of sprouts green mass and the synthesis of chlorophyll in them. The optimum temperature for germinating chia seedlings under given conditions was defined as 25 °C.

These conditions could be used for year-round cultivation of chia seedlings by the hydroponic method. However, in the further study, the established parameters would require clarification in particular the optimum experiment conditions, taking into account the realization of its multifactorial nature under hydroponic conditions.

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The impact of plant powders on acrylamide content in bakery products

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Abstract. This work is devoted to studying acrylamide (ACR) formation and the changes in its levels display during the storage in bakery products (BP) made of wheat flour enriched with plant powders (in the optimal amounts established earlier): blueberry – 3%; pine nut – 6%; rowan – 5%; sea buckthorn – 5%. BP were baked at two temperatures – 220 and 200 °C. ACR level was determined with the use of 'Kapel 105 M' capillary electrophoresis system in various BP parts (crust, sub-crust layer, crumb) 3 and 24 hours after baking. ACR formation differed in different BP layers. All plant powders slowed down its formation in the crust and the sub-crust layer. The process was influenced by formation of heterocyclic compounds (lactams) as a result of the Maillard reaction. In the crumb, ACR formation depended on the type of the used plant powder. In BP cooked with blueberry and rowan powders, the ACR level decreased, while in BP cooked with sea buckthorn and pine nut powders, it increased in comparison with other layers. Lowering the baking temperature helped to decrease acrylamide formation by 15–20% in the crumb and by 25–35% in the crust. After storing BP for 24 hours, a decrease in the ACR level was found, mainly in the crust and crumb. The intake of ACR in the human body of 70 kg when used with 100 g of BP enriched with plant powders will come to 0.16–0.2 μg. Lowering the baking temperature will decrease ACR level by 3–6%.

Key words: bakery products, acrylamide, crust, sub-crust layer, crumb, plant powders, storage.

INTRODUCTION

Healthy lifestyle trend has promoted the use of raw vegetable and plant supplements, rich in dietary fiber, biologically active substances and antioxidants, in production of bread and bakery products (BP) (Dziki et al., 2014; Kurek et al., 2015; Nilova & Pilipenko, 2016). The main idea is to maximize the level of plant supplements without compromising sensory properties of BP (Bagryantseva et al., 2010; Keramat et al., 2011). All BP, including the enriched ones, along with useful substances, may contain acrylamide (ACR) formed at the final production stage of baking, which has a toxic and carcinogenic effect (Bagryantseva et al., 2010; Keramat et al., 2011). The level of ACR in bread, BP and biscuits can reach up to 3,000 μg kg^{-1} (Mustafa, 2008; Bagryantseva et al., 2010; Krishnakumar & Visvanathan, 2014), with 7.5 times more in biscuits and crackers than in bread and BP (Friedman, 2003).

Formation of ACR depends on the type of flour and other prescription ingredients. Rye bread contains more ACR than wheat bread. Usage of higher yield flour, as well as multi-cereal, potato, and corn supplements in bread production stimulate formation of ACR (Claus et al., 2008; Capuano et al., 2009; Horszwald et al., 2010; Przygodzka et al., 2015). One can significantly reduce ACR levels in BP by regulating technological process of production (duration of the dough fermentation, usage of enzyme preparations and starters, regulation of temperature and steam during baking, selecting a specific type of oven) (Ahrné et al., 2007; Mustafa, 2008; Keramat et al., 2011; Kumar et al., 2014; Przygodzka et al., 2015; Bartkiene et al., 2017). Even long fermentation, typical of sourdough systems, can reduce levels of the amino-acid asparagine that is a precursor of acrylamide formation, by tens of times (Fredriksson et al., 2004).

Plant supplements have an ambiguous impact on ACR formation in BP. Fruits, berries, nuts and their products contain substances that are precursors of ACR. Fruits and berries are distinguished by a high content of glucose and fructose, the content of which varies from their type, place of growth and other factors. So, in lowbush blueberry cultivars the amount of glucose and fructose ranges from 3.69 to 10.35%, depending on the botanical variety (Kalt & McDonald, 1996). Their amount in cultivated (some varieties) and wild blueberries and can be the same (Klavins et al., 2015). But the protein content is very low and does not exceed 0.8%, so the study of the amino acid composition is usually not carried out. The content of glucose and fructose in sea buckthorn averages 3–6%, in some varieties up to 9% (Kuhkheil et al., 2017; Zemtsova, 2017), the asparagine content is 427 mg 100 g^{-1} (Oua et al., 2010, Bal et al., 2011). Nuts and their products contain минор saccharides include glucose (0%–0.27%), fructose (0% –0.17%), maltose (0% –0.2%), but a lot of proteins, in particular pine nut (13.7–15.2%) Acidic amino acids (aspartic acid + glutamic acid) predominate in tree nuts. Aspartic acid content varies from 0.3 to 2.7%, in pine nuts - 2.2% (Nergiz & Donmez, 2004; Chang et al., 2016). Unfortunately, the data on the content of free asparagine - the predecessor of ACR is not installed. Functional groups of polyphenols in raw plant materials, having a variety of structures, can react with ACR precursors, with chemicals formed at intermediate stages of reaction, or with ACR itself, leading to a decrease of ACR levels in BP or, conversely, stimulating its formation (Liu et al., 2015). There is evidence that the formation of ACR reduces to a greater degree oxidized antioxidant than non-oxidized (Oua et al., 2010).

Plant supplements that minimize formation of ACR do not always provide optimal sensory BP properties. For example, the epicatechingallat obtained from green tea, when added to wheat bread in the amount of 3.3–9.9 g kg^{-1} (i.e. less than 1%), reduced the formation of ACR by 34–37%, while impairing the color and texture of the product (Fua et al., 2018). The use of green tea extract in bread sticks contributed to minimizing ACR formation when added in the amount of 0.1%, and in bread crisps – 0.5%. At the same time sensory properties of the products, especially their texture, significantly worsened (Zhang & Zhang, 2007; Capuano et al., 2009). The use of pomegranate flower extract together with vitamin B3 in fried donuts production reduced formation of ACR. When used in the amount of 0.07 and 1.97% respectively, the above supplements did not alter sensory properties of the donuts but in this case, the ACR level was reduced only by 11.8% (Ashkezari & Salehifar, 2018). Replacing wheat flour with date seeds powder in pita bread enriched it with common phenolic compounds, including epicatechins. A slight decrease in ACR formation (in comparison with the regular pita bread) was

established only when the level of the date seeds powder was 5%. When the level of the date seeds powder reached 10–20%, ACR formation increased (Platat et al., 2015). Lack of a linear dependence between the amount of plant supplements and the formation of acrylamide is shown by examples of bamboo leaves powder, date seeds powder, green tea extract, and rosemary extract (Zhang & Zhang, 2007; Hedegaard et al., 2008; Platat et al., 2015).

One can obtain a zero ACR level in BP by adjusting the type and amount of plant supplements, baking temperature, and time of baking. For example, pita bread enriched with lyophilized mint fennel and turmeric extracts in different proportions may not contain ACR, but its organoleptic characteristics are worsened (Namir et al., 2018).

BP with plant supplements, suitable for commercial use, must have acceptable color, good porosity and taste. Thus, plant powders with high level of organic acids and ascorbic acid that strengthen gluten (i.e. made from mountain ash pomace and sea buckthorn powder) should be used in BP recipes with high sugar level (Nilova, 2012; Nilova & Malyutenkova, 2018). Blueberry and pine nut powders contain reducing sugars, so they can be used in production of BP with low sugar level. However, plant powders with high level of anthocyanins, such as blueberry powder (Nilova, 2012; Nilova et al., 2015), affect color quite strongly. Adding them in the amount of more than 3% leads to the appearance of a blue tint in BP, and this lowers its commercial value. At the same time, due to a higher level of reducing sugars in them (as well as in pine nut powder), they can be used in production of BP with low sugar levels. Therefore, when using plant powders in BP production it is very important to optimize sensory properties of BP in order to make a commercially suitable product.

This work was aimed at studying the effects of plant powders on the formation of acrylamide and its change during storage in BP of established recipes in terms of producing a valuable commercial product.

MATERIALS AND METHODS

Bakery products recipe and baking

The BP were produced by a straight dough method from wheat flour (gluten 28.9%, ash content 0.55) according to two recipes with different content of sugar and vegetable oil (i.e. control samples) and the bakery products (BP) made with the added different plant powders (in their optimal ratio, as calculated before) (Nilova, 2012; Nilova et al., 2015; Nilova & Malyutenkova, 2018). The following plant powders were used: blueberry powder, pine nut powder, rowan powder, and sea buckthorn powder. The powders of blueberry, rowan and sea buckthorn were obtained from squeezed berries, dried at 50 to 55 °C (to a moisture level of 6%) and milled into powder. The powders contained glucose and fructose in an amount, %, 11.5, 10.2, 5.6, respectively for blueberries, rowan and sea buckthorn (Shelenga et al., 2015). The content of aspartic acid was 0.62% for rowan and 2.6% for sea buckthorn. Pine nut powder produced by Specialist LLC, Russia, contained 1.2% of glucose and fructose, 34% of protein, and 3.5% of aspartic acid (Nilova, 2012; Nilova et al., 2017). BP recipes presented in Table 1. Baking of products weighing 100 g was made at two temperatures: 200 °C – 25 minutes and 220 °C – 20 minutes. After the bakery products were cooled down to the room temperature, identical samples underwent the specimen preparation procedure at once and after storage in polymer film during 24 hours. The crust, the layer

under the crust with a thickness of 1 cm and the central crumb were separated from the bakery products. Such different parts of the bakery products were dried separately in a cupboard drier at a temperature of 50 °C until the constant weight was reached.

Table 1. Dough recipes

Ingredient, g	Bakery products 4% fat			Bakery products 14.5 % fat		
	control	with powder		control	with powder	
		blueberry	pine nut		rowan	sea buckthorn
Wheat flour	1,000	970	940	1,000	950	950
Blueberry powder	-	30	-	-	-	-
Pine nut powder	-	-	60	-	-	-
Rowan powder	-	-	-	-	50	-
Sea buckthorn powder	-	-	-	-	-	50
Sugar	50	50	40	145	145	145
Sunflower oil	40	40	40	145	145	145
Yeast	20	20	20	20	20	20
Salt	15	15	15	15	15	15

Preparation of samples for the test

Acrylamide was extracted from the component parts of the bakery products: 50% (by volume) with ethanol – 5 mL per 300 mg of the grinded air-dried sample after homogenization – in the ultrasonic bath (250 Wt, 18 kHz) at 20 °C during 30 min. The extract was decanted and clarified by centrifugation (10 min., 3,000 g).

Determination of acrylamide in BP

For acrylamide identification, the 'Kapel 105 M' capillary electrophoresis system was used (NPF Lumex OJSC, Russia) with a quartz capillary having a diameter of 75 μm and total length of 60 cm, effective length of 50 cm (Kruchina-Bogdanov et al., 2018). 50 мM sodium tetraborate, pH 9.2, was used as background electrolyte (BGE). The capillary was washed before the sample was injected: 0.5 M HCl – 5 min., water – 5 min., 0.5 M NaOH – 5 min., water – 5 min., BGE – 10 min.

The capillary was thermostated at a temperature of 20 °C during 10 minutes. Detection was carried out at 202 nm. The detection threshold (signal: noise = 3:1) was 90 μg kg^{-1}. Hydrodynamic sample injection: 50 mbar 15 s.

Electrophoresis was conducted under the voltage of 10 kV.

E-O converter marker (electroosmotic flow) – benzyl alcohol (99.9%), UAHIM, Russia. Acrylamide produced by Reanal, Budapest, Hungary, was used as reference substance.

Calibration series with external standard was linear from 100 to 2,000 μg L^{-1} acrylamide (R^2 = 0.992). Lowest detection limit 30 μg L^{-1}. Slope coefficient for ACR concentration (in μg L^{-1}) vs. peak area (μV s) 0.000136. Recovery after addition of ACR to bread samples was no less than 97%.

The mass concentration of the component in the sample under analysis (X) was calculated by the formula:

$$\tilde{O} = \frac{k \cdot \tilde{N}}{m} \tag{1}$$

where k is the sample dilution coefficient; C is the acrylamide concentration determined by using the calibration graph, µg L^{-1}; m – sample weight, g.

The measurement result is represented in the following form: X$\pm\Delta$ µg kg^{-1} (X is the concentration of the component in the sample, µg kg^{-1}; Δ – the absolute error range in the identification, mg g^{-1}, with the confidential probability of P = 0.90). The arithmetic mean of the results from three parallel identifications was taken as the final result of the test.

Determination of cyclic amides (lactams) in BP

Cyclic amides (lactams) were explored using IR-Fourier spectroscopy in the area of 1,680–1,800 cm^{-1} (Bellami, 1971; Silverstein, 2011). The infrared spectra were determined using the IR-Fourier spectrometer 'ФСМ 1202' produced by Limited Liability Company 'Monitoring', Russia, with counting of automatic peaks relative to the baseline. Spectral registration parameters: spectral range – 400–4,000 cm^{-1}; number of scans – 20; resolution – 4 cm^{-1}; mode – interferogram. Absolute error in calibration of wavenumber scale – not more than ± 0.1 cm^{-1}. Deviation of 100% transmission line from the nominal value (1,950–2,050 cm^{-1}, resolution 4 cm^{-1}, 20 scans) – not more than % ± 0.5. Mean square deviation of the 100% transmission line (1,950–2,050 cm^{-1}, resolution 4 cm^{-1}, 20 scans) – no more than 0.025%. The obtained interferograms were transformed into transmission spectra. The samples for testing were prepared by pressing the crust or crumb of BP with potassium bromide. For the preparation of tablets, an accurate sample of potassium bromide and 2 g of BP were ground in an agate mortar. 100 mg of the mixture was selected and pressed in press moulds for 15 minutes on each side. The identification of lactams was made on the basis of the area of peaks in the region of 1,800–1,680 cm^{-1} (Bellami, 1971, Silverstein, 2011): cyclic amides (lactams), with large rings – near 1,680 cm^{-1}; monocyclic γ-lactams – in the interval of 1,700 cm^{-1}; polycyclic – in the interval of 1,700–1,750 cm^{-1}; monocyclic ß-lactams – in the interval of 1,760–1,730 cm^{-1}; polycyclic, condensed with other cycles - in the interval of 1,770–1,800 cm^{-1}.

The mean ACR level in 100g of BP was determined with account of proportions of the layers according to the following formula:

$$ACR = 0.32ACR_{ct} + 0.35ACR_{scl} + ACR0.33ACR_{cb} \qquad (2)$$

where 0.32; 0.35; 0.33 – layer proportions in BP; ACR_{ct} – ACR level in the crust per 100 g of BP; ACR_{scl} – ACR level in the sub-crust layer per 100 g of BP; ACR_{cb} – ACR level in the crumb per 100 g of BP.

The ACR was recalculated for 100 g of BP taking into account the mass fraction of moisture in the layers after their separation, which was determined by drying the layers to constant mass at 50 °C.

The research was made in triplicate. The reliability of the experimental data was evaluated by methods of mathematical statistics with the use of Microsoft Excel application for Windows 2007. All the results were expressed as means $\pm$ standard deviation and the statistical significance was assessed by Student's t test. To establish statistically significant differences between the values of the experimental BP samples compared to the control in the group, analysis of variance was used (ANOVA). Differences were considered significant at $p \pm 0.05$.

RESULTS AND DISCUSSION

Acrylamide was detected in all studied BP samples (Table 2); its amount depended on the recipe and the specific part of the product. The standard deviation from the mean did not exceed alleged ($P = 90$) and some value (*) correspond to the probabilities $P \geq 0.95$. The results did not contradict the previously published data (Mustafa, 2008; Bagryantseva et al., 2010; Krishnakumar & Visvanathan, 2014). According to the obtained results, the ACR levels in BP, in comparison with wheat flour bread, can exceed the average by 6–10 times due to the presence of sugar and vegetable oil in the recipes (Przygodzka et al., 2015; Bartkiene et al., 2017; Nachi et al., 2018).

Table 2. Content of acrylamide (ACR), µg kg⁻¹ DM, in bakery products, ± standard deviation. Asterisk (*) represents statistical significance of $p \leq 0.05$ (Student's t-test).

Object	Bakery products 4% fat			Bakery products 14.5% fat		
	control	with powder		control	with powder	
		blueberry	pine nut		rowan	sea buckthorn
Baking at 220 °C						
Crust	234.8 ± 10.3*	209.2 ± 9.3*	163.5 ± 7.3*	246.1 ± 21.0	203.0 ± 8,0*	186.8 ± 15.0
Sub-crust layer	210.0 ± 12.3	162.0 ± 7.0*	79.1 ± 6.5	213.6 ± 20.8	120.0 ± 10.5	91.4 ± 8.8
Crumb	226.4 ± 8.2*	150.5 ± 6.2*	265.2 ± 10.0*	244.5 ± 11.2*	110.0 ± 6.6	290.7 ± 23.8
Baking at 200 °C						
Crust	176.4 ± 11.0	136.8 ± 6.1*	117.2 ± 6.0	186.0 ± 14.2	140.6 ± 6.5*	122.5 ± 7.0
Sub-crust layer	166.7 ± 10.0	125,2 ± 8.0	64,5 ± 4.5	171.0 ± 15.0	94.2 ± 8.0	71.2 ± 5.0
Crumb	192.3 ± 10.0	120.0 ± 8.5	217.5 ± 12.0	212.7 ± 12.5	90.8 ± 7.0	232.5 ± 20.0[a]

a – there are no statistically significant differences between BP with plant powders and controls in the group ($p \leq 0.05$).

The highest ACR level was formed in BP which was cooked without plant powders. The amount of sunflower oil, used in the BP recipe, did not have a significant impact on the formation of ACR. The differences were minor and stayed within the error limit. The distribution of ACR in various layers of BP showed the following order: crust > crumb> sub-crust layer, despite the diverse heating temperatures they were exposed to during baking (Pashchenko & Zharkova, 2006).

Formation of ACR in BP, cooked with plant powders, showed fundamentally different results in comparison with the control samples. One of the main mechanisms of ACR formation is the Maillard reaction involving asparagine and hexoses which takes place when the temperature rises above 120 °C with maximum evaporation of water (Liu et al., 2015), which is typical for BP crust. Lacking sufficient data free asparagine content in plant powders, one can observe that ACR formation in BP correlates with free hexoses level in blueberry > rowanberry > seabuckthorn berry > pine nut. It was possible to distinguish two regularities that are associated with the presence of plant powders and do not depend on the amount of sunflower oil in the recipe. In BP cooked with blueberry

and rowan powders with different amounts of sunflower oil, distribution of ACR among the layers of BP was as follows: crust > sub-crust layer > crumb. The amount of ACR in the crust was almost the same. In the crumb and the sub-crust layer the amount of ACR was less in BP cooked with rowan powder by 27.0 and 25.9%, respectively. Perhaps this was due to the fact that the rowan powder level in the recipe was by 2% higher than the level of blueberry powder.

PB enriched with pine nut and sea buckthorn powders displayed the predominant formation of ACR in the crumb, which was the highest among all BP samples and their parts. The lowest percentage of ACR was found in the sub-crust layer; it was less than in the crust and crumb by 2 and 3 times respectively, both in BP enriched with sea buckthorn powder, and with pine nut powder. The principal differences between sea buckthorn and pine nut powders from blueberry and rowan powders are the absence of anthocyanins and the fat content (18.5% in sea buckthorn powder and 20% in cedar powder). Sea buckthorn and cedar fats contain many tocopherols and carotenoids. (Chang, 2016; Kuhkheil, 2017; Zemtsova, 2017). Due to the uneven heating of the dough during the crumb formation time, the highest baking temperature does not exceed 90–95 °C (Pashchenko & Zharkova, 2006). Such temperature does not destroy the fat-soluble antioxidants. As a result, they can prevent oxidation of sunflower oil, and formation of peroxides which can interact with ACR, reducing the ACR level (Hedegaard et al., 2008). Currently, data on the effect of peroxides on reducing ACR formation are controversial. In model systems, the addition of peroxides and oxidized antioxidants led to a decrease in the formation of ACR (Oua, 2010), in oxidized oil, the formation of ACR was lower than in non-oxidized, due to its polymerizatio (Hedegaard et al., 2008). Acrylamide concentration of potato chips ranged between 525 µg kg^{-1} (fried in oxidized oil, 12 h) and 722 µg kg^{-1} (fried in unoxidized oil) Karademira et al., 2019). At the same time, a direct relationship was established between the formation of peroxides in unsaturated vegetable oils and the formation of ACR. The highest amount of ACR was formed in model systems with soybean oil, heated at 180 °C, which had the highest peroxide value. More unsaturated corn oil formed less ACR and peroxides (Daniali, 2016).

As ACR is markedly volatile with steam (Dunovská et al., 2006), one can assume that it diffuses with water vapors in every direction from the crust: into both external atmosphere and internal layers of crumb, the ratio between these two processes being controlled by penetrability of outer crust layer and kinetics of formation of the latter. So, the physical diffusion rates can contribute into ACR allocation in BP strata, and into ACR losses with storage.

For the purpose of reducing formation of ACR in BP, a lower baking temperature of 200 °C was used (Przygodzka et al., 2015), which required an increase in baking time by 5 minutes to obtain the optimal sensory properties. In all types of BP, regardless of the recipe, formation of ACR decreased at different rates depending on the part of the product (Table 2). In the crust, formation of ACR decreased by 25–35%. Plant powders contributed to reduction of ACR formation by 28–35%, whereas in the BP cooked without plant powders, formation of ACR decreased by 25% only. In the crumb and the sub-crust layer decrease of ACR formation did not exceed 20–22% due to the uneven heating of the dough layers during the crumb formation time. The temperature of the central crumb layer and the layer bordering with the crust does not exceed 100 °C; but

the time it takes to reach this temperature differ, as well as the duration of the crust exposure during baking (Pashchenko & Zharkova, 2006).

One of the main mechanisms of ACR formation is the Maillard reaction which takes place when the temperature rises above 120 °C with maximum evaporation of water (Liu et al., 2015), which is typical of BP crust. Plant powders have changed the process of heterocyclic compounds (lactams) formation in different layers of BP; the results are presented in Fig. 1 as a sum of mono- and polylactams of γ- and β-forms, and lactams with large rings.
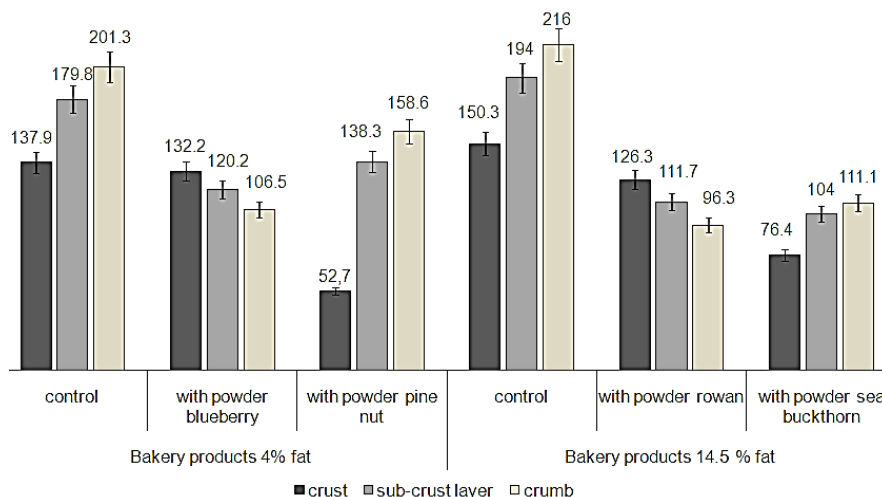

Figure 1. Content of total lactams in bakery products, relative standart units, ± standard deviation ($p \leq 0.05$, Student's t-test).

The nature of reaction of heterocyclic compounds formation depended on both in which BP part it took place, and which plant powders were used. In BP enriched with blueberry and rowan powders, which included anthocyanins, the amount of lactams in the crust was greater than in the sub-crust layer and in the crumb. BP enriched with sea buckthorn and pine nut powders as well as control samples, showed the opposite dependence: crumb > sub-crust layer > crust. In general, lactam formation in BP enriched with powders went slower in comparison with control samples. But a close relation (R^2) between the formation of lactams and ACR was established only in the crust (Table 3). We can assume that ACR formation depends on the Maillard reaction in the

Table 3. Relation (R^2) between acrylamide content and total lactams in bakery products after baking at two temperature regimes

Baking temperatures	Part of bakery product		
	crust	sub-crust layer	crumb
220 °C	0.871	0.635	0.219
200 °C	0.725	0.671	0.296

sub-crust layer ($R^2 > 0.6$) and does not depend on it in the crumb. However, it is impossible to firmly assert and draw conclusions, since the vegetable powders used for the production of BP are significantly different in chemical composition. When baking temperature was decreased, relation between the formation of lactams and ACR in the crust decreased, while increasing in the crumb and the sub-crust layer.

During storage, the decrease in ACR content is dependent on humidity and storage conditions of BP, such as packaging, temperature, and relative humidity. The results of the study of the content of ACR in BP after 24 hours of storage in the polymer packaging are presented in Table 4.

Table 4. Content of acrylamide (ACR), µg kg^{-1} DM, in bakery products in 24 hours after baking, ± standard deviation. Asterisk (*) represents average results with deviations $p \leq 0.05$ (Student's t-test)

Object	Bakery products 4% fat			Bakery products 14.5 % fat		
	control	with powder		control	with powder	
		blueberry	pine nut		rowan	sea buckthorn
Baking at 220 °C						
Crust	79.0 ± 4.0	67.6 ± 4.1	53.9 ± 4.5	85.2 ± 8.0	64.5 ± 6.1	61.1 ± 5.0
Sub-crust layer	115.3 ± 6.5	104.7 ± 6.8[a]	73.2 ± 4.0[b]	121.5 ± 10.8	94.8 ± 8.8	84.2 ± 8.0[b]
Crumb	51.4 ± 4.4	39.4 ± 4.0	57.3 ± 3.9[a]	54.9 ± 4.3	30.6 ± 3.0	65.0 ± 5.9[a]
Baking at 200 °C						
Crust	65.3 ± 4.0	45.4 ± 4.0	39.1 ± 3.0	64.0 ± 3.0*	45.5 ± 3.9	40.8 ± 4.0
Sub-crust layer	92.5 ± 8.2	80.7 ± 7.5[a]	60.8 ± 4.2[b]	95.2 ± 8.6	78.5 ± 4.5	66.5 ± 4.7[b]
Crumb	44.9 ± 3.8	32.5 ± 3.0	48.3 ± 4.1[a]	48.0 ± 3.9	27.2 ± 2.0	50.5 ± 3.8

a – there are no statistically significant differences between BP with plant powders and controls in the group ($p \leq 0.05$); b – there are no statistically significant differences between BP before and after storage in the group ($p \leq 0.05$).

After storage, BP contained less ACR in all layers compared to freshly baked products. The absence of statistically significant differences was found in the sub-crust layer BP with blueberries and in the crumb and in the sub-crust layer BP with pine nut powder and sea buckthorn compared with the control of the corresponding group. The decrease in ACR content had a general pattern: crumb > crust > sub-crust layer. In the crumb, its amount decreased 3.6–4.5 times, depending on the formulation of BP. The smallest amount of ACR in the crumb of BP is found in products with rowan powder, despite the fact that its amount during storage has decreased only 3.6 times.

The partial redistribution of moisture during starch retrogradation (Pashchenko & Zharkova, 2006), which occurs during the storage of BP, appears to dissolve free ACR, which gradually migrates from the crumb to the crust. In the sub-crust layer, the amount of ACR was 1.3 times more than in the crumb in BP with sea buckthorn and pine nut powders, 2.2 times in BP without plant components and 2.6 and 3 times in BP with blueberry and rowan powders respectively. The overall decrease in the amount of ACR in the sub-crust layer during storage was from 8–9% in the CIB with pine nut and sea buckthorn powders to 44–45% in BP without plant components.

At the same time, the decrease in ACR content in the crust was more significant than in the sub-crust layer – from 2.7 to 3.1 times, with a predominance in BP with plant powders. ACR may have volatilized from the surface of the crust. BP with plant powders contained less ACR in the crust than control samples. The influence of the type of plant powders on the content of ACR in the crust of BP was not established, there were no statistically significant differences.

During storage, the content of ACR in BP baked at 200 °C decreased similarly in BP baked at a higher temperature. ACR prevailed in the sub-crust layer of BP irrespective of their formulation; in the crust and in the crumb its predominance

depended on the plant powders used. The predominance of ACR in the crust compared to the crumb was determined in BP with blueberry and rowan powders, and in the crumb compared to the crust in all other BP.

The amount of ACR entering the human body with 100 g of BP, taking into account its content in different layers, is presented in Table 5. The proportion of the crust and sub-crust layer in BP depends on the mass of the product: the smaller the mass of BP, the greater the proportion of the crust and sub-crust layer. At the same time, the crust of freshly baked of BP has less moisture than the sub-crust layer by almost 4.5 times and 5 times than the central layer of the crumb. All this was taken into account when calculating the average content of ACR in BP.

Table 5. The amount of ACR entering the human body with 100 g of BP

| ACR content | Bakery products 4% fat | | | Bakery products 14.5% fat | | |
| | control | with powder | | control | with powder | |
		blueberry	pine nut		rowan	sea buckthorn
Baking at 220 °C						
µg 100 g^{-1} BP	16.55	13.08	12.23	17.33	11.03	13.73
µg per kg in a 70 kg-body	0.24	0.19	0.17	0.25	0.16	0.20
Baking at 200 °C						
µg 100 g^{-1} BP	13.10	9.45	9.47	13.92	8.24	10.14
µg per kg in a 70 kg-body	0.19	0.14	0.14	0.20	0.12	0.14

In BP baked at 220 °C, the content of ACR prevailed in products without the addition of plant powders; the reduction of sunflower oil in the formulation of BP decreases the content of ACR from 17.33 to 16.55 µg 100 g^{-1}. In other BP, the content of ACR decreased depending on the used plant powders: sea buckthorn > blueberry > pine nut > rowan.

If a person with an average weight of 70 kg-body consumes 100 g of BP, then the consumption of ACR will be 16–24% of the prescribed average amount of food (Bagryantseva et al., 2010). The use of lower baking temperatures reduces the amount of ACR in BP by 20–28%, and this process is more pronounced in BP with plant powders, especially in BP with blueberry powder (27.8%) and sea buckthorn powder (26.15%). 100 g of BP with vegetable powders, baked at 200 °C, will deliver 3–6% less ACR into the human body per kg of body weight.

CONCLUSIONS

ACR formation in BP enriched with plant powders occurs unevenly in different layers, predominantly in the crust in BP cooked with blueberry and rowan powders, and in the crumb in BP cooked with buckthorn and pine nut powders. There are no statistically significant differences between ACR formation in different layers of BP cooked without plant powders with different amounts of fat in the recipes. It is established that ACR formation in the crust has a close relation ($R^2 = 0.871$) with the Maillard reaction and the formation of heterocyclic compounds (lactams). One can assume that there exists a dependence of ACR formation on the Maillard reaction in the sub-crust layer ($R^2 = 0.635$), but no such dependence was displayed in the crumb due to the use of plant powders significantly differing in chemical composition. Lowering the

baking temperature leads to a decrease in ACR formation by 15–20% in the crumb and by 25–35% in the crust.

During storage of BP in polymer packaging for 24 hours, ACR is redistributed in its layers showing a tendency to reduce its level, which has a general pattern: crumb > crust > sub-crust layer. In the crumb, ACR level decreased by 3.6–4.5 times, depending on the BP recipe. Generally, ACR migration within BP can be attributed to physical diffusion with water in condensed or vaporized state.

When using 100 g of BP enriched with plant powders, the intake of ACR in the human body, calculated with account to its levels in different layers, will come to 0.16–0.20 µg per kg of the body weight, and when the baking temperature is reduced to 200 °C, it will also reduce to 0.12–0.14 µg.

The results obtained during the formation of ACR in different parts of BP using capillary electrophoresis require further research, both in model systems and using the LC-MS/MS method recommended by EFSA.

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Effect of ultrasonic treatment on the dissolution of milk solids during the reconstitution of skim milk powder

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Abstract. The producing reconstituted milk products that retain the same sensory properties as those of raw milk products is of high interest to the food industry In the technology of producing reconstituted milk processing products, the most significant factor that determines the component transition degree and the usefulness of the product being produced is the recombination process. It determines the possibility of bringing the organoleptic characteristics of reconstituted milk to the properties of the genuine one. One promising method to improve the process of milk powder recombination is ultrasonic exposure. The aim of the present study is to improve the process of milk powder recombination using ultrasonic exposure. The results of the conducted studies show that the ultrasonic treatment eliminates the agglomerates of dried milk particles in water and provides more accessible interaction between the particles and water, and as a result, improves the recombination process. The application of ultrasonic treatment during the reconstitution of the skim milk powder improved the dissolution of milk solids, as evidenced by around a 75% reduction in the amount of centrifuged insoluble sediment. The mass fractions of protein and lactose have increased by 4.8 and 6.5%, respectively.

Key words: reconstituted milk products, ultrasonic exposure, skim milk powder.

INTRODUCTION

The food and pharmaceutical processing industry is an important, system-forming sphere of the national economy, forming the agro-food market and the food and economic security of Russia. The modern dairy industry is characterized by significant changes towards smoothing the seasonality of the dairy production and the activation of the processes of creating large dairy farms implementing modern technological solutions for fodder preparation, feeding, handling and milking.

Currently, Russian dairy market is in a difficult situation. There are many problems, such as ireduction in the number of cows, significant proportion of semi-subsidence households in the production of raw milk, significant increase in the cost of milk production and processing, poor development of the raw material base, increase in the proportion of falsified dairy products in the diary market. All these factors restrain the ramp-up of the dairy production, do not allow modernizing the production and increase its efficiency, impede imports phase-out in the Russian dairy market.

At the same time, increased production of dried milk. Taking into account a large share of both domestic and imported dried milk in the production of milk products, the modernization of the technology for the production of dairy products based on or using dried milk remains an important issue (Andersson et al., 2018).

In the technology of producing reconstituted milk processing products, the most significant factor that determines the component transition degree and the usefulness of the product being produced is the recombination process. It determines the possibility of bringing the organoleptic characteristics of reconstituted milk to the properties of the genuine one.

The recombination process is a heterogeneous chemical reaction between a solid substance and a liquid and is accompanied by a transition of the substance to the solution.

The essence of the dissolution process lies in the interaction of dried milk products with water and includes several stages: dissolution of lactose and mineral substances, distribution of protein and fat in the solution, hydration of the dispersed phase, separation of excess air from the product. The intensity of the process and its effectiveness are determined by the properties of both dried milk and water.

The water that is a part of milk and dairy products is heterogeneous in its physicochemical properties, and its role is unequal. Most of the milk water (84.5–85%) is in a free state, i.e, it can participate in biochemical reactions. Free milk water is a solution of various organic and inorganic substances (sugar, salts, etc.). It can be easily turned into ice when milk is frozen or removed during thickening and drying.

A smaller portion (3–3.5%) of the water is in the bound state: the adsorption-bound water is retained by molecular forces at the surface of colloidal particles (proteins, phospholipids, polysaccharides). The stability of protein particles, as well as milk fat globules, depends on the properties of the hydrated shells. The subsequent layers of the water molecules are bound to protein by less strong bonds, and its properties do not differ from those of free water; chemically bound water is the water of crystalline hydrates, or crystallization water. In milk, the crystallization water is bound to the crystals of milk sugar. Bound water does not freeze at low temperatures (below –40 °C), does not dissolve salts and sugar. Bound water cannot be removed from milk during drying. The amount of bound water is usually used to judge on the hydrophility of proteins, i.e. the ability to bind the entire moisture (moisture of the first and subsequent layers).The forms of water bonds and the hydrophility of proteins determine the quality of reconstituted milk processing products.

The main processes that determine the recombination quality are the dissolution of lactose and mineral substances accompanied by the transition of fat and protein to the emulsion-colloidal state. As a result, a dispersion medium is formed, while the dispersity of proteins and fat should correspond to their dispersity in genuine milk. During the whole recombination process, excess air is released from the product particles, and the gas release rate influences the intensity of the course of other recombination stages (Chandan & Kilara 2011; Chalupa-Krebzdak et al., 2018).

Retrieved from www.scopus.comAt the first stage, when contacting water, lactose, mineral substances and whey proteins leach from the surface of the dried milk particle, then, water penetrates into the cracks and capillaries of the particle, displaces the air and leaches lactose and mineral substances from the inside of the dry matter. All these lead to the decay of the particle, and the insoluble components - fat and protein - are dispersed in the solution. However, particles in dried milk can be not only individual, but also in

the form of agglomerates, which are not dissolved for a long time. It has been established that when the agglomerates contact water, a liquid layer having a high concentration and viscosity is formed on their surface. This layer forms a shell that prevents penetration of water inside the agglomerate. Lactose and protein also determine the wetting properties of the dried milk particles: lactose is less subject to physical and chemical changes in production, so when reconstituted, it is well wetted with water and does not impede water impregnation of the milk powder layer. The wetting properties of protein depend on the degree of its denaturation: the less denatured the protein is, the worse it is wetted, however it has a high dissolution rate. Milk generally contains about 3.2% of proteins, the fluctuations range from 2.9% to 3.5%. These include casein, whey proteins and proteins of fat globule membranes (Liu et al., 2018).

Casein is actually a food protein that performs a new structure function in an organism. In addition, casein conveys calcium, phosphorus and magnesium in the composition of its particles. In milk, casein is in the form of specific particles, or micelles, which are complex aggregates of casein fractions with colloidal calcium phosphate (McCarthy et al., 2017; Lucey & Horne, 2018). The hydrophilic properties of casein depend on the structure, the charge value of the protein molecule, the pH of the medium, the concentration of salts and other factors. The stability of casein micelles in milk depends on the hydrophilic properties of casein. The hydrophilic properties of casein influence the ability of the acid and acid-rennet clot to retain and release moisture. Milk casein is contained in the form of a complex aggregate of calcium caseinate with colloidal calcium phosphate, the so-called caseinate calcium phosphate complex (CCPhC). The CCPhC also contains a small amount of citric acid, magnesium, potassium and sodium.

Whey proteins make up about 0.6% of proteins, they consist of |β-lactoglobulin, α-lactalbumin, immunoglobulins, serum albumin, lactoferrin.

Proteins of fat globule membranes are proteins, which are structural elements of fat globule membranes and contribute to their stability during processing.

To accelerate the recombination process, it is necessary to increase the penetrating ability of water, it can be done by mechanical and hydrodynamic methods. To intensify the agglomerate dissolution process, it is expedient to maintain a high viscosity of the dispersion medium. With an increase in the mixing process intensity, the efficiency of the dissolution process increases (Matignon et al., 2015; Hettiarachchi et al., 2018; Mercan et al., 2018; Liu et al., 2019; Wu et al., 2019).

The aim of the present study is to improve the process of milk powder reconstitution using ultrasonic exposure.

The improvement process based on ultrasonic cavitation energy takes place during implementation of mechanisms belonging to high-energy chemistry and is epithermal. Shestakov et al proved that epithermal cavitation treatment of water may destroy its intermolecular hydrogen bonds of cluster structure (thus causing declusterization of water) and may increase its solvency and capacity for dissociation (Shestakov et al., 2010).

Ultrasonic exposure is characterized by the presence of elastic vibrations and waves with the frequency above 15–20 kHz, which determine its specific features in various media. The most important nonlinear effect in the ultrasonic field is cavitation - the appearance of a mass of pulsating bubbles filled with steam, gas or their mixture in the liquid. The movement of bubbles in different directions, their collapse, merging with

each other, etc. generate compression pulses (micro-shock waves) and microflows in the liquid, which contributes to local heating of the medium and the appearance of ionization. As the result of these effects, solid bodies in the liquid are destroyed (cavitation erosion), the liquid is mixed, various physical and chemical processes are initiated or accelerated. The degree and depth of the cavitation processes are determined by the ultrasonic exposure conditions (Gogate, 2011).

The aim of the present study is to improve the process of milk powder reconstitution using ultrasonic exposure.

MATERIALS AND METHODS

Model samples of raw milk were reconstructed by traditional technology and using ultrasound treatment.

Traditional recovery technology: powdered milk is introduced into water at a temperature of 38–45 °C in the ratio of 1:10, actively mixed and aged for 3 hours.

Dry milk samples had the following characteristics: mass fraction of moisture - $2.85 \pm 0.02\%$, fat – $1.1 \pm 0.02\%$, protein – $37.4 \pm 0.1\%$, lactose – $50 \pm 0.1\%$.

Ultrasonic exposure on the sample was performed taking into account the variations in power and duration (120, 180 and 240 W for 1, 3 and 5 minutes) at various stages: 1) treatment of water (before adding dried milk); 2) jointly dried milk and water, and 3) two-stage treatment (first water and then jointly water and milk powder) (Fig. 1). After adding dry milk to the water treated with ultrasound (option 1), it has been mixing actively for 1 minute. Additional mixing of samples 2 and 3 was not carried out, since ultrasound, when exposed to a mixture of dry milk and water, also contributed to their dispersion.
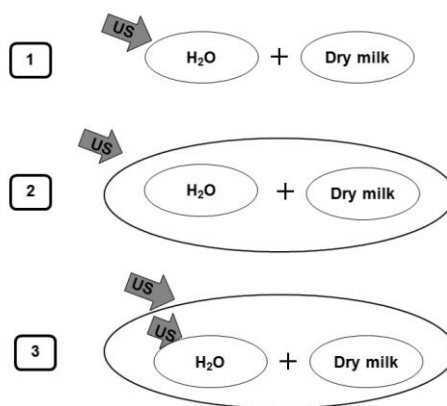
Figure 1. Experiment model of milk powder reconstitution.

To process the studied samples based on ultrasonic exposure, Volna-M ultrasonic technological device (model UZTA-04/22-OM) with the following characteristics was used:

frequency of mechanical oscillations – 22 ± 1.65 kHz;
power – 400 VA;
ultrasonic exposure intensity, no less than 10 W cm^{-2}
radiating surface diameter – 25 mm.

The ultrasonic oscillatory system is built on piezoelectric ring elements and is made of VT5 titanium alloy. The engineering solutions used are protected by the RF patent No. 2141386.

The insolubility indices were determined in a graduated tube after holding the reconstituted sample and centrifuging it for 5 minutes. It is characterized by the amount of insoluble sediment in the reconstituted dry milk product sample.

Thermogravimetric analysis was performed by mass spectrometry analysis of volatile products of thermal decomposition of liquid materials using NetzchSTA 449 'Jupiter' at temperatures from 20 °C to 400 °C. The method is based on recording the thermal effects of transformations occurring under the conditions of a programmed exposure to temperature, and makes it possible to follow the course of the transformation of a substance during the heating process (Duckworth, 1980; Nilova et al., 2017).

Ratio of free and bound water in the samples of recombined raw milk, % was determined by thermogravimetric method, based on the change in mass of the sample of the analyzed products under the influence of temperature 125 ± 2 °C.

The mass fraction of lactose was determined by the refractometric method, based on the determination of the refractive index of protein-free whey.

The mass fraction of skim solids was defined as the difference between the mass fractions of solids and fat. The mass fraction of solids was determined by drying the analyzed sample at a temperature of (102 ± 2) °C. The method of determining the mass fraction of fat is based on the selection of fat from a milk drink under the action of concentrated sulfuric acid and isoamyl alcohol, followed by centrifugation and measuring the amount of released fat in the graduated part of the fat meter.

The mass fraction of protein was determined by the method based on the burning of the organic components of the milk sample in the Kjeldahl flask in the presence of sulfuric acid and catalysts (GOST R 53951-2010).

The statistically significant differences amongst the groups were established using the Kruskal-Wallis criteria. In order to be able to detect statistically significant differences between the two compared groups, the Manna-Whitney criteria (U) were used. The differences were deemed significant where $p < 0.05$. The statistical interconnections were studied using a non-parametric correlational analysis calculating the coefficients of the correlation of rankings according to Spearman (Rs).

RESULTS AND DISCUSSION

The results of evaluating the ratio of free and bound water in reconstituted raw milk (Fig. 2) showed a large proportion of water in the free state (38.01%) in the control sample (produced using the traditional dried milk recombination technology), which in turn is preconditioned by the processes of the ability of the dried milk particles to interact with clusters of water, and protein substances - to the hydration processes. Ultrasonic cavitation through internal explosions improves the separation of water clusters into individual molecules and agglomerates of dried milk into individual particles, which determines the increase in the ability of individual particles to interact with water molecules, promoting their swelling. This fact is illustrated by the data in Fig. 2, which indicates a smaller amount of free water in the raw milk sample processed by ultrasound at the stage of a mechanical mixture of dried fat-free milk and water – 30.9%, which is 18.7% less than the control sample obtained using the traditional technology. Water treatment before adding dried milk allows separating water clusters, however, the agglomerates of dried milk particles that are not broken into individual particles are

probably less accessible to interaction, which increases the free water content in the sample obtained on the water exposed to ultrasonic trratment, to 32.1%.
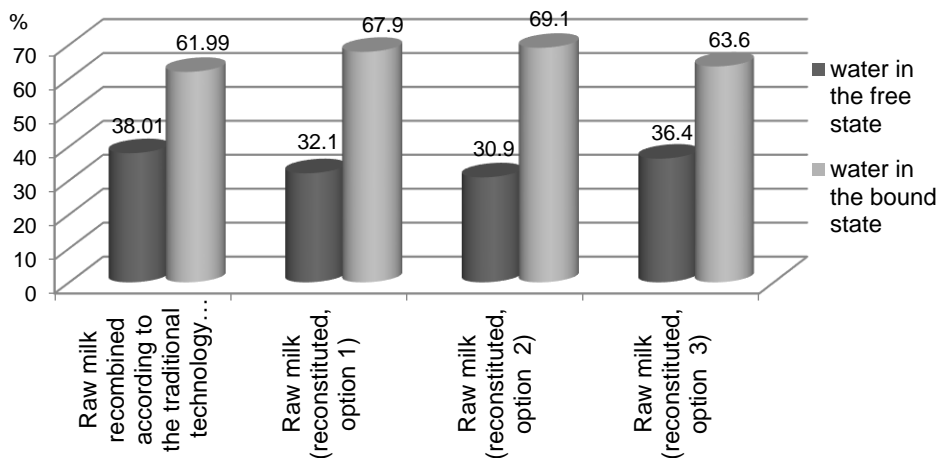

Figure 2. Ratio of free and bound water in the samples of reconstituted raw milk, %.

The state and forms of water bonds in the structure predetermine the uniformity of the composition, resistance of the milk system to various impacts (temperature drops, mechanical treatment, processing with a simultaneous exposure to several factors, etc.). Digestibility and other properties of milk are also characterized, as it has already been noted, by the state and forms of the water bond in the structure. Water in the bound state determines the degree of protein hydration and, as a consequence, its nutritional value (Tsirulnichenko & Popova, 2016).

The aforesaid was confirmed in the subsequent evaluation of the mass fraction of protein in the test samples.

The results of evaluating protein and lactose mass fractions, skim solids of milk drinks made according to the processing schemes modified by ultrasonic exposure, versus the control sample are shown in Fig. 3.

The protein content of the milk produced on the raw milk (control) recombined according to the traditional scheme was 2.69%, which does not meet the requirements of the standard, and one of the reasons for this may be a low reconstitution rate.

The ultrasonic treatment of the mixture of dried fat-free milk and water confirms the previous conclusions on the positive tendencies in the destruction of agglomerates of dried milk particles and water dissociates, which makes them more accessible for the interaction, and as a result, intensifies the reconstitution process (the volume of the insoluble precipitate twice decreased), and confirms the data on increasing water-bearing capacity of the protein fraction (Fatkullin et al., 2017; Silva et al., 2018).

The most intense changes covered the mass fractions of protein and lactose in the milk sample made from raw milk reconstituted according to version 2 (joint ultrasonic treatment of the mixture of fat-free milk and water) by 4.8 and 6.5%, respectively. This eventually led to an increase in the mass fraction of skim solids by 11.6%.
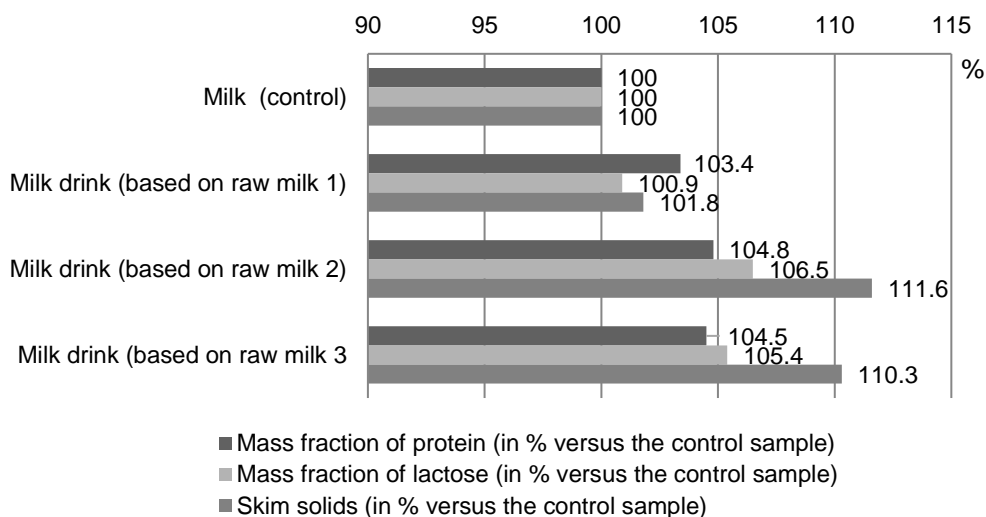
Figure 3. Results of evaluating the mass fraction of protein, lactose, skim solids versus the control sample in test samples, %.

Table 1. Results of evaluating the mass fraction of protein, lactose, skim solids in milk drink samples, % ($P < 0.05$)

	Mass fraction of protein, %	Mass fraction of lactose, %	Skim solids, %
Milk drink (control)	2.69 ± 0.03	3.52 ± 0.03	7.79 ± 0.03
Milk drink (based on raw milk 1)	2.78 ± 0.03	3.55 ± 0.03	7.93 ± 0.03
Milk drink (based on raw milk 2)	2.82 ± 0.03	3.75 ± 0.03	8.69 ± 0.03
Milk drink (based on raw milk 3)	2.81 ± 0.03	3.71 ± 0.03	8.59 ± 0.03

When processing the mechanical mixture of dried fat-free milk and water at the recombination phase, the mass fraction of protein in the milk drink was $2.82 \pm 0.03\%$ (see Table 1). The physical essence of the ultrasonic effect, namely the formation of cavitation bubbles, the rupture of which leads to the development of enormous pressures, is a source of a powerful impact and intensification of physicochemical processes.

Water treatment before adding dried fat-free milk thereto, as well as the two-stage ultrasonic treatment, also yield positive results, intensifying the process of milk powder recombination, which is reflected in the content of the protein fraction in the milk drink – $2.78 \pm 0.03\%$ and $2.81 \pm 0,03\%$ respectively.

The difference in the mass fractions of protein and lactose in samples 2 and 3 is statistically insignificant, fits into the range of experimental error.

The difference in values between skim solids and the sum of mass fractions of protein and lactose may be due to the better recovery of carbohydrates during ultrasound exposure. As it is known, milk contains monoses (glucose, galactose, fructose, etc.), deoxysugar, aminosugar, phosphosugar. The share of mineral substances increases as well (Popova et al., 2014; Potoroko et al., 2018).

The results of increasing the mass fraction of protein in the milk drink samples correlate with the results of decreasing the insolubility index in raw milk (Fig. 4).
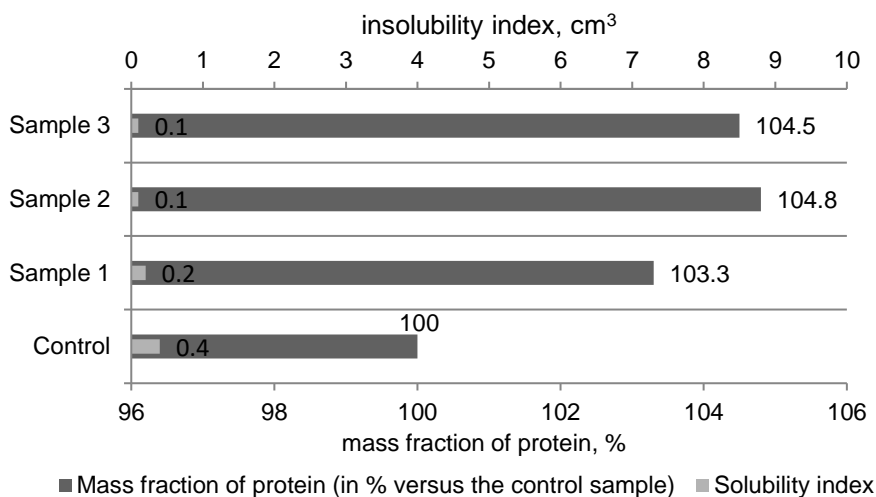
Figure 4. Insolubility indices and relative change in the mass fraction of protein in the samples obtained under different conditions, %.

The correlation coefficient is –0.97, which characterizes a strong feedback. The figure shows that when ultrasonic treatment is introduced into the dried milk reconstitution technology, the insolubility index decreases within the range of 62.5–75% versus the control sample, while the increase in the mass fraction of protein is from 3.3 to 4.8%.

The data on the change in the protein mass fraction content in the samples are correlated with the results of determining the mass fraction of lactose. During reconstitution water leaches the main components from the surface of the dried milk particles in the following sequence: lactose → mineral substances → whey proteins. Then, it penetrates into the agglomerates and leaches these components from the inside of the dry matter. Ultrasonic exposure of dried milk and water in addition to the rupture of the hydrogen bonds in the water also destroys the agglomerates of the particles, which accelerates the interaction of water with individual particles and facilitates the lactose release process. Therefore, the treatment at the stage of the mechanical mixture of dried fat-free milk and water gives a better lactose reconstitution effect ($3.75 \pm 0.02\%$).

Water treatment before the reconstitution of dried fat-free milk due to the rupture of the hydrogen bonds and the occurrence of cavitation processes tends to intensify the process of lactose leaching from the particles surface, which increases the lactose content in the milk drink to $3.55 \pm 0.03\%$.

This index also correlates with the insolubility index -0.8. The increase in the mass fraction of lactose during the ultrasonic treatment is 0.9–6.5% on average, depending on the ultrasonic treatment conditions.

CONCLUSIONS

The application of ultrasonic treatment during the reconstitution of the skim milk powder improved the dissolution of milk solids, as evidenced by around a 75% reduction in the amount of centrifuged insoluble sediment.

The mass fractions of protein and lactose increased by 4.8 and 6.5%, respectively. This eventually led to an increase in the mass fraction of skim solids by 11.6%.

Thus, the results of the conducted studies show for the influence of the ultrasonic treatment, taking into account the exposure stage, on the quality indicators of reconstituted raw milk and its technological characteristics, which are further reflected in the improvement of the quality of milk products including fermented milk products.

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Enrichment of the grains from rye wort after shock-activator-disintegrating processing

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Abstract. In this study**,** the mode of obtaining grains (pellets) from wort prepared from rye processed by the shock-activator-disintegrator (SAD), has been developed. Additionally, the enrichment of the grains by proteins using the strain of yeast producing proteins was carried out. For cultivation of a pure culture of a strain of yeasts producing proteins, grains with a concentration of 43.00 g 100 g^{-1} reducing substances and 62.71 mg g^{-1} of the total amount of amino acid were used. Different concentrations (10%, 20%, 30% and 40% by weight of the grains) of filtrate 24.90% dry matter, 21.00 g 100 g^{-1} reducing substances and 10.82 mg g^{-1} of the total amount of free amino acid was added to the grains. As nitrogen and phosphorus-containing mineral feed, diammonium phosphate was added to the nutrient medium. To obtain a pure culture of *Candida tropicalis, SK-4* yeast strain was isolated. The content of crude protein and the concentration of amino acids were determined. The morphological state of the cells was assessed. The results of this study show that to prepare the nutrient medium for a pure culture of a strain of protein microorganisms-producers and its cultivation, it is necessary to add 30% of the filtrate to the grains, while the proportion of crude protein in the protein-containing additive reaches more than 40%. The resulting protein-enriched product has a balanced amino acid composition, which is of interest as a protein-containing feed additive and its fine particle size distribution allows its use for feeding farm animals.

Key words: shock-activator-disintegrator (SAD), rye filtrate and grains (pellets), proteolytic enzyme complex, *Candida tropicalis*, protein-enriched product.

INTRODUCTION

Shock-disintegrator-activator processing of grain is a promising way of preparing grain raw materials in the process of obtaining the wort. This type of processing allows to carry out deep destruction of starch and to activate own enzymes of grain crops (Alimova, 2018).

By Electron microscopy, it has been shown that during processing there is a deep destruction of the endosperm of the grain. The starchy grains of the endosperm acquire oval-rounded and eye forms, their sizes varying from 25 to 40 microns.

After SDA-processing, protein matrices have a more developed surface. The intermediate protein is separated much easier, freeing more starch granules and partially leaving starch grains in the protein matrix. Moreover, the intermediate protein of the vitreous endosperm is destroyed during grinding together with starch grains strongly attached to it.

According to the electropherograms presented by Sabirov et al. (2018), the flour obtained by grinding on a disintegrator is rich in water-soluble albumin fraction of 17–28 kDa. Herewith, SAD-processing of the grains does not lead to a decrease in the total content of amino acids, the biological value of flour.

From the flour, obtained by processing the grains on the SDA-equipment, the wort is obtained. In order to further enrich the wort and increase its biological value, it is advisable to add proteolytic enzymes (Sabirov et al., 2017b).

Grains or pellets are the residual products obtained in the production of wort. The composition of the grains (pellets) is dominated by cellulose, hemicellulose and indigestible protein. It is the source of nutrients, such as carbohydrates and minerals (Kuznetsov & Ruchai, 2010). However, due to the fact that during the preparation of the wort from the SDA-processed grain, the most complete dissolution of the dry substances of the raw material is achieved. Thus, the energy and biological value of the solid fraction, the pellets, decrease. For this reason, the enrichment of these pellets with nutrients is important. One of such methods is cultivation, on the basis of grains (pellets), of microorganism's strains that produce protein.

The production of feed protein is currently widely used by the yeast of the main *Saccharomycetaceae* family of the genus Candida. They are able to grow on a variety of substrates and give a high biomass yield. Yeast strains, Candida, are commercially introduced, providing a high biomass yield (Azoulay et al., 1980).

Candida is facultative anaerobes. With aeration, the yeast oxidizes sugar in the nutrient medium to water and carbon dioxide (aerobic respiration). The released heat energy is used by yeast for the synthesis of cellular matter and metabolic processes. Under aerobic conditions, much more biomass accumulates in the substrate than during anaerobic respiration. Therefore, cultivation is recommended for continuous aeration of the medium (James et al., 2003; Hosiyev & Plieva, 2014).

The main nutrients for microbial cells are sugars, as a source of energy, and compounds of such macro-elements as nitrogen and phosphorus, which are part of proteins.

Nitrogen and phosphorus can be added to the nutrient medium in the form of mineral additives, for example, diammonium phosphate. As a source of sugar in the nutrient medium, the wort filtrate can be added. It is noteworthy that the wort prepared from SDA-processed grains, with the addition of proteases, will also contain amino acids and low molecular weight peptides that can be absorbed by the cells of inoculated microorganisms.

For cultivation of a pure culture of a strain of a microorganism of a protein producer, for the purpose of preparing a seed material, a grain mash filtrate diluted with water with a mass fraction of dry substances not more than 10% can be used. It is known (Plieva et al., 2015) that enzymatic hydrolysis of a protein contributes to the dissolution of grain components, enriching the wort with biogenic nitrogen: soluble peptides and free amino acids. This allows intensifying the growth of cells of the strain of

microorganisms cultured on a nutrient substrate from the filtrate and wort grains, by introducing a dose of proteolytic enzymes during its preparation.

Thus, it is possible to create an integrated technology for the processing of grains, including cereals with a high content of non-starch polysaccharides. To obtain grains and protein product, the cultivation of strains of microorganisms producing protein on the basis of grains must be carried out. Those grains can be used to create new functional foods or to intensify fermentation in the alcohol industry (Alimova et al., 2014). It is recommended to add the enriched protein product into the diet of farm animals.

Therefore, the aim of this work is to investigate ways of enrichment of grains obtained after SDA-processing of rye with proteolytic enzymes and protein strain *Candida tropicalis.*

MATERIALS AND METHODS

The object of the study was the rye first-class crop harvested in 2018. The used rye contains 8% moisture content, 53% starch content and trash impurities up to 1%.

The moisture content was determined by using Shimadzu MOC-120H moisture analyser (Sabirov et al., 2017a).

The starch content of the barley was determined by using Polarimeter (PolAAFF55). The determination of the starch content of barley was conducted according to ISO/TC 93- Ewers polarimetric method (ISO/TC 93, 1997).

The rye grains were milled using a DESI-15 shock-activator-disintegrator with a five-row rotor (Disintegrator, Estonia). Then, the milled flour was investigated using a Malvern Mastersizer 2000 laser particle size analyser (Malvern Panalytical Ltd, UK). The average integral particle size of flour was 158.1 μm.

Preparation of rye wort

Milled flour (375 g) was measured and transferred into hand-made mash tuns filled with 1,125 mL of warm water (45 °C). The mixture flour and water was then placed in a water bath a 'LOIP LB-163' (Russia) equipped with temperature regulators and a heating system with the constant stirring. Enzyme preparation was then done by adding an enzyme preparation of the thermostable α-amylase 'AmiloLux-ATS' (0.2 units g^{-1} of starch) for the partial hydrolysis of starch. The hydrolysis of starch was carried out for 1 hour at 60 °C.

After that, an enzyme preparation containing glucoamylase 'GlucoLux-A' enzymes (0.9 units g^{-1} of starch) was added for saccharification. The process of saccharification was carried out for 1.5 hours at 60 °C.

Further, the temperature was decreased to 55 °C, and 'Protosubtilin GZx A-120' enzymes (0.5 units g^{-1} of raw material) were added. The mixture was heated again for 1 hour at 55 °C.

Finally, the enzyme preparation of the acid protease 'Pro100L' (0.3 units g^{-1} of raw material) was added, and the proteolysis process was carried out at 55 °C for 1 h. 'Sibbiopharm Ltd' (Berdsk, Russia) manufactured all used enzymes.

The wort was centrifuged for 60 min at 4,600 rpm. The filtrate was separated from a solid fraction (pellets or grains). The concentration of dry matter (%) of the filtrate was measured similarly to that of Nsengumuremyi et al. (2019). The concentration of dry

matter of filtrate was 24.9%. The solid fraction (grains) was dried at 60 °C to 10% moisture content.

Medium preparation and growth of starter culture

The medium used to grow starter culture was prepared by the same procedure as the preparation of rye wort described above with modification. The same dose of amylolytic enzymes was added while different doses of fungal and bacterial proteolytic enzymes were applied: (a) 0.25 units PS (bacterial protease) g^{-1} of raw material + 0.15 units PS (fungal protease) g^{-1} of raw materials; (b) 0.5 units PS (bacterial protease) g^{-1} of raw material + 0.30 units PS (fungal protease) g^{-1} of raw materials; (c) 1 unit PS (bacterial protease) g^{-1} of raw material + 0.6 units PS (fungal protease) g^{-1} of raw materials. The hydrolyzate was centrifuged for 60 min at 4,600 rpm. The liquid fraction (filtrate) was separated from the solid fraction (grains). The filtrate was diluted to $6 \pm 0.1\%$ of dry matter and was used in the preparation of nutrient medium.

As an additional source of mineral elements, diammonium phosphate with a calculation of 0.4% nitrogen and 0.06% per 1 g of the grain was added to the nutrient medium.

The prepared nutrient medium was sterilized at 1 atm., 121 °C for 30 min. The medium was cooled down to 35 °C.

The inoculation of strains of *Candida tropicalis* was carried out in sterile conditions in the laminar cabinet. The growth of strains of *Candida tropicalis* was carried out under aerobic conditions in incubator-shaker (ES-20) at 35 °C at 220 rpm.

Enrichment of biomass with proteins

4 samples were prepared for cultivation. 4 conical flasks were prepared and in each conical flask, 50 g of dried rye solid sediment (grains) were dissolved in 450 mL of water. Then, 1.5 g of carbamide (urea) was added to each sample and acidified with orthophosphoric acid to pH 5.5. As a source of carbohydrate for yeasts, different concentrations of the filtrate were added to every sample. In the 1st sample, 10% (5 mL of filtrate) was added; 20% (10 mL of filtrate) was added to the 2nd sample; 30% (15 mL of filtrate) was added to the 3rd sample and 40% (20 mL of filtrate) was added to the 4th sample. All samples were sterilized in an autoclave (Tuttnauer 2540MK, USA) at 1 atm. for 30 min. The pH of all samples was adjusted to 6 ± 0.1 by adding a 10% solution of orthophosphoric acid. A strain of the yeast *Candida* CK-4, derived by ecological selection, was taken as the protein-producing microorganisms. Cultivation was carried out under conditions of continuous aeration of the medium with an air flow rate of 100 m^3 h^{-1} per medium volume of 1 m^3. Aeration was performed using a Rocker 420 laboratory compressor. After 2 days, the biomass was centrifuged. The supernatant was discarded and the precipitate was dried to $9 \pm 0.3\%$ moisture content.

In the process of cultivation, the control was carried out by counting the cells of the microorganism strain.

Determination of parameters

Alpha-amino nitrogen was determined by the Ninhydrin colourimetric method (Lie, 1973).

The mass fraction of glucose and maltose in the filtrate were determined after the method of oxidation of the aldose group of sugars with iodine (Barakova & Tishin, 2010).

The content of crude protein was determined by the Kjeldahl method on an automated Vadopest installation. The installation includes a burning unit, a distiller and a titrator. The absolute error of the device is not more than 0.5% (Ibatullin et al., 2015).

The haemocytometer was used to count cells of yeasts. The total; budding and dead cell count were determined (Thomson et al., 2015).

The concentration of amino acids was determined using the 'KNAUER' amino acid analyzer: the calculation of aminogram was carried out by comparing the areas of the standard and the sample (Kondratenko et al., 2015).

Data analysis

Data generated were subjected to analysis of variance (ANOVA) using Origin statistical software (version 8.1) at 5% significance. All measurements were made in at least triplicate. Results were reported as means ± standard deviations.

RESULTS AND DISCUSSION

For the growth of microbial culture cells, carbohydrates are used as an energy source. Therefore, the nitrogen-containing substances are needed. In the process of preparing the wort from milled flour, proteins are hydrolysed into peptides and free amino acids. Depending on the complex of proteolytic enzymes activity and applied dose, it is possible to produce wort with various degrees of protein hydrolysis into low molecular weight compounds, which can be estimated by the content of α-amino nitrogen. According to the study of Amelyakina et al. (2012), the data show that the most complete hydrolysis of proteins can be achieved by the combined use of bacterial and fungal proteases. Therefore, it is important to determine the dose of this proteolytic complex that can be added in the preparation of wort. The latter is used as the basis of the nutrient medium for the growth of microorganisms, which will contribute to the maximum growth of cells. Therefore, three samples of filtrate were prepared using different doses of fungal and bacterial proteolytic enzymes.

The concentration of α-amino nitrogen, glucose content, maltose content and the osmolality of filtrate were recorded in Table 1. This filtrate has been used in the preparation of the liquid nutrient medium, in which the protein-producing yeasts were grown.

Table 1. Qualitative parameters of the filtrate used in the preparation of the liquid nutrient medium

Samples	α-amino nitrogen, mg L^{-1}	Glucose content g 100 mL^{-1}	Maltose content G 100 mL^{-1}	Osmolality, mmol L^{-1}
Sample A*	402.7 ± 0.3	10.87 ± 0.06	20.66 ± 0.07	$1,104.66 \pm 0.06$
Sample B**	441.7 ± 0.2	11.03 ± 0.04	20.95 ± 0.05	$1,172.66 \pm 0.02$
Sample C***	503.9 ± 0.2	11.06 ± 0.03	21.02 ± 0.06	$1,265.33 \pm 0.04$

*Bacterial protease (0.25 unit g^{-1} of raw material) + fungal protease (0.15 unit g^{-1} of raw materials); **Bacterial protease (0.50 unit g^{-1} of raw material) + fungal protease (0.30 unit g^{-1} of raw materials); ***Bacterial protease (1 unit g^{-1} of raw material) + fungal protease (0.60 unit g^{-1} of raw materials).

Statistically at the 0.05 significance level, the concentrations of α-amino nitrogen are significantly different. By increasing the dose of bacterial and fungal protease, the concentration of α-amino nitrogen increases. On the other side, the increment of bacterial of fungal protease has not affect the concentration of glucose and maltose. Statistically, at the 0.05 significance level, glucose or maltose contents of 3 samples are NOT significantly different. Regarding the osmolarity, stastically the means are NOT significantly different at 0.05 level.

At the next stage, the filtrate was used to prepare the nutrient medium for a pure culture of *Candida tropicalis*. The concentration of dry matter of filtrate was $24.90 \pm 0.02\%$. Therefore, to dilute pure cultures of microorganism strains of protein producers, the filtrate was diluted with water to a $6 \pm 0.1\%$ of dry matter. Data on the growth of microbial cells after 24 hours are presented in Fig. 1.

The figure above represents how the cells of yeasts strains that produce protein have grown in a nutrient medium. The media are based on the filtrate of wort

Figure 1. The growth of cells of microorganism-producing protein strains in the process of cultivation of pure culture after 24 hours (millions mL^{-1}).

obtained by adding different doses of bacterial and fungal proteases. Sample 1; 2 and 3 represent cell count of protein-producing strains on a nutrient medium based on the filtrate of wort with a dose of Bacterial protease (0.25 unit g^{-1} of raw material) + fungal protease (0.15 unit g^{-1} of raw materials); bacterial protease (0.50 unit g^{-1} of raw material) + fungal protease (0.30 unit g^{-1} of raw materials) and bacterial protease (1 unit g^{-1} of raw material) + fungal protease (0.60 unit g^{-1} of raw materials) respectively.

In all three samples of the pure culture of strains of microorganisms producing protein, dead cells were not detected. The most active cell growth is observed in samples 2 and 3, in which the same indicator is higher than 2–3 times compared to sample 1. However, to obtain a sample of nutrient medium 2, a smaller dose of protease enzymes was applied compared to sample 3.

Therefore, from the point of view of decreasing the cost of ancillary materials and the provision of the most active cell growth, it is important to prepare wort, as a nutrient medium for isolation and cultivation of a culture of microorganisms, with a dose of bacterial protease 0.50 unit g^{-1} of raw material and fungal protease 0.30 activity unit g^{-1} of raw material.
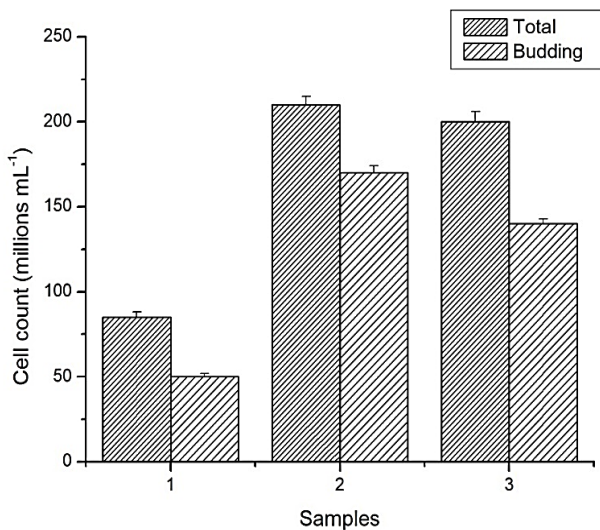
For the cultivation of microorganisms, such basic groups of substances as carbohydrates and available nitrogen-containing substances are needed. Based on this, an analysis of the carbohydrate composition and free amino acids in the filtrate and the grains was carried out. The data are presented in Tables 2 and 3, % of absolutely dry substance.

Statistically (at 0.05 significance), the concentration of reducing substances of filtrate and grains are significantly different.

From the data presented in Tables 2 and 3, a significant part of sugars and amino acids that were contained in the raw material was transferred to the filtrate, thereby reducing their concentration in the grains. Therefore, in the preparation of a nutrient medium for the cultivation of a strain of a microorganism producing protein, a partial addition of the filtrate to the mass of the grains (pellet) is required. In order to establish the quantitative fraction of return of the filtrate to the mass of the pellet (grains), nutrient media were prepared with the addition of different concentrations of the filtrate to the grains, exactly 10%, 20%, 30% and 40% by weight. In the obtained samples of nutrient media was carried out the cultivation of a strain of the microorganism. The results of the experiments are presented in Figs 2–5.

According to the data presented in Figs 2 to 5, the most active cell growth was observed in the first 24 hours, and the maximum of budding cell count was reached. The greatest cell count is at 24 hours of cultivation, after which comes the stage of attenuation of the vital activity of microorganisms. The total duration of cultivation was 48 hours.

Thus, by cultivating the strain of the microorganism of the protein producer on the nutrient medium, dry samples of the protein product were obtained from grains and filtrate of the wort. To determine the nutritional value of the samples, the crude protein content was determined. The results are shown in Fig. 6.

Table 2. The carbohydrate composition of the filtrate and grains of rye wort (% of absolutely dry substance)

Sample	Reducing substances, %	Total reducing substances, %
The filtrate of rye wort	84.41 ± 1.10	-
Grains of rye wort	4.78 ± 0.01	6.07 ± 0.01

Table 3. The content of free amino acids in the filtrate and grains of rye wort, % of absolutely dry substance

Name of amino acid	Free amino acid content (%)	
	Grains (Pellet)	Filtrate
Aspartic acid	0.0390	0.1365
Serine	0.0449	0.1486
Threonine	0.1807	0.6466
Glutamic acid	0.0770	0.3332
Proline	0.1496	0.6466
Glycine	0.0196	0.0643
Alanine	0.0587	0.1526
Valine	0.0414	0.1526
Methionine	0.0219	0.0763
Isoleucine	0.0426	0.1446
Leucine	0.1001	0.3334
Tyrosine	0.0311	0.1205
Phenylalanine	0.0702	0.2369
Histidine	0.0679	0.2249
Lysine	0.0564	0.1647
Tryptophan	0.2048	0.4900
Arginine	0.0875	0.2431
Total amount of free (unbound) amino acids	1.2934	4.3454

Figure 2. Cultivation of strains of microorganisms-protein producers on the nutrient medium on the basis of fractions with the introduction of grain wort filtrate in the amount of 10% by weight of the used solid fraction; cell growth, millions of cells mL^{-1}.
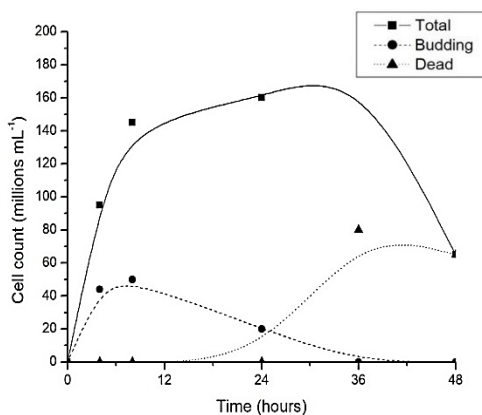
Figure 3. Cultivation of strains of microorganisms-protein producers on the nutrient medium on the basis of fractions with the introduction of grain wort filtrate in an amount of 20% by weight of the used solid fraction; cell growth, million mL^{-1}.
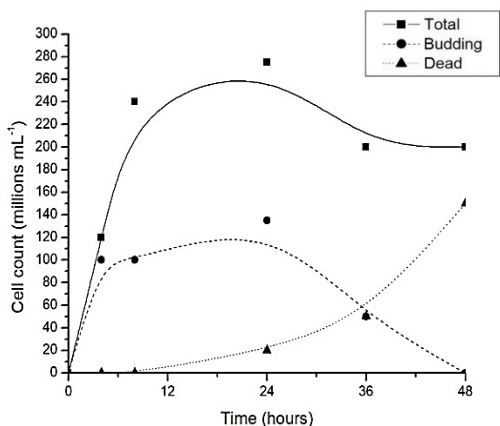
Figure 4. Cultivation of strains of microorganisms-protein producers on the nutrient medium on the basis of fractions with the introduction of grain wort filtrate in an amount of 30% by weight of the used solid fraction; cell growth, millions of cells mL^{-1}.
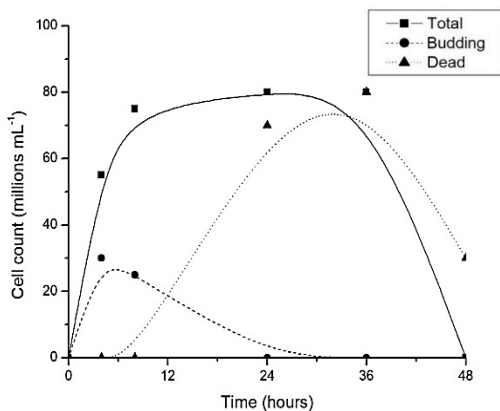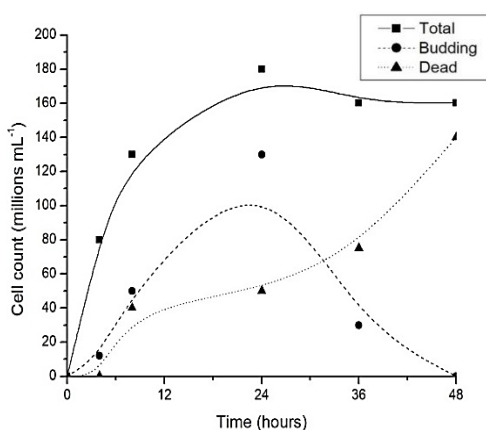

Figure 5. Cultivation of strains of microorganisms-protein producers on the nutrient medium on the basis of fractions with the introduction of grain wort filtrate in the amount of 40% by weight of the used solid fraction; cell growth, millions of cells mL^{-1}.

Where 1 is a sample of grains; 2 – a sample of the protein product, obtained in a nutrient medium with a 10% addition of rye wort filtrate by weight of the solid fraction, % in terms of a.d.s.; 3 – a sample of the protein product obtained in a nutrient medium with a 20% addition of rye wort filtrate from the mass of the solid fraction,% in terms of a.d.s.; 4 – sample of a protein product obtained in a nutrient medium with 30% addition of rye wort filtrate by weight of the solid fraction,% in terms of a.d.s.; 5 – a sample of

the protein product obtained in a nutrient medium with 40% of the filtrate of rye wort from the mass of the solid fraction, % of absolutely dry substance.

As shown in Fig. 6, there is an increase in the content of crude protein in samples 2, 3 and 4 with an increase in the amount of nutrient media in the wort filtrate. The maximum content of crude protein is in sample 4, where this parameter is above 40% of a.d.s. Thus, under these cultivation conditions, the greatest accumulation of crude protein can be achieved with a 30% addition of the filtrate to the culture medium.

The biological value of both food and feed products is assessed by their amino acid composition, exactly, the content of essential amino acids. In order to establish the nutritional value of the obtained protein product, the amino acid composition of sample 4 was examined. The results of the analyses are presented in Table 4.

According to the data presented in Table 4, the protein product has a complete amino acid composition. The total content of essential amino acids in the protein product, which includes valine, isoleucine, leucine, lysine, methionine, threonine, tryptophan and phenylalanine, is more than three times higher than that of the grains (pellet). And the content of lysine, which is considered the main limiting amino acid in the diet of pigs, is higher in the protein product more than 3 times than in the grains. The resulting product can be recommended as a protein
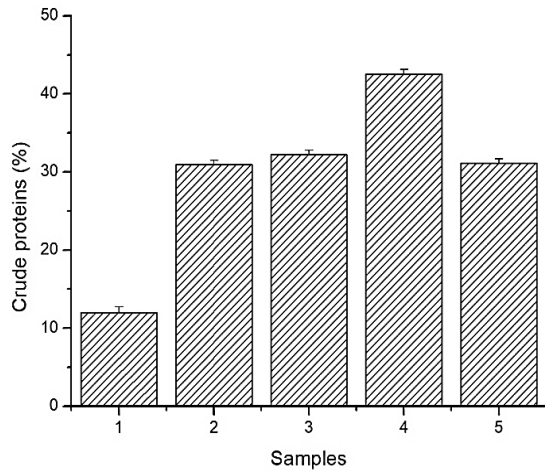
Figure 6. The content of crude protein in samples of the protein product, % in terms of absolutely dry substance, *a.d.s (absolutely dry substance).

Table 4. The amino acid composition of grain mash and protein product grains obtained on a nutrient medium with 30% addition of rye wort filtrate by weight of the solid fraction, mg g^{-1}

The name of the amino acid	The total content of amino acids, mg g^{-1}	
	Protein product	Grains (pellet)
Aspartic acid	15.79	4.99
Serine	10.26	3.37
Threonine	8.48	2.51
Glutamic acid	26.12	11.87
Proline	13.21	6.42
Glycine	10.20	3.61
Alanine	11.16	3.71
Valine	8.11	2.80
Methionine	1.74	0.61
Isoleucine	7.61	2.01
Leucine	13.00	4.39
Tyrosine	3.37	0.84
Phenylalanine	8.09	2.97
Histidine	4.75	1.90
Lysine	7.66	2.28
Tryptophan	15.12	5.71
Arginine	8.96	2.72
The total content of essential amino acids	69.81	23.28
Total quantities	173.63	62.71

supplement in the diet of farm animals. A fine particle size distribution of such a feed additive may be promising in the poultry industry (Rimareva et al., 2001).

CONCLUSIONS

As a result of the experiments, it was found that for the enrichment of the protein components of the grain obtained from the wort prepared from rye processed at the SDA-installation, it is necessary to add an acidic and neutral protease in the process of preparing the wort. It is also advisable to carry out the enrichment of the grain protein yeast strain Candida.

The results of cultivation of Candida yeast strain on this nutrient medium makes it promising to search for new microorganisms of protein producers with high biomass yield and non-pathogenic for animals and humans.

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Impact of using the developed starter culture on the quality of sourdough, dough and wheat bread

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Abstract. There is no technological necessity of sourdough usage when preparing wheat bread as it can be prepared without sourdough but only with yeast using. However, sourdough helps to solve such problems as fast microbial spoilage, unexpressed taste and smell, crumbling crumb. The use of sourdough prepared with directional cultivation of microorganisms allows to produce high-quality competitive bread. Developing a starter culture with an optimized microbial composition was the purpose of this study, allowing the quality and the microbiological stability of wheat bread improving. A new starter microbial composition for the sourdough was developed. Lactic acid bacteria strains *L. plantarum E90, L. brevis E120* and yeast *S. cerevisiae Y139* were selected for the new composition. It was proven that the rice products using to microorganism immobilization allows saving the largest number of living cells after drying and during storage. The rate of acid accumulation in sourdough was established. The sourdough dynamic viscosity decrease at the end of fermentation by 2.2 times was established, which means that the fermentation process leads to the sourdough liquefactio. The optimal dosage was established (5–10% flour in sourdough). This dosage provided good physico-chemical and organoleptic quality indicators of bread. It was proved that the sourdough usage allows getting good-quality bread even when the flour with unsatisfactory amylolytic activity (high drop number) is used. Slowing down the microbial spoilage in sourdough bread was proven. In general, the developed sourdough wheat bread biotechnology improves bread quality and its resistance to the ropy-bread disease.

Key words: wheat sourdough, sourdough bread, yeast, lactic fermentation, microbial spoilage.

INTRODUCTION

In the past decade, the tendency to return to sourdough technology has been noted. It was found out that the sourdough influences sensorial, technological, nutritional, and functional features of bread (Arendt et al., 2007; Corsetti et al., 2007; Gobbetti et al., 2014; Messia et al., 2016).

Sourdough is a mixture of flour and water fermented with lactic acid bacteria and yeasts (Corsetti et al., 2001; Succi et al., 2003; Iacumin et al., 2009; Reale et al., 2005; Reale et al., 2007; Minervini et al., 2015). These microorganisms may originate from

flours, water and equipment or may be inoculated as industrial starter containing pure cultures of lactic acid bacteria and yeasts (Gobbetti et al., 2008; Kosovan, 2008; Auerman, 2009; Huyset al., 2013; De Vuyst et al., 2014; Nionelli & Rizzello, 2016).

The metabolites of microorganisms affect the quality of sourdough and bread. Sourdough contains lactic acid and acetic acid (and some other organic acids) produced by lactic acid bacteria and causes a specific sour taste of bread (Afanasjeva, 2003 Espinosa et al., 2011). Depending on the level of lactic acidification, sourdough fermentation leads to the increase in bread extensibility, softness and volume (Rinaldi, 2015). Acidification impacts the solubility of the structure-forming components such as gluten, starch, protein and arabinoxylans, and positively interferes with the activity of endogenous enzymes (Gobbetti et al., 2008, Sandra et al., 2012). Acid production retard starch digestibility and adjust pH to a range, which favors the action of certain endogenous enzymes, thus, changing the bioavailability pattern of minerals and phytochemicals (Reale et al., 2007; Poutanen et al., 2009).

Organic acids, alcohols, esters, carbonyls, carbon dioxide, diacetyl, hydrogen peroxide and exopolysaccharides produced by lactobacilli and yeasts can improve the flavor and aroma (Gamel et al., 2015).

Sourdough fermentation is also associated with anti-fungal and anti-bacterial properties that can improve bread shelf-life. Addition of 30% of wheat sourdough provides a protective effect against bread staling and extends the bread shelf-life (Torrieri et al., 2014; Rinaldi et al., 2015). Control of staling and keeping the bread quality for longer periods can lead to important economic benefits (Gamel et al., 2015).

The aim of this work is to develop a new microbial starter composition as well as to evaluate the impact of the starter on the properties of the dough and of bread.

MATERIALS AND METHODS

Microbial cultures and ingredients

Microorganisms from the collection 'Lactic acid bacteria and yeast for the baking industry' of St. Petersburg branch State Research Institute of Baking Industry were used. In detail, 5 strains of lactic bacteria belonging to the genus *Lactobacillus (L. plantarum E90, L. plantarum E104, L. plantarum E96, L. plantarum E94 and L. brevis E120)* and 8 strains of yeast belonging to the species *Saccharomyces cerevisiae (Y155, Y129, Y146, Y139, Y151, K1, Y152, Y168).*

To study the effect of wheat sourdough on the dough rheological properties and the bread quality, different quality wheat flour of 1 grade (in accordance with the Russian classification) was used (Table 1).

Table 1. Quality indicators for wheat flour

Indicators	No. 1	No. 2
Moisture content, %	13.8	13.4
Whiteness, unit of the device	45	48
Falling number, s	343	216
Acidity, degrees N.	3.4	3.4
Mass fraction of raw gluten,%	29.5	25
The quality of raw gluten, unit of the device	60	60

Characterization of lactic acid bacteria strains

Acidifying activity. Strains were cultured in standard liquid medium MRS (BioMerieux, France). 1 mL of lactic acid bacteria cultural liquid was inoculated into 100 g mixture of water and flour (humidity 65%) and was kept at 24 ± 1 °C for 2 h. The

titratable acidity was calculated according to Di Renzo et al. (2018).The content of volatile acids was determined by neutralizing the evaporated volatile acid using a 0. n. solution of NaOH (Afanasjeva, 2003).

Antagonistic activity. The antagonistic activity of lactic acid bacteria against pathogens of ropy bread disease was determined by the method of agar slab method (Polak-Berecka et al., 2009; Dec et al., 2016). Test culture of lactic acid bacteria was inoculated in a deep way on the MRS agar (BioMerieux, France) in the Petri dishes and was incubated at the optimum temperature of 30 °C for 3 days for the formation and accumulation of inhibitory compounds in agar. Then, the agar slab with a grown culture of lactic acid bacteria was cut with a sterile cork borer (diameter 7 mm) and transferred to another Petri dish on the surface of the meet-peptone agar, freshly inoculated with the *B. subtilis* test strain.

The *B.subtilis* test strain was grown on meat-peptone medium with agar and a suspension containing 10^8 cells mL^{-1} was prepared using a densitometer DEN-1 (BioSan, Latvia – England).

The plates were kept for 3 hours in a refrigerator at a temperature of 4 °C (in order to avoid premature growth of the test strain) to diffuse antibiotic substances from the slab into the agar with the test strain, and then incubated at a temperature favorable for the development of the *B. subtilis* test strain (37 °C). The degree of antagonistic activity of the test culture of lactic acid bacteria was judged by the size of the zone of growth inhibition of the *B. subtilis* test strain around the agar slab.

Characterization of yeast strains

The fermentation activity of yeasts in a mixture of water and flour (humidity 65%) was studied.

Yeast fermentation activity was determined by the amount of released carbon dioxide. 1 mL of yeast cultural liquid containing 10^8 cells mL^{-1} were added at 100 g of flour and water mixture (humidity 65%). Cells number was determined using a densitometer DEN-1 (BioSan, Latvia – England).

The flasks were tightly capped with a container filled with 96% sulfuric acid (special glass device – Muller valve). Sulfuric acid prevents the evaporation of water. Only CO_2 is removed from the flask. Flasks were left to ferment for 24 hours at 24 ± 1°C, measuring the amount of CO_2 released. From the difference in mass, before and after fermentation, the fermentation activity of each yeast strain was judged (Kurtzman & Fell, 1998).

Dried microbial composition preparation

The lactic acid bacteria (*L. plantarum E90* and *L. brevis E120, see results)* characterized by the best acidifying and antagonistic activity and the yeast (*S. cerevisiae Y139, see results)* characterized for the best CO2 production, were used for the preparation of the dried starter culture.

The strains of lactobacilli were grown on malt wort (density of 12° Balling). The culture of the yeast was grown on malt extract (density of 8° Balling). Grown monocultures of lactobacilli were mixed in a 1:2 ratio (*L. plantarum E90: L. brevis E120*). Than mixed with various whole grain flour (corn, rice, oats and wheat) in a ratio of 1:1.2. The mixture was dried in an IR-drier (LOIP L3-120/300-VG1, Russia) at a temperature of 50 ± 2 °C to a moisture content of 16–20%. The drying temperature was chosen experimentally at previous stages of research not presented in this article.

The content of lactobacilli cells in mix immediately after mixing before drying was $5 \cdot 10^7$ CFU g^{-1} and the content of yeasts cells was $5 \cdot 10^5$ CFU g^{-1}.

After drying, the starter was stored in a tightly closed plastic container at a temperature of 4–6 °C.

The cultural liquid of the *S. cerevisiae Y139* yeast was mixed in a ratio of 1:1.2 with the products of processing of various grain crops — corn, rice, oats, and wheat. The mixture was dried in an IR-drier (LOIP L3-120/300-VG1, Russia) at a temperature 40 ± 2 °C to a moisture content of 16–20%.

The obtained dry mixtures of lactobacilli and yeast were mixed in a ratio of 1:3 and were used to prepare wheat sourdough. Colony forming units were determined by plating serial dilutions on malt extract agar for yeasts and MRS agar for lactobacilli. Incubation temperature was 25 °C for yeasts and 30 °C for lactobacilli.

Sourdough preparation

When developing a new wheat sourdough for the conditions of discrete production, the following technological parameters were taken as initial:

– humidity – 65%;

– 1st step – the preparation of a sourdough: mix flour, water and a new starting composition (mixture humidity 65%) and keep this mixture at a temperature of 24 ± 1 °C for 16–18 h to achieve a pH of 4.0–5.5.

The quantity of lactobacilli in starter composition was $(3–4) \ 10^8$ CFU g^{-1} and the quantity of yeast was $(1–1.2)10^8$ CFU g^{-1}.

– 2nd step: the subsequent maintenance of the leaven implies refreshing once a day.

The ratio of fermented sourdough: nutrient mixture – 1:5. Sourdough ferments for 24 h in two stages: Stage I at a temperature of 24 ± 1 °C for 2–3 h and Stage II at a temperature of 10–12 °C for 21–22 h. Cooling is necessary to preserve the sourdough during a break in the work of the bakery. The wheat sourdough formulation is presented in Table 2.

Table 2. Formulations used to prepare sourdough at the first and second step

Ingredients, g	I step	II step
New starter composition	20.0	-
Wheat flour	394.0	2,047.0
Water	586.0	2,953.0
Fermented sourdough of I step	-	1,000.0
Total	1,000.0	6,000.0

Bread making procedure

Wheat bread formulations used in this study are presented in Table 3. Percentages of ingredients were based on 100 g of flour. Part of the wheat flour was replaced by flour in the composition of sourdough in accordance with the traditional Russian bread making way of dosage (Kosovan, 2008).

Required quantity of sourdough was mixed with the rest of the flour in the recipe, yeast, salt and water until dough humidity of 45%. Control dough was prepared by mixing all the components without sourdough. After mixing, the dough was fermented for 60 min. After that dough were shaped into 400-g loaves, placed in aluminium to calculate to a moisture content test 45% pans, and leavened at 30 °C until the volume was twice the initial volume. The leavened dough were cooked in an oven SvebaDahlen (Sweden) at 230 °C for 20 min.

Table 3. Formulations of the dough

Ingredients, g	Control	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Wheat flour	100.0	95.0	90.0	85.0	80.00	75.0
Wheat flour inside the sourdough	-	5.0	10.0	15.0	20.0	25.0
Sourdough	-	12.3	24.6	36.9	49.2	61.5
Salt	1.3					
Bakery yeast	1.0					
Water	66.9	59.1	51.2	43.1	35.1	25.4

Sourdough and dough assessments

Sourdoughs and the doughs samples were evaluated for different parameters. Moisture of the dough and sourdough was determined by drying it at a temperature of 130 °C for a period of forty minutes in drier (SHS-1M, Russia). Acidity was determined by titration, using a 0.1 N. solution of NaOH (State Standard of the Russian Federation, 1996). The lifting capacity was determined by the rate at which it rose in a glass of water at a temperature of 32 °C for a 10 g mass of dough shaped into a ball and with a humidity level of 45% (Puchkova, 2004). The increase in volume was calculated by the ratio between the final volume and the initial volume multiplied by 100%. To determine the the lactobacilli and yeast proportion in a leaven, the method of microscopy and counting in a fixed colored preparation in 50 fields of view was used (Afanasjeva, 2003).

The study of the sourdough rheological properties (dynamic viscosity) was carried out on the rotational viscometer (Reotest-2, Germany). The strain rate was varied from 0.333 to 27 s^{-1}. The measurements were carried out in a cylinder-measuring device according to Couette (measuring device S3) at a temperature of 28–30 °C, the weight of the starter was 50 g. The viscosity was calculated using the formula given in the device instructions (in Pa s).

The gas-forming and gas-holding capacity of the dough were determined using a F3 Chopin Reo-fermentometer. Dough samples weighing 315 g were placed on the bottom of the drum, preheated to 28.5 °C. Installed on the dough piston and tightly closed the system lid. The duration of the experiment was 300 minutes. The movement of the piston, which was mounted directly on the dough, estimated the rise of the dough during the fermentation.

Assessment of baked bread

Assessment of quality. The quality of bread was ascertained evaluating the following parameters. Moisture and acidity (as reported above). 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 bread, compressibility was determined on the automatic penetrometer Labor (Hungary) (Puchkova, 2004). The content of volatile acids was determined by neutralizing the evaporated volatile acid using a 0.1 n. solution of NaOH. The alcohol content was determined by using the iodometric method, which is based on the quantity of sodium thiosulfate spent in titration.

Assessment of sensory characteristics. A panel of 10 non-specialists was used to evaluate the sensory characteristics of the sourdough bread produced. Then, they were asked to evaluate separately smell, taste, texture of crumb, color of crust and porosity.

The ranking scale ranged from 1 to 5 (5–like extremely, 4.5–like very much, 4–like moderately, 3.5–like slightly, 3–neither like nor dislike, 2.5–dislike slightly, 2–dislike moderately, 1.5–dislike very much, 1–dislike extremely).

Molds spoilage assessment

The impact of the sourdough on mold disease of wheat bread was investigated. The model experiments with contamination of sterile bread slices of a pure culture of the mold *Penicillium chrysogenum* were carried out (Dubrovskaya, 2018). Immediately after baking in the oven opening, the loaves were packed into sterile paper, placed in a sterile room, and cooled to a temperature of between 25–28 °C. After cooling the bread was cut in a sterile environment, with slices being taken at a size of 3.5 × 6.5 cm and at a thickness of 0.3–0.4 cm. The slices were placed in sterile Petri dishes. An aqueous suspension of a pure culture of the mold, *Penicillium chrysogenum*, was prepared for the infection of slices of bread. The biomaterial of *Penicillium chrysogenum* was transferred from a tube containing a pure culture of mold grown on malt agar to 1ml of sterile water using 'Tween-80' and was thoroughly suspended. The suspension was inoculated into each slice of bread in three shots using a microbiological needle. Petri dishes with infected slices were incubated at a temperature of 25 ± 1 °C until the first signs appeared of a growth of mold colonies. Mold growth was monitored in a first 16 hours and every 2 hours.

Ropy disease assessment

To determine the effect of starter on microbial resistance the bread was infected with a strain of *B. subtilis* specie (Dubrovskaya, 2018). To contaminate the bread, bread crumbs with spores were prepared next way. Spore-forming bacteria on meat-peptone medium was added to the surface of the sliced bread and cultured at a temperature of 37 °C for 96 hours or until signs of disease. Diseased bread dried in an oven at a temperature of 50 ± 2 °C and milled to obtain crumbs. Crumb contained 10^8 spores·g^{-1}. 1% of infected crumbs were added while kneading the dough for wheat bread. Ready bread was stored at 37 °C before the appearance of symptoms of the ropy disease (Afanasjeva, 2003). Ropy bread disease appirience was monitored in a first 16 hours and after that every 4 hours.

Statistical analysis of the data

All of the experiments were carried out a total of five times. Statistical analysis was performed using Excel software with significance tested at the 95% confidence level and differences among means were determined using the least significant difference and Duncan's test. The confidence intervals shown in the histograms and in the table reflect the accuracy of the used methods.

RESULTS AND DISCUSSION

Lactic acid bacteria were characterized for some technological properties. *L. plantarum E90* and *L. brevis E120* had the highest acidifying activity. The greatest amount of volatile acids, which together with other aroma-forming substances make a significant contribution to the formation of taste and smell of finished bakery products, was produced by *L. brevis E120* strain (Table 4).

Studies of the lactobacilli antagonistic activity have shown that all strains inhibited the growth of *B. subtilis*. The strongest antimicrobial effect *L. plantarum* species have had. The inhibition zones did not differ significantly for all *L. plantarum* strains (Fig. 1). The comparison of the antagonistic activity and the total acidity, showed that strains: E 85, E 94, E 104, despite their low acidifying activity had high inhibitory activity.

Table 4. Acid-forming activity of lactic acid bacteria

Lactobacilli strains	Acidity, degrees N	Volatile acids, %
L. plantarum E85	6.4 ± 0.3^a	0.56 ± 0.03^a
L. plantarum E94	5.8 ± 0.3^a	0.53 ± 0.03^a
L. plantarum E104	6.5 ± 0.3^a	0.45 ± 0.02^b
L. plantarum E90	10.0 ± 0.5^b	0.43 ± 0.02^b
L. brevis E120	10.0 ± 0.3^b	1.08 ± 0.05^c

a-c = Means $\pm$ SD within the same column with different lowercase superscript letters are significantly different ($P \leq 0.05$).

Hence, it can be assumed that these bacteria are capable of synthesizing other inhibitors, e.g. bacteriocins.

The findings from the study were in line with the findings from similar studies conducted, where lactobacilli showed significant antimicrobial activities against *Bacillus* (Klewicka & Libudzisz, 2004; Denkova et al., 2013; Khandakar et al., 2014)
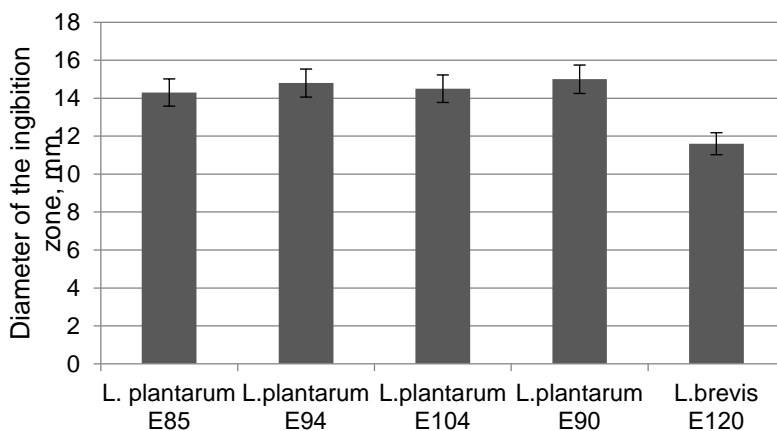

Figure 1. Antagonistic activity of lactic acid bacteria.

The yeast fermentation activity was investigated. Yeast strains had different fermentative activity. The strain *S. cerevisiae Y139* had the highest fermentative activity after 24 hours of fermentation (Fig. 2). The difference in fermentation activity may be due to the peculiarities of the enzyme systems of each strain. The variations in fermentation efficiencies of yeast strains were also noted the when studied wines and juice fermentation (Wahab et al., 2005; Joshi et al., 2009; Sharma et al., 2011). Fermentation efficiency is totally dependent up on the ability of yeast strain to respond over various stress conditions subjected during fermentation, such as pH, high ethanol concentration, osmotic pressure, nutrient availability (Bauer & Pretorius, 2000, Sharma et al., 2011).

The lactobacilli strains *L. plantarum E90* and *L. brevis E120* and the yeast *S. cerevisiae Y139* were used for the new starter microbial composition.
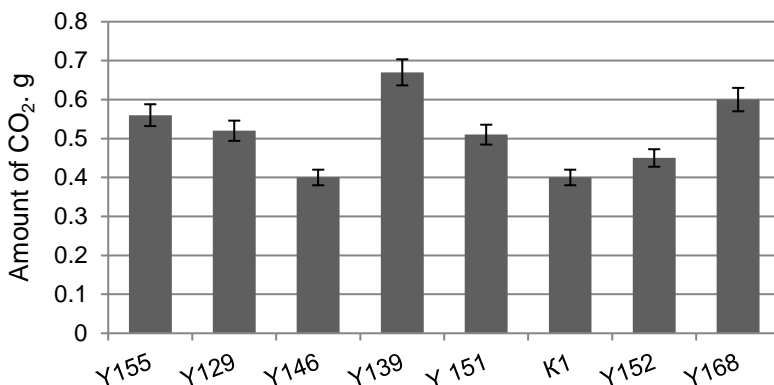
Figure 2. Fermenting activity of yeast

The effect of grains products on the survival of lactic acid bacteria and yeast was investigated in dry microbial compositions. Immediately after drying, the largest number of lactobacilli cells was detected in the microbial composition with rice product (Fig. 3). The highest number of viable lactobacilli cells during storage was detected in compositions with rice and oats products. After 1 month of storage it was 95.5% and 80% of the initial quantity, respectively. Two months later, it was 68% and 63%, respectively.
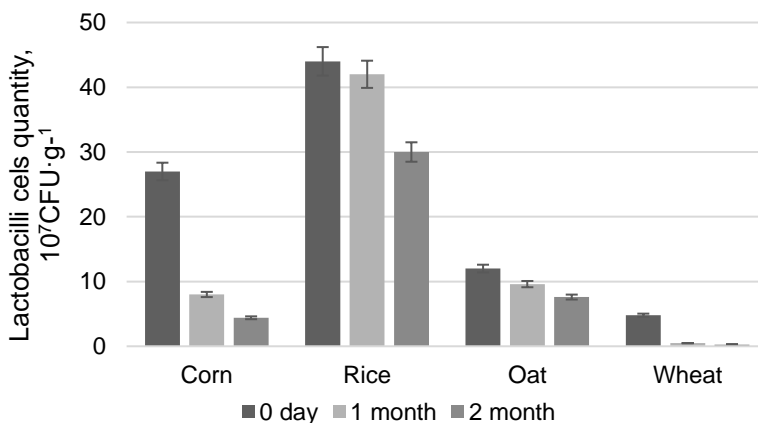
Figure 3. Survival of lactobacilli cells during storage.

The largest number of viable yeast cells was detected in the dried mix with rice products. Immediately after drying it was 35–38% higher than in other samples (Fig. 4). The smallest number of living cells during storage was in mix with wheat products. After 2 months of storage, the number of viable yeast cells in mix with wheat products decreased to 48% compared with initial number. It may be because used rice products have more fibers to immobilize and to protect yeast cells.

Figure 4. Survival of yeast cells during storage.

A new starter composition was developed. It includes lactobacilli *(L. plantarum E90* and *L.brevis E120*) and yeast *(S. cerevisiae Y139)* mixed with rice products in a ratio of 1:3.

The effect of the new starter culture on the quality of the sourdough was investigated. Indicators of a new sourdough at the step I and step II during 24 hours of fermentation were established (Table 5).

It was established that sourdough had lower acidity at the step I than at step II as it needs time to lactobacilli from the starter composition grow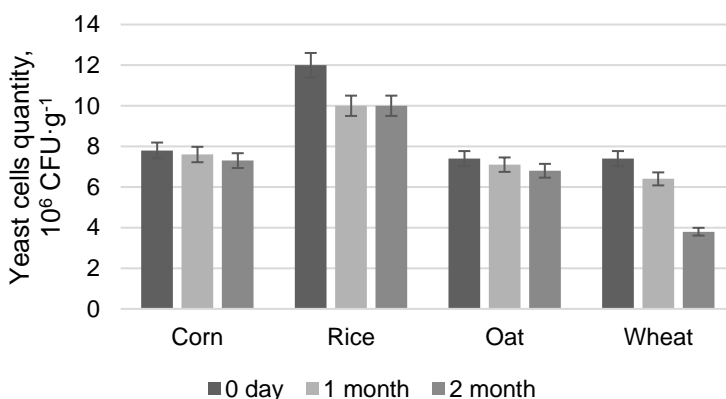 up at the dominant quantity. At the second step acid accumulation was most intense in the first 2–3 h at the temperature of 23–25 °C. The rate

Table 5. Biotechnological indicators of sourdough at the step I and step II

Indicators	Step I	Step II
Acidity, deg. N:		
at the temperature 23–25 °C	5.2 ± 0.3^a	5.4 ± 1.2^b
at the temperature 10–12 °C	-	9.5 ± 1.0
Lifting capacity, min:		
at the temperature 23–25 °C	26 ± 12^a	63 ± 7^b
at the temperature 10–12 °C	-	27 ± 8
Lactobacilli: yeast proportion		
at the temperature 23–25 °C	`1:13	1:100
at the temperature 10–12 °C	-	1:22

a-b = Means ± SD within the same row with different lowercase superscript letters are significantly different ($P \leq 0.05$).

of acid accumulation was 2.1–2.2 deg h^{-1}. At the temperature of 10–12 °C, the rate of acid accumulation slowed down and was 0.19–0.2 deg h^{-1}. It indicates that the lactic acid fermentation proceeded more intensively in the first period of fermentation at the step II. Sourdough lifting capacity reached optimal values by the end of the second fermentation period at the temperature of 10–12 °C. It means that yeast cells accumulate in sufficient quantity only by the end of fermentation. And it confirms that the sourdough quality will be good enough for dough preparing once a day.

The effect of fermentation on the sourdough texture was studied. It was established (Fig. 5) that the sourdough dynamic viscosity at the beginning and at the end of fermentation at a temperature of 25 °C was 16 and 17 times higher respectively when a strain rate was 0.33 s^{-1} than when it was 27 s^{-1}. The sourdough dynamic viscosity at the end of fermentation at a 10–12 °C was 9.4 times higher when deformation rate was 0.33 s^{-1} than when it was of 27 s^{-1} Studies have shown that the sourdough dynamic

viscosity at a temperature of 10–12 °C at the lowest strain rate was 2.2 times lower than in sourdough fermented at 25 °C. It showed that the fermentation process leads to the liquefaction of the sourdough. It may be due to the enzymes action of lactic acid bacteria and yeast on the dough biopolymers. Acidification and the reduction of disulfide bonds of gluten by lactobacilli promote the primary activity of cereal proteases, which lead to the liberation of various sized polypeptides. Intracellular peptidases of sourdough lactic acid bacteria complete proteolysis and liberated free amino acids (Loponen et al., 2004; Gobbetti et al., 2014).
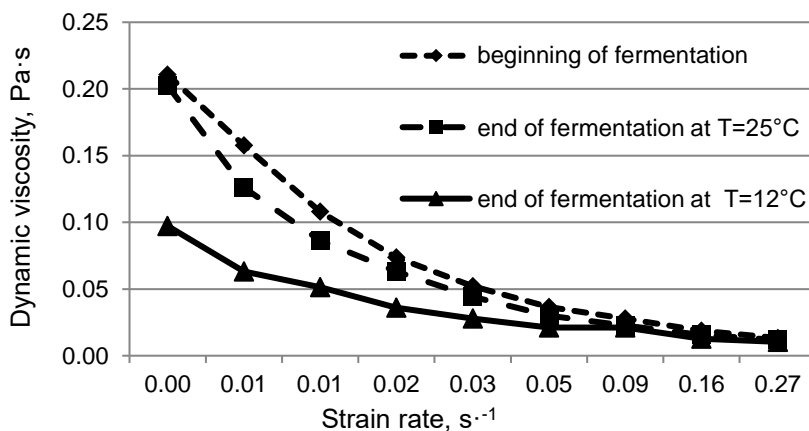

Figure 5. Indicators of the sourdough dynamic viscosity, depending on the strain rate.

The effect of the sourdough on the dough quality was studied. It was established that the acidity increased while sourdough dosage increase (Fig. 6, a), as was expected. Acidity increased twice when 25% of flour was replaced by sourdough (sample 5). It is obvious because of the sourdough acidity. The dough lifting capacity (Fig. 6, b) was worse than without sourdough. It may be because sourdough acidity inhibits the bakery yeast fermentation.

The influence of sourdough on the bread quality was studied. It was established that the acidity in the samples of sourdough bread (Fig. 7) expectedly increased. In samples 4 and 5 the acidity was 0.2 and 0.4 degrees higher than normative indicator for such kind of bread in accordance with Russian Federal normative documentation (State Standard of the Russian Federation GOST 27842–88). These samples were indicated as non-standard breads.

Specific volume (Fig. 7) of samples 1–4 was 11.5–15.4% higher compared to the control. Sandra et al. (2012) reported that the addition of 20% of wheat sourdough increases CO_2 production, which may influence on the bread volume. But specific volume of sample 5 was 7.7% lower compared to the control. It may be because such a big quantity of sourdough inhibits yeasts (Torrieri et al., 2014; Gamel et al., 2015).
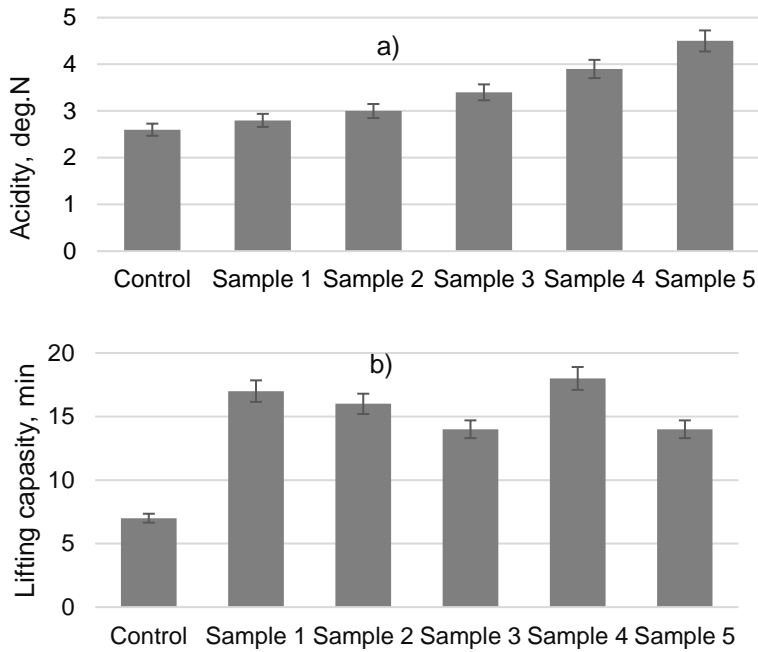
Figure 6. The effect of wheat yeast on the quality of dough.

* Examples of samples are given in Table 3.

The alcohol content in the sourdough bread (Fig. 7) was lower compared to the control, which is obviously associated with the suppression of the development of yeast cells because of the dough acidity. The content of volatile acids in bread increased with sourdough dosage increasing (Fig. 7). It contributed to the improvement in the taste and smell of bread. However, the taste of samples 4 and 5 was too sour. Gamel et al. (2015) and others also reported than organic acids and alcohols produced by lectobacilli and yeasts influence the flavor and aroma.
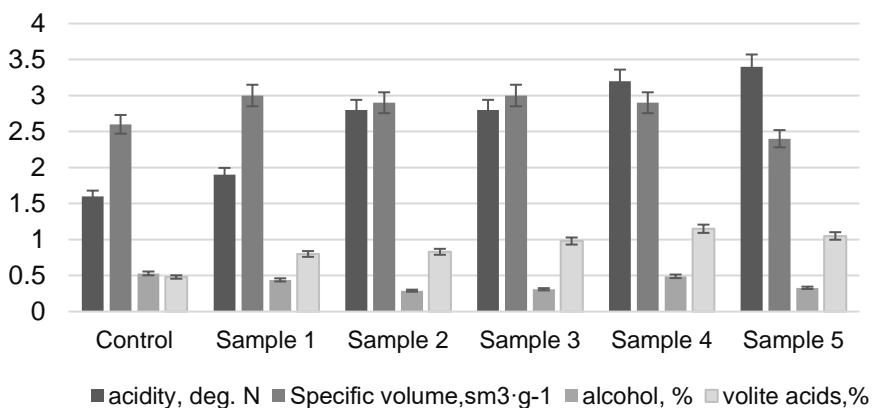
Figure 7. The effect of wheat sourdough on the quality of bread.

The data obtained confirm other studies. Depending on the quantity of using sourdough the fermentation process leads to the chances in bread quality (Gobbetti et al., 2008, Sandra et al., 2012; Therdthai & Jitrakbumrung, 2014; Rinaldi, 2015; Nionelli et al., 2016).

Sensory characteristics of bread are presented in Fig. 8. Sample 1 and sample 2 had the better crust color, crumb texture and porosity than the control. Samples 1, 2 and 3 had the best taste and smell. The smell was more intense, pleasant. Samples 4 and 5 had too sour taste and smell, and its crumbs had gray or grayish surface which is usually due to the high acidity (Gobbetti et al., 2014; Torrieri et al., 2014)
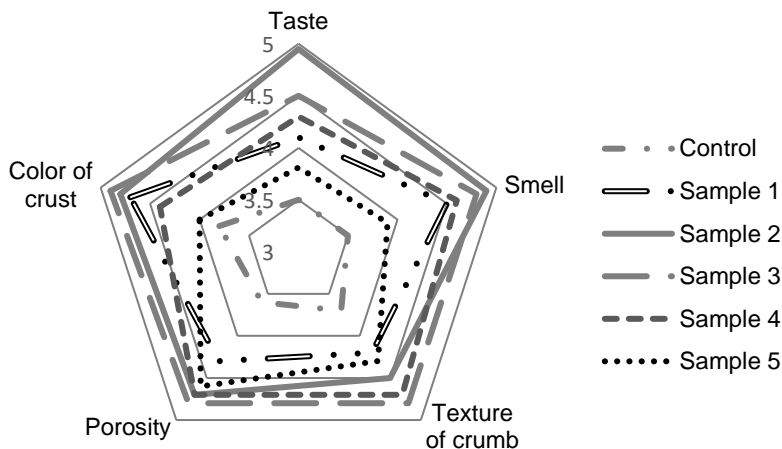
**Figure 8.** Sensory characteristics of wheat bread.

The effect of wheat sourdough on the bread shelf-life was studied. It was established that with increasing sourdough dosage, resistance to mold was increased for 16–56 h compared with the control. Moreover, it was found that samples No. 1 became moldy 36 hours later than the control. Samples No. 2–5 did not exhibit ropy disease during the entire storage period. Increasing the microbial safety and shelf life of wheat sourdough bread has also been reported by Gamel et al. (2015) and Katina et al. (2009).

Considering the data obtained above, for further studies were chosen sourdough dosages 5 and 10%.

The effect of wheat sourdough on the gas-forming and gas-holding capacity of dough was studied. Reofermentometric characteristics of dough samples are presented in Table 6.

Table 6. Dough reofermentometric characteristics

Indicators	control	Sample 1	Sample 2
Total volume of released CO_2, sm^3	$1{,}756.0 \pm 88.0^a$	$2{,}051.0 \pm 103.0^b$	$1{,}946.0 \pm 97.0^b$
Volume of CO_2 retained, sm^3	$1{,}706.0 \pm 85.0^a$	$2{,}026.0 \pm 101.0^b$	$1{,}919.0 \pm 96.0^b$
Volume of lost CO_2, sm^3	50 ± 3^a	25 ± 1^b	27 ± 1^b
Gas retention coefficient,%	97.2 ± 4.9^a	98.8 ± 4.9^a	98.6 ± 4.9^a

a-e = Means ± SD within the same row with different lowercase superscript letters are significantly different ($P \le 0.05$).

The effect of the sourdough on the quality indicators of the dough prepared with different flour types was investigated. It was established that dough acidity were higher when used flour with falling number of 216 s than when used flour with falling number 343 s (Fig. 9, a). The dough lifting capacity was worse when used flour with the falling number 216 (Fig. 9, b). The falling number shows the activity of amylase. The greater the falling number, the lower the activity of amylase. Low amylase activity leads to less production of maltose and glucose. Consequently, the deterioration of lifting capacity was because of lower content of maltose and glucose for lactobacilli and yeast feeding when used flour with a falling number of 343 s.
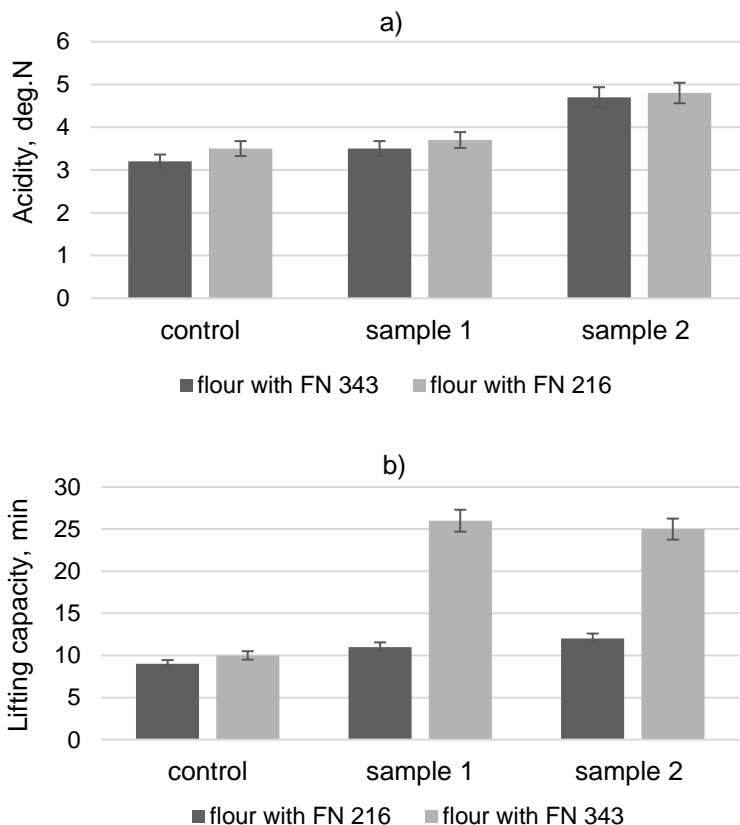
Figure 9. Quality of sourdough dough with different types of flour.

The effect of wheat sourdough was studied on the physic-chemical quality indicators of the bread prepared with different quality flour. It was found that the acidity was lower when using flour with a falling number of 343 s (Fig. 10, a). It was expected, since the acidity of this dough was also lower. The specific volume of the dough samples (Fig. 10, b) was lower when used flour with a falling number of 343 s than when used flour with falling number of 216 s. Sourdough bread made with flour with a falling number of 343 s had slightly lower compressibility of the crumb (Fig. 10, c) than bread prepared with flour with a falling number 216 s and was almost the same as compared with the control prepared without sourdough. Obtained data showed that the sourdough

allowed bread quality improving even when flour with unsatisfactory amylolytic activity (high falling number) was used.
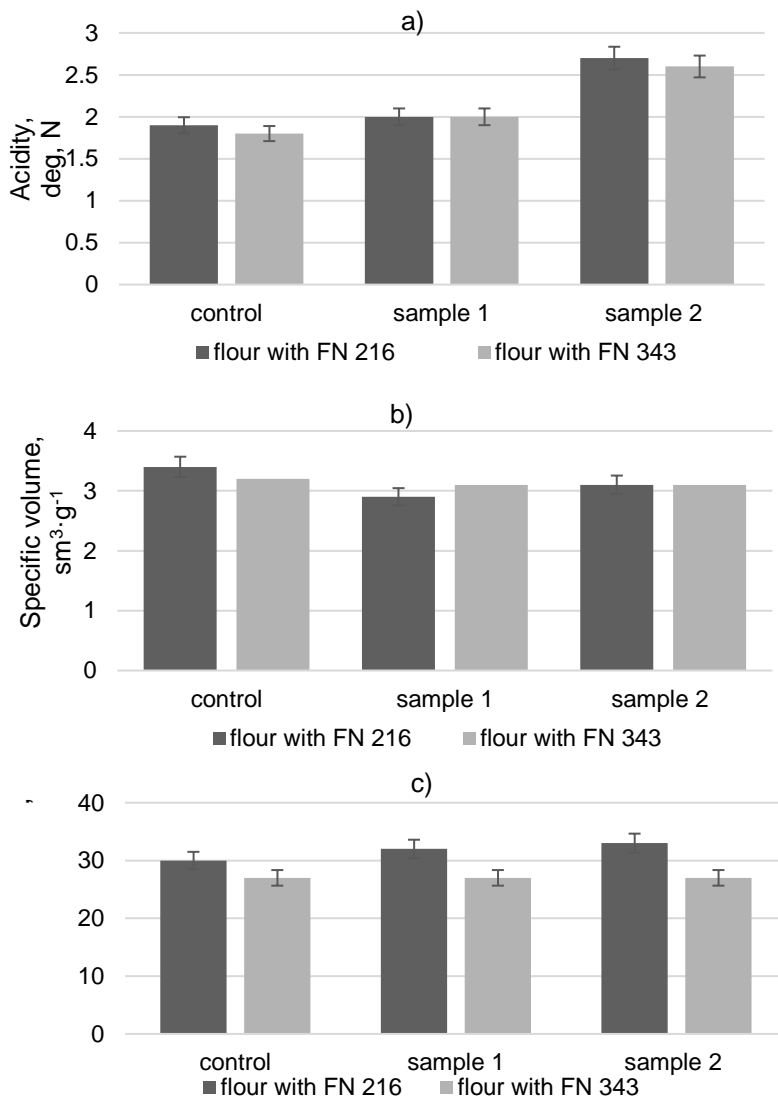
Figure 10. Quality of sourdough bread from different types of flour.

CONCLUSIONS

To develop a new microbial starter composition, lactic acid bacteria strains *L. plantarum E90,* possessing the greatest antagonistic activity, and *L. brevis E120* strain, producing the largest amount of volatile acids, as well as the yeast *S. cerevisiae Y139*, having the best fermentative activity, were selected. It was proven that the use of rice products in the composition makes it possible to obtain highest number of viable cells. It was established that the acid addition rate in sourdough during the first 2–3 h at

a temperature of 23–25 °C was 2.1–2.2 deg h^{-1}. Subsequently, at the temperature of 10–12 °C, the rate of acid accumulation slows down to 0.19–0.2 deg h^{-1}. The decrease in the sourdough dynamic viscosity by the end of fermentation by 2.2 times was established, i.e. the fermentation process leads to the liquefaction of the sourdough. The optimal dosage was established (5–10% flour in sourdough). This dosage provided good physico-chemical and organoleptic quality indicators of bread. In the dough samples with 5 and 10% of flour in the sourdough, the volume of the released and retained gas was higher than in the control one. The volume of gas lost in the leaven dough samples was 1.7–2 times less compared to the control one, as evidenced by the large specific volume of bread samples. The use of sourdough allowed slowing the mold disease and completely inhibiting the ropy-bread disease. It was proven that the use of sourdough allowed getting good quality bread even when using flour with unsatisfactory amylolytic activity (high falling number).

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Effect of high pressure processing on raw pork microstructure and water holding capacity

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Abstract. High pressure processing (HPP) is widely used as an alternative to thermal food preservation technologies, including processed meats treatment. This technology affects food texture and water-holding capacity, which may have beneficial effect on product yield. After thermal treatment, meat partially releases water together with water-soluble proteins, which is concerned as a loss. It is very important not only because of changes in taste properties, but also economic aspects such as reduced final product weight. The aim of the study was to evaluate changes in the meat microstructure and water-holding capacity upon high pressure treatment. Pork samples were treated at various pressures and holding times, namely, 300 and 600 MPa with a 1 and 15 minutes holding time at each pressure. Untreated sample was regarded as a control. Microstructure of pork meat was evaluated after the paraffination of the samples. Fibre cross section area and space between fibres were measured and reported. Water-holding capacity was measured by centrifugation of meat samples over filter and calculating released amount of juice. Results indicated that fibre size did not change significantly after treatment at 300–600 MPa pressure comparing to the control sample – untreated meat. However, high pressure can affect hydrophobic properties of myofibrillar protein. The experimental results showed that water-holding capacity increases with the high pressure treatment. It is an important issue in meat processing industry, because HPP treatment allows reducing the water loss in fresh pork.

Key words: high pressure processing, histology, expressible water, pork.

INTRODUCTION

The structural changes in food systems caused by high pressure processing (HPP) depend on the pressure effect on specific food compounds. Thus, HPP can modify macromolecules or biopolymers, disrupting hydrogen bonds, resulting in the loss of enzyme and membrane activity of proteins (Farkas, 2016). It has been demonstrated that pressurization induce coagulation of proteins without drastic chemical changes, which are observed in heat treated products (Cao et al., 2012). HPP would not break covalent bonds, retaining flavours, pigments and other nutritionally important compounds.

From a physical point of view pressurization moves molecules closer to each other, leading to phase transitions which may be reversible after treatment (Hugas et al., 2002). An increased hydrostatic pressure induces structural changes in protein molecules. These structural changes may lead to conformations of molecules, resulting in new functional

properties of proteins such as gelation, coagulation, association, and dissociation (Chapleau et al., 2004), therefore enhancing the stability of meat gels. Pressure affects the functional properties of myofibrillar proteins related to meat texture, changing their solubility and water binding capacity (Duranton et al., 2012).

Meat is mainly constituted by water (approximately 75%). Lean meat contains also protein (15–21%), fat (0.5–25%), oligonutrients and vitamins, especially B group vitamins (Hugas et al., 2002). The most variable compound is fat. There exist close negative correlation ($r = -0.99$) between fat and water, while protein content has positive correlation with water. The structural organization of the muscle proteins is decisive for the distribution of the water within the meat.

An increased water retention results in reduced cooking loss without application of such additives as starch or phosphates, thus giving higher yield of final product (Ma et al., 2012). Additionally, changes in protein and starch structures may create natural compounds which possess emulsifying, stabilizing, texturizing properties used for water retention (Farkas, 2016). Water holding capacity (WHC) is closely related to other attributes such as meat texture, colour, juice loss etc. (Warner, 2017). On the other hand, low WHC can cause big water losses from meat and meat products due to exudation and evaporation, resulting in weight loss and reduced quality of the product. A thorough understanding of the water holding capacity is important as it affects quality, safety, and profitability.

Water in meat can be in three forms – bound, immobilized or free. Bound water makes only 1–2%, being bound to proteins it stays within meat through all processing steps. Immobilized water can make up to 80% from whole water. It has weaker link to proteins and other cell structures. Immobilized water may be lost or transferred to free water category. In meat processing it is important to immobilize as much water as possible, thus increasing WHC. Free water is easily lost in meat processing. However it may be trapped in meat structures such as cell membranes and capillaries, which are related to the space between myofibrils. Any type of processing which damages these structure will lead to increased loss of water. Although adequate processing allows changing free form to immobilized, which includes reduction in protein denaturation, increase in meat pH, increasing sarcomere length, minimizing damage of muscle structure, maintaining low storage temperatures (Huff-Lonergan & Sosnicki, 2005).

The aim of the study was to examine changes in pork microstructure and water holding capacity depending on the applied pressure and treatment time.

MATERIALS AND METHODS

The study was done in the scientific laboratories of the Faculty of Food Technology, Latvia University of Life Sciences and Technologies.

Chilled pork obtained from *Musculus longissimus lumborum* was purchased from the meat processing company 'Kurzemes Gaļsaimnieks' (Latvia) unpackaged; stored under chilled condition at temperature 3 ± 1 °C ; maximal storage time 24 h. No breed, age, sex or premortal handling was recorded.

Meat treatment

The obtained chilled pork meat was cut in 2.0 ± 0.2 cm thick slices across the muscle fibre and slices were divided into portions with the weight of 80.0 ± 0.2 g each,

packed in the vacuum pouches made from polyamide/polyethylene film (film thickness 60 ± 3 μm). The final pressure in the vacuum-packaging was 8 mbar. Samples were stored in the refrigerator at 4 ± 2 °C till experiment. The pH of chilled pork immediately after purchase was 5.51 ± 0.06. Samples of pork meat were treated in a high-pressure processor ISO-Lab S-FL-100-250-09-W (Stansted Fluid Power Ltd., UK) with a pressure chamber of 2 L and a maximum operating pressure of 900 MPa. The pressure transmitting medium was a mix of propylene glycol with water (1:2 v/v) at room temperature.

Vacuum-packed meat samples were randomly assigned to one of the treatment pressures (300 and 600 MPa), each pressure level was applied for three meat samples for durations of 1 and 15 min. while untreated sample served as the control. Totally we had five batches of samples: 1) control – raw meat; 2) 300 MPa 1 min; 3) 300 MPa 15 min; 4) 600 MPa 1 min; 5) 600 MPa 15 min. The pressurisation experiment was repeated 4 times.

Expressible water

The amount of expressible water of pork samples was determined according to the modified centrifugation method described by Januškevičienė et al. (2012). 10.00 ± 0.01 g of minced meat were placed on a plastic funnel, which was lined with large – pore filter (0.45 μm) of known weight. The funnel with the sample was placed into centrifugation test-tube. Centrifugation in a centrifuge Z 206 A (Hermle Labortechnik GmbH, Germany) was carried out for 20 min at a speed – 6000 revolutions per min. Then, the sample was weighed together with a filter and the amount of expressible water was calculated. The reported results are average of 12 independent measurements.

Preparation of histological samples for light microscopy

Meat samples were cut into $5 \times 5 \times 5$ mm pieces along muscle fibres. Then samples were placed into the perforated cassettes for treatment in a 10% formalin solution for 24 h at room temperature, followed by holding at 37 °C 2 h in 70% ethanol, 2 h in 80% ethanol, and 24 h in 98% ethanol. The next preparation step was soaking of sample in xylene for 0.5 h and another 0.5 h in fresh xylene. Next, the sample was moved to solution of xylene and paraffin (ratio 1 : 1) and held for 1.5 h at 57 °C, then transferred to melted paraffin for 1 h, and another time to new liquid paraffin for 1 h. After this, the samples were removed from cassettes, placed in the plastic moulds and poured with paraffin. After paraffin solidified, samples were cut into thin slices (5 μm) by Microtom (Microtom GmbH, Germany) and dropped into water bath. Prepared samples were placed on the glass slides and dried for 10 min at 37 °C. For removal of paraffin, the slide with the sample was soaked in xylene for 10 min, then in 98% ethanol for 10 min and let dry (Kondratovics, 1976; Ramane et al., 2008). Ten slides per condition were observed using a microscope Leica DM300 LED (magnification 10×40), photos were taken by camera Leica DFC 290 HD and ten measurements per sample were completed using software Leica Application System (LAS) V4.2. (Leica Microsystems, Germany).

Environmental scanning electronic microscopy (ESEM)

Sample for ESEM were prepared according to Das Murtey M. & Ramasamy P. (2016) with some modifications. First, small samples of meat are placed in PE containers

and fully coated with 4% formaldehyde solution. After 2 h, the formaldehyde solution was replaced by deionised water, which has been exchanged 3 times every 5 minutes. The sample were dehydrated by immersion in ethanol baths of increasing content until 96% EtOH, then samples were frozen in liquid nitrogen and then lyophilised for 72 h.

In order to improve the electrical conductivity of the surface of the samples, their surface shall be coated with a thin layer of Au (~ 15 nm), using the Emitech K550X materials. A surface inspection of samples has been performed with the Schottky-type field emission electron microscope Tescan Mira/LMU, under high vacuum conditions, at an electron acceleration voltage of 15 kV using a back-scattered electron detector.

Data processing
Microsoft Excel v16.0 for Windows was used to process the obtained data; mean ± standard deviation was calculated. Cross-comparison of data was performed using ANOVA and Tukey's test. For data analysis, confidence level was 95% ($\alpha = 0.05$). The factors have been evaluated as significant, if P-value $< \alpha_{0.05}$.

RESULTS AND DISCUSSION

Water holding capacity for high-pressure treated meat
Water holding capacity was significantly affected by high pressure treatment. Centrifugation of pork samples demonstrated that the highest amount of expressible water was in the control sample ($16.00 \pm 2.98\%$), which was about 5-fold higher than from HPP samples (Fig. 1).
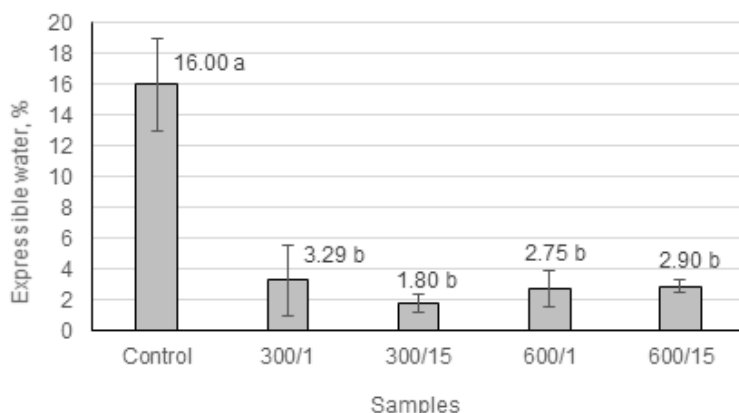
Figure 1. Expressible water in pork detected by centrifugation method.
Different letters indicate significant differences ($P < 0.05$), n = 12.

Among HPP samples the highest WHC exhibited pork treated at 300 MPa for 15 min, having the smallest released water amount ($1.8 \pm 0.55\%$), but the lowest WHC was for pork treated at 300 MPa for 1 min. Expressible amount of water for pork treated at 600 MPa was not affected by treatment time.

The differences between untreated pork and HPP pork samples were statistically significant ($P < 0.05$), which coincides with other research results (Ros-Polski et al., 2015; Xue et al., 2017) who found that water became more tightly bound to the meat

matrix. Pressure greatly influences functional properties of myofibrillar proteins, such as solubility and their water binding and gelling ability. These properties are related to meat ability hold water (Chapleau et al., 2003). It may be influenced also by pH, which is changed during HPP treatment, as it was described in our earlier research (Sazonova et al., 2017). There was not established significant differences among expressible water in HPP treated samples ($P > 0.05$) irrespective of applied pressure or time.

According to Huff-Lonergan & Lonergan (2005) the majority of water in muscle is held within the myofibrils, between them and within other structural elements of muscle – sarcolemma, cells, muscle bundles. When pressure is applied at ambient temperature, little to no changes to connective tissue is observed, probably due to collagen stabilization by hydrogen bonds (Warner et al., 2017). However, HPP treatment can cause destabilization of non-covalent interactions between proteins, also little unfolding occurs, with formation of hydrophobic and disulphide bonds after pressure release (Chapleau et al., 2004; Sun & Holley, 2010). Thus, non-covalent interactions between amino acid residues, which support the protein tertiary structure is first destabilized and then replaced by protein-water interaction. Our previous study (Sazonova et al., 2019) indicated that FTIR spectra showed the intensity decrease in bands representing collagen type I, which was proportional to the pressure and to the treatment time. Thus suggesting denaturation of collagen and release into meat juice, which could bind water.

Microstructure of high pressure treated pork

Microscopy of histological samples (Fig. 2) showed that the fibre size in untreated pork was slightly smaller compared to treated samples (Table 1). After HPP treatment fibre cross section area was slightly increased which correlates with improved water holding capacity. It can be related to protein denaturation. Fig. 2 shows the connective tissue in a transverse section of meat (the connective tissue is light, the fibre are dark).

There was not observed statistically significant ($P > 0.05$) differences in fibre cross section area and extracellular space. It indicates that observed muscle structures retained their shape after pressurization. Similar conclusion was drawn by Jemeljanovs et al. (2007), who described nucleus, which were retained under sarcolemma after HPP. Researchers have described changes in the protein

Table 1. Fibre area and extracellular space in pork

Sample	Fibre area, μm²	Extracellular spaces line length, μm
Control	1,074.58 ± 453.97 a	12.79 ± 5.32 a
300/1	1,159.06 ± 454.73 a	10.32 ± 4.19 a
300/15	1,395.00 ± 599.53 a	15.84 ± 6.45 a
600/1	1,501.13 ± 595.76 a	18.41 ± 8.46 a
600/15	1,267.58 ± 510.90 a	11.57 ± 4.67 a

Values within the same column sharing the same letters are not significantly different ($P > 0.05$), $n = 100$.

secondary, tertiary and quaternary structures, which brings modifications in both structure and function of proteins (Zhang et al., 2018). These effects typically are magnified with increased pressure.

ESEM's observations (Fig. 2) were helpful in supplementing histology data and providing evidence that the samples retained their fibre shape and size.

Transmission electron microscopy completed by Kaur et al. (2016) showed the presence of aggregates, resulting probably from protein denaturation of sarcoplasmic proteins, in the subcellular space and between myofibrils.

400 × (A)	500 × (B)	1,000 × (C)

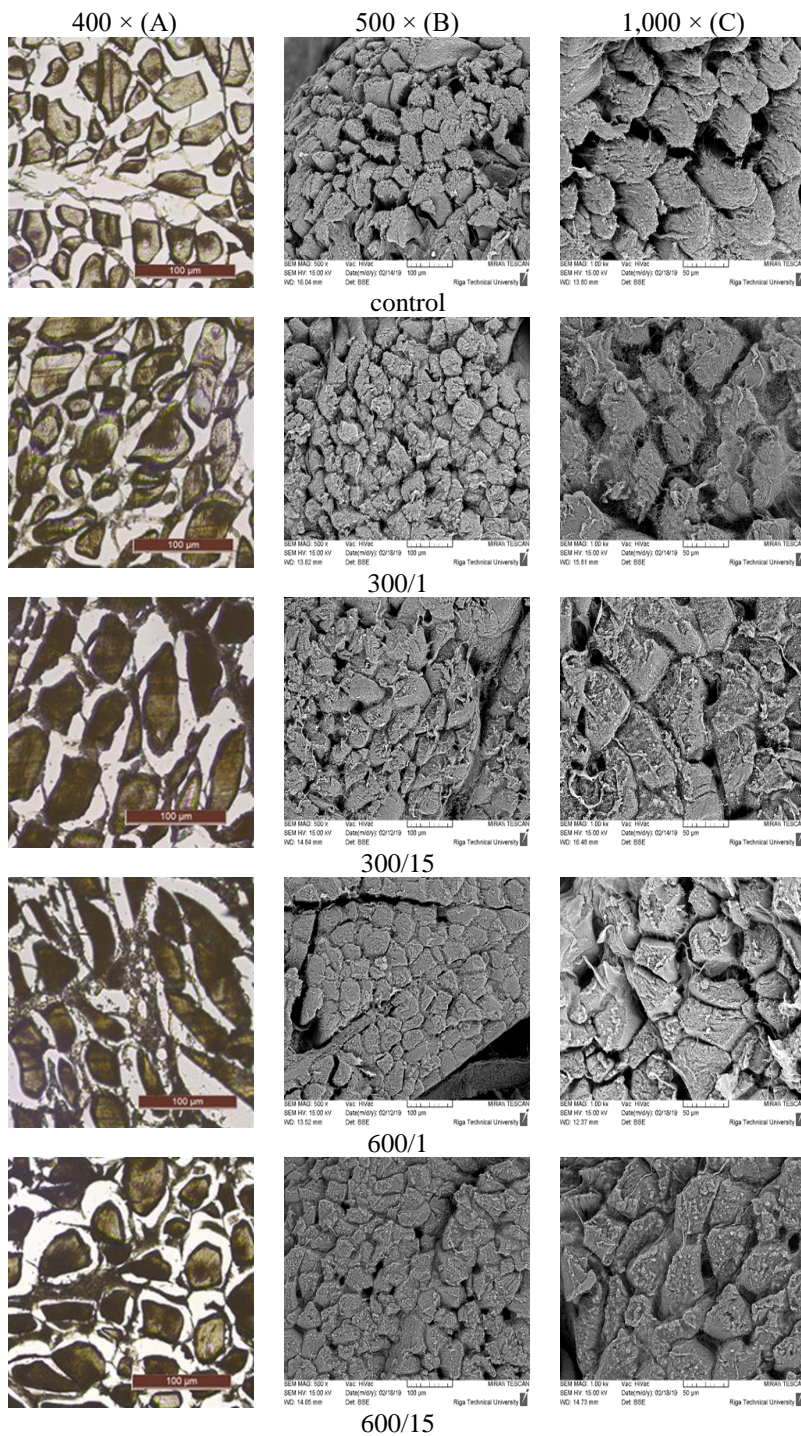control

300/1

300/15

600/1

600/15

Figure 2. Cross section of pork muscle (*Musculus longissimus lumborum*) tissue after HHP treatment. The Histological cross sections (column A) were observed at 400 × magnification. ESEM cross sections were observed at 500 × magnification (column B). ESEM cross sections were observed at 1,000 × magnification (column C).

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There were no statistically significant differences among samples, although the untreated sample had slightly smaller fibre cross section area. Literature analysis revealed that there is a lack of data on changes in raw meat upon HPP. Majority of the recent researches deal with meat supplemented with various additives (Duranton et al., 2012; Ma et al., 2012). For more advanced understanding of changes occurring in HPP, further studies on cross sections would be suggested.

CONCLUSIONS

The study revealed, that HPP treatment at 300–600 MPa for 1 and 15 min influenced pork meat water holding capacity, increasing it approximately 5-fold comparing to untreated meat, irrespective of applied pressure and time. Since microstructure analysis indicated that fibres in treated samples were not different from untreated pork, the increased water holding capacity could be due to macromolecular changes caused by HPP treatment.

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Biosynthesis of glycosidase inhibitors on wheat bread wastes hydrolysate medium by *Streptomyces sp.* 170

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Abstract. The aim of the present study is to investigate the potential effect of bread hydrolysate as a novel nutrient medium for cultivating *Streptomyces sp.* 170 (*S.*170). Moreover, it evaluates the productivity and inhibitory activity of pancreatic α-amylase inhibitors (PAAI). Bread hydrolysate medium (BHM) and corn starch hydrolysate medium (CHM) prepared with α-amylase enzyme concentrations (1.5 and 2.5 units g^{-1} bread) and (1.5 units g^{-1} corn starch), respectively were utilized in the study. The Seherde-Blair and modified Akulova methods were applied to evaluate the carbohydrates concentration and the inhibitory activity of the media respectively. Results of bread and corn media were compared to each other. Furthermore, the activity of PAAI synthesized by *S.*170 was compared to other *Streptomyces* species. The results showed a significant difference ($P < 0.05$) between the total simple sugars (glucose + maltose) concentration produced in CHM (27.5%) and BHM prepared with α-amylase 1.5 units (45.1%). Besides, BHM produced by α-amylase 2.5 units demonstrated the maximum total concentration of simple sugars (49.9%). In addition, 48 h of *S.*170 incubation were quite enough to exhibit the highest inhibitory activity (2,632 IU mL^{-1}) in BHM prepared with α-amylase 2.5 units. The analysis demonstrated a non-significant difference in the inhibitory activity of PAAI in CMH (1,300 IU mL^{-1}) and BMH with α-amylase 1.5 units (1,111 IU mL^{-1}). Also, compared to other *Streptomyces* species, *S.*170 conferred highly active PAAI. In conclusion, BHM showed its efficiency to a great extent in the cultivation of *S.*170 and production of PAAI with a notable high activity.

Key words: bread hydrolysate medium, corn starch medium, *Streptomyces sp.* 170, inhibitory activity, pancreatic α-amylase inhibitors.

INTRODUCTION

Glucosidase enzymes are a group of enzymes, which hydrolyse the complex carbohydrates as starch into simple sugars such as dextrin, maltose and glucose. The pancreatic α-amylase enzyme is considered as one of the glucosidase enzymes. It is

secreted by human pancreas for digesting starch into glucose which is absorbed into the bloodstream. This process elevates the postprandial glucose level in the blood (Selvaraj et al., 2012). α- amylase inhibitors aid blocking the carbohydrate digestion through inhibiting the activity of α- amylase enzymes in the small intestine. Thus, it can delay the digestion of carbohydrates and reduce the postprandial glucose level in blood. Several researchers stated that α-amylase inhibitors can be applied in the biomedical field for treating diabetes millets type 2 and obesity management, also in agriculture field for pests controlling and in the biotechnological field (Paloma et al., 2012; Sujatha et al., 2013; Elizabeth et al., 2017).

α- amylase inhibitors can be obtained from different sources such as medicinal plants, animal and microbial sources, also it can be synthesized chemically. Among all the sources, the microbial source was reported as the best source because of its plasticity for genetic manipulation and potential for economical bulk production. The principal producers for α-amylase inhibitors are some bacteria such as *Streptomyces, Bacillus, Stenotrophomonas maltrophilia* and *Actinoplanes sp. SE*-50, as well as some fungi (Sharova, 2015; Tayyaba et al., 2017; Van Bon et al., 2017). The growth conditions of *Streptomyces* species in liquid media are key factors for controlling the production of secondary metabolites. These metabolites can be antibiotics, antitumor, antifungals, pesticides and enzyme inhibitors (Ferial, 2015). Most of the secondary metabolites are synthesized in the stationary phase of *Streptomyces* growth (Lelia, 1998). The synthesis of secondary metabolites by *Streptomyces* differs quantitatively and qualitatively depending on the composition of liquid medium utilized during the cultivation. The main nutritional requirements for *Streptomyces* cultivation are nitrogen sources such as ammonium salts (ammonium lactate, ammonium nitrate or ammonium chloride) in combination with carbon source such as glucose, mannose, starch (corn or potato) or dextrin and mineral salts such as phosphorus salts (Robert & Hubert, 1954; Linda & Rabab, 2017). However, finding the most desirable liquid medium for cultivating *Streptomyces* is a hurdle, since it varies according to the purpose of their cultivation. The common types of liquid media employed Corn-Glucose broth, Tryptic Soy-Broth (TSB), R2YE and YEME media (Micah et al., 2010; English et al., 2017).

Industrial wheat bread wastes have been skyrocketing over the past decades. They represent 10% of the total production of wheat bread annually, which can provoke massive environmental and economic problems. These bread wastes come from unsold bread in retail stores and substandard produced bread from the industries (Yuji et al., 1997; Ahmet et al., 2016; Sükrü et al., 2017). Wheat bread wastes were reported to contain wide myriad of nutrients such as moisture content – 36.5%; protein – 17%; fat – 0.5%; carbohydrate – 46% (contains 80–82% starch); minerals approximately 1.68% (Ca, P, Mg, Fe) and vitamins (B_1, B_2, Niacin) (Abede et al., 1992; Mohammed & Mohsen, 2009; Shalaby et al., 2014).

In recent years, the biotechnological recycling of wheat bread wastes has been proposed as an interesting solution for managing the wastes of bread by using them as a raw material in preparing a novel liquid medium for the production of valuable secondary products. The main step in bread recycling is the hydrolysis using enzymes to produce hydrolysate media containing simple nutrients like glucose, free amino nitrogen and phosphate. The hydrolysate media can be used in cultivating different microorganisms to produce secondary products like bioethanol, methane, lactic acid, succinic acid, amylase, protease, anaerobic bio-hydrogen and some aroma compounds

(Daniel & Carol, 2013; Ahmet et al., 2016; Saima et al., 2016). Bread hydrolysis entails two main steps: 1 – liquefaction for hydrolysing starch into dextrin and small amounts of maltose and glucose using thermostable α-amylase enzyme; 2 – saccharification for hydrolysing dextrin and maltose into glucose using the glucoamylase enzyme (Fatemeh et al., 2008; Marijana et al., 2014). Many factors can influence the hydrolysis rate, quality of hydrolysed media and yield of hydrolysed nutrients in the media and consequently change the productivity of secondary products. Substrate quantity, substrate particle size, the temperature of hydrolysis, hydro-module, the viscosity of hydrolysed media, enzymes concentration, pH of hydrolysed media and others are the most influential factors (Helena et al., 2017; Sükrü et al., 2017).

Therefore, the objective of this research is to produce a novel nutrient medium by replacing the traditional carbon source of corn starch with bread starch through bread hydrolysis using different enzyme concentrations. Besides, this study evaluates the activity of pancreatic α-amylase inhibitors produced by *Streptomyces sp.* 170 in bread nutrient medium. In addition, it investigates the effect of different enzyme concentrations on the yield of hydrolysed nutrients, as well on the activity and the yield of pancreatic α-amylase inhibitors. The bread hydrolysis was performed using an only thermostable α-amylase enzyme, based on the fact that the *Streptomyces* species are able to produce the glucoamylase enzyme (Razieh, 1993).

MATERIAL AND METHOD

Materials
Enzymes preparations (Table 1) were obtained from Erbsloh Company, German.

Table 1. Enzymes preparations and their properties

Enzymes types	Preparation contents	Activity	Temperature range	pH range
Dystistim BA-T	Bacterial thermostable α-amylase produced from *B. licheniformis stearothermophilus*	950 units AC mL^{-1}	30 to 110 °C	4.5 to 8.0
Dystistim GL	Fungal xylanase produced from *Penicillium funiculosum* and some species of *Trichoderma reesei*	730 units KC mL^{-1}	30 to 90 °C	3.5 to 6.0

Streptomyces sp. 170 (*S.*170) was sourced from the collection VNIIPD – filial of FSFSE 'FSC of food systems of V. M. Gorbatov' RAS, Russia; and stored at low temperature (-12 °C) for 7 months, suspended in 15% glycerol solution.

Wheat bread 'Moskovskii Company, Russia' and corn starch 'Ibred'krakh Malpatoka, Russia' were purchased from local supermarkets. Soy flour was supplied by Partnior-M Company, Russia. Pancreatin preparation was received from Sigma Company, USA.

Methods
Preparation of wheat bread and its hydrolysis
Method of wheat bread preparation for its hydrolysis is presented in the following scheme of bread preparation (Fig. 1).
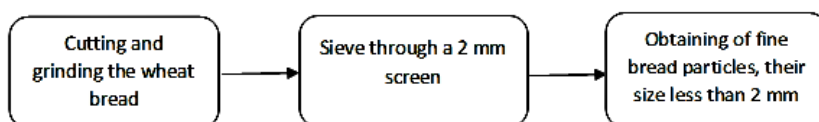
Figure 1. Scheme of wheat bread preparation for its hydrolysis.

The hydrolysis of bread was carried out using thermostable α-amylase enzyme with two different concentrations (2.5 or 1.5 units g^{-1} bread) and xylanase enzyme to produce bread hydrolysate medium (BHM). The technological steps of the bread hydrolysis process are depicted in Fig. 2.

Hydrolysis process of corn starch
Corn starch was hydrolysed using thermostable α-amylase enzyme with a concentration of 1.5 units g^{-1} corn starch and xylanase enzyme. The method of hydrolysis to produce corn starch hydrolysate medium (CHM) is the same as described above.

Physicochemical analysis of bread hydrolysate medium and corn starch hydrolysate medium
Individual analysis of carbohydrates concentration (glucose, maltose, and dextrin) of each medium were quantified using Seherde-Blair method which is a modification of Smirnov method (Tregubov & Kostenko, 1991), whereas the total dry matter concentration (°Brix) and the refractive index were measured with the aid of refractometer PTR46 Index Instruments (Lembe & Umezuruike, 2015).

Media modification and sterilization methods
Corn and bread hydrolysate media were modified, adding soy flour (5.0 g L^{-1}), NaCl (3.0 g L^{-1}), KH_2PO_4 (1.0 g L^{-1}), and $MgSO_4 \times 7H_2O$ (0.5 g L^{-1}), to allow growth of *Streptomyces sp.*170 (*S.*170) and production of pancreatic α-amylase inhibitors (PAAI). The pH of the media was adjusted to 7.0. The media were then sterilized using an autoclave (Tuttnauer, MK 2540, USA), at temperature 121 °C under atmospheric pressure 1 bar (15 psi) for 15 min.

Inoculum preparation
The frozen *Streptomyces sp.* 170 culture was prepared for inoculation by thawing it at temperature 37 °C for 3 min. The titre of the thawed bacterial culture was 10^7–10^8 CFU mL^{-1}.

Bacterial inoculation and its incubation
Periodical deep inoculation of *Streptomyces sp.*170 was carried out under aerobic sterilized conditions with cell titre 10^7–10^8 cells mL^{-1} culture medium. The incubation

was performed in a thermostatic shaker incubator (Multitron, INFORS Company, Switzerland), with speed 230 ± 20 rpm at temperature 29 ± 1 °C for 96 h (Pozdnjakova et al., 2009; Sharova et al., 2009).
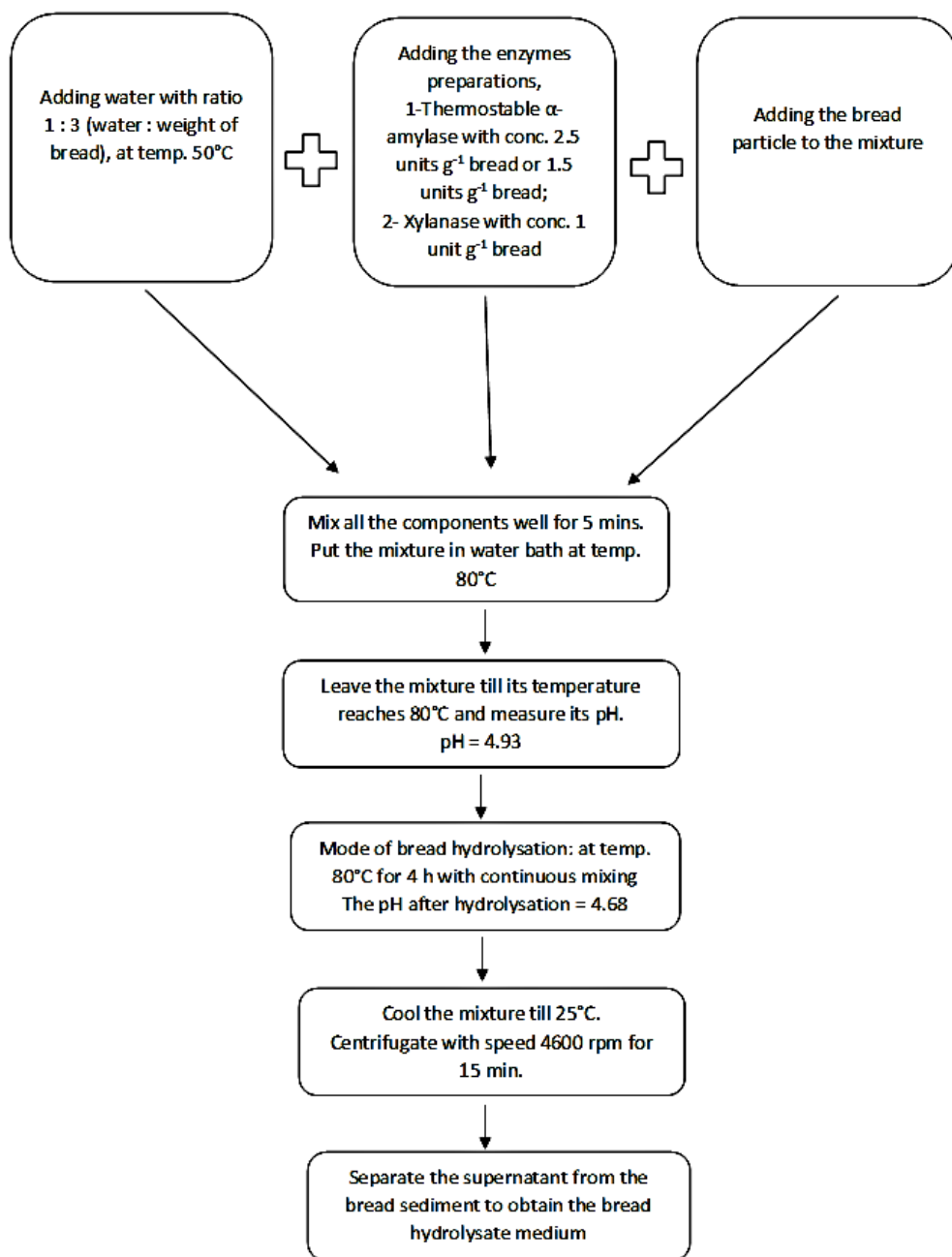

Figure 2. The technological flow chart of bread hydrolysis using thermostable α-amylase and xylanase enzymes.

Determination of inhibitors activity

Samples were taken after 24, 48, 72 and 96 h of incubation and biomass separated via filter element cartridge (Sartorius, Germany) with a polyethersulfone membrane (300 μm) at a pressure of 0.2 MPa and temperature 25 °C. The filtrate was further filtered through a membrane with pores size of 0.45/0.20 μm to remove residual bacterial cells.

The inhibitors activity (IA) was quantified with a spectrophotometer (CF-46, LOMO, Russia) at wavelength 660 nm using a modified Akulova method (Pozdnjakova et al., 2009; Sharova, 2015). Pancreatin preparation consists of pancreatic α-amylase (EC 3.2.1.1; 1,4-α-D-glucan glucanohydrolase) was utilized as the standard.

The inhibitory activity unit (IU) was expressed as IU mL^{-1}. This unit means the amount of inhibitor which can suppress the activity of 1 unit of pancreatic α-amylase by 50% for 10 min at 37 °C and pH 7.0.

The inhibitory activity was calculated by the following equation (1):

$$IA = \frac{D_{00.} - D_2 \cdot 100}{D_1 - D_2 \cdot 50} \cdot K \qquad (1)$$

where D_{oo} – is the optical absorption of the experimental sample, nm; D_1 – is the optical absorption of the control sample 1, nm; D_2 – is the optical absorption of the control sample 2, nm; $\frac{D_{00.} - D_2}{D_1 - D_2} \cdot 100$ – is the extent of inhibition, %; K – is the dilution factor of the experimental sample; and 50 – is the coefficient of the calculation of the inhibitory extent by 50%. The extent of inhibition should be in the range of 40–55% (Sharova, 2015).

Statistical analysis

All experimental measurements were performed in triplicate to avoid experimental error. The generated data were subjected to analysis of variance (ANOVA one-way) using Origin 61 statistical software with a significant difference at $P \leq 0.05$. The graphical analysis was performed using Microsoft Excel 2013.

RESULTS AND DISCUSSION

The physicochemical parameters of corn starch and bread hydrolysate media

The chemical analysis of corn starch and bread hydrolysate nutrient media shows the presence of glucose, maltose and dextrin with different concentrations according to the type of raw material.

The results analysis of both nutrient media are represented in Table 2 and significant difference ($P < 0.05$) was established between the total concentration of simple sugars (glucose + maltose) produced by corn starch or bread starch hydrolysis using 1.5 units of α-amylase enzyme. In corn starch hydrolysate media (CHM), it was found that the total concentration of simple sugars was 27.5 ± 5.0%. On the other side, the total concentration of simple sugars in bread hydrolysate media (BHM) prepared with α-amylase 1.5 units g^{-1} bread was 45.1 ± 8.2%.

The results could be ascribed to the starch hydrolysis process. Before starch liquefaction using an α-amylase enzyme, starch gelatinization must occur. Starch gelatinization means absorption of water by starch granules at high temperature, which

differs with respect to the type of grain. This can help in physical disruption of starch granules and its exposure to the enzymatic hydrolysis (Uthumporn et al., 2010). The corn gelatinization temperature is between 66–72 °C and the gelatinization temperature for wheat is between 52–66 °C (Marek et al., 2010; Ubwa et al., 2012). The difference in gelatinization temperature between corn and wheat can help the wheat bread begins its hydrolysis faster than the corn hydrolysis, consequently, the wheat bread hydrolysis produces more simple sugars in the hydrolysate nutrient media. This result agrees with the previous observations of Ubwa et al. (2012) which concern the rapid hydrolysis of cereal grains starch with low gelatinization temperature and its conversion into simple sugars.

The results of total dry matter concentration and refractive index presented in Table 2 prove the non-significant difference ($P > 0.05$) between CHM and BHM prepared with 1.5 units of α-amylase enzyme. Therefore, the variance in *Streptomyces sp*. 170 growth rate will depend on the difference of total simple sugars concentration more than the difference of total dry matter concentration in both hydrolysate media.

In addition, the chemical analysis of the bread hydrolysate medium (BHM) prepared with 2.5 units of α-amylase enzyme for each gram bread was evaluated and its results were presented in Table 2. The ANOVA analysis was established between the result of total simple sugars concentration in BHM prepared with 1.5 units of α-amylase enzyme (Table 2) and its result in BHM prepared with 2.5 units of α-amylase enzyme (Table 2), for studying the significant effect of α-amylase enzyme concentration utilized during the BHM preparation on the yield of total simple sugars.

Table 2. Physicochemical parameters of corn starch and bread hydrolysate media

Type of media	Simple sugars (glucose + maltose), %	Dextrin, %	DM, %	RI
CHM with α-amylase 1.5 units	27.5 ± 5.06	72.5 ± 7.39	12.0 ± 4.58	1.3560 ± 3.18
BHM with α-amylase 1.5 units	45.1 ± 8.25	54.8 ± 6.01	15.6 ± 4.72	1.3572 ± 3.47
BHM with α-amylase 2.5 units	49.9 ± 7.11	50.1 ± 4.18	16.0 ± 2.29	1.3576 ± 4.15

The statistical analysis indicates that there is no significant different ($P > 0.05$) between the results of total simple sugars concentration in both BHM prepared by two different concentrations of α-amylase enzyme. This result proves the non-significant effect of utilizing the α-amylase enzyme with different concentrations in BHM preparation on the yield of total simple sugars.

The concentration of total simple sugars in BHM prepared with α-amylase 1.5 units g^{-1} bread was 45.1 ± 8.25%, whereas in BHM prepared with α-amylase 2.5 units g^{-1} bread was 49.9 ± 7.11%. These results were in agreement with the previous work, that the effect of different α-amylase enzyme concentrations had no significant effect on glucose yield during the liquefaction step of bread hydrolysis. Moreover, without the addition of α-amylase enzyme, less glucose yield can be obtained, which demonstrates a synergistic action between α-amylase and glucoamylase enzymes (Sükrü et al., 2017).

A non-significant difference ($P > 0.05$) was observed between the results of total dry matter concentration and refractive index in both hydrolysate media. The non-significant difference ($P > 0.05$) in both concentrations of total simple sugars and total dry matter within hydrolysate media can indicate non-variation in the growth rate of *Streptomyces sp.* 170 and the productivity of secondary metabolites.

Inhibitory activity of pancreatic α-amylase inhibitors produced by *Streptomyces sp.* 170 in corn starch and bread hydrolysate media

The activity of pancreatic α-amylase inhibitors (PAAI) was represented in Fig. 3 and expressed by inhibitory unit per mL nutrient media (IU mL^{-1})

	24 h	48 h	72 h	96 h
■ CHM	500	970	1,050	1,300
▨ BHM (with α amylase 1,5 units g-1 bread)	0	950	1,111	500
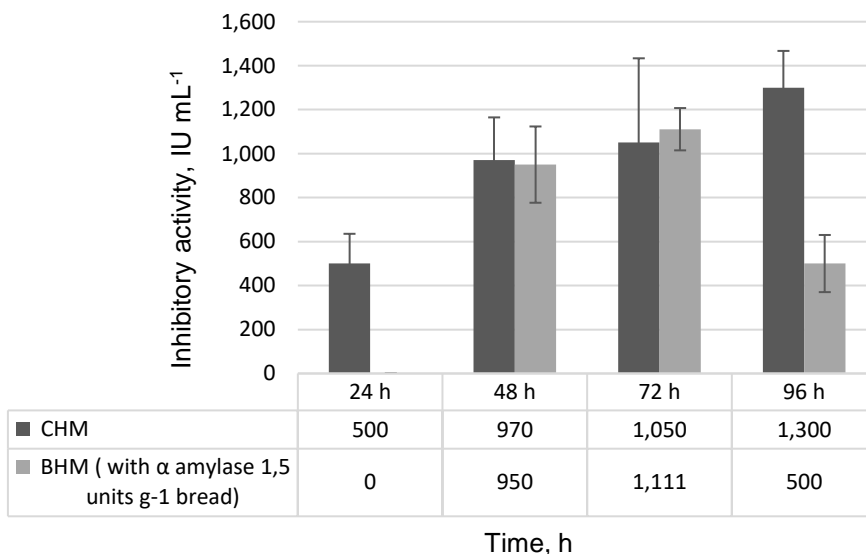
Figure 3. Inhibitory activity of pancreatic α-amylase inhibitors in CHM and BHM.

The results show that the difference between the maximum inhibitory activity in CHM ($1,300 \pm 100$ IU mL^{-1}) and the maximum inhibitory activity in BHM produced with α-amylase enzyme 1.5 units g^{-1} bread ($1,111 \pm 50$ IU mL^{-1}) is not significantly different ($P > 0.05$). Besides, the maximum inhibitory activity of PAAI was obtained after 96 h and 72 h of bacterial incubation in CHM and BHM respectively. According to literature, *Streptomyces* species can utilize glucose and maltose more than dextrin during their growth, thus can increase the production rate of secondary metabolites (Robert & Hubert, 1954; Sharova, 2015). The previous results of carbohydrates analysis revealed more glucose and maltose in BHM when compared to CHM, hence the reason for the rapid production of PAAI in BHM than in CHM. The same results were noticed previously that the highest glucose and maltose concentrations in hydrolysate media aid to rapid production of inhibitors by *Streptomyces* species (Pozdnjakova et al., 2009; Natalya, 2015).

Inhibitory activity of pancreatic α-amylase inhibitors produced by Streptomyces sp. 170 in bread hydrolysate media prepared with two different concentrations of α-amylase enzyme

Fig. 4 shows the inhibitory activity of pancreatic α-amylase inhibitors (PAAI) in BHM prepared with two different α-amylase enzyme concentrations (1.5 and 2.5 units g^{-1} bread).

	24 h	48 h	72 h	96 h
■ BHM (with α amylase 1,5 unit g-1 bread)	0	950	1,111	500
■ BHM (with α amylase 2,5 unit g-1 bread)	2,000	2,632	2,105	2,105

Time, h
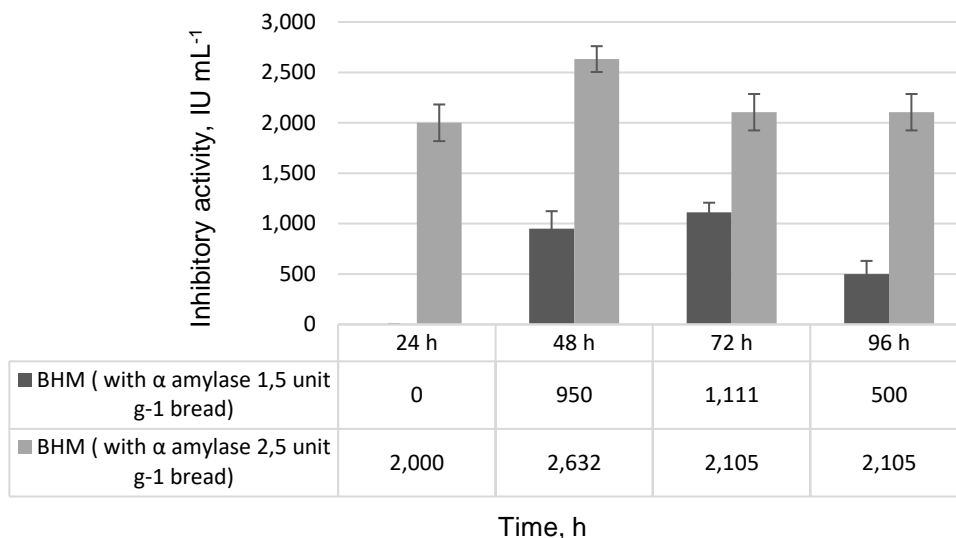
Figure 4. Inhibitory activity of pancreatic α-amylase inhibitors in BHM prepared with two different α-amylase enzyme concentrations.

The diagram shows a significant difference ($P > 0.05$) between the maximum inhibitory activity in BHM prepared with α-amylase 1.5 units g^{-1} bread ($1,111 \pm 50$ IU mL^{-1}) and the maximum inhibitory activity in BHM prepared with α-amylase 2.5 units g^{-1} bread ($2,632 \pm 100$ IU mL^{-1}). Furthermore, the maximum inhibitory activity was obtained after 72 h and 48 h of bacterial incubation in BHM prepared with α-amylase 1.5 unit g^{-1} bread and 2.5 unit g^{-1} bread respectively. The reason of these significant changes in the inhibitory activity can be justified by the slight increment of total simple sugars concentration in BHM prepared with α-amylase 2.5 units g^{-1} bread ($49.9 \pm 7.11\%$) than its concentration in BHM prepared with α-amylase 1.5 units g^{-1} bread ($45.1 \pm 8.25\%$). The prior investigation also showed a significant effect of glucose concentration in growth media on the productivity and activity of bacterial secondary metabolites (Bharathiraja et al., 2016). The same observation was established by Natalya (2015), which proved the significant effect of increment the α-amylase enzyme concentration during the hydrolysate media preparation to increase the activity of inhibitors produced by *Streptomyces sp.* 170.

The final result proves the ability of *Streptomyces sp.* 170 to produce PAAI with high activity ($2,632 \pm 100$ IU mL^{-1}) in BHM prepared with α-amylase 2.5 unit g^{-1} bread. The inhibitory activity of PAAI produced by different *Streptomyces* species was compared in Table 3.

Table 3. Comparison of the inhibitory activity of pancreatic α-amylase inhibitors produced by different *Streptomyces* species

Bacterial species	Inhibitory activity in the initial media, IU mL^{-1}	Conditions of α-amylase inhibitors production	Reference
Streptomyces lucensis ВКПМ Ac-1743[**]	1,600 ± 100	Starch hydrolysate medium, incubation at 32 °C for 120 h	(Sharova et al., 2018)
Streptomyces violaceus ВКПМ Ac-1734[**]	2,400 ± 100	Starch hydrolysate medium, incubation at 32 °C for 120 h	(Sharova et al., 2018)
Streptomyces species *K*-20	3,200 ± 200	Potato starch hydrolysate medium, incubation at 29 °C for 120 h	(Kolodyaznaya et al., 2014)
Actinoplanaceen S/E 50/13	2,800 ± 280	Sucrose medium, incubation at 30 °C for 120 h	(Xiaolong et al., 2006)
Streptomyces dimorphogenes nov. sp. NR-320-*OM 7HB*	1,240 ± 120	Glucose medium, incubation at 30 °C for 120 h	(Natalya, 2015)
Streptomyces sp. 170	2,632 ± 100	Bread hydrolysate medium, incubation at 29 °C for 48 h	Current study

[**]: the storage temperature of *Streptomyces lucensis* and *Streptomyces violaceus* was at -12 °C.

CONCLUSION

This article shows that the wheat bread hydrolysate medium could replace the traditional corn starch hydrolysate medium in cultivating *Streptomyces sp.* 170. In addition, the bread hydrolysis could produce higher simple sugars concentration than in corn starch hydrolysis. The different enzyme concentrations had no significant effect on the simple sugars concentration. Nevertheless, a non-significant change in the simple sugars concentration using different enzyme concentrations had a significant effect on the inhibitory activity of pancreatic α-amylase inhibitors. The maximum inhibitory activity of pancreatic α-amylase inhibitors was obtained in bread hydrolysate medium prepared with α-amylase 2.5 units g^{-1} bread after 48 h of bacterial incubation. There was no significant difference between the inhibitory activity of pancreatic α-amylase inhibitors produced in corn starch and bread hydrolysate media. Furthermore, the inhibitory activity reached its peak in bread hydrolysate medium faster than in corn starch hydrolysate medium. Comparing literature data with the current study revealed that *Streptomyces sp.* 170 has the ability to produce pancreatic α-amylase inhibitors with high activity. Therefore, the bread hydrolysate could be employed as a rich nutrient media in cultivating *Streptomyces sp.* 170 for synthesizing pancreatic α-amylase inhibitors with high activity.

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The effect of ageing on chosen quality characteristics of skeletal muscles of Aberdeen Angus bulls

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Abstract. The objective of the trial was to study the qualitative parameters of two muscles of Aberdeen Angus bulls with 250–300 kg carcass weight. After slaughter, *longissimus thoracis et lumborum* (LD) muscle and unseparated *semimembranosus* and *adductor femoris* (SMA) muscles were removed from the chilled carcasses. Muscles were vacuum-packed and wet aged at +2 °C for 10, 14, 18 and 20 days. Meat pH, electrical conductivity, shear force and colour were measured in all ageing times. Two thermal treatment methods (sous-vide (SV) and grilling) were used to determine cooking losses. The effects of muscles, ageing times and muscles by ageing times interaction was found with two-factorial analysis of variance. The effects of muscles, ageing times and muscle groups by ageing time interaction for raw and SV treated meat shear force was significant. Ageing decreased SV treated meat shear force from day 10 (40.8 N) to 18 (29.7 N). Fresh and SV treated LD muscle was tougher compared to the SMA muscle group, but SM showed a better response to the tenderness within 20 days of ageing. Redness and yellowness value was higher in the SM group in comparison to LD. Muscles showed good colour (lightness, redness and yellowness) stability within ageing for 20 days. No interactions were found between muscle groups and ageing times for SV treated and grilled beef cooking losses. However, SV treated meat lost more weight than grilled meat slices. The present study suggests that the optimal ageing time for meat is 18 days when the grilled meat cooking loss is the lowest.

Key words: beef cattle, *semimembranosus*, *adductor femoris, longissimus,* muscle, colour, shear force, cooking loss, Aberdeen Angus.

INTRODUCTION

Aberdeen Angus is one of the most numerous beef cattle breed raised in Estonia. The interest in the quality of the beef is increasing with its growing popularity. Within the finishing period, local beef production based on the system where animals were reared extensively in the pastures. Tenderness is one of the challenging factors that affect the consumer's satisfaction in beef (Egan et al., 2001; Cassens et al., 2018). Meat ageing under controlled refrigerated conditions is the most common method used by meat retailers to increase the tenderness of the beef (Guelker et al., 2013; Piao et al., 2015).

Meat tenderness increases within the ageing period because of the enzyme activities, which lead to myofibrillar degradation (Guerrero et al., 2013). Changes in the biochemical and microbiological characteristics take place during this process – meat colour, flavour, texture are altered – all these improve meat quality (Wicklund et al., 2005). Wet-aging of the beef is one of the cheapest methods to improve meat tenderness (Sitz et al., 2006). To improve meat tenderness, beef should age at least 14 days (Hanzelková et al., 2011). However, the tenderness of the aged meat depends also on the location of the muscle as some of them have greater physical activity than others (Simonetti et al., 2015). Monson et al. (2005) concluded that the various muscles need different time for ageing. Most of the active muscles myoglobin content is higher because of the increased oxygen requirement, which contains more pigments (Mancini & Hunt, 2005). Oxygenation of the myoglobin (blooming) takes place as the meat exposed to the oxygen (AMSA, 2012) and this process affects meat colour (Lee et al., 2008).

Most of the beef offered for retail sale are vacuum-packed fresh or thermally treated meat. Meat loses water during the ageing and processing because of the changes in the charges and structure of the myofibrillar protein. Production yield is associated with the level of weight loss during meat processing and affect an economic outcome. The level of water holding capacity of fresh meat is usually estimated through the time and labour consuming analyses. However, a limited number of studies are using other time efficient and real-time analyses. For example, Florek et al. (2007) used electrical conductivity measurements to determine the tenderness of the beef. The electrical conductivity characterizes the concentration and mobility of ions (Shi et al., 2014) and therefore, shows the level of destruction in the cell membranes. Increased conductivity point to the leaked water in the intra- and extracellular space (Byrne et al., 2000).

As consumers are using less and less time for food preparation, then the retailers are demanding more ready-to-eat products. Thermally treated meat products are offered usually as boiled, grilled or roasted. An aim of the meat processing industry is to improve processing to decrease weight loss of the product during cooking. Schellekens (1996) defines sous-vide as cooking of the raw products inside vacuum bags under controlled temperature and time. Several authors have been found that meat cooked this way have better textural properties (Christensen et al., 2012; Mortensen, 2012) and popular among consumers (Bañón et al., 2007). However, grilling involves direct, radiant heat and can be used for cooking meat quickly. Hotplate direct contact to the meat surface closes the space between the muscle fibres because of crust formation (Sánchez-Muniz & Bastida, 2006) and this help to keep the moisture inside the meat. Grilling and frying resulted in the lowest cooking losses compared to the various cooking methods in the Serrano et al. (2007), Alfaia et al. (2010) and Domínguez et al. (2014) studies. Contrary to these findings, Juárez et al. (2010) discovered that frying and grilling showed the highest moisture loss compared to boiling.

Therefore, final product quality and meat processing economic output depends on the skills to find optimal ageing time for muscles and thermal treatment method. Considering this, the objective of this study was to evaluate some physicochemical and textural properties of beef of Aberdeen Angus. The effects of different ageing times and cooking methods on the quality traits of the two muscle groups were studied. The emphasis of the study was to find optimal ageing time for the grass-fed beef muscles by analyzing muscle and ageing time interaction.

MATERIALS AND METHODS

The **carcasses** were obtained from free-ranged Aberdeen Angus bulls that had been slaughtered at a commercial abattoir in Arke Lihatööstus Ltd. The animal's transport time from the farm to the slaughterhouse was 1–2 h and the pre-slaughter resting duration in the lairage pens was approximately 3 h. Slaughtering was carried out to the accordance with good animal welfare practices and slaughtering procedures followed the Veterinary and Food Board of Estonia. The electrical stimulation was not used in the slaughterhouse to tenderize meat. Three randomly selected carcasses from the different batches were used. The age of the animals were 20–24 months and weight of the carcasses 250–330 kg.

After the dressing, carcasses were split by sawing centrally down the vertebral column, weighed and transported to the cold store to cool down at 0 °C for approximately 24 h. After the chill period, carcasses were deboned in accordance with the scheme used by the abattoir, whereas no primal cuts removed prior deboning. During this process, samples of the *longissimus thoracis et lumborum* (LD) muscle (loin) and a group of unseparated muscles, *semimembranosus* and *adductor femoris* (SMA) (round), were removed. The intramuscular fat content of the samples was 0.7–1.7%. Four samples were taken from each muscle group and vacuum-packed to determine changes in the meat quality within a 20-day ageing period. Bags were marked with a permanent marker with muscle name and packing date. The samples were wet aged at +2 °C for 10, 14, 18 and 20 days in the Memmert climate chamber ICH110 (Memmert GmbH, Schwabach, Germany). The climate chamber was equipped with two PT100 platinum resistance thermometers (Pico Technology, Cambridgeshire, UK) for mutual monitoring, which allows temperature stability ± 0.3 °C.

Two **cooking methods**, sous-vide and electric contact grill, were used for the preparation of samples. The muscles were cooked in the rotated order within the study period. For the sous-vide cooking, steaks with 2 cm thickness were vacuum-packed and submerged immediately in the water bath for 180 min at 65 °C. The bags were removed from the water bath and cooled down in icy water (2 °C) for 1 h. The meat samples were removed from the bags afterwards and used for the analyses.

The raw meat samples were removed from the bags and let shortly stay in the room temperature. Steaks (2 cm) were grilled in an electric contact grill Sage Smart Grill™ Pro BGR840 (Sage Appliances GmbH, Düsseldorf, Germany) preheated at 230 °C. The steaks core temperature were monitored by thermocouples attached with a digital monitor. Before the removal from the grill, an internal temperature of the meat samples was allowed to reach 65 °C (medium).

The **pH** was determined to the accordance with reference method ISO 2917:1999 on the all aged muscles using a pH-meter Mettler Toledo Seven Excellence (Mettler-Toledo LLC, Columbus, Ohio, USA). A device was equipped with an electrode Inlab Expert Pro-ISM with a built-in temperature sensor for temperature compensation during the measurement. Five grams of grounded meat was homogenized in 50 mL of 0.1 M KCL solution. The device was calibrated before use in the standard solutions pH 4.0 and 7.0 at the 23 °C. The calibration procedure and measuring samples were carried through at the room temperature (23 °C). The pH-meter probe was cleaned with distilled water and paper tissue between the measurements.

Electrical conductivity was measured respectively to the method from Yao et al. (2011). Five grams of beef sample was ground by blender and homogenized with 50 mL distilled water for 1 minute (6,000 revolutions per minute). The electrical conductivity of the beef sample was measured with a temperature compensated electrode Inlab 731-ISM attached to a device Mettler Toledo Seven Excellence (Mettler-Toledo LLC, Columbus, Ohio, USA). An electrical conductivity meter probe calibration procedure was performed with the potassium chloride standard buffer at 1413 μS cm⁻¹ and 12880 μS cm⁻¹.

Shear force analysis on raw meat and sous-vide treated samples was performed using a TA.XTplus Texture Analyser (Stable Micro System Ltd., Surrey, UK) equipped with Warner-Bratzler blade. Fresh meat slices were held for 120 min at –18 °C in the freezer and sous-vide cooked steaks were chilled for 24 h at 4 °C (AMSA, 2016). Freezing of the raw meat was necessary to ensure the uniformity of the drilled samples. Ten round cores with the 11 mm diameter were drilled parallel to the muscle fibres from each steak. Meat cores were held on the room temperature and sheared through the centre, perpendicular to the fibre direction at the constant speed 120 mm min⁻¹ and with the 50 N force.

The determination of meat surface **colour** measurements was carried through with a handheld spectrophotometer X-Rite 964 (X-Rite Inc., Grand Rapids, Michigan, USA). An assessment was conducted in the CIE L* (lightness) a* (redness) b* (yellowness) colour model using measurement geometry 0°/45°, D65 illuminant and 10° observer. The spectrophotometer was calibrated before the usage in accordance with the standard reference with a ceramic disk for white calibration measurements and a trap opening for black. 2 cm meat slices were cut perpendicular to the muscle fibres and hold in the open plastic bags at +2 °C for 2 h (blooming time) to ensure optimal myoglobin oxygenation (Lee et al., 2008; AMSA, 2012). Colour measurements were taken from the three different locations at the cutting surface of the meat steaks.

Sous-vide treated and grilled meat samples **cooking losses** were estimated to determine the loss of water and soluble substances of meat during thermal treatment. Sous-vide treated meat slices were cooled down in the ice slurry to stop the temperature increase, then removed from the bags and blotted with the paper towel to remove excessive moisture. Grilled steaks were let to cool down for 5 minutes under the foil to stop movements of water. All thermally treated samples were reweighed, wherein two cooking losses measurements (hot and cooled) were obtained for the grilled steaks. The total cooking loss was calculated in accordance with Eq. 1:

$$Cooking\ loss, \% = \frac{(raw\ weight, g - cooked\ weight, g)}{raw\ weight, g} \times 100 \qquad (1)$$

Statistical analysis. Analysis of variance was used to test the effects of muscles, ageing times and muscles by ageing time interaction, the fixed blocking effect of bull and the random effect of the muscle portion assigned to each ageing duration were also considered. The least square means corresponding to factors' levels were estimated and compared with the *Tukey* method in SAS 9.4. Relationships between variables are reported as Pearson correlation coefficients.

RESULTS AND DISCUSSION

No significant differences ($P > 0.05$) in pH mean values were found between muscles and ageing times, either interaction between ageing times and muscles was not detected (Table 1). However, the pH-value of LD muscles was slightly higher (5.55) than in SMA group of muscles (5.47) and effect of the muscle as a factor was found close to the statistical significance ($P = 0.114$). The ageing increased the pH-values of beef ($P > 0.05$) from day 10 (5.46) to day 18 (5.45), dropped slightly afterwards on the day 20 (5.52). The LD muscle acted a similar way regarding the interaction between the muscles and ageing times ($P > 0.05$). SMA muscle group pH decreased on day 14 (5.43) and showed an increase again on day 18, reached to the same level as on day 10 (5.46) and gained to the level of 5.52 on day 20.

Table 1. The effects of muscle groups, ageing times and muscle groups by ageing times interaction on technological parameters of beef

Factor / Levels	Technological parameters			
	pH (raw)	Electrical conductivity, µS cm^{-1} (raw)	Shear force, N (raw thawed)	Shear force, N (sous-vide)
Muscles (M)	$P = 0.114$	$P = 0.074$	$P < 0.001$	$P = 0.538$
LD	5.55	1081.6	27.6 [a]	38.7
SMA	5.47	1103.7	23.1 [b]	30.7
SE	0.051	16.2	2.24	9.68
Ageing time (AT)	$P = 0.398$	$P = 0.305$	$P = 0.094$	$P < 0.001$
Day 10	5.46	1087.6	27.9	40.8 [a]
Day 14	5.50	1110.1	24.1	34.7 [b]
Day 18	5.54	1081.3	25.1	29.7 [b]
Day 20	5.52	1091.6	24.3	33.7 [b]
SE	0.033	10.20	1.20	6.80
Interaction M x AT	$P = 0.302$	$P = 0.857$	$P = 0.002$	$P < 0.001$
LD				
Day 10	5.47	1069.9	29.0 [a]	41.5 [ab]
Day 14	5.56	1098.3	23.0 [ab]	37.9 [abc]
Day 18	5.62	1076.0	30.0 [a]	32.3 [acd]
Day 20	5.53	1082.3	28.2 [a]	43.2 [b]
SM				
Day 10	5.46	1105.2	26.8 [ab]	40.0 [abc]
Day 14	5.43	1121.9	25.1 [ab]	31.5 [cd]
Day 18	5.46	1086.7	20.2 [b]	27.1 [d]
Day 20	5.52	1101.0	20.4 [b]	24.2 [d]
SE	0.048	15.50	1.72	9.61

LD – *longissimus thoracis et lumborum* muscle, SMA – *semimembranosus* and *adductor femoris* muscle group; Factors' *P*-values and levels' least square means (*LSM*) with standard errors (*SE*) are presented; *LSM* of the same factor's levels without common superscript letter are statistically significantly different ($P < 0.05$, pairwise comparison of *LSM* followed by multiple comparison adjustment with *Tukey* method).

Meat pH-value is often related to technological and visual quality indicators, such as tenderness, colour, water holding capacity (Valero et al., 2014; Khan et al., 2016). Muscle pH in the living animal is around 7.0, but after the slaughter, it drops and 18–40 h later remains between 5.30–5.80 in the normal meat (Savell et al., 2005; Farmer

& Farell, 2018; Zhang et al., 2018). Muscles pHs has not exceeded values that correspond to the normal meat pH within the ageing period, which indicates an absence of the defective muscles. Small standard error value point to the limited variability of the pH-values. Over 80% of the carcasses pH fell into the narrow range (5.40–5.59) in Page et al. (2001) study. Simonetti et al. (2015) found that the pH of the Nellore bulls four muscles not differed significantly and was between 5.48–5.55. However, only *Trapezius thoracis* muscle showed significantly higher pH-value (5.66) compared to other muscles. They justified the existence of such difference with the lower glycogen level in the muscle, which was induced by the muscle extensive physical activity and shorter rest period.

Although ageing of the beef was performed in the vacuum-sealed bags, which promotes the growth of lactic bacteria due to anaerobic conditions and presumably increases acidity, the pH was not significantly affected by ageing time. Li et al. (2014) observed no changes in the muscle pH despite the significant increase in the count of the lactic acid bacteria between ageing day 8 and day 19 in the bag and vacuum ageing study. Contrary, Simonetti et al. (2015) found that the pH of the meat drops significantly on day 7 and increases on day 14. They inferred that the lower pH was observed especially due to the growth of the lactic bacteria, however, the microbial count was not observed. Tänavots et al. (2013) found a significant pH decrease after 28 days of ageing in the *longissimus* and *teres major* muscles. Constant pH within ageing may refer to the proper processing of the fresh meat, where the contamination with the bacteria is minimal (Jones, 2004). Therefore, a low count of lactic acid bacteria was not able to increase meat pH during a short ageing period, despite the favourable conditions in chill-stored and vacuum-packaged meat (low temperature and absence of oxygen).

Protein degradation intensifies in the lower pH conditions and free water augments in the muscle during this process. Dissolved substances hold charges that increases conductivity in meat (Schmitten et al., 1987). However, the present study showed that a relationship between pH and electrical conductivity was weak and non-significant (Table 4). The similar weak relationship between impedance and pH was found also by Page et al. (2001). As defective muscles were not detected, the pH-values remained in a narrow range and influenced the relationship between pH and electrical conductivity. Another reason for a weak relationship could be explained by the uneven moisture distribution and concentration inside the muscles.

Electrical conductivity not differed between muscles and ageing times and no interactions were found between muscles and ageing times (Table 1). However, the tendency to the lower electrical conductivity was observed in LD muscle, which indicates that the LD steaks contained less free fluid.

The fresh LD exhibited significantly ($P < 0.05$) higher value in the shear force assessment (27.6 N), compared to the SMA (23.1 N) (Table 1). The tenderness of the fresh steaks increased notably from day 10 to 14, but the change was not significant. However, a significant interaction was found between muscles and ageing times for the raw meat shear force ($P = 0.002$). LD muscle did not show a notable change in the shear force within 20 days of ageing, on the other hand, SMA tenderness improved from day 14 to 18 ($P > 0.05$). Raw LD muscle was significantly tougher on day 18 and 20, compared to the SMA at the same ageing times. Tänavots et al. (2013) observed tenderness escalation in LD muscle from day 14 to 28. Baldwin (2012) referred that the toughness of the muscle is related to the presence of the intramuscular connective tissue

and the myofibrillar component. Muscles with higher activity contain more connective tissue and are therefore tougher. Either increased α-white fibres in these muscles makes them less tender (Calkins et al., 1981). Kirchofer et al. (2002) classified *longissimus dorsi, semimembranosus* and *adductor* muscles as 'white', which indicate to their increased toughness. The results from the present study reflect their findings that LD muscle red and white fibres diameter and area is larger. Hence, more force was needed to cut through LD muscle.

The meat slices were heated up to 65 °C at which point proteins aggregate and makes cutting easier (Tornberg, 2005). Thermal treatment with the sous-vide method turned both muscles tougher compared to the fresh meat (Table 1). Slightly more force ($P > 0.05$) was applied to cut LD muscle (38.7 N), whereas the SMA group was tenderer (30.7 N). The significant effect of the muscles to the shear force was observed in Simonetti et al. (2015) and Cho et al. (2016) study.

Sullivan and Calkins (2011) classified LD muscle in their review article the toughest (WBSF > 46 N) and *semimembranosus* and *adductor* muscles tenderness were intermediate (WBSF > 39 N). However, they referred that some differences related to the toughness of the various muscles may exist among the authors. Stolowski et al. (2006) characterized LD muscle as 'tender with slow but continued response to ageing up to 42 d' and SMA as 'slightly tender with ageing response up to 14 d'. An interaction between the muscles and ageing times corresponded to the previous categories as the sous-vide treated SMA muscle group shear force was dropped notably since ageing day 14 (Table 1). LD response to ageing was modest and agreed with the Simonetti et al. (2015) results where they did not found a significant decrease in shear force from day 7 to 14. Tänavots et al. (2013) observed significant gain in LD tenderness from day 14 to 28 and later from day 35 to 60, which confirms that longer ageing period is necessary to improve this muscle tenderness. According to Shackelford et al. (1991), the beef is tender if its shear force remains below 45 N. The present study showed that both muscle groups could be considered as tender already on the tenth day of ageing so that the further ageing is not necessary. However, the shear force decline significantly from day 10 (40.8 N) to 18 (29.7 N) (Table 1).

Muscles, ageing times and their interaction did not show a difference in L* values (Table 2). Simonetti et al. (2015) found no interaction between ageing times and muscles and a difference was not existed in ageing from day 7 to 14, whereas only *Semitendinosus* muscle lightness was significantly higher from four others. Kadim et al. (2013) claimed that light diffusion properties during the ageing were related to the level of protein degradation due to the changes in the meat pH. This process intensifies the release of the free water, which flows to the surface of the meat through the spaces between the muscle fibres. The presence of excessive fluid on the surfaces leads to an increase in L* (Pereira et al., 2008). The previous finding was not confirmed in the present study, probably due to constant pH value within ageing time. Contrary, Sosin-Bzducha & Puchała (2017) found that beef lightness decreased within the ageing period up to 14 days. A relationship between meat L* and pH was not found either in the present study (Table 4). However, Page et al. (2001) found that L* is moderately related to pH ($r = –0.40$). At the same time, the negative relationship confirms previous claims that meat with lower acidity is lighter.

'White' muscles are less red as the myoglobin concentration in these muscles is lower (Cezar & Sousa, 2007). Myoglobin degradation is responsible for the red appearance of the meat, whereas darker colour is less favourable for consumers (Bass et al., 2008), while lipid peroxidation turns the colour of meat yellower (Sosin-Bzducha & Puchała, 2017). The a* (redness) and b* (yellowness) intensities showed the difference between muscles (Table 2). The highest values for both variables were found in SMA (a* = 20.3; b* = 19.1) and lowest in LD (a* = 14.3; b* = 15.4) ($P < 0.05$). Bass et al. (2008) observed also that high a* value was related to *semimembranosus* and *adductor* muscles, but a* value appeared to be lower in *longissimus*.

Table 2. The effects of muscle groups, ageing times and muscle groups by ageing times interaction on colour values of beef

Factor / Levels	Colour		
	L* (lightness)	a* (redness)	b* (yellowness)
Muscles (M)	$P = 0.644$	$P = 0.029$	$P = 0.021$
LD	38.0	14.3 [a]	15.4 [a]
SMA	39.8	20.3 [b]	19.1 [b]
SE	2.61	1.47	0.85
Ageing time (AT)	$P = 0.213$	$P = 0.214$	$P = 0.642$
Day 10	37.4	18.1	17.6
Day 14	38.3	17.8	18.0
Day 18	38.5	17.4	17.1
Day 20	41.3	15.9	16.4
SE	2.11	1.20	0.97
Interaction M x AT	$P = 0.298$	$P = 0.140$	$P = 0.319$
LD			
Day 10	37.4	15.3	15.8
Day 14	36.7	16.2	17.4
Day 18	39.1	13.0	13.9
Day 20	38.5	12.9	14.4
SMA			
Day 10	37.3	20.9	19.3
Day 14	39.9	19.4	18.5
Day 18	38.0	21.7	20.3
Day 20	43.9	18.9	18.4
SE	2.98	1.70	1.37

LD – *longissimus thoracis et lumborum* muscle, SM – *semimembranosus* and *adductor femoris* muscle group; Factors' *P*-values and levels' least square means *(LSM)* with standard errors (*SE*) are presented; *LSM* of the same factor's levels without common superscript letter are statistically significantly different ($P < 0.05$, pairwise comparison of *LSM* followed by multiple comparison adjustment with *Tukey* method).

Ageing decreased slightly intensities of the redness (a*) and yellowness (b*) colour ($P > 0.05$), which corresponds to the findings in *gluteus medius* steaks used in Lee et al. (2008) study. Contrary, in the research conducted by Simonetti et al. (2015) and Sosin-Bzducha & Puchała (2017), the a* and b* increased within the 14 days ageing period. Differences in the results can be explained by the longer blooming time in the present study. Lee et al. (2008) proved that blooming development related deoxygenation and pigment reduction processes (AMSA, 2012), were not fully stabilized on 120 min. They indicated also that the differences in the extent of the blooming might exist between

various muscle types. An absence of oxygen in the vacuum bags may also slow down the process of discolouration.

Strong positive correlation was found between a* and b* value ($r = 0.69$; $P = 0.003$) in the present study (Table 4) and even stronger relationship ($r = 0.95$) was observed for *longissimus* muscle by Page et al. (2001) and Giaretta et al. (2018), showing that discolouration processes are related to the same direction. A negative moderate relationship between colour measurements and pH ($r_{a*;pH} = -0.59$; $P_{a*;pH} = 0.016$ and $r_{b*;pH} = -0.46$; $P_{b*;pH} = 0.071$) indicate that acidity of the muscle plays important role in muscle colour by altering hue.

Table 3. The effects of muscle groups, ageing times and muscle groups by ageing times interaction on cooking losses

Factor / Levels	Cooking losses related to the treatment methods		
	Sous-vide	Grilled (hot)	Grilled (cooled)
Muscles (M)	$P = 0.995$	$P = 0.908$	$P = 0.977$
LD	28.2	14.1	19.0
SMA	28.1	14.5	18.8
SE	3.88	2.41	2.64
Ageing time (AT)	$P = 0.646$	$P = 0.105$	$P = 0.058$
Day 10	29.3	18.9	25.7
Day 14	27.8	15.5	19.7
Day 18	28.1	11.5	14.8
Day 20	27.4	11.3	15.3
SE	2.90	2.46	2.73
Interaction M x AT	$P = 0.754$	$P = 0.445$	$P = 0.313$
LD			
Day 10	29.0	21.6	29.9
Day 14	27.0	15.5	19.1
Day 18	28.9	10.2	13.5
Day 20	27.9	9.2	13.4
SMA			
Day 10	29.6	16.3	21.5
Day 14	28.6	15.6	20.4
Day 18	27.4	12.8	16.2
Day 20	27.0	13.4	17.3
SE	4.10	3.47	3.86

LD – *longissimus thoracis et lumborum* muscle, SMA – *semimembranosus* and *adductor femoris* muscle group; Factors' *P*-values and levels' least square means (*LSM*) with standard errors (*SE*) are presented.

No interaction was detected between muscles and ageing times for cooking losses, both factors, muscles and ageing times, was not significant. Sous-vide treated and grilled muscle groups showed relatively equal weight losses. Grilling is the preferred thermal treatment method compared to the sous-vide, as the LD muscle lost 14.1% and the SMA group 13.6% less weight. A relatively big difference could be explained by circumstances that the longitudinal muscle fibres shrinking as muscle temperature increases above 60–65 °C and this causes substantial water loss (Baldwin, 2012). Ismail et al. (2019) confirmed that beef *Semitendinosus* muscle water loss was significantly smaller at a lower temperature (45 °C), compared to sous-vide treated meat slices at 65 °C. On the other hand, grill hotplate direct contact with the meat slice helps crust formation (Sánchez-Muniz & Bastida, 2006) and prevents the release of the fluids from

the meat. Fabre et al. (2018) observed a significant difference between *longissimus* and *semimembranosus* muscle cooking losses by using oven and griddle plate treatment, but there was no difference in water bath cooking.

Grilled meat (hot and cooled) cooking loss decreased within the ageing period from day 10 to day 14 and day 14 to day 18 (Table 3) probably due to increased proteolytic activity, which caused swelling of myofibrils due to the uptake of water. Grilled meat tend to hold moisture better, which makes it juicier and more acceptable to consumers.

Table 4. Correlations between beef quality properties

	Traits	1.	2.	3.	4.	5.	6.	7.	8.	9.
1.	SF (raw), N	1.00	–0.33	–0.18	–0.38	0.01	–0.22	0.33	0.18	0.22
2.	pH	–0.33	1.00	0.16	–0.13	–0.59[*]	–0.46[#]	–0.48[#]	–0.58[*]	–0.50[#]
3.	EC, µS/cm (raw)	–0.18	0.16	1.00	0.11	0.24	0.44[#]	–0.35	–0.21	–0.20
4.	L* (lightness)	–0.38	–0.13	0.11	1.00	–0.09	0.39	0.24	0.04	0.01
5.	a* (redness)	0.01	–0.59[*]	0.24	–0.09	1.00	0.69[**]	–0.05	0.21	0.13
6.	b* (yellowness)	–0.22	–0.46[#]	0.44[#]	0.39	0.69[**]	1.00	0.05	0.21	0.16
7.	CL (sous-vide), %	0.33	–0.48[#]	–0.35	0.24	–0.05	0.05	1.00	0.453[#]	0.40
8.	CL (grilled, hot), %	0.18	–0.58[*]	–0.21	0.04	0.21	0.21	0.45[#]	1.00	0.97[***]
9.	CL (grilled, cooled), %	0.22	–0.50[#]	–0.20	0.01	0.13	0.16	0.40	0.97[***]	1.00

SF – shear force, EC – electrical conductivity, CL – cooking loss; # indicates that the correlation is different at level 0.1; * indicates that the correlation is significantly different at the level 0.05; ** indicates that the correlation is significantly different at the level 0.01; *** indicates that the correlation is significantly different at the level 0.001.

CONCLUSIONS

Ageing time has an effect on the technological and qualitative parameter of beef, by improving tenderness and colour. Muscle effect in ageing must consider as *longissimus thoracis et lumborum* muscle was tougher than *semimembranosus* and *adductor femoris* muscle group and therefore need longer ageing. Although both muscle groups were tender on the ageing day 10, the optimal ageing time for the vacuum-packed *semimembranosus* and *adductor femoris* muscle group is 18 days.

The *semimembranosus* and *adductor femoris* muscle group has a higher colour value, which attracts consumers more, compared to the *longissimus thoracis et lumborum* muscle. Muscles showed good colour (lightness, redness and yellowness) stability within ageing for 20 days.

Ageing has a slight positive effect on the grilled beef cooking loss, but there are no changes in sous-vide treated meat.

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The determination of impact of malt grist moisture on porosity and permeability using measurement of differential air pressure

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Abstract. This article is focused on determination of malt grist and spent grains porosity and permeability using measurement of differential pressure of the air passing through the layer of malt grist and spent grains. For preparation of malt grist were used different disintegration equipment (two roller mill and disc mill). The method of differential pressure measuring is used for the determination of porosity of malt grist layer, defined as fraction of the volume of voids over the total volume. Measurement confirmed the logical assumption; the higher-pressure difference is above and below the spent grain layer, the lower value of porosity.

Key words: malt grist, special surface area, porosity, permeability, spent grains.

INTRODUCTION

The topic of this article was the determination of impact of malt grist fineness moisture on porosity and permeability using measurement of differential pressure. Both parameters are of great importance for the way of disintegration having significant impact on final structure of malt grist, on quality of filtered first wort and, of course, for final beer. For determination of permeability and porosity of malt grist was used the equipment for measurement of differential pressure above and below the layer of the sample, its flow schema is shown and described in Fig. 3. The topic of porosity and permeability of cereal grist with different roughness was studied and published f.e. by Chládek (Chládek, 1977).

Before the actual mashing process, the malt is mechanically grinded using malt mills (grinders) that work on the principle of grinding between two, four, six pairs counter-rotating roller mills, grinding using rotating hammers and grinding between two discs (so called dispersing reactor Dispax) and wet milling. The product of malt grinding is called a malt crush, which is the basic material for the actual beer brewing (Kunze, 2010; Vaculík et al., 2013, Smejtková et al., 2016).

Basic aspects of milling

In order to give the malt enzymes, the opportunity, during mashing, to act on the malt contents and break them down, the malt must be broken into small fragments. This

process is called milling. The disintegrated malt used for a brew is called the malt grist and its amount used is called the charge.

Milling is a mechanical process and within it the husks should be disintegrated carefully because they are lately used in lauter tun as a filter material. A whole series of consideration must be taken into account when fragmenting the malt. But before the malt is milled the amount used is weighed with a weighing machine. The malt is fragmented in a grist mill. Depending on the process used a distinction is made between: dry milling, wet milling, hammer milling, dispersion milling.

In Table 1 and 2 are shown optimal composition of malt grist both for lauter tun mash filter, using both Pfungstädter and MEBAK Plansifter For mash filter is possible to use finer disintegration of malt, allowing shorter time of lauter process.

Table 1. Composition of malt grist using Pfungstädter plansifter

Sieve no.	Fraction	Wire thickness (mm)	Mesh hole size (mm)	
1	husks	0.31 (0.800)	1.270	(1.25)
2	coarse grists	0.26 (0.630)	1.010	(1.00)
3	fine grits I	0.15 (0.315)	0.547	(0.50)
4	fine grits II	0.07 (0.160)	0.253	(0.25)
5	flour	0.04 (0.080)	0.152	(0.125)
Sieve bottom fine flour		-	-	

The mesh hole size slightly changed by MEBAK (Mitteleuropäische Brautechnische Analysenkommission e. V) lead to the same results. The following grading can be taken as normal values for a good tun or mash vessel grist (Table 2).

Table 2. Composition of malt grist for lauter tun and for mash filter using MEBAK equipment

	Lauter tun grist (%)	Mash filter grist Conventional and Type 2001	
		Conventional, (%)	Type 2001
Sieve 1	18–25	11	1
Sieve 2	< 10	4	2
Sieve 3	35	16	15
Sieve 4	21	43	29
Sieve 5	7	10	24
Sieve bottom	< 1 –15	16	29
Husk volume (mL 100 g^{-1})	> 700	> 650	

During mashing, the enzymes must be able to get at the malt contents in order to degrade them. For this the malt must be broken into smaller pieces. The greater the extent of comminution, the larger the surface area available for enzymic attack and the better the breakdown of the malt material. But after mashing the wort must be run off.

This is a filtration process in which - depending on the mash separation equipment used - the husks are needed as a filter material. Because the husks are required for mash separation (lautering), they must be disintegrated as little as possible during milling. A dry husk fragments easily and filterability is greatly reduced by the small fragments produced by disintegration of the husk. On the other hand, the husk is more elastic the

wetter it is and it is then easier to protect. Consequently, lautering is more rapid. The process of wetting the husks is known as conditioning. If a lot of water is added, however, the entire contents of the grain become moist and are then squeezed out of the husk during comminution. This process is known as wet milling. Nowadays the general preference is for a dry endosperm which can be ground as required during milling and, if possible, a moist, elastically deformable husk.

Comminution of the malt depends on its modification. A more modified malt presents little resistance to the grinding roller during milling since the interior of the com is friable and loosely packed. Therefore, the fraction of flour and fine grits is high in the case of well-modified malts. These grits and the flour from well-modified malt sample contain enzymes and readily dissolve later in the brewhouse. Badly modified corn tips and badly modified malt corns are harder and not so easily comminuted. This can be seen from the larger fraction of coarse grits. Because their internal conversion has been retarded they still require extensive enzyme degradation. They release their extract only with difficulty. A smaller yield must therefore be expected if these coarse grits are not completely degraded in the brewhouse:

- milling must be finer the less well modified the malt;
- the degree of comminution has a decisive influence on the volume and filtration efficiency of the spent grains.

ln the case of commonly used lauter vessels the finer the milling the smaller the volume of spent grains, but the finer the grist, the less porous the filter bed, the sooner it becomes pressed together and so the longer the filtration takes.

It can even happen that the wort no longer flows through at all. Consequently, when using a lauter tun, milling must not be too fine, or else the spent grain depth must be reduced if finer milling is used. When a modem mash filter is used none of this applies because the filtration is performed through a very mail pore polypropylene cloth. Therefore, when using such a mash filter the malt can be very finely ground by a hammer mill and very good yields are consequently obtained. The most commonly used mills in breweries are dry grist mills. ln them the malt is crushed in a dry state between rollers arranged in pair. Depending on the number of rollers these are classified as two roller mills, four roller mills, five roller mills, six roller mills, conditioned dry milling equipment and wet milling (Kunze, 2010).

Spent grains

On spent grain discharge, about 100 to 120 kg of spent grains containing 70 to 80% water are obtained from 100 kg of malt grist; this is 21 to 22 kg spent grains h L^{-1} beer. Dried spent grains have approximately the following composition: protein 28.0%, fat 8.2%, nitrogen-free extract 41.0%, cellulose 17.5%, inorganic material 5.3%. The spent grains are sold as cattle food if possible. The nutritional value of spent grain is approximately one-fifth of that of the same amount of barley. This is understandable if one remembers that as much extract as possible is removed during mashing. The advantage of spent grain is its better digestibility compared with the original material. Spent grains do not contain any vitamins and so they cannot be used on their own as animal feed. The spent grains still contain sugar and a lot of protein. Consequently, in warm weather they can easily become sour. There is however not always, or everywhere, a demand for spent grains by neighbouring farmers. In that case, their rapid and

satisfactory disposal must be arranged before they turn sour and start to smell. lt is however becoming increasingly difficult for breweries to dispose of spent grains. More and more breweries are therefore starting to dry the spent grains and bum them. As a result of this, a considerable part of the energy used in the brew-house can be recovered. Another way is in fermenting the spent grains and producing biogas, the burning of which can also result in the recovery of energy (Kunze, 2010).

MATERIAL AND METHODS

Procedure for processing samples of malt and malt scrap for measurement purposes

Malt were crushed using following equipment: two roller mill VKM 130/150 with grinding gap adjustments of this mill in the range 0.4 mm (the capacity of the mill is 200.0 kg h^{-1}) and disc mill Skiold SK 2500 (the capacity of the mill is 12,000 kg h^{-1}) (Figs 1, 2 and Table 3).

Figure 1. Two roller mill VKM 130/150.

Figure 2. Disc mill Skiold SK 2500.

Two roller mills for dry milling are used in craft breweries only (Chládek, 2007; Chládek et al., 2013). Because no??? further differentiation of the grist can be obtained with only one pair of rollers during dry milling, optimal yields cannot be obtained (Bogdan & Kordialik-Bogacka, 2017; Chládek et al., 2018). This does not apply to two roller mills used for wet milling or with conditioned malt (Chohan & Garson, 2009; Kunze, 2010).

Table 3. Technical parameters of mills

Two roller mill VKM 130/150		
Type	(-)	two roller mill
Made in	(-)	Czech republic
Grinding gap	(mm)	0.4
Capacity	(kg h^{-1})	200
Disc mill Skiold SK 2500		
Type	(-)	disc mill
Made in	(-)	Denmark
Grinding gap	(mm)	0.0; 0.4
Capacity	(kg h^{-1})	12,000

Next equipment used for measurement was disc mill Skiold SK 2500, supplied by company Skiold Sæby, Denmark (Fig. 2).

The disc mills are mainly used for preliminary and fine grinding of medium-hard and hard-brittle substances (Vaculík et al., 2013; Smejtková et al., 2016).

The experimental measurements were carried out at constant temperature 25 °C and air pressure 968,9 hPa.

Every experiment was repeated 10 times. The average value was calculated and the results are shown in following tables.

Measurement of porosity and permeability of malt grist and spent grains prepared from different malts

Measurement of porosity and permeability of malt grist and spent grains prepared from different malts was published by Chladek 1977.

Definition of porosity is following: porosity or void fraction is a measure of the void (i.e. 'empty') spaces in a material, and is a fraction of the volume of voids over the total volume, between 0 and 1, or as a percentage between 0% and 100% (Basařová, 2010; Kunze, 2010; Kosař & Procházka, 2000).

Parameters of the barley malt shown in the Table 4.

Table 4. Parameters of the barley malt

Variety	(-)	Sladek
Harvest	(year)	2018
Friction angle	(°)	32
Size of whole grain	(mm)	3.65 ± 0.142

The individual weights of the samples in the measuring chamber (Table 8).

Density of the spent grains sample (Feynman et al., 2011):

$$\bar{m} = \frac{m_1 + m_2 + m_3}{3} \ \text{(g)} \qquad (1)$$

$$\rho' = \frac{\bar{m}}{V} \ \text{(g cm}^{-3}) \qquad (2)$$

$$\varepsilon = 1 - \frac{\rho'}{\rho} \ \text{(-)} \qquad (3)$$

$$Q = S \cdot v \ \text{(m}^3 \text{ s}^{-1}) \qquad (4)$$

where $\bar{m}$ – average mass of the sample of the spent grains in the chamber (g); m_1, m_2, m_3 – the individual weights of the samples in the measuring chamber (g); ρ' – volume weight of the spent grains sample (g.cm^{-3}); ρ – density of the spent grains sample (g.cm^{-3}); ε – porosity of dry malt grist (-); S – area of cross-sectional the sample chamber (m^2); v – airflow velocity (m s^{-1}); Q – volume flow of air through the sample (m^3 s^{-1}).

Definition of permeability is following: permeability is the ability of a substance to allow another substance to pass through it, especially the ability of a porous rock, sediment, soil, or material to transmit fluid through pores and cracks. Darcy's law is an equation that describes the flow of a fluid through a porous medium (Feynman et al., 2011; Diyokeugwu & Glover, 2018).

The average dynamic viscosity value $\eta = 18.25.10^{-6}$ Pa.s was used for the calculations. Differential pressures for the dry wheat sample (100% Light barley malt) were experimentally determined for individual samples (Table 5).

Table 5. The resulting values for the calculation of the density of the spent grains sample

Sample no.	Δp_1 (Pa)	Δp_2 (Pa)	Δp_3 (Pa)
1	28.1	27.1	26.2
2	28.2	25.8	26.4
3	28.6	26.6	26.5
4	28.6	25.8	26.1
5	28.9	26.1	26.2
6	29.4	27.7	26.3
7	29.3	27.5	26.3
8	29.5	26.2	26.5
9	28.0	26.4	26.2
10	29.0	26.7	26.1
Mean	28.761	26.59	26.28
Standard deviation.	0.54659451	0.66741625	0.14757296
Coefficient of variation	1.90047115	2.51002725	0.56154093
$\Delta\bar{p}$ (Pa)	27.21		

Permeability of the spent grains sample is defined by following equation:

$$\Delta\bar{p} = \frac{\Delta p_1 + \Delta p_2 + \Delta p_3}{3} \quad \text{(Pa)} \tag{5}$$

$$A = \pi \cdot r^2 \quad \text{(m}^2\text{)} \tag{6}$$

$$K = \frac{Q \cdot \eta \cdot L}{\Delta\bar{p} \cdot A} \quad \text{(m}^2\text{)} \tag{7}$$

where Δp_1; Δp_2; Δp_3 – differential pressures of dry samples (100% barley malt) experimentally determined for individual samples (Pa); $\Delta\bar{p}$ – average value of the differential pressure of the dry barley malt (Pa); r – inside diameter of the measuring chamber (Table 6) (m); A – area of cross section (Table 6) (m²); L – height of the measuring chamber (Table 6) (m); Q – volume flow of air through the sample (m³ s⁻¹); η – dynamic air viscosity (10^{-6} Pa.s); K – permeability according to Darcy's relationship (Table 6) (m²) (Feynman et al., 2011).

Table 6. The resulting values for the calculation of the density of the spent grains sample

r (m)	A (m²)	L (m)	K (m²)
0.133	0.056	0.00098	$3.9.10^{-7}$

The description of laboratory measuring instrument for differential pressure determination

The laboratory measuring instrument was connected according to assembly diagram as shown in Figs 3 and 4. From pressure balloon (1) with pressure reducing valve (2) was pipeline divided, one branch of it was connected with the differential pressure gauge (4), the second branch was connected with the bottom of sample chamber (3). Above the top of sample chamber (3) the pipe-line was again divided, one branch led to the differential pressure gauge (4), second one was connected with the flowmeter (5), from which the measured air escaped free into space. For measurement of the values of differential pressure of the air below and above sample chamber was used differential manometer type Testo 512 (Table 7), supplied by company Conrad (Germany).
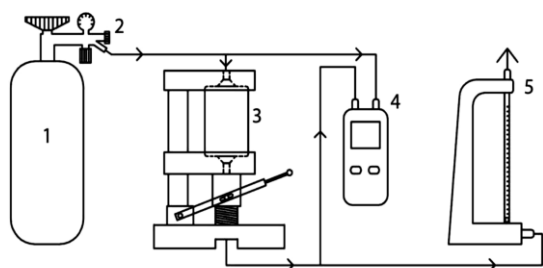
Figure 3. The scheme of the laboratory measuring instrument for differential pressure determination above and below the sample layer: 1 – pressure balloon with compressed air; 2 – pressure reducing valve; 3 – measuring chamber; 4 – differential pressure gauge; 5 – flowmeter.

Figure 4. Measuring assembly for determining differential pressure in samples: 1 – pressure balloon with compressed air; 2 – pressure reducing valve; 3 – measuring chamber; 4 – differential pressure gauge; 5 – flowmeter.

Table 7. Technical parameters of differential manometer Testo 512

Sensor type	(-)	integrated pressure transducer
Measuring range – pressure	(hPa)	0.0–20.0
Measuring range – speed	(m s⁻¹)	+ 5.0 – + 55.0
Distinction – pressure	(hPa)	0.001
Distinction – speed	(m s⁻¹)	0.1
Dimension (w x d x h)	(mm)	202.0 x 57.0 x 42.0
Weight	(g)	300.0

As the source of the pressured air was used pressure balloon with pressure reducing valve, supplied by company MedX5, its parameters are described in Table 8.

Measurement method:

- before measurement the testing chamber was weighed,
- chamber filling by measured sample up to upper edge, cleaning the upper edge,
- the desired air pressure was adjusted by pressure regulator,
- due to air pressure was sample inside of testing chamber pressed down, that why was chamber refilled up to upper edge,

Table 8. Technical parameters of a pressure bottle with a regulator

Volume	(l)	9.0
Weight	(kg)	4.8
Operating pressure	(MPa)	30,0
Length	(mm)	628.

- after new cleaning the upper edge was the testing chamber was again weighed and put in measurement system,
- the pressure difference at inlet and outlet of air from sample chamber was measured.

RESULTS AND DISCUSSION

According to moisture contentin malt grist and in spent grains, the values of permeability and porosity significantly changed (Table 12). During moisture content varying in measured samples were not found any linear dependence of permeability on water content.

For experimental activities had been used following samples (Table 9):

Table 9. Basic parameters of used equipments

Sample	Mill	Grinding gap (mm)	Distributor roller (-)	Rotation speed (r min^{-1})
Spent grains (100% light barley malt – malt 1)	two roller	0.40	-	200
Spent grains (75% wheat barely malt, 25% dark barley malt - malt 2)	two roller	0.40	-	200
Light barley malt	two roller	0.40	-	200
Light barley malt	disc	0.40	1/3	2,800
Light barley malt	disc	0.15	1/3	2,800
Light barley malt	disc	0.15	2/3	2,800

The individual weights of the samples in the measuring chamber: $m_1 = 10.61$ g; $m_2 = 9.93$ g; $m_3 = 9.49$ g (Table 10).

Table 10. Dimensions of the measuring chamber and resulting values for the calculation of the density of the spent grains sample

Diameter d (mm)	Height h (mm)	Volume V (mm^3)	
17.57	56.8	55,086.0	
Average mass $\bar{m}$ (g)	Volume weight ρ' (g cm^{-3})	Porosity ε (-)	Volume flow Q (m^3 s^{-1})
10.00	0.176	0.863	0.0095

As shown in the Table 12 there is a comparison of two samples of brewery-spent grains. The values of permeability of measured samples did not differ from one another. The higher value of permeability $3.09.10^{-8}$ m^2 was shown, only by spent grains gained from light malt (spent grain 1), while the permeability of second sample from spent grains brewed the combination of light and Bavarian malt was determined $2.86.10^{-8}$ m^2. This explains the significantly longer time of lautering process of sweet wort, using as a raw material next to light malt as well Bavarian, caramel and colour ones. Due to higher temperature of kilning in malt plant its texture is more fragile causing the decrease its permeability.

Every experiment was repeated 10 times. The average value was calculated and the results are listed in tables (Table 11 and 12).

Measurement confirmed the logical assumption; the higher-pressure difference above and below the spent grain layer, the lower value of porosity and permeability.

The values of permeability of sample No. 1 were in the range $4.11.10^{-7}$ m^2 up to $3.09.10^{-7}$ and porosity 0.85 up to 0.45. The values of permeability of sample No. 2 were in the range $3.69.10^{-7}$ m^2 up to $2.86.10^{-7}$ and porosity 0.83 up to 0.36 (all those figures are the average of tens measurements).

Measured values correspond with the results published in article 'Alternatives to malt in brewing' (Bogdan & Kordialik-Bogacka, 2017), 'Pivovarství' (Basařová, 2010) and others.

Table 11. Permeability and porosity values for different moisture content in malt grist

Type of mill	Two roller mill	Disc Mill	Disc mill	Disc mill	Disc mill
Grinding gap	0.4 mm	0.4 mm	0.4 mm	0.0 mm	0.0 mm
Distributor roller	-	1/3	2/3	1/3	2/3
Permeability and porosity values for dry malt grist (moisture content 4%)					
Porosity	0.64	0.55	0.62	0.55	0.48
Permeability	$1.17.10^{-7}$	$2.72.10^{-9}$	$3.73.10^{-9}$	$1.14.10^{-9}$	$5.81.10^{-9}$
Permeability and porosity values for malt grist (moisture content 45%)					
Porosity	0.51	0.54	0.42	0.38	0.43
Permeability	$1.14.10^{-7}$	$1.52.10^{-7}$	$1.70.10^{-7}$	$1.79.10^{-7}$	$9.73.10^{-8}$
Permeability and porosity values for malt grist (moisture content 50%)					
Porosity	0.31	0.33	0.29	0.27	0.28
Permeability	$1.05.10^{-8}$	$1.24.10^{-8}$	$2.08.10^{-8}$	$2.12.10^{-8}$	$2.38.10^{-8}$
Permeability and porosity values for malt grist (moisture content 60%)					
Porosity	0.30	0.32	0.28	0.26	0.27
Permeability	$1.32.10^{-8}$	$1.58.10^{-8}$	$2.56.10^{-8}$	$3.21.10^{-8}$	$3.52.10^{-8}$
Permeability and porosity values for malt grist (moisture content 65%)					
Porosity	0.39	0.31	0.22	0.24	0.22
Permeability	$3.48.10^{-8}$	$2.14.10^{-8}$	$3.11.10^{-8}$	$3.56.10^{-8}$	$4.24.10^{-8}$

Table 12. Comparison of permeability, porosity, moisture content and differential pressures of samples of grain

Malt (Table 9)			Moisture content in the malt					
			0	45	50	60	65	80
			(%)					
Sample 1	Δp	(hPa)	0.26	0.30	0.32	0.39	0.42	0.57
	Porosity	(-)	0.85	0.74	0.81	0.69	0.72	0.45
	Permeability	(m^2)	$4.11.10^{-7}$	$3.76.10^{-7}$	$3.62.10^{-7}$	$.3.50.10^{-7}$	$3.25.10^{-7}$	$3.09.10^{-7}$
Sample 2	Δp	(hPa)	0.27	0.31	0.38	0.57	0.68	0.74
	Porosity	(-)	0.83	0.70	0.73	0.65	0.62	0.36
	Permeability	(m^2)	$3.69.10^{-7}$	$3.59.10^{-7}$	$3.46.10^{-7}$	$3.19.10^{-7}$	$3.02.10^{-7}$	$2.86.10^{-7}$

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Utilization of image analysis for description of drying characteristics of selected tropical fruits

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Abstract. This study is focused on the utilization of image analysis for description of dimensions, and colours changes of fruits during drying process. Selected tropical fruits such are banana (*Musa acuminata*), mango (*Magnifera indica*) and pineapple (*Ananas comosus*) originally from North Sumatera in Indonesia were used in this experiment. Sliced pieces of the fruits were dried in experimental oven under temperature 90 °C for period of time 180 min and image of fruits samples were recorded by digital camera with HD resolution continuously throughout drying process. With aid of image analysis using Image J software and regarding to drying characteristics the colours and dimensions of the samples were analysed.

Key words: shape, dimensions, properties, mango, banana, pineapple.

INTRODUCTION

Understanding to the drying behaviour of tropical fruits is one of key factors which can strongly influence the quality and hence the sale price of final product (Mohsenin, 1970; Agrawal & Methekar, 2017). Tropical fruits such are banana (*Musa acuminata*), mango (*Magnifera indica*) and pineapple (*Ananas comosus*) are one of the main agricultural products of Southest Asia which are used in the production of drying commodities (Nakasone & Paull, 1998) and thus they are of great interest to food processors using ideas and theories of Industry 4.0 respectively Agriculture 4.0 with regard to high level quality of final products.

Nowadays in the era of Industry 4.0 it is necessary to use more accurate information as input data into virtual models or into artificial intelligence machines. Knowledge of the usual drying properties of agricultural products are not entirely sufficient and it is necessary to better understand to the changes of the shapes, dimensions, colours of dried fruits during whole drying process (Mongpraneet et al., 2002; Sharma et al., 2005; Adak et al., 2017). This precision information is also utilized in simulation models for the results` specification and verification (Petrů et al., 2012; Petrů et al., 2014; Mizera et al., 2017). In the current literature there are published studies focused on the drying characteristics (Karathanos & Belessiotis, 1997; Krokida et al., 2003; Soomro, 2014)

and several studies related to the dimensions' changes (Herák, 2016; Herák et al., 2018) however only few publications (Mizera et al., 2018; Salehi & Kashaninejad, 2018) are related to the description of the dimension, shape or colour changes in dependency on drying time.

Thus the aim of this article is to determine dependency between dimensions` changes, colour`s changes and drying time of banana (Musa acuminata), mango (Magnifera indica) and pineapple (Ananas comosus) with aid of image analysis.

MATERIALS AND METHODS

Sample
Banana (*Musa acuminata*), mango (*Magnifera indica*) and pineapple (*Ananas comosus*) obtained from Medan, Indonesia, were used for this experiment. Samples with diameter 20 mm and thickness 3 mm with following initial moisture content values, $77.7 \pm 0.8\%$ (w.b.) - banana, $87.5 \pm 1.4\%$ (w.b.) - pineapple, $86.6 \pm 1.2\%$ (w.b.) - mango, were prepared from peeled and sliced pieces of fruits (Fig. 1).

Drying experiments
Drying of samples were carried out in the experimental drying oven (CZU TF, Czech Republic) equipped with HD camera (EOS M10, Canon, Tokyo, Japan). Samples of all three fruits were placed on sieve in the oven (Fig. 1). To explore the effect of drying on the dimensions and colour of the samples the temperature of drying was set to 90 ± 1 °C. The process of drying was continuously recorded by HD camera with frame rates 25 fps for 180 min. Experiment was repeated three times.

Figure 1. Experimental drying oven equipped wit HD camera.

Image analysis
Acquired pictures were analysed by the digital image analyses using Image J software, version 1.50b, that utilises java based image processing. The default threshold method based on IsoData algorithm was used in this experiment. Using Image J software dimensions of the samples, X (mm), Y (mm), and colour RGB histogram with mean of image intensity were determined for each picture (Fig. 2). Geometric mean diameter D_g (mm) was calculated using following equation (Eq. 1), (Mohsenin, 1970).
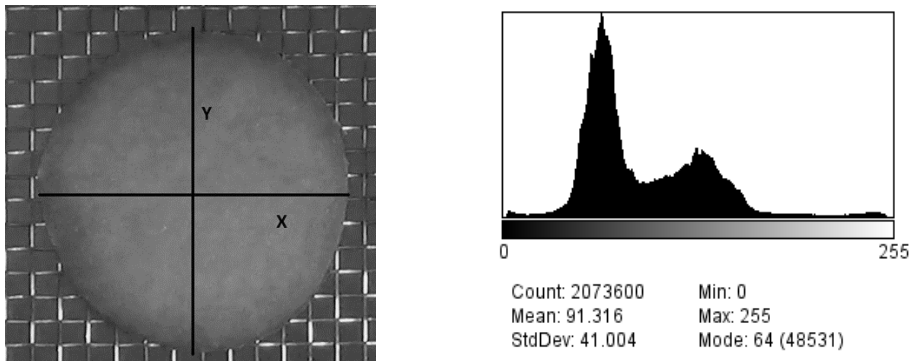
$$D_g = \sqrt{X \times Y} \qquad (1)$$

Figure 2. Determined dimensions of fruits` sample, image histogram.

RESULTS AND DISCUSSION

Selected images recorded at drying time of 0 min, 20 min, 40 min and 180 min are shown in Fig. 3. Individual samples are presented in the following order mango, banana, pineaple.
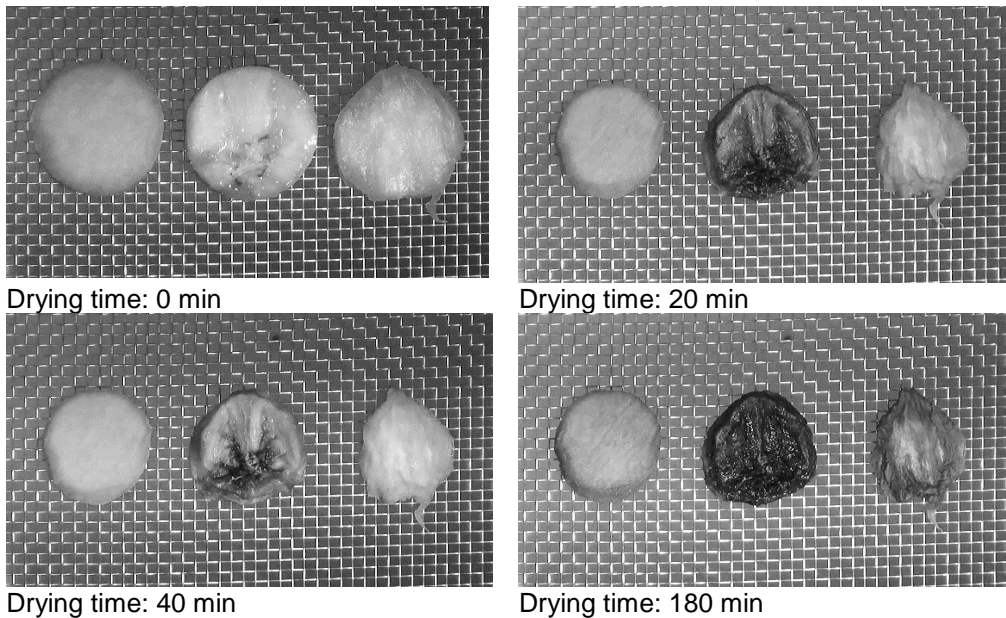

Figure 3. Selected images at given drying time. Order from left side: mango, banana, pineapple.

From determined dependencies between geometric diameter and drying time (Fig. 4) it follows that banana is more sensitive to dimension`s changes than pineapple or mango (Fig. 3). It is also evident that at drying time about 40 min the changes of dimensions were almost stopped and they can be considered as constant value. From the recorded images (Fig. 3) and dependency between image intensity and drying time

(Fig. 5) it follows that the colour`s changes were rapidly changed in interval from 0 to 40 min and after it the changes of colour became linear dependency.
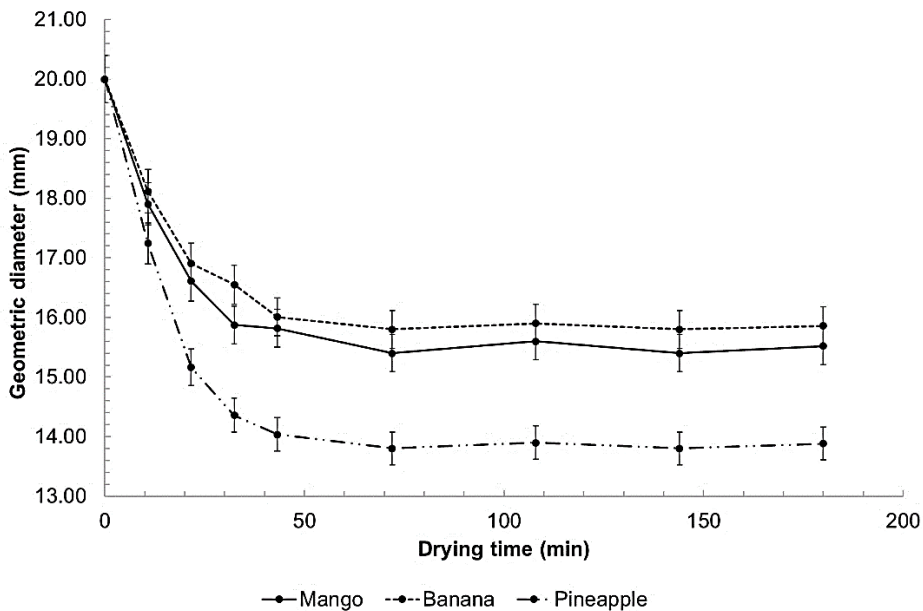

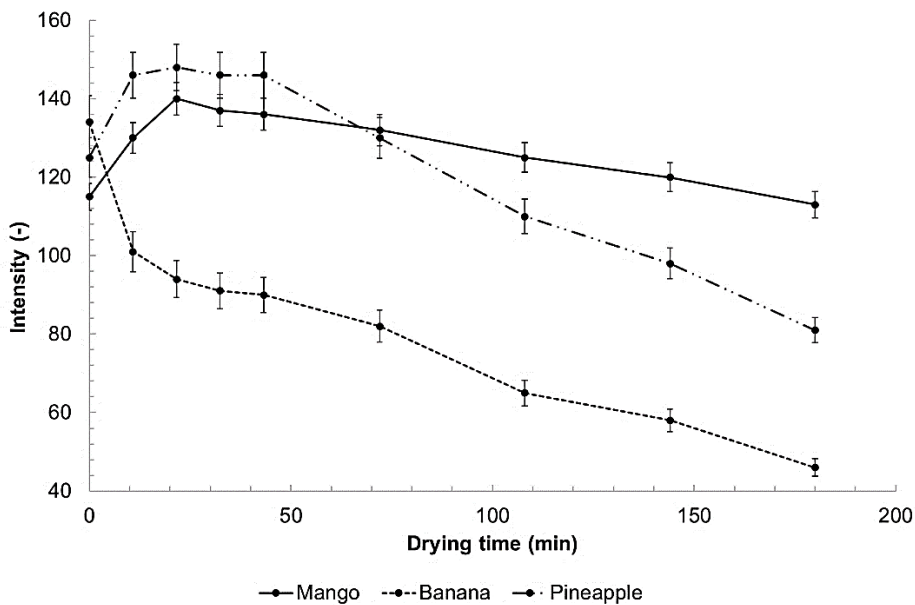**Figure 4. D**ependency between geometric diameter and drying time.

Figure 5. Dependency between image intensity and drying time.

From the beginning of the drying process the image intensity of mango and pineapple were increased and image intensity of banana was decreased (Fig. 3, Fig. 4). At drying time about 30 min the image intensity of mango and pineapple changed to linear declining trend and image intensity of banana continued at linear declining trend. Practically it means that during first 30 min of drying process the colour of mango and pineapple were very quickly brightened and the colour of banana was very quickly darkened. During last 150 min of drying process the colour of all fruits were slowly darkened. From determined results implies that conducted drying process can be divided into two intervals, first interval where the changes of dimensions and colours have nonlinear dependencies on the drying time and they occur very quickly, and second interval where the changes of dimensions are almost unobservable and changes of colour become linear dependency on drying time. This statement is in accordance with already published study related to the colour changes of lemon slice during the combined infrared-vacuum drying (Salehi & Kashaninejad, 2018).

According to the already published studies the differences in individual characteristic of colour and dimension changes are given by the structure of the sample being dried and the mechanism of internal liquid migration and it is strongly linked to the initial moisture content (Simović et al., 2016). Some previous authors determined, that moisture diffusion represents the dominant physical mechanism affecting drying characteristics (Singh & Gupta, 2007; Xiao et al., 2010). This effect can also be seen in the drying of different agricultural products indicating that the absence of constant water supply to the sample surface lowered the drying rate, which is expressed through the rapid decline of drying rate values (Stamboulis et al., 2001; Mizera et al., 2017). The gained results and knowledge in this study can be used as background for further research related to the modelling of drying behaviour under different drying temperatures, modelling of mass changes or modelling of heat transfers.

CONCLUSIONS

Dependencies between colour`s changes, dimension`s changes of mango, banana and pineapple and drying time were determined with aid of image analysis.

From determined results implies that conducted drying process can be divided into two intervals, which are given by drying time (0 to 40 min), (40 min to 180 min) for dimension`s changes and (0 to 30 min), (30 min to 180 min) for colour`s changes.

First interval where the changes of dimensions and colours have nonlinear dependencies on the drying time and they occur very quickly, and second interval where the changes of dimensions are almost unobservable and changes of colour become linear dependency on drying time.

Gained results in this study could be used for the development of further models of fruits` drying process and to design technology in the new era of Industry 4.0 respectively Agriculture 4.0.

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The study of physical properties of spray dried whey and milk permeates lactose

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Abstract. The aim of this study was to investigate substrate and environment effect on the physical state of lactose crystals, their stability and behaviour comparing with pure lactose which traditionally used in an analysis. Sweet and acid whey permeate as well as milk permeate were analysed. Mini spray-drier (BÜCHI B-290, Labortechnik AG, Switzerland) was used for the study. Lactose optical rotation was measured with a polarimeter, structural characteristic was carried out by X-ray diffractometer and glass transition analyses was made by TGA/DSC. α-Lactose monohydrate (Sigma-Aldrich, Germany) was used as a control. All spray-dried permeates samples showed amorphous state lactose crystals. The DSC analysis demonstrated a glass transition in the interval of 85–95 °C, melting 202–204 °C for spray-dried permeates lactose. In turn, the control sample showed crystallization at 158 ± 0.5 °C and a melting peak at 226 ± 0.5 °C. Optical rotation of spray-dried lactose obtained from sweet and acid whey permeate and milk permeate was in the range from 18 to 28°, control sample 52°. The study results showed that substrate, sample pH, ingredients and their derivatives impact lactose glass transition and mutarotation. The current study highlights the essential physical properties of spray-dried permeates lactose and the importance of its purity in food, cosmetic and pharmaceutical industry.

Key words: lactose, milk permeate, whey permeate, glass transition, mutarotation.

INTRODUCTION

Permeate is considered as a suitable substrate for lactose hydrolysis. The outcome of final sugars and its concentration depends on permeate type, solid content and medium environment. That gives a propose to analyse more closely properties and behaviours of lactose from permeate and build a new strategy to raise glucose-galactose syrup sweetness. Lactose is a disaccharide which consists of galactose and glucose. The main source of lactose is bovine milk containing approximately 4.8% carbohydrate (Jenab et al., 2018). Lactose is considering as a problem for whey utilization since it crystallizes at low temperatures. As one of the possibilities in food industry, lactose can hydrolyse by enzymatic or acid method (Das et al., 2015). Lactose is used for investigation and product formation in various biotechnological processes (Ryan & Walsh, 2016) obtaining lactose derivatives such as galacto-oligosacchrides, lactulose, lactitol, and lactobionic acid (Huppertz & Gazi, 2016). Whey can be fractionated into retentate (rich with protein) and permeate (rich with lactose) using ultrafiltration (Jenab et al., 2018).

The major compound in whey permeate and milk permeate is lactose in addition with a small number of soluble minerals and proteins. Whey permeates solids consist of 76 to 85% lactose, and 11 to 16% proteins and minerals, whereas milk permeate solid contains 78 to 88% lactose and 11 to 16% proteins and minerals (Pandalaneni & Amamcharla, 2018). In aqueous medium, lactose may exist in α and β forms but after drying process only anhydrous form. Crystallization of lactose is important in complex dairy systems under industrial process conditions, such as spray-drying, freeze-drying, and storage, such as ice-cream, evaporated milk and whey. In this case, the different crystals exist together with amorphous lactose and changing in overall the functional properties of lactose. A-Lactose monohydrate is the main lactose form in spray-dried lactose/whey permeate and lactose/gelatine mixtures (Gänzle et al., 2008). The most common method is spray-drying for dehydration of milk and whey products. During this process, moisture is removed and leads to the formation of lactose at amorphous state. Notable to highlight that the presence of protein(s), fat, minerals and lactic acid can largely influence the physical and chemical behaviours as well as water absorption, glass transition temperature and crystallization of the lactose (Shrestha et al., 2007). In particular, the focus of the study was to analyse the spray-dried sweet and acid whey, as well as milk permeates powders to gain the useful information of powders physical properties which could be taken in consideration as a prospective information in food products production and physical properties assessment. The aim of this study was to investigate substrate and environment effect on lactose crystals, their stability and behaviour comparing with pure lactose which traditionally used in an analysis.

MATERIALS AND METHODS

Materials
Acid whey and milk permeate were kindly donated by JSC 'Tukuma piens'. Sweet whey was derived from dairy Ltd 'Latvijas Piens' where further ultrafiltration was done in the laboratories of Latvia University of Life Sciences and Technologies. α-Lactose monohydrate ≥ 99% as a control was purchased from Sigma-Aldrich (Latvia).

Preparation of permeate
Sweet whey was treated using cross-flow membrane filtration Armfield FT17 (UK) and polymer membrane filter GKSP, 92 mm, Sterlitech (USA) with molecular weight cut-off of 3 kDa was used in the study, all process was operated at temperature 4 ± 2 °C and pressure 28 ± 2 bar. Sweet whey was stored in the fridge. Acid whey and milk permeates were produced on an industrial scale using ultrafiltration UF Unit, GEA (Germany) equipped with spiral membranes. Sweet, acid and milk permeate was analysed by MilkoScan^{TM} Mars, Foss Analytical A/S (Denmark) for lactose, protein and fat, total solids content determination and pH measurment.

Preparation of spray-dried lactose and permeates powders
To obtain a powder from sweet and acid whey as well as milk permeate and 5% w/v aqueous α-lactose monohydrate solution was used BÜCHI mini spray-drier B-290 (Labortechnik AG (Switzerland)), experiment was done based on Chandrapala & Vasiljevic (2017), also Islam & Langrish (2010) works with some modifications. To objectively analyse the impact of permeate origin on lactose physical and chemical

parameters under the same conditions was spray-dried 5% w/v aqueous α-lactose monohydrate solution (initial material α-lactose monohydrate purity ≥ 99%) and compared with spray-dried permeate powders lactose.

The spray-dryer was used with following conditions: aspirator rate 100%, the flow rate of the feed solution 40–50 mL min^{-1}, an inlet air temperature 170 °C and an outlet temperature 115–120 °C. The collected powders were immediately placed in 50 mL tube and stored in a desiccator with 0–3% relative humidity at room temperature till further analysis.

Instrumental analysis
Optical rotation
Polarimeter Polax-2L, Atago (USA) was used for measurement of lactose optical rotation. Each sample was dissolved in deionized water at a concentration of 1% (w/v) and placed into 100 mm cuvette. To calculate sugar optical rotation, the following equation was used:

$$[\alpha]D = \frac{\alpha}{I \cdot C} \tag{1}$$

where I – length of cuvette, dm; C – the sample concentration in g 100 mL^{-1} (Chandrapala & Vasiljevic, 2017).

X-ray powder diffraction
Structural characterization of samples was carried out using diffractometer Bruker AXS, D8 Advance (Germany) with CuKα1 radiation at λ = 1.5418 Å and a position sensitive detector (PSD). The tube was operated at voltage 40 kV and current 40 mA. Scan range 5°–60° on 2θ scale at rate 5° min^{-1} was used according to Wu et al. (2014) method. The database ICDD PDF2 was used for patterns analysis.

Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA)
Analyses were performed on a Mettler Toledo TGA/DSC (Switzerland) operating with the STARe System Software. In one sample measurement, instrument was performing thermal transition and thermogravimetric analysis. Sample 5–10 mg was weighted into an aluminium pan and heated at the temperature range 30 to 300 °C within heating rate of 10 °C min^{-1}. An empty aluminium pan was used as a reference in every test (Badal Tejedor et al., 2018; Veldre et al., 2011).

Scanning Electron Microscopy (SEM)
The morphology of the lactose crystal samples was examined using scanning electron microscopy SEM-FIB Tescan Lyra with an accelerating voltage of 12 kV. Each sample was placed onto a carbon tape on an aluminium sample disc and a compressed gas was used to remove unfixed powder particles. Lactose samples were coated with a 27 nm gold layer using Quorum Q150R coating unit at 25 mA for 45 s (Kougoulos et al., 2010).

Data analysis

Results were expressed as mean ± standard deviation (SD) of three replicates for composition measurments. Statistical analyses were used with analysis of *One-Way ANOVA* and *Tukey* test at the significance level $P < 0.05$.

RESULTS AND DISCUSSION

Ultrafiltration was used to remove low molecular weight components but keep lactose in the same concentration. Table 1 represents the amount of main components and medium pH of each permeate before spray-drying.

Table 1. Proximate composition and pH of permeates before spray-drying

Permeate	Fat, %	Proteins, %	Lactose, %	Total solid, %	pH
Sweet whey	0[a]	0.2 ± 0.1[a]	3.8 ± 0.1[a]	4,6 ± 0.1[a]	6.1 ± 0.1[a]
Acid whey	0[a]	0.5 ± 0.1[b]	4.2 ± 0.2[b]	5.2 ± 0.2[b]	4.6 ± 0.1[b]
Milk	0[a]	0.5 ± 0.1[b]	4.7 ± 0.1[c]	6.0 ± 0.1[c]	5.8 ± 0.1[c]

Results indicated with the same letter within a column do not differ significantly ($P > 0.05$).

Permeate composition varies depending on initial product for ultrafiltration and the condition of ultrafiltration in terms of membrane type, concentration rate and other factors (Barile et al., 2009). Sweet whey storage conditions could activate lactose crystallisation gaining molecular weight therefor part of sugar during ultrafiltration was not able to get through membranes as it showed in Table 1. Parameters of each membrane also affected the condition of what size of the particle was removed. Besides of minimal amount of protein, permeate solid contains also mineral salts. Salts are considering as an important factor which associates with lactose and affects the crystallisation of spray-dried lactose. The effect of salts differs with their types and amount (Imtiaz-Ul-Islam & Langrish, 2008). This should be taken into consideration if spray-dried lactose and permeate powder intended to use in enzymatic lactose hydrolysis. The ionic environment affects enzymatic activity and evolution of lactose hydrolysis reaction (Demirhan et al., 2008).

Sugar optical rotation [α]D measurements (Table 2) showed that [α]D of α-lactose monohydrate was 52 ± 0.1° and its corresponding to the information on sample specification provided by producer. All spray-dried samples had lower [α]D than α-lactose monohydrate but significant difference was not observed among powder from acid whey and milk permeates a well as 5% (w/v) lactose solution. The low [α]D of sweet whey permeate powder could be related to the solid content which left during ultrafiltration.

Table 2. Optical rotation of α-lactose monohydrate (control) and lactose in spray-dried permeates powders

Samples	Optical rotation, (°)
α-lactose monohydrate	52 ± 0.1[a]
Spray-dried:	
5% (w/v) lactose solution	34 ± 0.1[b]
Sweet whey permeate	20 ± 0.3[c]
Acid whey permeate	30 ± 0.3[b]
Milk permeate	27 ± 0.2[b]

Results indicated with the same letter within a column do not differ significantly ($P > 0.05$).

The mutarotation rate of lactose is greatly affected by spray-drying temperature and pH as well as presence of other sugars and salts reported by Huppertz & Gazi (2016). During spray-drying in a small part of time substrate was heated up till 170 °C and outlet from a nozzle at 115–120 °C that may consider as one of the factors which caused the lactose transformation as well as intensive mutarotation between α and β lactose (Hynes & Zalazar, 2008).

The morphology of lactose crystals in spray-dried permeates powder samples and α-lactose monohydrate crystals is showed in Fig. 2.
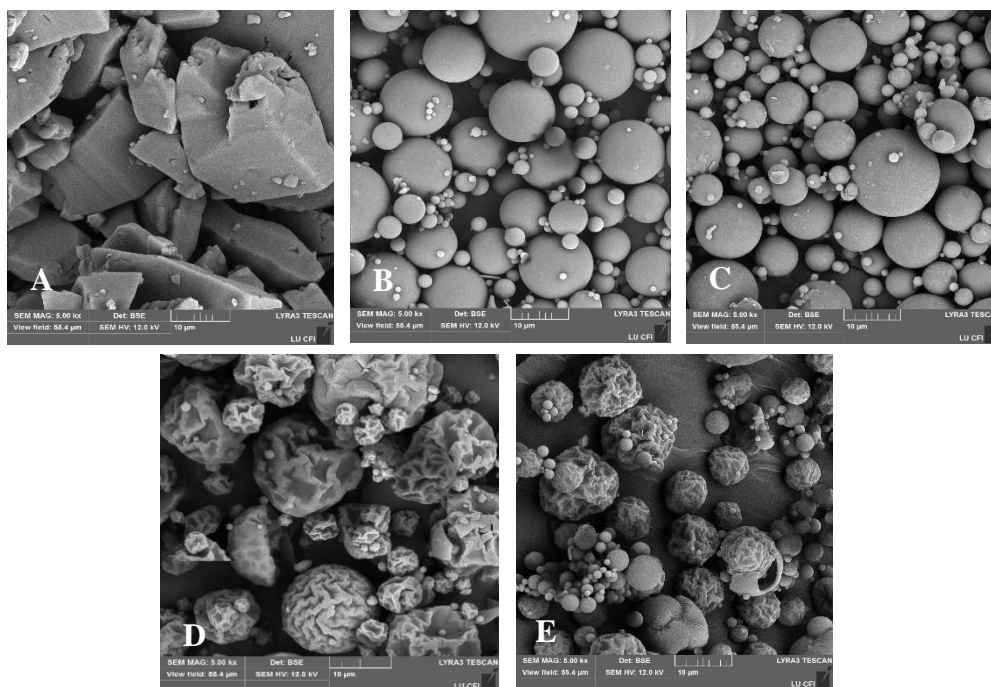

Figure 2. SEM surface morphology of α-lactose monohydrate crystals (A) and spray dried lactose obtained from 5% lactose solution (B); from sweet whey permeate (C); from acid whey permeate (D); from milk permeate (E). All micrographs are shown at magnification 5,000×.

Fig. 2, A shows the typical crystal shapes of α-lactose monohydrate, such as diamond-shaped plate and pyramids. Spray-dried lactose powder and permeate powders have an amorphous lactose form and can be observed in Fig. 2, B–E. During spray-drying of substrates, water evaporation and substrate viscosity increase so fast that crystal formation cannot happen and lactose forms into amorphous form (Gänzle et al., 2008). In Fig. 2, B is a standard crystalline form in a fresh spray-dried powder. According to the findings in the literature spray-dried conditions have the significant impact on powder properties, such as initial product solid content, drying temperature, initial droplet size and others (Kim et al., 2009). In this study, the appearance of droplets obtained from different permeate powders was not similar Fig. 2, B–E. Droplet surface of spray-dried lactose obtained from 5% lactose solution Fig. 2, B and from sweet whey permeate Fig. 2, C was spherical in shape with a smooth surface and the size of particles in both figures was relatively similar. However, the surface of the spray-dried droplets

obtained from acid whey Fig. 2, D and milk Fig. 2, C has various shapes. One part of droplets has shrivelled appearance, another part has fissures or breakages and another part was smooth. This can be explained by the findings of Kim et al. (2009) where the solid content and drying temperature affected droplet surface and coverage. These observations are confirmed by the results in Table 1, which clearly shows the significant difference of the total solids content among permeates as well as pH. Acid whey and milk permeates in Table 1 showed that pH was below 6 and might affected more strongly the surface appearance of droplets. Initial product solid content (fat, protein, salts, lactose) and pH are responsible for the dried droplet surface formation. Depending on content and pH in permeate during spray-drying, substances are interacting with each other that reflects on the surface, particles having lactose coverage and lactose crystallinity (Ebrahimi et al., 2015).
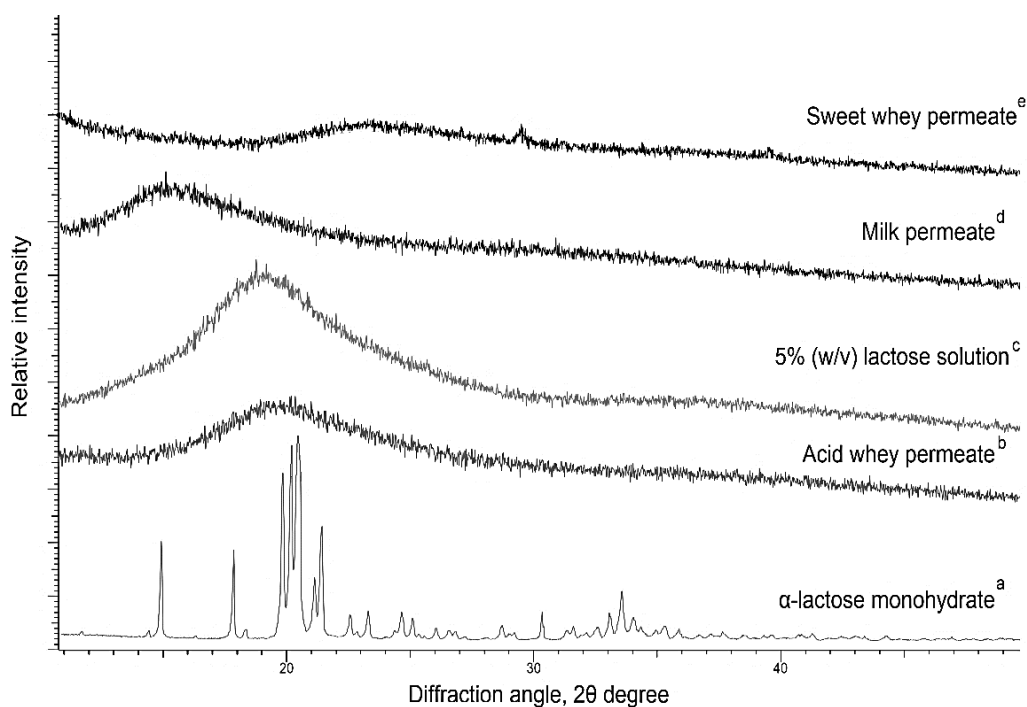

Figure 3. X-ray diffraction patterns of α-lactose monohydrate crystals and spray-dried lactose obtained from 5% (w/v) lactose solution; sweet whey permeate; acid whey permeate; milk permeate. Results indicated with the different letter differ significantly (*P* < 0.05).

The X-ray analysis was used to determine the crystalline form of commercial α-lactose monohydrate and spray-dried lactose from different substrates. Crystal properties of all samples were observed in Fig. 3. All patterns of spray-dried powders approved that using particular spray-drying conditions lactose obtained an amorphous (non-crystalline) form. It was not possible to identify the polymorphs in these certain patterns. To the same observations came up Price & Young (2004) in the study was used inlet temperature 185 °C and pattern of spray-dried lactose was without any major crystalline long-sharp peak instead of α-lactose monohydrate. The database ICDD PDF2

identified in pattern of spray-dried sweet whey permeate powder the presence of potassium chloride at diffraction angle $28 \pm 0.5°$ and $40 \pm 0.5°$ 2θ degree. That could be related to the interaction between lactose and this particular salt under certain conditions. The pattern differences might depend on the composition and amount of elements in each substrate, drying temperature and medium pH (Chen et al., 2015). In contrast, α-lactose monohydrate showed sharp diffraction peaks which allowed to identify the types of lactose crystal forms. In product, specification was mentioned the presentence of β-lactose which amount is so less that it was difficult to identify this crystal.
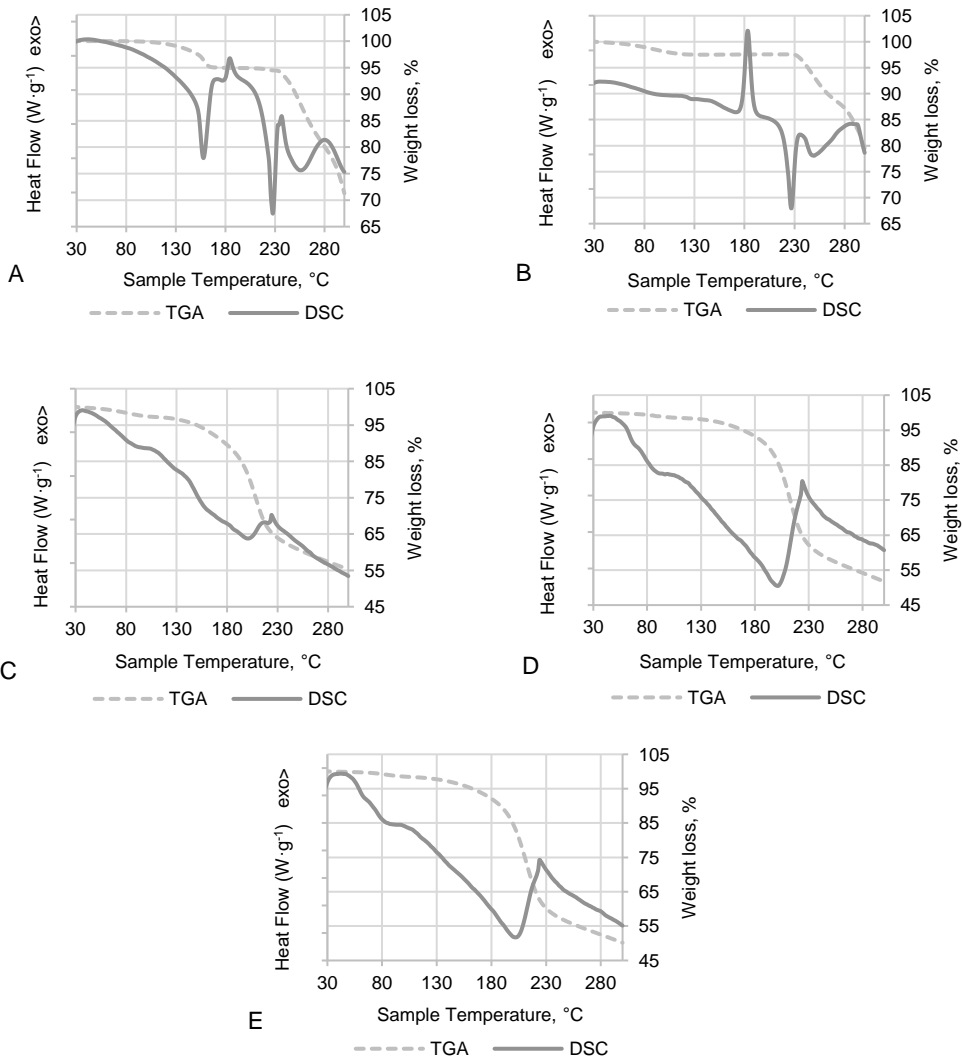

Figure 4. DSC thermal profile and TGA weight loss profile for α-lactose monohydrate crystals (A) and spray–dried lactose obtained from 5% lactose solution (B); from sweet whey permeate (C); from acid whey permeate (D); from milk permeate (E).

The crystallinity was analysed by measuring thermal properties of the sample upon heating using DSC and the loss of moisture by TGA. Both profiles of the lactose samples are showed in Fig. 4. Samples were heated at the temperature range from 30 to 300 °C showing the difference among samples. The two endothermic peaks were showed in DSC profile of α-lactose monohydrate (see Fig. 4, A). The first peak at 158 ± 0.5 °C expressed the evaporation of the crystallized water, the second peak at 226 ± 0.5 °C showed that lactose sample melted before decomposition and the total weight loss was $27.5 \pm 0.5\%$. The amorphous spray-dried lactose Fig. 4 B glass transition started at 58 ± 1 °C where weight loss was $2 \pm 0.5\%$, recrystallization started at 183.5 ± 0.5 °C and melting peak at 227 ± 0.5 °C where also occurred a rapid weight loss of $18 \pm 1\%$. It should be noted that α-lactose monohydrate crystal contains approximately 5% of water and water is less strongly bounded. During recrystallization at 184 ± 1 °C α-lactose monohydrate (Fig. 4, A) and amorphous spray-dried lactose (Fig. 4, B) according to Badal Tejedor et al. (2018) study converted into the same physical form which led to the same melting temperature. Lactose transformation into α and β forms during thermal treatment are not excluded. The DSC profiles of spray-dried permeate powders Fig. 4, C–E were similar ($F_{crit} = 5.14$, $P > 0.05$). The glass transition to permeate samples Fig. 4, C–E started in the interval of 85–95 °C and melting of samples were observed at 202 ± 2 °C. During the melting phase the weight loss to lactose samples from permeates Fig. 4, C–E were in the range of 30–45%. Results indicate that presence of other substances such as fat, protein, salts, lactic acid and medium environment decrease lactose thermal resistance. The DSC profiles highlight the physical properties and the differences between lactose from permeates and pure lactose.

CONCLUSIONS

The present study showed that the physical properties of spray-dried permeate powders are strongly influenced by the presentence of substances and condition. Lactose is very sensitive sugar and environment conditions can easily affect its behaviour. Study results reveals permeate powders capability of lactose crystal transformation and thermal transition behaviour where each of powder showed slightly different results. Research showed that permeate powders do not contain highly pure lactose, also the presence of other substances is established which effect their physical properties. Knowing lactose physical properties in permeate powders, they could help to avoid and prevent difficulties during food processing and storage, as well as in permeates quality evaluation.

Further studies are necessary to evaluate spray-dried permeate powders as an option in the food industry to use their as a substrate in enzymatic lactose hydrolysis. Permeate powders contain mineral salts which can act as an activators for β-galactosidases and contribute the hydrolysis reaction.

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