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CONTENTS

Effect of simultaneous inoculation of commercial yeast starter cultures on Kombucha fermentation

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Abstract. Kombucha – a spontaneously fermented tea beverage, produced by SCOBY (a symbiont of bacteria and yeasts), has become popular in recent years. Its functional properties and features for industrial production and treatment remain largely unknown, however. Our aim of using additional yeast cultures during the primary fermentation was to modify and ultimately improve the sensory properties of the kombucha beverage. During five fermentation experiments the total viable count (TVC) of microbes was determined both in Kombucha and SCOBY samples. The first four fermentation experiments were conducted to stabilize the growth of SCOBYs that were initially of different origin. The last (5th) fermentation contained the simultaneous inoculation of three different active *S. cerevisiae* cultures into the sweetened black tea together with the SCOBY and was followed by a sensory analysis. Two samples remained as control samples without additional yeast starter culture. The yeast starter cultures affected the microbial counts of Kombucha, but the effect on the microbial count of SCOBY was not statistically significant ($p > 0.05$). The Kombucha containing wine yeast culture had the lowest sensory quality, while Kombucha containing brewer's yeast had the most pleasant flavour and aroma. In conclusion, the simultaneous fermentation with commercial yeast cultures had a minor effect on the microbial counts in SCOBY when compared to the fermentation time, but all added cultures clearly modified the taste and aroma properties of the Kombucha drinks.

Key words: kombucha, SCOBY, tea, simultaneous fermentation, *Saccharomyces cerevisiae*.

INTRODUCTION

Kombucha is a beverage obtained by fermentation of sugar-sweetened tea with Symbiotic Culture of Bacteria and Yeasts (SCOBY) (Greenwalt et al., 2000; Jayabalan et al., 2011; Leal et al., 2018; Zhao et al., 2018). Kombucha has become very popular in recent years and both its production and consumption have increased. Especially the production of flavoured Kombucha drinks and the improvement of Kombucha's probiotic properties by the addition of lactic acid bacteria during simultaneous fermentation have recently gained more interest (Cvetković et al., 2019).

Kombucha and SCOBY contain acetic acid bacteria (e.g. from genera *Acetobacter*, *Bacterium*, *Gluconoacetobacter*, *Gluconobacter*, *Halomonas*, *Herbaspirillum*, *Komagataeibacter*), lactic acid bacteria (e.g. *Oenococcus oeni*, *Lactobacillus satsumensis*, *Lactobacillus nagelii*) and yeasts (e.g. from genera *Brettanomyces,*

Candida, *Dekkera*, *Hanseniaspora*, *Kloeckera*, *Kluyveromyces*, *Mycoderma*, *Mycotorula*, *Rhodotorula*, *Saccharomyces*, *Saccharomycodes*, *Schizosaccharomyces*, *Zygosaccharomyces*, *Zygotorulaspora*, *Pichia*, *Torula*, *Torulaspora, Torulopsis*) (Kozaki et al., 1972; Jankovic & Stojanovic, 1994; Markov et al., 2001; Dogan et al., 2002; Teoh et al., 2004; Jayabalan et al., 2010; Yapar, 2010; Reva et al., 2015; Coton et al., 2017) that live in tight symbiosis. The microbial species in different SCOBYs and Kombuchas may vary and due to this it is not possible to identify a uniform microbial community (Jayabalan et al., 2014). The most important acetic acid bacterium in Kombucha is *Komagataeibacter xylinus*, which synthesizes cellulose during fermentation (Jayabalan et al., 2010). The cellulose appears as a thin film on the surface of the tea solution and cells of other bacteria and yeasts attach to it (Jayabalan et al., 2010).

To make Kombucha, tea leaves are first put into a container, then hot boiled water is added and sucrose is dissolved in this hot tea beverage. Traditionally black tea is used for making Kombucha, but other teas can also be used – e.g. white tea, green tea, oolong tea and different herb teas (thyme, mint, sage, lemon balm, echinacea) (Markov et al., 2001; Malbaša et al., 2002; Markov et al., 2006; Jayabalan et al., 2010; Velićanski et al., 2013; Adzadogo, 2015). The sugared tea beverage is then cooled down to room temperature and SCOBY is finally added to start the fermentation process (Teoh et al., 2004; Malbaša et al., 2005; Vázquez-Cabral et al., 2014; Leal et al., 2018). The container used for growing SCOBY is covered with paper or other suitable material to prevent airborne microbes or insects from getting into the drink (Dutta & Paul, 2019). To obtain a pleasant sour beverage, primary fermentation is stopped once the titratable acidity has been reached 44.5 g L^{-1} (Velićanski et al., 2013).

Fermented Kombucha beverage contains alcohols, aldehydes, ketones, esters, amino acids, tannins, terpenoids, saponins, flavonoids, phenols, alkaloids, CO_2, enzymes, catechins, caffeine and other compounds (Adzadogo, 2015; Kumar & Joshi 2016). It is claimed that the chemical composition, mainly polyphenols and secondary metabolites formed during fermentation of Kombucha, add a therapeutic effect to the beverage (Watawana et al., 2015). Malbaša et al. (2011) and Watawana et al. (2015) state that Kombucha helps in improving digestion, gives relief against arthritis, prevents microbial infections, helps in combating cancer and removes toxic substances from the body.

Kombucha is described as a refreshing and slightly sweet-sour beverage whose taste is similar to that of effervescent apple cider (Jayabalan et al., 2014; Kumar & Joshi, 2016). After a short fermentation time, the beverage should acquire a pleasant fruity and sour taste, which tends to become slightly vinegary during prolonged fermentation (Reiss, 1994; Jayabalan et al., 2014). The taste of Kombucha depends on the concentration levels of residual sugar, carbon dioxide and organic acids (especially the concentration of acetic and gluconic acid) (Leal et al., 2018). Acetic acid gives the tea beverage an astringent and sour taste and gluconic acid gives it a mild taste (Chen & Liu, 2001). As a result of CO_2 formation, gas bubbles are formed in the tea solution (Mukadam et al., 2016).

Flavourings are usually added to Kombucha after primary and secondary fermentation have taken place in aerobic conditions or in a sealed or air-locked vessel (Bleam et al., 2016). Secondary fermentation may be carried out with or without SCOBY (Dutta & Paul, 2019). Whereas in an open vessel the new SCOBY is formed and a

domineering growth of acetic acid bacteria can be observed, in a sealed vessel more alcohol and lactic acid are produced (Bleam et al., 2016). To improve the probiotic properties of Kombucha lactic acid bacteria are used for the simultaneous fermentation (Nguyen et al., 2015; Cvetković et al., 2019).

There is a lack of scientific research about the effect of simultaneous fermentation on the sensory properties and microbial loads of Kombucha. Traditionally the taste properties of Kombucha are altered or improved during secondary fermentation by adding for example different juices to Kombucha. Modification of sensory properties with yeasts during primary fermentation would be an easier and more time-efficient method, however, especially in a commercial context, i.e. the food industries. Accordingly, the aim of this study was to evaluate the effect of different commercial *Saccharomyces cerevisiae* starter cultures on the microbial abundance of both SCOBY and Kombucha as well as on the sensory properties of the fermented drinks.

MATERIALS AND METHODS

Materials

SCOBYs used in the experiments were originally obtained from two different households in Estonia: one was grown in green tea (TF 1, TF referring to 'tea fungus') and the second was grown in black tea (TF 2).

For simultaneous Kombucha fermentation the *Saccharomyces cerevisiae* commercial starter cultures for dry wine (Enovini dry wine yeast, Browin, Poland), dry cider (Dry cider yeast Ciderini Dry, Browin, Poland) and ale-type beer (BrewGo- O2, Browin, Poland) were obtained from the local market in Tartu, Estonia.

Preparation of black tea

According to proportions suggested by AL-Kalifawi & Hassan (2013), tea solutions containing 5 g of black tea (Ceylon Jambo Grand black tea, Ranfer, Sri Lanka), 0.5 L of hot water and 50 g of sucrose (Polski Cukier, Poland) were prepared in eight sterilized 720 mL glass jars for each of the five fermentation test series. Tea leaves were removed after 30 minutes of soaking and the solutions were then left to cool in closed jars at room temperature (20–24 °C).

Growing SCOBYs

Four circular pieces with a diameter of 2.8 cm each were cut out of both TF 1 and TF 2 'mother' SCOBYs (Fig. 1, a) obtained from two different households. These samples were marked as TF 1.0, TF 1.1, TF 1.2, TF 1.3 and TF 2.0, TF 2.1, TF 2.2, TF 2.3, respectively. All pieces of SCOBYs were then inoculated in pre-made black tea solutions and grown at room temperature (20–24 °C) in glass jars (Fig. 1, b), covered with sterile gauze and fixed with a rubber band in a room with restricted access. In the following experiments, the daughter cultures of the SCOBYs from the previous test series (TS) were used. Before inoculation into the sugared tea beverage, an additional 10 g piece of the daughter SCOBY was taken for microbiological analysis.
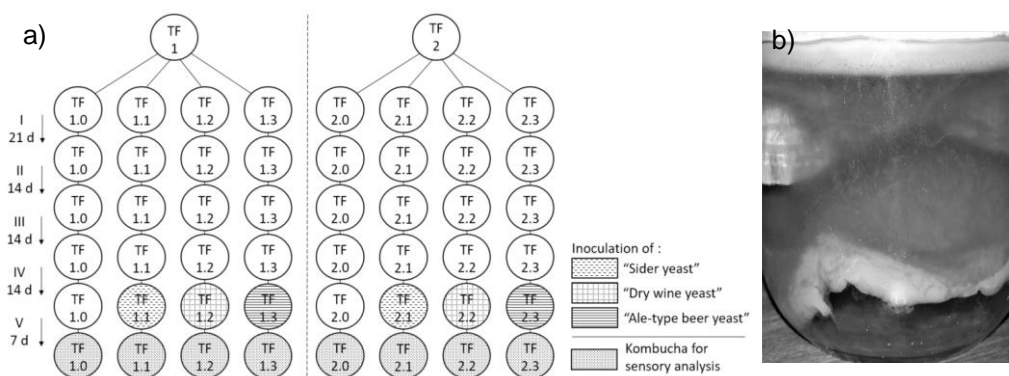
Figure 1. a) Scheme of SCOBY sampling through the five test series of fermentation. TF-SCOBY; TF 1 and TF 2 – 'mother' SCOBYs previously grown in green or black tea, respectively; TF 1.0–1.3 and TF 2.0–2.3 – daughter SCOBYs originating either from TF 1 or TF 2, respectively, I–V – five test series, d – fermentation time in days.b) Photo of SCOBY growing in sugared black tea, taken by M. Abel.

The fermentation time varied through the different test series. Initially it was 21 days in the first test series for initial growth activation and then 14 days in the next three experiments that were performed for growth stabilization of SCOBYs. Shortening the duration of the fermentation process to 7 days for the final (V) test series prior to sensory analysis was important as the acidity levels of the Kombucha drink may become potentially harmful for consumers if its fermentation lasts more than 10 days (Nummer, 2013).

Measuring the pH

During all experiments, the pH value was measured in sugared tea solutions before fermentation and in Kombucha (TFL 1.0–1.3 and 2.0–2.3, TFL referring to 'tea fungus liquid' according to the TF that was grown in it) after fermentation periods of 21, 14 or 7 days with a pH meter (SevenGo pro, Mettler Toledo, Switzerland).

Addition of starter cultures for simultaneous Kombucha fermentation

At the beginning of the last (V) test series, *S. cerevisiae* starter cultures were added to the sugared black tea solutions in duplicates (Table 1, Fig. 1, a) together with the SCOBY according to instructions by the manufacturer. 1 g of yeasts were previously dissolved in sterile distilled water, pre-incubated and added to the black tea.

Yeast cultures were not added to samples TF 1.0 and TF 2.0 that remained control samples.

Table 1. *S. cerevisiae* starter cultures used for simultaneous Kombucha fermentation

Starter culture	Pre-incubation*	Samples
Yeast for making dry cider 'Ciderini dry'	20 min, 25 °C	TF 1.1/ TF 2.1
Yeast for making dry wine 'Enovini'	20 min, 30 °C	TF 1.2/ TF 2.2
Yeast for making ale-type beer 'BrewGo- O2'	10 min, 35 °C	TF 1.3/ TF 2.3

*Pre-incubation conditions of yeasts were conducted according to manufacturer's instructions.

Sensory analysis of Kombucha

Sensory analysis of Kombucha fermented for 7 days (containing wine, beer and cider yeast starter cultures as well as control samples) was carried out at the end of the last (V) test series. Ten randomly selected untrained assessors took part in the sensory analysis.

20 mL of each sample (TFL 1.0, TFL 1.1, TFL 1.2, TFL 1.3, TFL 2.0, TFL 2.1, TFL 2.2, and TFL 2.3) was given for each assessor. During the sensory analysis appearance, aromatic and taste properties, consistency and acceptability of the samples were assessed.

A descriptive method was used for the sensory analysis. The properties to be evaluated were selected by the authors through external observation, prior tasting and based on literature sources (Gramza-Michałowska et al., 2016; Neffe-Skocińska et al., 2017). During the evaluation, the assessors had the opportunity to point out additional tastes and aromas of each Kombucha beverage under review and graded predetermined properties of a beverage on an unstructured linear scale with a length of 10 cm. The grades on the linear scale ranged from 0 to 10 with 0 being the lowest and 10 being the highest grade. One point on the linear scale corresponded to 1 cm. The intensity of colour, aroma and taste was rated on a scale of not perceptible to intense. Flocculation and gaseousness were evaluated based on the presence of flakes and bubbles in the liquid. The acceptability of the beverages was evaluated on a scale 0–5 where 0 was considered as 'unfit for consumption' and 5 was considered as 'very pleasant' The evaluation results were averaged for each of the eight samples.

Enumeration of bacteria and yeasts

For the microbial analysis of Kombucha, five ten-fold serial dilutions of a 1 mL sample were performed in 9 mL of 0.1% sterile peptone water. For the microbial analysis of SCOBY, a 10 g sample was placed in a sterile plastic bag with 90 mL of 0.1% sterile peptone water. The samples were then homogenized in a Stomacher®400 Circulator (Seward Ltd., England) at 300 rpm for 10 min, after which the ten-fold serial dilutions up to 10^{-7} were finally prepared.

In order to enumerate the total viable count of bacteria and yeasts, 1 mL of dilutions were pour plated in duplicates in either PCA (Milk Plate Count Agar LAB 115, LabM Ltd., England) or in SDA (Sabouraud Dextrose Agar, Biolife, Italy), respectively. The PCA plates were then incubated for 72 h at 30 °C and SDA plates for 5 days at 25 °C. After the incubation, the colonies on the growth media were counted, averaged and transformed into $\log_{10}$-scale, displayed as log CFU mL^{-1} (or g^{-1}).

Additionally, the decimal dilutions of Kombucha and SCOBY were spread plated using a sterile 10 µl inoculation loop on Violet Red Bile Agar LAB 31 (LabM Ltd., England) followed by an incubation for 72 hours at 37 °C for the inspection of the presence of *coli*-like bacteria.

Statistical analysis

Microsoft Office Excel 2013 was used to analyse the data collected during the experiments. To analyse variations in the observed parameters among the samples ANOVA (Analysis of Variance) and Student's *t-test* were used, where a *P* value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The pH value of Kombucha

Monitoring the pH value of Kombucha during the fermentation process is important, because if the pH has not reached ≤ 4.2 within 7 days, the Kombucha is either contaminated or the fermentation temperature is too low (Nummer, 2013). In both cases, the fermentation process must be restarted with a new SCOBY. If the pH of Kombucha drops below 2.5, it is unsafe for consumers, because long-term fermentation will raise the amount of acetic acid to a level which may adversely affect their health (Nummer, 2013). pH values of all Kombucha samples tested during experiments (Table 2) were safe for consuming, however.

Table 2. pH of Kombucha during five series of fermentation

TS (d)*	1.0	1.1	1.2	1.3	Mean[1]	2.0	2.1	2.2	2.3	Mean[2]
I (21)	2.66	2.67	2.86	2.71	2.73 ± 0.08	2.88	2.84	2.79	3.01	2.88 ± 0.08
II (14)	2.79	3	2.93	2.89	2.90 ± 0.08	2.69	2.82	2.83	2.73	2.77 ± 0.06
III (14)	2.91	2.8	2.84	2.84	2.85 ± 0.04	2.87	2.91	2.82	2.94	2.89 ± 0.05
IV (14)	2.88	2.94	3.05	2.79	2.92 ± 0.09	3.03	2.96	2.95	3.03	2.99 ± 0.04
V (7)	3.03	3.05	3.02	3.07	3.04 ± 0.02	3.39	3.17	3.06	3.12	3.19 ± 0.12

*test series (duration in days); [1]averaged values of TFL 1.0–1.3 Kombucha samples, originating from TF 1 'mother' SCOBY ± standard deviation; [2]averaged values of TFL 2.0–2.3 Kombucha samples, originating from TF 2 'mother' SCOBY ± standard deviation.

The average pH of the initial sugared black tea solutions was 6.77, which is consistent with earlier scientific studies where their pH ranged from 6.62–7.8 (Blanc, 1996; Lončar et al., 2000; Velićanski et al., 2013; Gramza-Michałowska et al., 2016). The pH of the fermented solution, Kombucha, in the current study was 2.66–3.39 which in turn is in good concordance with previously reported pH ranges of 2.2–3.4 (Teoh et al., 2004; Gramza-Michałowska et al., 2016; Coton et al., 2017).

The analysis of the results showed that the history of previous growth conditions of SCOBY (samples TF 1.0–1.3 vs samples TF 2.0–2.3 originally grown in sweetened green or black tea, respectively) did not have a significant effect on the overall pH of the fermented solution ($P > 0.05$). Comparison of the pH differences of samples TFL 1.0–1.3 and TFL 2.0–2.3 in different test series revealed that the pH of samples TFL 2.0–2.3 in TS II was statistically significantly lower than that of samples TFL 1.0–1.3 ($P < 0.05$). There were no statistically significant differences ($P > 0.05$) on the pH of Kombucha in the remaining test series, including the last fermentation (TS V) where the yeast starter cultures were included, however. Compared to other samples where the starter cultures were added, the pH was lowest in samples that contained wine yeast starter cultures, i.e. samples TFL 1.2 and TFL 2.2 (for which the pH was 3.02 and 3.06 respectively).

Similar to previous studies by Lončar et al. (2000), Velićanski et al. (2013) and Gramza-Michałowska et al. (2016), the pH of the Kombucha was found to be most affected by the length of time of the fermentation - the longer the fermentation time (7 *vs* 14 *vs* 21 days), the lower the pH value ($P < 0.001$).

Microbial dynamics in SCOBY and Kombucha

As SCOBYs used in experiments were of different origin, the first four fermentation series were conducted to stabilize the growth of SCOBYs. It was important to observe whether and how the SCOBY previously grown in green tea would adapt to the new growth conditions in black tea. To monitor the change in microbial loads over time, the total viable count (TVC) of bacteria and yeasts was determined in all Kombucha ($n = 48$) and SCOBY ($n = 48$) samples used during the experiments. An additional goal was to observe how inoculation with yeast starter cultures affected the microbial growth in SCOBY and Kombucha during the last test series.

The initial values of bacteria in two SCOBYs before fermentation experiments were 7.15 log CFU g^{-1} in 'mother' TF 1 and 5.53 log CFU g^{-1} in 'mother' TF 2. There was a statistically significant ($P < 0.05$) decrease in bacterial loads in the two following fermentation series in samples taken from TF 1 (TF 1.0–1.3 in TS I and II) possibly due to the stress caused by the replacement of the green tea with the black tea (Table 3). The number of bacteria in this SCOBY line was back to the initial level (6.15–7.26 log CFU g^{-1}) by the end of the 3rd fermentation in sugared black tea solution. Such dynamics in bacterial counts was not seen in TF 2.0–2.3 samples and neither in TVC of yeasts where the initial numbers in TF 1 and TF 2 were 5.2 and 6.11 log CFU g^{-1}, respectively. Overall, the TVCs of microbes in SCOBY during the various stages of the fermentation process were similar with bacteria ranging between 5.28–8.81 log CFU g^{-1} and yeasts ranging between 5.08-8.04 log CFU g^{-1}. Our results were somewhat lower for bacteria, but well within the range of previous findings in which the counts of bacteria and yeasts in SCOBY were 7.90–9.11 log CFU g^{-1} (Coton et al., 2017) and 6.32–7.40 log CFU g^{-1} (Chen & Liu, 2001), respectively.

Table 3. Number of bacteria (B) and yeasts (Y) in SCOBY samples (TF 1.0–2.3) during five fermentation test series (I-V)

Sample	Microbes	Total viable count (log CFU g^{-1})*				
		I	II	III	IV	V
TF 1.0	B	5.43	5.80	7.26	7.18	6.68
	Y	5.62	5.56	7.11	7.20	6.65
TF 1.1	B	5.69	6.53	7.20	< 5.48	6.57
	Y	5.66	6.65	7.15	< 5.48	6.38
TF 1.2	B	5.90	5.98	5.83	7.72	7.95
	Y	5.92	5.82	5.74	7.73	7.85
TF 1.3	B	5.28	6.36	6.15	6.52	6.99
	Y	5.08	6.28	6.15	6.57	7.00
TF 2.0	B	6.68	7.23	5.72	6.04	8.11
	Y	5.65	7.18	5.75	5.91	8.04
TF 2.1	B	7.61	6.89	6.66	8.04	6.89
	Y	7.66	6.92	7.63	7.99	6.83
TF 2.2	B	5.72	6.15	6.86	7.61	8.81
	Y	5.58	6.08	6.88	7.64	7.92
TF 2.3	B	5.90	6.59	< 5.48	5.80	7.11
	Y	5.90	6.52	< 5.48	5.82	6.94

*values < 5.48 refer to possible errors during the cultivation and were excluded from the analysis.

Interestingly, there was no statistically significant difference between the total bacterial counts of samples TF 1.0–1.3 when compared to the total bacterial counts of samples TF 2.0–2.3 in the respective test series, suggesting that the SCOBY, previously grown in green tea (TF 1.0–1.3), adapted rapidly to the new growth conditions. Yet, there was a significant variance ($P < 0.05$) in the counts of bacteria and yeasts that grew slowly with each fermentation and was most significant after the final fermentation, however. Nevertheless, there was no statistical difference ($P > 0.05$) in counts between the last two fermentations. This could be explained with either the effect of added yeast starter cultures or alternatively with the shorter fermentation period at the last fermentation.

In Kombucha the TVC of bacteria and yeasts was, like in SCOBY samples, similar to each other: 3.56–6.26 and 3.72–6.08 log CFU mL^{-1}, respectively (Table 4). In previous studies the TVC of bacteria and yeasts in Kombucha has been higher, ranging between 4.11–7.90 log CFU mL^{-1} in bacterial (Jayabalan et al., 2007; Coton et al., 2017) and 5.97–7.90 log CFU mL^{-1} in yeasts counts (Chen & Liu, 2001).

Table 4. Number of bacteria (B) and yeasts (Y) in Kombucha samples (TFL 1.0–2.3) during five fermentation experiments (I-V)

Sample	Microbes	Total viable count (log CFU mL^{-1})				
		I	II	III	IV	V
TFL 1.0	B	3.75	5.00	5.15	5.08	5.51
	Y	3.72	4.93	5.11	4.95	5.52
TFL 1.1	B	4.64	5.43	4.65	4.76	6.23
	Y	4.63	5.51	4.64	4.72	6.08
TFL 1.2	B	4.57	4.48	4.04	5.69	> 5.48
	Y	4.48	4.52	4.00	5.67	> 5.48
TFL 1.3	B	4.04	6.26	4.71	4.61	6.15
	Y	3.96	5.36	4.79	4.61	6.04
TFL 2.0	B	4.69	5.30	4.11	3.56	5.60
	Y	4.59	5.26	4.66	3.97	5.32
TFL 2.1	B	4.41	4.78	4.72	5.18	6.04
	Y	4.57	4.88	5.04	5.15	5.80
TFL 2.2	B	4.64	5.08	5.11	5.15	> 5.48
	Y	4.61	4.11	5.49	5.18	> 5.48
TFL 2.3	B	4.65	4.75	4.48	4.18	4.32
	Y	4.71	4.71	4.81	4.32	4.49

*values > 5.48 refer to possible errors during the cultivation and were excluded from the analysis.

As in SCOBY samples, the dynamics of microbial counts during the five test series in Kombucha (TFL) 1.0–1.3 and 2.0–2.3 samples was similar ($P > 0.05$). It was also noted by Kaewkod et al. (2019) that the total counts of bacteria and yeast cells in Kombucha prepared from either green or black tea were not significantly different.

There was a significant increase ($P < 0.05$) in microbial loads in the Kombucha samples when yeast starter cultures were added when compared to the previous two (TS III and IV) and the first (TS I) fermentation. In addition, there was a statistically significant increase ($P < 0.05$) in counts of bacteria and yeasts at the end of the second fermentation. Again, these results could be explained by the effect of the shortened

fermentation time from 21 to 14 and then to 7 days and/or with the simultaneous fermentation with added yeast cultures.

Sensory analysis of Kombucha

In this study the effect of different *S. cerevisiae* starter cultures on the organoleptic properties of Kombucha was evaluated by sensory analysis. For that purpose, first initial four fermentations were carried out to stabilize the entire fermentation process by giving time for SCOBY, obtained from different households, to adapt to the new growth environment and treatments. To ensure the safety of the assessors during the consumption of Kombucha, absence of *coli*-like bacteria both in Kombucha and in SCOBY was tested prior to the first and then for the two last fermentation(s). The commercial yeast cultures were added together with SCOBY to the black tea solutions to start the 5th fermentation experiment (TS V). To our knowledge, Kombucha fermentation has not yet been carried out in this way before. To improve the probiotic properties of Kombucha, lactic acid bacteria have been added to the black tea solution during the first fermentation with SCOBY (Nguyen et al., 2015; Cvetković et al., 2019); the flavour of the Kombucha has also been improved by means of the second fermentation.

During the sensory analysis, the appearance, taste, aroma and overall acceptability of the Kombucha drinks were evaluated. The results show that yeasts added to the black tea solutions had different effects on the properties of the Kombucha. The characteristics of the taste were most influenced by the different yeasts. Samples TFL 1.2 and TFL 2.2 to which wine yeast was added, had the most vinegary and sour taste ($P < 0.05$). This sensory result was supported by their low pH values (pH was 3.02 and 3.06 respectively). In contrast, samples TFL 1.3 and TFL 2.3, to which brewer's yeast was added, had a much sweeter taste than the rest of the samples (with the exception of sample TFL 2.0, which was assessed to be the sweetest sample) ($P < 0.05$) and thus a less sour and vinegary taste than the samples with cider and wine yeasts (TFL samples 1.1, 2.1 and 1.2, 2.2) ($P < 0.05$).

Most of the beverages tested had sweet, sour and vinegary taste (Fig. 2). Traces of mould and a slight bitterness were also perceived in all of the samples. Honey flavours were also detected. Previously, Neffe-Skocińska et al. (2017) noted the taste of tea, lemon and sour tastes in their study, a sensory notion supported by Gramza-Michałowska et al. (2016), who noted that sweet, sour and citrus flavours were most evident in Kombucha. In addition, Gramza-Michałowska et al. (2016) also found that their Kombucha samples had a slight taste of tea and beer.

The overall acceptability of Kombucha (as part of the sensory evaluation) was considered low (in average ± SD, the rating was 2.19 ± 1.44) (Fig. 2). Most of the samples (27.9%) were evaluated with a grade 1 (on a scale of 0–5). The most acceptable Kombucha beverage was sample TFL 1.3 to which brewer's yeast was added ($P < 0.05$, but the difference was not statistically significant when compared to samples TFL 2.0 and 2.3), while TFL samples 1.2 and 2.2, to which wine yeast was added, had the lowest acceptability ($P < 0.05$). Samples TFL 1.2 and TFL 2.2 were described as being too sour and vinegary in taste. Gramza-Michałowska et al. (2016) found that traditionally fermented Kombucha samples evaluated in their experiment were of moderate acceptability.
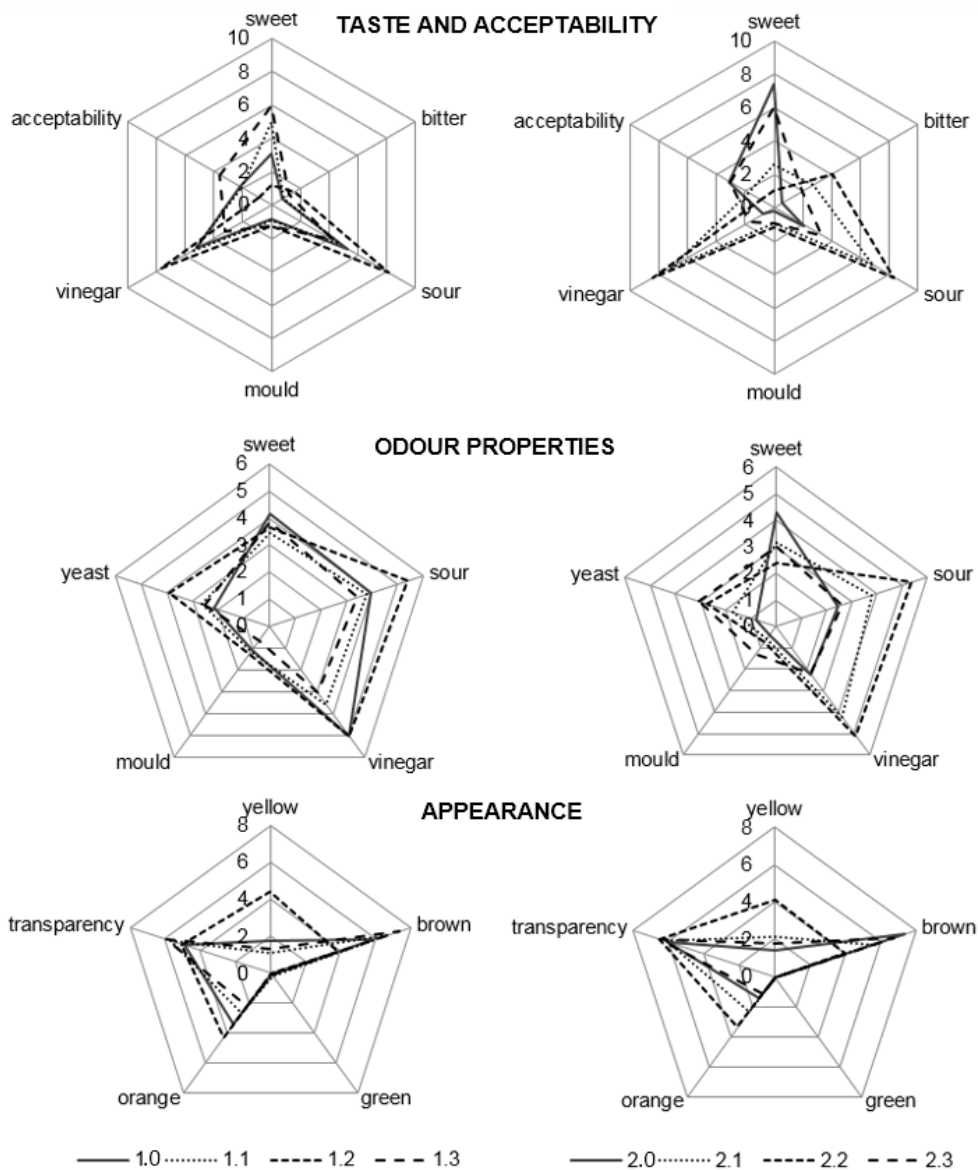
Figure 2. Taste and acceptability, odour properties and appearance of Kombucha samples. TFL 1.0 & 2.0 (control samples), TFL 1.1 & 2.1 (added cider yeast), TFL 1.2 & 2.2 (added wine yeast) and TFL 1.3 & 2.3 (added brewer's yeast). Kombucha drinks in first column originate from SCOBY previously (pre experiments) grown in sugared green tea (TF 1) and drinks in second column originate from SCOBY always grown in sugared black tea (TF 2).

Sweet, sour and vinegar scents were the most noticeable fragrances (Fig. 2). In addition, the smell of yeast and apple were also perceived. In earlier studies, the most commonly perceived scents were those of tea, lemon, sour, acetic acid, yeast and beer (Gramza-Michałowska et al., 2016; Neffe-Skocińska et al., 2017).

All samples evaluated showed medium transparency (Fig. 2). The dominant colour of all the samples was brown ($P < 0.05$), but orange and yellow were also perceived. An earlier study also found Kombucha to be brown in colour with amber and yellow (Gramza-Michałowska et al., 2016). The results show that added yeasts did not affect the external properties of the solutions. All samples to which *S. cerevisiae* was added (TFL 1.1, TFL 1.2, TFL 1.3, TFL 2.1, TFL 2.2 and TFL 2.3) were similar in appearance to control samples (TF 1.0 and TFL 2.0).

CONCLUSION

The addition of commercial yeast cultures to Kombucha did not significantly affect the overall microbial count in SCOBY, but it affected the overall microbial count in Kombucha; however the length of time of the fermentation process might play a more essential role in this respect. Therefore, the interplay of different parameters during the simultaneous fermentation process should be further and more thoroughly studied. In this study it was shown that all added commercial yeast cultures modified the sensory properties of the individual Kombucha drink. Detailed research is further needed to explore the precise influence, the possible advantages as well as the optimal conditions for simultaneous inoculation of various microbial strains, including probiotics, to the Kombucha fermentation process.

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Shelf life extension of minimally processed vegetables using combinations of bacterial bioprotection and modified atmosphere packaging

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Abstract. The objective of the work: to study the effect of combination of bacterial bioprotective cultures and modified atmosphere packaging for prolonging the refrigerated storage period of minimally processed vegetables. Sweet pepper, zucchini, eggplant, celery stalks were used for preparation of minimally processed vegetables. SafePro® bio-products from Chr. Hansen (Denmark) containing strains of *Lactobacillus sakei, Pediococcus acidilactici, Lactobacillus curvatus, Leuconostoc carnosum* were used as bioconservatives. For packaging minimally processed vegetables, the bags made of flat multilayer PA/adhesive/PE films and composite PET/A1/PE film material were used. The bags were filled with gas mixtures including nitrogen and carbon dioxide. Storage of packaged minimally processed vegetables was carried out in a refrigerator at a temperature of 4 ± 2 °C for 16 days. The viability of cultures *Lactobacillus sakei, Pediococcus acidilactici, Lactobacillus curvatus, Leuconostoc carnosum* in modified atmosphere packaging was studied. It was revealed that the gas mixture of 60% nitrogen and 40% carbon dioxide and the culture of *Lactobacillus sakei* contribute to the preservation of the quality of fresh-cut sweet pepper, eggplant and zucchini, and *Leuconostoc carnosum* is the more effective for celery storing. In the process of refrigerated storage for 14 days, the solids content in the experimental samples increased 1.3–2.1 times, the loss of organic substances was 26–50%, depending on the type of vegetables. The developed technology for the refrigeration preservation of minimally processed vegetables using bio-products treatment and in modified atmosphere packaging made it possible to increase the shelf life of fresh-cut vegetables by 2 times.

Key words: minimally processed vegetables, bacterial bioprotection, *Lactobacillus sakei*, modified atmosphere packaging, shelf life extension, refrigerated storage, vegetable microbiota.

INTRODUCTION

Nowadays the HoReCa segment (Hotel, Restaurant, Cafe/Catering) is rapidly developing around the world. The HoReCa trading channel is an association of enterprises of food industry, services, suppliers of B2B (Business to Business) products, which are targeted for resale at HoReCa sale outlets, as well as representatives of information and marketing services.

In high demand are convenient food sets including meat, fish and minimally processed vegetables (MPV) packaged using combined technologies, including vacuum and modified atmosphere packaging (MAP) (Preeti et al., 2011; Bouletis et al., 2017; Bazarnova et al., 2018b) for fast cooking at home.

In food industry, the production of MPV is the most labor-intensive. Moreover, the methods used for vegetables processing should not only prevent their spoilage, but also contribute to the preservation of vitamins, mineral and biologically active substances in them (Hounsome et al., 2008; Sun & Holley, 2012; Evstigneeva et al., 2016; Eliseeva et al., 2018; Pankina et al., 2019).

A promising technology for preserving minimally processed vegetables is the combined usage of various biotechnological techniques, which gives a pronounced synergistic effect and allows prolonging the shelf life of culinary products without the use of chemical preservation agents (Sun & Holley, 2012; Oluwafemi et al., 2013).

Many bacteria synthesize protein-peptide antibiotic substances, which suppress affined species or strains that inhibit their growth, or have a wider spectrum of antibacterial action (Durango et al., 2006). These substances with a very specific action are called bacteriocins, their biosynthesis is encoded by specific plasmids and in most cases occurs on ribosomes. In food industry bacteriocinogenic strains have found application as biological preservatives. The main advantage of using bio-preservatives is safety. Some of the microorganisms used as bio conservatives are probiotic for the human intestinal microbiota, including *Lactobacillus, Bifidobacterium, Propionibacterium, Streptococcus thermophilus, Lactococcus* (Vinderola & Reinheimer, 2000).

The main cultures used in food technology are lactic acid bacteria (lactobacillus and pediococcus) (Axelsson, 2004), which reduce pH, so that the development of pathogenic microbiota in acidified media slows down (Timoshenkova et al., 2019). Micrococcaceae, homofermentative lactic acid bacteria and pediococcus, yeast and atypical lactic acid bacteria mostly in the form of pure cultures are also used as bioprotective ones (Lucera et al., 2012).

The MAP technology for long keeping products is successfully used for products in portion consumer packaging. It is the most modern way to maintain the quality and freshness of food products and increase their shelf life for several times without freezing and also to exclude of use of chemical additives and preservatives (Oluwafemi et al., 2013; Moor et al., 2014; Kirse et al., 2017). In MAP packaging, air is replaced by a mixture of inert gases, which eliminates or slows down the oxidation (spoilage) of food. The presence of carbon dioxide prevents the development and reproduction of fungi, bacteria and other microorganisms. Oxygen (O_2), carbon dioxide (CO_2) and nitrogen (N_2) are the three main gases used for MAP. The gas choice depends on the type of product being packaged (Speranza et al., 2009; Ahmed et al., 2011). These gases are used individually or in combination with each other to achieve a balance between the product shelf life and its optimal organoleptic properties (Brandenburg & Zagory, 2009).

To determine the efficiency of bioprotective cultures' impact on the microbiota caused by biodeterioration of vegetables, it is necessary to study the tolerance of bio protective cultures in inert gas mixtures based on nitrogen and carbon dioxide. The other task is to develop a technology for product processing by these cultures in order to obtain a synergistic effect from the combined usage of several biological barriers (Gialamas et al., 2010; Bartolini & Ducci, 2017; Bazarnova et al., 2018b), as well as to assess the

dynamics of changes in biochemical and microbiological quality indicators of minimally processed vegetables during cold storage.

The objective of the work: to study the effect of combination of bacterial bioprotective cultures and modified atmosphere packaging for prolonging the refrigerated storage period of minimally processed vegetables.

MATERIALS AND METHODS

SafePro® microorganism specimen (Chr. Hansen, Denmark) containing strains of *Lactobacillus sakei, Pediococcus acidilactici, Lactobacillus curvatus, Leuconostoc carnosum* (Table 1) were used as bio protective cultures.

There were used red and yellow sweet pepper, zucchini, eggplant, celery stalks for preparation of MPV.

For packaging MPV prepared from fresh vegetables there were used bags made from flat multilayer PA/adhesive/PE films and composite PET/A1/PE film material (Lion Company LLC, Russia), as well as food gas mixtures: Biogon ®NC20 (80% nitrogen, 20% carbon dioxide by volume) and Biogon ®NC40 (60% nitrogen, 40% carbon dioxide by volume) from Linde Gas, Russia. For spraying of bio-products' suspension onto the surface of MPV, the 4000-B1 finely dispersed spray nozzle was used (VKT, Russia).

Table 1. Characteristics of bio products*

Characteristics	Name			
	SafePro B-SF-43	B-2 SafePro	B-LC-48 SafePro	B-LC-20 SafePro
Content	*Leuconostoc carnosum*	*Lactobacillus sakei*	*Lactobacillus curvatus*	*Pediococcus acidilactici*
Type	*Microaerophilic*	*Facultative anaerobic*	*Microaerophilic*	*Microaerophilic*
Total amount of cells, CFU g^{-1}	1×10^9	1×10^9	1×10^9	1×10^9
Produced substances	*D(-)-lactic acid*	*L (+)-lactic acid*	*L (+)-lactic acid*	*DL (+/-)-lactic acid*
Produced bacteriocins	*Leukacin C*	*Sakacin A*	*Kurvacin*	*Pediocin*
Temperature Opt/Max/Min, °C	20/30/-4	25/40/-4	37/40/4	43/52/15

* Data according to Vinderola & Reinheimer (2000).

For microscopic analysis of cultures' activity, there were lyophilized preparations with addition of nutrient substratum activated in 1 l of warm water (37 °C). To determine the activity of the studied cultures, there were activated biological products, microfilmed and counted the number of rods and coccus of cultures with using statistical methods. For microscopic analysis of cultures, there were samples activated with nutrient substratum in 1 L of warm water (37 °C). There was estimated degree of development of cocci bacteria by the number of cocci in staphylococci and streptococci (Belyaev, 2016).

There was determined influence of the modified gas mixture on the cultures' vitality with the microscopy method by staining with methylene blue to detect dead cells.

The Eclipse binocular microscope with the TC-100 camera (100× magnification), the IS-500 digital camera, and photo microanalysis program were used to study the microscopic image of cultures during storage.

To study the tolerance of cultures in MAP packaging during cold storage, the foil substrate was sprayed with suspensions of activated cultures. Further the substrates were placed in a gas barrier film with mixtures of Biogon ® NC20, Biogon ® NC40 and stored at a temperature of 4 ± 2 °C. The control sample was packaged in a film with atmospheric air and stored under similar conditions until the microorganisms stopped functioning.

To study the combined effect of biological products and inert gas mixture on the dynamic of shelf life of MPV, the samples of raw vegetables were treated with suspensions of activated bio cultures. To prepare a suspension of a biological product the sample weighing 2 g was set into a container and diluted by filtered water (0.2 L) with a temperature of 4 ± 2 °C. After thoroughly shaking the container, there was another 0.8 L of water with a temperature of 4 ± 2 °C added. The lyophilized biological product was placed in a spray container.

The raw materials are subjected to primary processing and sorting, i.e. removal of spoiled and damaged vegetables. After that vegetables are washed in cold water to remove contaminants, after which they are laid out in perforated containers to remove excess moisture from the surface of vegetables. Further the vegetables are cut into pieces of the required size depending on their type and put on sheets.

The prepared vegetables were sprayed with the suspension 4 ± 2 °C. Spraying is carried out at a nozzle operating pressure of 7 bar. After spraying the suspension, the fresh-cut vegetables are dried or centrifuged at 300–600 rpm, depending on the type of vegetables, to remove droplet-liquid moisture. The processed vegetables are packaged by polypropylene gas-barrier bags using Biogon ®NC20, Biogon ®NC40 gas mixtures. There were packed control samples of MPV in the film with use MAP packaging without any treatment by bio products.

Packed experimental and control samples of MPV were stored in a refrigerator at a temperature of 4 ± 2 °C for 16 days.

During storage, organoleptic, biochemical, physicochemical and sanitary-significant microbiological indicators of MPV were determined.

To analyze fresh-cut vegetables for sensory characteristics there was used standardized method (ISO 13299: 2003). Sensory analyzes (5-point grading scale) were carried out over 14 days of storage by the 5 member sensory panel. Sensory profiles were developed for each sensory characteristic of fresh-cut vegetables, and the main sensory differences were assessed using a standardized technique. Sensory characteristics included assessment of appearance, color, texture, taste and smell.

The mass fraction of solids in MPV was determined by drying to a constant mass at a temperature of (135 ± 1) °C (Nilova et al., 2017).

To determine the sum of phenolic compounds, the Folin-Ciocalteu reagent was used (Bazarnova et al., 2018a). The content of organic acids in vegetables was determined by titration (Bazarnova & Ivanchenko, 2016).

The barrier properties of bio protective cultures relative to the microbiota of vegetables were determined by analyzing the sanitary-indicative microbiota of fresh-cut vegetables using official methods of analysis (AOAC, 2015). There was investigated yeast and mold count (AOAC method 997.02), *Escherichia coli* count (E-coli) (AOAC

method 991.14), Aerobic plate count (APC kfu g^{-1}) (AOAC method 986.32), *Staphylococcus aureus* count (AOAC method 975.55).

To assess the microbiological parameters, there were used experimental and control samples from fresh-cut vegetables and unprocessed ones samples before their storage set and on the 7th and 14th days of refrigerated keeping.

The each sample was undergone researched in triplicate. The accuracy of the experimental data was evaluated by using mathematical statistical methods in Microsoft Excel. The data gained through this process are presented with a confidence coefficient of 0.95.

RESULTS AND DISCUSSION

Fig. 1, (a–d) shows influence of MAP-packaging on the vitality of bio-barrier microorganisms at 4 ± 2 °C. The microscopic examination showed that the culture of *Lactobacillus sakei* (B-2 SafePro preparation) had the greatest growth potential. The 0.05 mL of its suspension had the highest concentration of microorganisms from streptobacilli, consisting on average from 20 to 40 rods, which explains their active development. A good development of the *Leuconostoc carnosum* culture (SafePro B-SF-43 preparation) is also noticed which has about 20 rods in 0.05 mL of its suspension. Cultures of *Lactobacillus curvatus* (B-LC-48 SafePro), *Pediococcus acidilactici* (B-LC-20 SafePro) (c and d) showed low activity, which is expressed by low concentration of microorganisms in suspension. In addition, it is worth noting that the storage temperatures of minimally processed vegetables are outside the range of active growth of *Pediococcus acidilactici*.
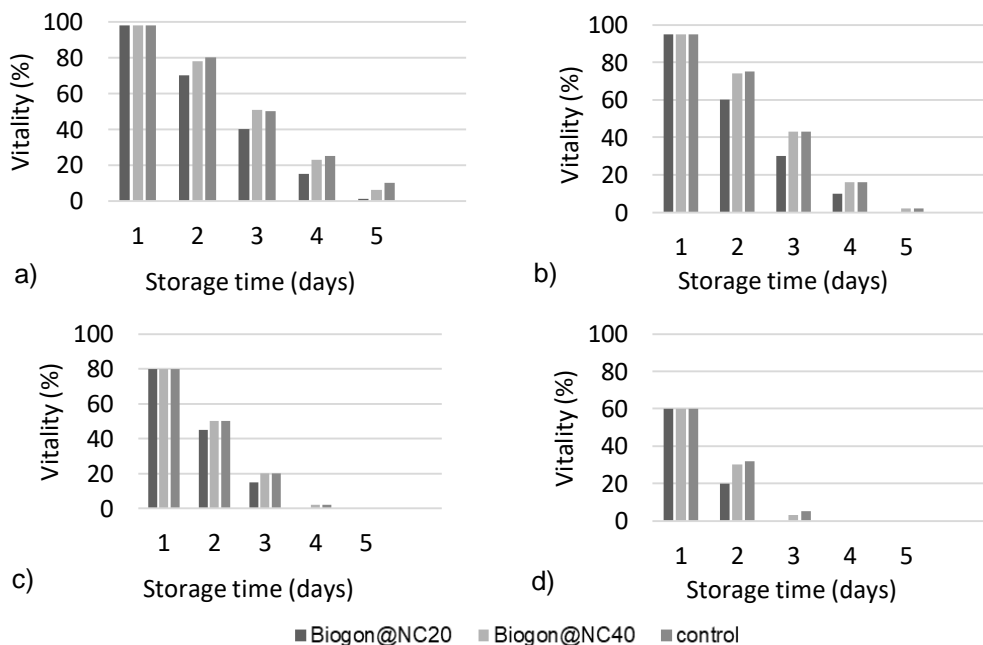
Figure 1. Influence of MAP-packaging on the vitality of bio-barrier microorganisms at 4. ± 2 °C: a – B-2 SafePro; b – SafePro B-SF-43; c – B-LC-48 SafePro; d – B-LC-20 SafePro.

The results presented in Fig. 1 show that *Lactobacillus sakei* culture (B-2 SafePro), remained viable up to 5 days, has the highest vitality in the Biogon ®NC40 gas medium. The same culture showed less viability in Biogon ®NC20 gas medium.

The other cultures show low tolerance in Biogon ®NC20 and Biogon ®NC40 gas media. This is due to a lack of oxygen in gas mixtures and a significant content of carbon dioxide.

Table 2. The sanitary-indicative microbiota of minimally processed vegetables during cold storage for 14 days; 4 ± 2 °C

Minimally processed vegetables	Bioprotective cultures	The sanitary-indicative microbiota				
		APC, CFU g^{-1}	Yeast, CFU g^{-1}	Mold, CFU g^{-1}	*E. coli*	*S. aureus*
		Limits				
		no more than $1 \cdot 10^4$	no more than 500	no more than 500	absence in 1 g	absence in 1 g
		Values after 14 days of storage				
Red sweet pepper	Control	$5 \cdot 10^5$	120	300		
	Lactobacillus sakei	$2 \cdot 10^2$	8	20		
	Leuconostoc carnosum	$2 \cdot 10^2$	12	22	absence	absence
	Lactobacillus curvatus	$2 \cdot 10^2$	14	20		
	Pediococcus acidilactici	$2 \cdot 10^2$	16	20		
Yellow sweet pepper	Control	$1 \cdot 10^5$	140	510		
	Lactobacillus sakei	$2 \cdot 10^2$	10	20		
	Leuconostoc carnosum	$2 \cdot 10^2$	15	22	absence	absence
	Lactobacillus curvatus	$2 \cdot 10^2$	14	20		
	Pediococcus acidilactici	$2 \cdot 10^2$	16	20		
Zucchini	Control	$1 \cdot 10^5$	80	502		
	Lactobacillus sakei	$6 \cdot 10^2$	10	36		
	Leuconostoc carnosum	$6 \cdot 10^2$	14	40	absence	absence
	Lactobacillus curvatus	$6 \cdot 10^2$	14	44		
	Pediococcus acidilactici	$6 \cdot 10^2$	18	50		
Eggplant	Control	$2 \cdot 10^5$	120	500		
	Lactobacillus sakei	$3 \cdot 10^2$	12	44		
	Leuconostoc carnosum	$3 \cdot 10^2$	14	56	absence	absence
	Lactobacillus curvatus	$3 \cdot 10^2$	18	56		
	Pediococcus acidilactici	$3 \cdot 10^2$	18	56		
Celery stalks	Control	$1 \cdot 10^5$	140	80		
	Lactobacillus sakei	$3 \cdot 10^2$	22	60		
	Leuconostoc carnosum	$3 \cdot 10^2$	18	38	absence	absence
	Lactobacillus curvatus	$3 \cdot 10^2$	18	68		
	Pediococcus acidilactici	$3 \cdot 10^2$	30	70		

Table 2 shows the results of studies of sanitary-indicative microbiota of the experimental and the control samples of fresh-cut vegetables after their refrigerated storage in MAP packaging (Biogon ®NC40). The results were compared with the limits set by the Commission Regulation (EC) on microbiological criteria for foodstuffs (2005). It was established all processed samples of fresh-cut vegetables had a safe level

of studied microbiological parameters, in contrast to untreated control samples, the APC level of which was exceeded already for 7 days of refrigerated storage. Proper peeling and washing of vegetables before treatment by bio protective cultures, as well as the absence of risks of recontamination during their packaging, determined their microbiological safety in relation to *Escherichia coli* and *Staphylococcus aureus*.

The deterioration of organoleptic properties goes slower in the samples treated by bio protective cultures in contrast to the control samples (Fig. 2).
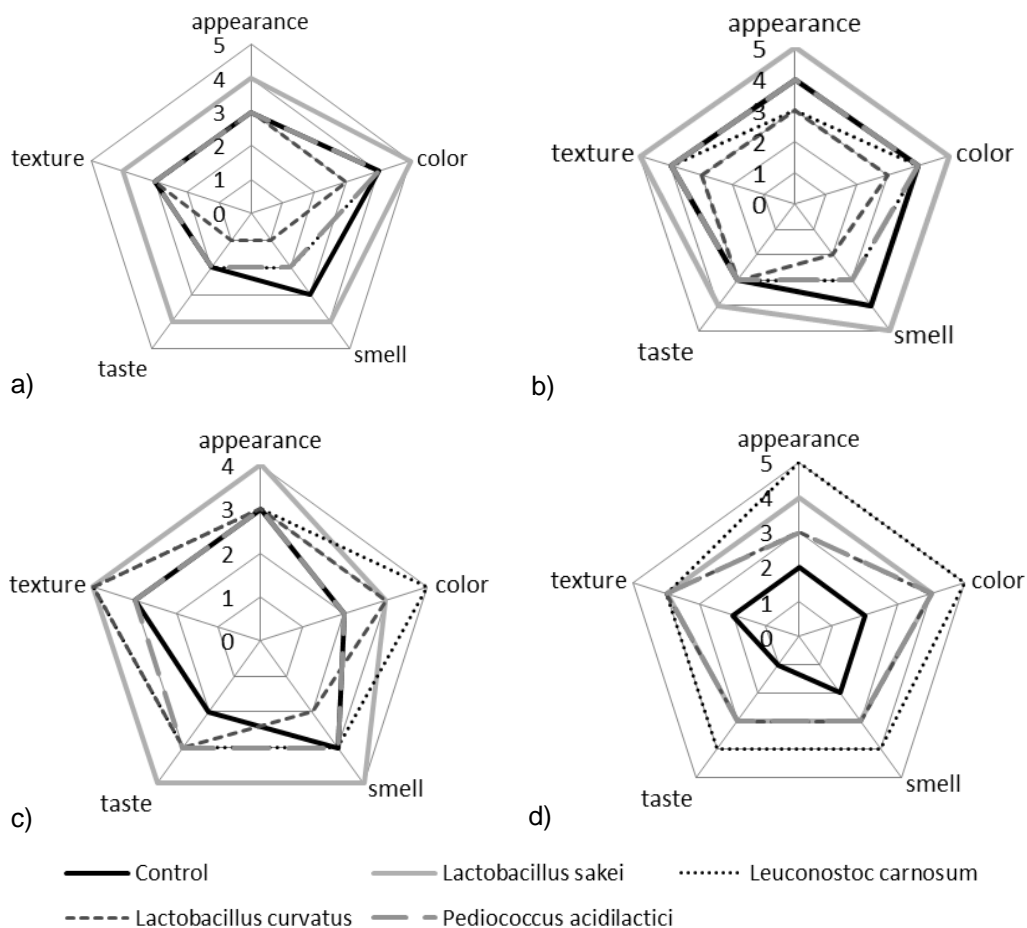
Figure 2. Sensory characteristics of minimally processed vegetables during cold storage for 14 days; 4 ± 2 °C: a – red sweet pepper; b – zucchini; c – eggplant; d – celery stalks.

The total quality level of the minimally processed vegetables from red pepper and zucchini treated by *Lactobacillus sakei* during 14 days of refrigerating storage can be described as 'good', and for eggplant and celery as 'satisfactory'. At the same time, unprocessed fresh-cut vegetables from pepper, eggplant and celery were rated as 'unsatisfactory', since at least one of the signs they had was rated at 2 marks.

It was established the culture of *Lactobacillus sakei* (B-2 SafePro preparation) has the greatest barrier effect during the refrigerated storage of fresh-cut red and yellow

pepper, eggplant, zucchini in Biogon ®NC40 medium. This allows preserving the organoleptic and microbiological parameters of the studied MPV up to 14 days at a temperature of 4 ± 2 °C at the required level. The most effective culture for celery was *Leuconostoc carnosum* (SafePro B-SF-43). The shelf life of untreated (control) samples of MPV under the same conditions was 7 days, which is 2 times less.

There were presented results of studies of dynamics of content of dry substances and organic acids of fresh-cut vegetables from eggplant, red pepper, zucchini and celery during refrigerated storage in the Table 3.

Table 3. Dynamics of the mass fraction of solids and organic acids in test MPV during the refrigeration storage

Minimally processed vegetables	Duration of storage, days	Mass fraction of solid, %		Content of organic acids, mg g^{-1}	
		control	test	control	test
Eggplant	1	7.11 ± 0.35	7.11 ± 0.35	0.70 ± 0.05	0.80 ± 0.05
	7	7.23 ± 0.35	7.12 ± 0.30	0.70 ± 0.05	0.80 ± 0.05
	10	8.84 ± 0.41	7.73 ± 0.35	0.50 ± 0.04	0.60 ± 0.04
	14	16.29 ± 0.80	9.88 ± 0.51	0.30 ± 0.02	0.40 ± 0.02
	16	18.21 ± 0.91	11.78 ± 0.55	0.20 ± 0.01	0.30 ± 0.02
Red sweet pepper	1	4.12 ± 0.08	4.11 ± 0.08	2.10 ± 0.20	2.00 ± 0.20
	7	5.24 ± 0.06	4.79 ± 0.08	1.60 ± 0.15	1.70 ± 0.15
	10	5.87 ± 0.19	5.13 ± 0.15	1.30 ± 0.15	1.40 ± 0.15
	14	8.63 ± 0.10	5.33 ± 0.11	0.90 ± 0.10	1.20 ± 0.10
	16	10.81 ± 0.20	6.44 ± 0.22	0.80 ± 0.10	1.10 ± 0.10
Celery stalks	1	2.24 ± 0.09	2.24 ± 0.09	0.20 ± 0.05	0.20 ± 0.05
	7	3.83 ± 0.07	3.22 ± 0.08	0.20 ± 0.05	0.20 ± 0.05
	10	5.18 ± 0.12	3.47 ± 0.09	0.10 ± 0.02	0.10 ± 0.02
	14	6.34 ± 0.06	4.01 ± 0.13	0.10 ± 0.02	0.10 ± 0.02
	16	7.32 ± 0.12	4.84 ± 0.08	not determined	not determined
Zucchini	1	5.61 ± 0.08	5.61 ± 0.09	4.00 ± 0.50	4.20 ± 0.50
	7	7.82 ± 0.07	6.81 ± 0.09	3.80 ± 0.50	5.10 ± 0.60
	10	12.49 ± 0.28	7.89 ± 0.09	2.90 ± 0.30	4.60 ± 0.50
	14	16.32 ± 0.11	12.32 ± 0.12	2.10 ± 0.20	3.10 ± 0.40
	16	20.91 ± 0.17	15.82 ± 0.10	1.60 ± 0.15	2.60 ± 0.30

It was registered that in control samples of MPV the solids content increased by 2.5–3 times over the 16 days, which is explained by moisture loss due to shrinking of vegetables (Barsukova et al., 2019). This process proceeded 1.5 times slower in the experimental samples.

In this case, processed fresh-cut red pepper and eggplant were characterized by the lowest degree of shrinkage. The solids content in them on the 16th day of storage increased by 56 and 65%, respectively.

The highest content of organic acids before storage was registered in red pepper and zucchini: 2.1 and 4.2 mg g^{-1}, respectively. During refrigerated storage, their amount decreases. On the 7th day, there is a noticeable difference between the content of organic acids in control samples and samples treated by bio-products, which ranges from 5 to 10%, depending on the vegetables type. This difference increases with long time storage and reaches 20% on the 14th day.

Fig. 3 presents the results of studies of the content of phenolic compounds in minimally processed eggplant during storage.

Loss of phenolic compounds over the entire period of refrigerated storage for the experimental samples is about 30%, in comparison with 50% for the control samples. In experimental samples, the dynamics of phenolic substances slows down by about 15%.
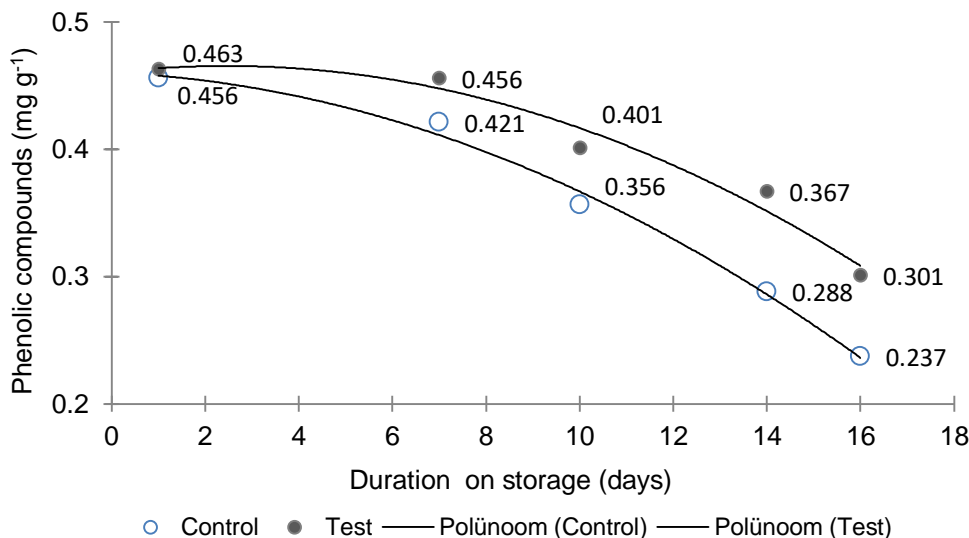
Figure 3. Dynamics of phenolic compounds of eggplant during the refrigeration storage.

The determining factor in the duration of the heat treatment of vegetables is their texture – one of the important indicators of culinary readiness. It was experimentally established that the control samples of vegetables reached culinary readiness faster than the experimental samples subjected processed by the biological products. Table 4 shows the estimated duration of frying of the experimental and the control samples of fresh-cut vegetable at $t = 180 \pm 2\ °C$ at the end of their shelf life in MAP packaging. The criterion of a paired comparative test between the test samples and the control were the duration of frying vegetables until culinary readiness and the desired texture of ready vegetables.

Table 4. Duration of frying vegetables until culinary readiness

Vegetables	Duration of frying, sec	
	control	test
Eggplant	290 ± 10	360 ± 10
Red sweet pepper	220 ± 10	260 ± 10
Celery stalks	390 ± 15	440 ± 15
Zucchini	150 ± 10	210 ± 10

The obtained experimental data formed the basis for the development of technological recommendations for the culinary processing of fresh-cut vegetables treated by bio-protective cultures, which we recommend to use as part of sets for preparing first and second courses, from which consumers can prepare hot dishes according to the developed instructions enclosed in consumer packaging.

The technological scheme for production of sets with indicated critical control points (CCP) at which it is necessary to carry out safety control at the factory is presented in Fig. 4.
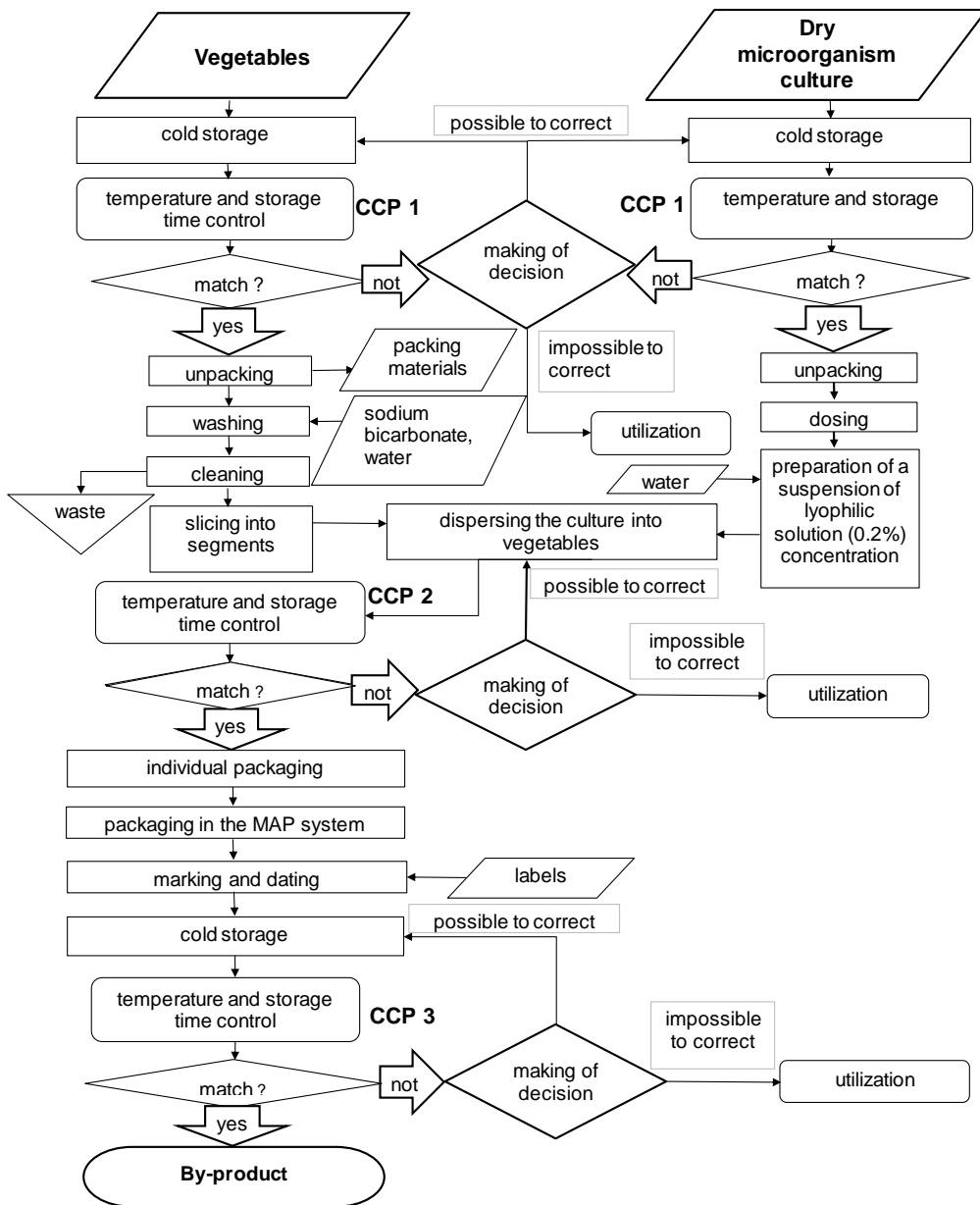
Figure 4. Technological scheme for production of fresh-cut vegetables with indicated critical control points.

CONCLUSIONS

There was studied impact of modified gas mixtures Biogon ®NC40 and Biogon ®NC20 on the vitality of cultures of *Lactobacillus sakei*, *Pediococcus acidilactici*, *Lactobacillus curvatus*, *Leuconostoc carnosum*. It was set that culture of *Lactobacillus sakei* (B-2 SafePro) was able to active growth in the Biogon ®NC40 gas environment up to 5 days including. The other studied cultures showed low resistance in gas mixtures Biogon ®NC20 and Biogon ®NC40. It can be due to the lack of oxygen and a significant content of carbon dioxide in the tested gas mixtures.

It was found that minimally processed zucchini, eggplants, red pepper treated with *Lactobacillus sakei* culture and celery stems treated with *Leuconostoc carnosum* culture are preserved under the temprature 2–4 °C for up to 14 days, which is 2 times longer than the minimally processed vegetables untreated by cultures under the same conditions.

There has been developed method of processing minimally processed vegetables with a suspension of bio-protective cultures before packaging in a polymer film using a gas mixture, including 60% nitrogen and 40% carbon dioxide by volume (Gnilitskij et al., 2019).

Treated with bio-protective cultures fresh-cut vegetables retain organoleptic, biochemical and microbiological parameters in accordance to the regulated norms for 14 days in MAP packaging and after this storage period do not lose their suitability for further heat treatment.

The technology of minimally processed vegetables using of combination of biological products and MAP packaging for refrigerated storage indicating critical control points in the technological cycle of their production has been introduced into the production of the food company 'Velikoross' (St. Petersburg).

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Biochemical composition and quality of herring preserves with addition of bio-protective cultures

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Abstract. Herrings rich in vitamins B12, A, D, minerals, including calcium, potassium, magnesium, iodine, possess high levels of lysine, tyrosine, phenylalanine and tryptophan, as well as omega-3 unsaturated fatty acids, namely eicosapentanoic, docosahexoenoic and docosapentoenoic. To suppress the microbiological spoilage of fish preserves, it is promising to use bio-protective cultures that have minimal impact on the production process and product properties. Bacterial strains are able to exert a static effect on the microflora, which causes biodeterioration of food products. Microorganisms as part of bio-protective cultures are included in the fermentation process, so they can be attributed to ordinary food ingredients, so there is no need to put separate information on the packaging. The chemical composition of the frozen Atlantic and Pacific herring fillet, the amino acid composition of proteins, and the fatty acid composition of lipids were studied. The difference between Atlantic and Pacific herrings was detected, which consists in a significantly higher content of docosahexaenoic acid. The difference in the fractional composition of triacylglycerols for the Atlantic and Pacific herring was established. The microflora of SafePro B-2 biological product (Chr. Hansen GmbH), containing multiple strains of *Lactobacillus sakei*, and the viability of the culture in preserves fillings were studied. The experimental development of canned food with SafePro B-2 additives was carried out. Microbiological, organoleptic and biochemical indicators of the preserves quality during cold storage were studied. The influence of introduced culture on the dynamics of preserves curing period and their shelf life was established.

Key words: frozen fillet of Atlantic and Pacific herrings, fatty acid composition, triglycerides, bio-protective cultures, preserves, fillings, buffering, curing period.

INTRODUCTION

Fish raw materials are characterized by diversity in size, mass composition, biochemical properties and nutritional value.

Herrings are important products of fishing industry as it counts about 20% of the global fish catch. Herring in Russia is captured in the northern Atlantic Ocean, the Black, Azov, Caspian, Barents Seas and in the Far East.

As a result of ripening the herring meat in a salty form acquires a pleasant taste and aroma, so the major volume of catch is salted, some part of which is then smoked cold and marinated. Another part of catch is used for production of canned food, a small amount of small herring is sent for hot smoking and is sold freshly frozen.

Herring is a popular component of traditional fish dishes in the Russian cuisine. It is rich in vitamins B12, A, D, minerals, including calcium, potassium, magnesium, iodine (Olsen, 2001). Herrings possess high levels of lysine, tyrosine, phenylalanine and tryptophan, as well as omega-3 unsaturated fatty acids, namely eicosapentanoic, docosahexoenoic and docosapentoenoic. The linoleic acid is the omega-6 fatty acid of herring (Olsen, 2001; Timberg et al., 2011). Atlantic herring (*Clupea harengus*) is widespread in the northern Atlantic Ocean, and also lives in the vast water area of the Greenland, Norwegian and Barents Seas (Gritsenko et al., 2006). Pacific herring (*Clupea pallasii*) is found in the Pacific Ocean from Korea to the Anadyr Gulf and from California to the Bering Strait. It is catched in the Bering, Okhotsk and Japan Seas (Laakkonen et al., 2013).

Analysis of modern technologies for herring processing shows an increase in the relative share of the natural fish preserves output (Bocharova-Leskina et al., 2015; Maksimova et al., 2018; Timoshenkova et al., 2019). However, statistics shows a weak saturation of this segment (Naujmin, 2017). The assortment of herring preserves is limited to products in oil and wine fillings possessing long shelf life.

To suppress the microbiological spoilage of fish preserves, it is promising to use bio-protective cultures that have minimal impact on the production process and product properties (Abdrakhmanova & Zaitseva, 2012). Bacterial strains with antagonistic activity against pathogens of microbiological food spoilage belong to the Lactobacillus genus and are able to exert a static effect on the microflora, which causes biodeterioration of food products (Axelsson, 2004; Bazarnova et al., 2018). Microorganisms as part of bio-protective cultures are included in the fermentation process, so they can be attributed to ordinary food ingredients, so there is no need to put separate information on the packaging.

It is also advisable to establish threshold values of biochemical markers of spoilage of frozen herring, which undergo a long-term storage process. These markers will make it possible to assess the content of protein and lipid decomposition products and establish a correlation with normalized physical-chemical quality indicators of frozen fish (Rehbein & Orlick, 1990).

Based on the aforesaid, chemical-technological and biochemical studies of Russian herring and development of technology of preserves using bio-protective cultures are relevant.

The aim of this work is to study the biochemical composition of Atlantic and Pacific herring fillets and the quality of herring preserves with addition of bio-protective cultures.

MATERIALS AND METHODS

Samples of frozen Atlantic herring *Clupea harengus* (Greenland, plant GL 5778) and Pacific herring *Clupea pallasii* (Murmansk Trawl Fleet, ship Vladimir Sibirtsev); samples of salted Atlantic and Pacific herring fillet ('Baltic coas' JSC) were used as objects of study.

Samples of apple and citrus pectin manufactured by Herbsreith & Fox KG Pektin-Fabriken (Germany) and B-2 SAFEPRO (CHR Hansen, Denmark) containing strain *Lactobacillus sakei* (1×10^9 CFU g^{-1}) strains were used for developing preserves fillings.

To obtain the composite sample, blocks of frozen herring fillet with a size range of frozen herring fillet from six to ten pieces per 1 kg were used. Samples were taken after defrosting in blocks, the fillet temperature on the block surface was 9 °C, inside the block it was –1 °C. Prior to testing samples were stored at -22 °C in transport packaging from the manufacturer. Samples were delivered to the laboratory in a vacuum bag without complete disruption.

The mass fractions of protein, fat, and moisture in the herring fillet, as well as the mass fraction of salt in salted fish and the filling of preserves were determined according to AOAC (Association of Official Analytical Chemists).

The composition of fatty acids in herring fillets was determined by gas-liquid chromatography of methyl esters according to the procedure for pharmacopoeia fish oil (Bazarnova et al., 2019).

When determining the composition of triglycerides (TAG), the lipid fraction was separated by the BUME method (Lofgren et al., 2012) by a 4-fold excess (volume: weight) of a mixture of n-butanol and methanol (3:1 by volume), followed by extraction with a mixture of n-heptane and ethyl acetate (3:1 by volume) in the presence of 1% acetic acid. Phospholipids were then precipitated by acetone in the cold, after which the content and distribution of triglycerides and free fatty acids were determined by short-column high-temperature gas-liquid chromatography (Hooper & Parrish, 2009). The analysis conditions were the following: Agilent DB-HT + SimDis column (5 m × 0.54 mm × 0.15 μm); the volume of the injected sample is 1 μl, the inlet temperature is 380 °C, the flow divider is 1:25; carrier gas is nitrogen, 20 cm s^{-1}; column temperature: 2 min. at 80 °C – 25 °C min^{-1}; 5 min. at 380 °C. The AOC20i automatic sample feeder ensured high reproducibility of results (within ± 5% of the peak area), which made it possible to use the method of external standards.

The degree of raw materials preservation by the content of biogenic amines was evaluated after extraction with 0.5 M of perchloric acid (4 °C, 16 h), with subsequent centrifugation at 3,000 rpm min^{-1} for 5 minutes to remove fat. The content of free biogenic amines in deproteinized muscle tissue extracts was determined by ion chromatography using mobile phase with strong acid and acetonitrile (Erupe et al., 2010). The analysis conditions were the following: Dionex CS10 column, 4×125 mm, 40 °C; conductometric detector; mobile phase is 5 mM perchloric acid + 6% acetonitrile, 0.6 mL min^{-1}.

Herring ageing during salting and in preserves was determined by the buffer value in grad. The solids content in preserves fillings during storage was according to (Tülsner & Koch, 2010).

Pilot production of salted herring fillet and preserves was carried out on the basis of 'Baltic coast' JSC (St. Petersburg). Analysis of sanitary-significant microflora of frozen herring fillet, experimental and control samples of 'Spicy herring in jelly' preserves during refrigerated storage for 25 days at 4 ± 2 °C was carried out according to methods recommended by AOAC (2015). In particular, we determined the amount of mould and yeasts (AOAC 997.02), *Escherichia coli* (AOAC 991.14), APC kfu g^{-1} (AOAC 986.32), *Staphylococcus aureus* (AOAC 975.55), Bacteria of the *Clostridium* genus (AOAC 974.38).

The introduction of bio-protective cultures in preserves fillings was carried out as follows. The lyophilized B-2 SAFEPRO preparation was diluted in distilled water in a ratio of 1:15. The resulting suspension was evenly distributed over the surface of herring

cut and put into cans in a ratio of 0.02% to the fish weight, after which they were filled with fillings, hermetically sealed, and stored at $4 \pm 2\,°C$ for 25 days. Preservatives produced without addition of bio-protective cultures were used as control samples.

Statistical processing of research results was carried out using the Microsoft Office Excel software and the one-way analysis of variance Analysis of Variance (ANOVA). The obtained experimental data are presented with the reference to confidence interval calculated using the t-criteria. The confidence probability is 0.95 and statistical significance of the given results is $p < 0.05$. The samples were examined in 3-fold repeatability mode. The difference between Atlantic and Pacific herring fillets is statistically significant.

RESULTS AND DISCUSSION

The process of herring filleting can lead to damage accelerating unwanted changes during fish freezing and storage. Reduced herring cutting quality affects the fillet texture when stored in frozen state. The results of organoleptic assessment of quality and sanitary significant microflora of the studied samples of frozen Atlantic and Pacific herring fillets showed that all fillet samples comply with the requirements for quality and safety of fish (Bremner, 2002; Blackburn, 2006).

Fatty fish species are known to contain more than 5% of lipids localized in tissues as triglycerides. Seasonal fluctuations in the fat content of the Atlantic herring are significant and can vary from 1 to 25% of the total fat content. The tendency of frozen fish to rancid depends on the fatty acid composition and localization of adipose tissue (Rehbein & Orlick, 1990).

It was found that the fat content of Pacific herring, which is caught in the winter period, is higher than that of the Atlantic by 3.2%, and the moisture content is less by 5%. The protein content in both types of herring is approximately the same (Table 1).

Table 1. Total chemical composition of the studied herring fillets

Characteristics	Atlantic		Pacific	
	Winter catch	USFDA* data	Winter catch	USFDA* data
Protein, %	16.9 ± 0.2	17.7	16.3 ± 0.2	14.0
Fat, %	16.3 ± 0.3	12.5	19.5 ± 0.4	15.0
Moisture, %	68.3 ± 2.0	61.3	63.3 ± 1.8	69.5

$*$ − USFDA − *Food and Drug Administration*is the federal agency of the United States Department of Health and Human Services.

It was found that saturated fatty acids in the studied fillet samples are represented by palmitic (C16:0) and myristic (C14:0) acids, the content of stearic (C18:0) acid is very small (Table 2).

Monounsaturated fatty acids are represented by the ω-9 family: oleic (C18:1), the sum of gadoleic (C20:1(n11) and gondoic (C20: (n9), eruca (cis-13-docosenoic) 22:1(n9) acids.

Polyunsaturated fatty acids are represented by acids of the ω-3 family: docosahexaenoic (C22:6(n3), eicosapentaenoic, stioric acid (C18:4(n3), alpha-linolenic (C18:3(n3), clupadonic (C22:5(n3) acids.

Table 2. Fatty acid composition of lipids for Atlantic and Pacific herring fillet

Designation	Fatty acid composition of herring fillet		USFDA data***	
	Atlantic	Pacific	Atlantic	Pacific
Saturated Fatty Acids (SFAs)				
8:0	- *	-	-	-
10:0	0.24 ± 0.02	0.32 ± 0.03	0.06	-
12:0	-	-	0.15	-
14:0	- *	4.73 ± 0.25	7.04	7.34
16:0	12.07 ± 0.60	12.50 ± 0.65	17.20	16.27
18:0	0.87 ± 0.04	1.13 ± 0.05	1.39	2.07
20:0	0.14 ± 0.01	0.16 ± 0.01	-	-
22:0	0.03 ± 0.01	0.04 ± 0.01	-	-
Monounsaturated fatty acids (MUFAs)				
16:1	4.82 ± 0.25	6.51 ± 0.30	7.91	8.50
18:1n9	8.12 ± 0.40	12.91 ± 0.65	19.28**	23.42**
18:1n11	1.07 ± 0.05	3.12 ± 0.16	-	-
20:1**	10.87 ± 0.50	12.76 ± 0.65	9.17	10.93
22:1n9	16.44 ± 0.80	19.54 ± 0.95	10.68	12.00
Polyunsaturated fatty acids (PUFA)				
18:2n6	1.60 ± 0.08	0.57 ± 0.03	1.65	1.54
18:3n3	1.55 ± 0.08	0.18 ± 0.01	1.31	0.46
18:4n3	4.45 ± 0.20	0.82 ± 0.04	2.72	1.99
20:2n6	0.22 ± 0.01	0.08 ± 0.01	-	-
20:3n3	0.36 ± 0.02	0.20 ± 0.01	-	-
20:4n3	0.70 ± 0.03	0.23 ± 0.01	0.76**	0.77**
20:4n6	0.17 ± 0.01	0.04 ± 0.01	-	-
20:5n3	7.26 ± 0.40	6.51 ± 0.30	9.01	10.93
22:5n3	0.70 ± 0.03	0.53 ± 0.03	0.70	1.38
22:6n3	9.32 ± 0.50	5.25 ± 0.30	10.96	5.54

* – The content of fatty acid is less than 0.1%; ****** – I someresum; ******* – USFDA – *Food and Drug Administration*is thefederal agency of the United States Department of Health and Human Services.

It was proved that polyunsaturated fatty acids have antioxidant properties, preventing premature aging, participate in the synthesis of prostaglandins, remove excess cholesterol in the form of unsaturated esters from the human body, thus contributing to the prevention of cardiovascular diseases, joint inflammation, and vision improvement (Hooper et al., 2019).

A characteristic statistically significant difference between the fatty acid composition of the Atlantic herring fillet sample and the Pacific herring consists in a significantly higher content of docosahexaenoic acid: about 10% and 5–5.5%, respectively. This difference is observed for the studied samples.

Fig. 1 shows the TAG composition for samples of Atlantic and Pacific herring fillets. The number after the letter T denotes the total number of carbon atoms in the acyl chains of TAG. For example, T48 corresponds not only to tripalmitin with three residues with a chain length of 16 carbon atoms, but also to all isomers containing saturated and unsaturated acid residues with a total chain length of 48 carbon atoms - lauryl distearin, oleyl palmityl myristine, etc.
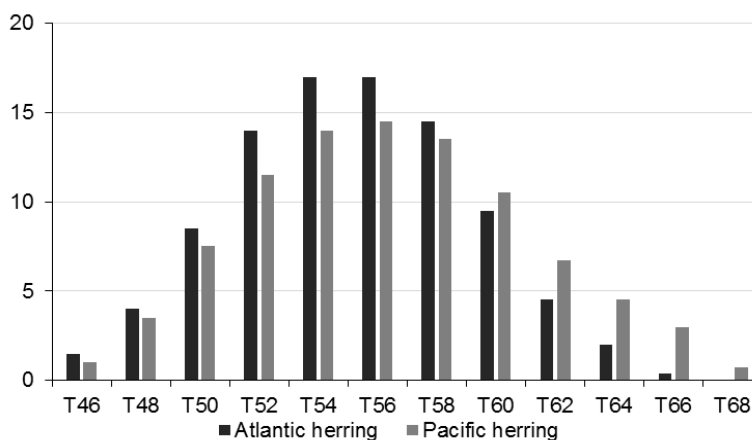
Figure 1. Distribution of triacylglycerols (TAG) in herring samples, %. T is the total number of carbon atoms in acyl chains of TAG.

The fillet samples were found to have different fractional composition of triacylglycerols. For the Atlantic herring fillet sample, the maximum in the T54–T56 region is characterized by a sharper decline to the high molecular weight region, while noticeable amounts of triglycerides with a total length of acyl chains T66 and T68 are present in fat of Pacific herring fillet.

It was revealed that the level of lipid decomposition products (free fatty acids) in herring fillet is 3–5% of neutral fat (Table 3). The total content of free fatty acids (FFA) in the Pacific herring fillet is 1.5 times higher than that in the Atlantic herring fillet.

Table 3. The content of lipid hydrolysis products (FFA) in herring, g kg^{-1}

Herring type	Amount of carbon atoms in FFA						
	C12	C14	C16	C18	C20	C22	Total
Atlantic	0.11 ± 0.01	1.61 ± 0.08	7.09 ± 0.35	5.36 ± 0.30	8.06 ± 0.40	11.31 ± 0.60	33.54
Pacific	0.24 ± 0.01	1.44 ± 0.07	11.80 ± 0.60	8.32 ± 0.40	12.22 ± 0.60	16.25 ± 0.80	50.27

The amino acid composition of the Atlantic herring is presented in Table 4. It was found that the content of indispensable amino acids (AA) in the herring fillet of winter catch is about 5 mg g^{-1} higher than that in the fillet of autumn catch, but their percentage in the total composition of amino acids does not depend on the catch season. The differences between the obtained values and the data provided by USFDA (The federal agency of the United States Department of Health and Human Services) are attributed to environmental factors in the habitat of Russian herring.

Table 5 presents the content of biogenic amines in samples of Atlantic and Pacific herring fillets. It was revealed that reduction and demethylation of TMAO is almost limiting (methylamine predominates, TMA and TMAO are absent).

Table 4. The amino acid composition of the Atlantic herring fillet

Designation by nomenclature	Season of catch				USFDA data	
	Autumn		Winter			
	mg g^{-1}	%	mg g^{-1}	%	mg g^{-1}	%
Asp	13.4 ± 0.7	10.8 ± 0.6	14.2 ± 0.7	10.8 ± 0.6	18.39	11.3
Glu	22.3 ± 1.0	17.9 ± 0.9	24.2 ± 1.1	18.4 ± 0.9	26.81	16.5
Ser	5.2 ± 0.3	4.1 ± 0.2	4.8 ± 0.3	3.6 ± 0.2	7.33	4.5
His	3.0 ± 0.2	2.4 ± 0.1	4.1 ± 0.2	3.1 ± 0.2	5.29	3.2
Gly	6.7 ± 0.3	5.4 ± 0.3	7.8 ± 0.4	6.0 ± 0.3	8.62	5.3
Thr*	6.4 ± 0.3	5.1 ± 0.3	6.1 ± 0.3	4.6 ± 0.2	7.87	4.8
Ala	9.0 ± 0.5	7.2 ± 0.3	9.6 ± 0.5	7.3 ± 0.3	10.90	6.7
Arg	8.3 ± 0.4	6.7 ± 0.3	8.4 ± 0.4	6.4 ± 0.3	10.80	6.6
Tyr*	3.8 ± 0.2	3.1 ± 0.2	4.6 ± 0.2	3.5 ± 0.2	6.06	3.7
Val*	7.3 ± 0.4	5.8 ± 0.3	7.0 ± 0.4	5.3 ± 0.3	9.25	5.7
Met*	4.4 ± 0.2	3.5 ± 0.2	4.4 ± 0.2	3.4 ± 0.2	5.32	3.3
Phe*	4.9 ± 0.3	3.9 ± 0.2	5.5 ± 0.3	4.2 ± 0.2	7.01	4.3
Ile*	5.5 ± 0.3	4.4 ± 0.2	7.3 ± 0.4	5.6 ± 0.3	8.28	5.1
Leu*	11.0 ± 0.6	8.9 ± 0.5	12.2 ± 0.6	9.3 ± 0.5	14.60	9.0
Lys*	13.1 ± 0.7	10.5 ± 0.5	14.1 ± 0.7	10.7 ± 0.6	16.50	10.1
The sum of indispensable amino acids	56.4	45.2	61.2	46.6	74.9	46.0

* – indispensable amino acids.

Thus, we can conclude that, despite the microbiological and organoleptic characteristics of frozen herring fillet meet the regulatory requirements, the studied samples of Atlantic and Pacific herring fillets contain a significant amount of hydrolytic decomposition products of proteins and lipids.

The Atlantic and Pacific herring fillet salting was carried out for 5 days at a temperature of $0 \pm 2\,°C$. Salt and moisture content in the herring fillet and brine were measured with a frequency of 0.5 days.

Table 5. The content of free amines in extracts of herring fillet samples, g kg^{-1} (relative inaccuracy is 10%)

Herring fillet	Methylamine	Dimethylamine	Trimethylamineoxide	Trimethylamine
Atlantic	2.59	0.12	Less than 0.01	Less than 0.01
Pacific	1.56	1.41	Less than 0.01	Less than 0.01

Salted semi-finished product of the Atlantic herring with a salt content of 4% and Pacific herring with a salt content of 4.5% are used for preparation of preserves. It was found that the salting duration of Atlantic herring fillets to the required salt content in fish is about 72 hours, and the duration of salting of Pacific herring fillets is about 120 hours.

It was revealed that the moisture-holding capacity of herring fillets during salting increases, which is explained by salt action on the muscle proteins of fish. The total percentage of bound moisture in slightly salted herring fillets increases by 3–4% by weight (Ozerova et al., 2017).

The studies of herring fillet buffering shows an increase in this indicator during salting to 70 degrees.

For the preparation of preserves, filling mixtures containing gelling components based on apple (0.85–1.40%) and citrus (0.87–1.33%) pectin, apple (3.0–3.5%) and wine (3.0–3.47%) vinegar with sugar and flavoring compositions from dry spices were developed.

Table 6 presents the nutritional and energy value of preserves from herring fillet in jelly. It was found that 100 g of 'Spicy herring in jelly' preserves allows one to fully cover the daily needs for polyunsaturated fatty acids of the omega-3 group.

Table 6. Nutritional and energy value for 100 g of 'Spicy herring in jelly' preserves

Protein, g	Fat, g	Carbohydrates, g	Calorific value, kcal
9.5	5.0	4.5	100
PUFAs content			
Name	g $100g^{-1}$	% of daily needs per day	
ω 3	1.05	105	
ω 6	0.07	2.75	

Table 7 presents the results of study of sanitary-indicative microflora of experimental and control samples of fish preserves 'Spicy herring in jelly' before and after refrigerated storage.

The results were compared with the standards established by the Commission Regulation (EC) on microbiological criteria for foodstuffs (2005). It was found that samples with addition of bio-protective cultures had a safe level of microbiological parameters, in contrast to control samples without addition of bio-protective cultures.

Table 7. Microbiological indicators of preserves 'Spicy herring in jelly' during storage at 4 ± 2 ºC for 25 days

Indicator	Control sample of the 'Spicy herring in jelly'		Experimental sample of the 'Spicy herring in jelly'		Standards
	Before storage	After storage	Before storage	After storage	
APC kfu·g^{-1}	1.0×10^2	1.2×10^3	1.0×10^2	2.0×10^2	1.0×10^5
Escherichia coli in 0.001 g	Not detected	Not detected	Not detected	Not detected	Are not allowed
Staphylococcus aureus in 0.01g	Not detected	Not detected	Not detected	Not detected	Are not allowed
Bacteria of the *Clostridium* genus in 0.01 g	Not detected	Not detected	Not detected	Not detected	Are not allowed
Mould, CFU·g^{-1}	Less than 10	10	Less than 10	Less than 10	Not more than 10
Yeasts, CFU·g^{-1}	Less than 10	11	Less than 10	Less than 10	Not more than 100

The microflora of preserves is represented by a significant number of rod-shaped bacterial cells, cocciform and diplobacteria are less observed (Ozerova et al., 2018). After 25 days of storage, a slight stratification of herring meat was observed in the control sample of preserves. One to two rods and diplobacteria were present in smears

from muscle deep layers within the field of view, and decomposed fibers of muscle tissue were visible on the glass slide. Extraneous microflora was absent in the experimental samples of preserves.

It was found that addition of *Lactobacillus sakei* to the fillings helps to soften the fish, which is associated with the acceleration of its maturation, which is confirmed by data shown in Fig. 2.
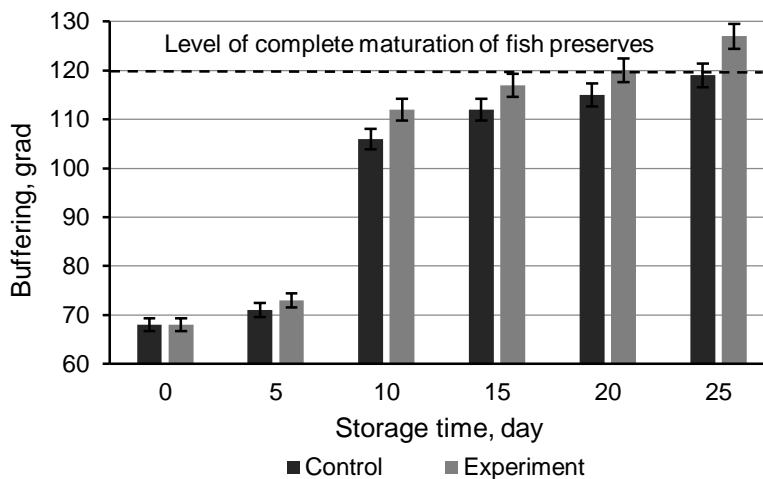
Figure 2. The influence of bio-protective cultures on maturation of 'Spicy herring in jelly' preserves during refrigerated storage. 4 ± 2 °C, 25 days. The storage relevance between the control and experimental samples are statistically significant ($p < 0.05$).

It was also established that the sanitary-indicative microflora of 'Spicy herring in jelly' preserves during the entire storage period met the requirements of the Regulation standards.

It was found that the shelf life of preserves at a storage temperature of 4 ± 2 °C is 20 days taking into account the reserve ratio.

CONCLUSIONS

The studies of biochemical composition of the frozen fillet of Pacific and Atlantic herring showed that the fat content in the fillet during the winter period of catch in the Pacific herring is 3.2% higher than that of the Atlantic; the moisture content is 5% less, and the protein content is practically the same.

The PUFA content in the Atlantic herring fillet is 26.3%, and in the Pacific herring fillet it is 33.4% of the total fatty acid composition. Omega-3 fatty acids are represented by eicosatrienic (20:3n3), eicosapentanoic (20:5n3), docosa-pentaenoic (22: 5n3) and docosahexaenoic (22: 6n3) acids, and omega-6-fatty acids are linoleic (18:2n6), eicosadiene (20:2n6) and arachidonic (20:4n6) acids. A statistically significant difference between the fatty acid composition of the Atlantic herring fillet sample and the Pacific herring was revealed, which consists in a significantly higher content of docosahexaenoic acid: about 10% versus 5–5.5%, respectively.

It was established that the total content of PUFAs does not depend on the herring catch season and is somewhat different from the Food and Drug Administration data, which states for the influence of the herring habitat region on the ratio of fatty acids in herring lipids.

The difference between the samples of Atlantic and Pacific herring fillets by the fractional composition of TAG was established. For Atlantic *C. harengus* herring samples, the maximum in the T54–T56 region is characterized by a sharper decline to the high molecular weight region, while noticeable amounts of triglycerides with total acyl chain length of T66 and T68 are present in the Pacific herring fat.

The total content of free fatty acids in the Pacific herring fillet is 1.5 times higher than that in the Atlantic herring fillet, which indicates a more intensive process of lipid hydrolytic decomposition.

The studies of biogenic amines in frozen herring fillet showed almost complete reduction and demethylation of trimethylamino oxide, which is proved by predominance of methylamine.

The study of quality indicators of 'Spicy herring in jelly' preserves during refrigerated storage for 25 days revealed a slight decrease of solids content in fillings (by 0.4%). The indicator of buffering of preserves during storage increased to 127 grad, which indicates the completion of the ripening process of preserves. It was found that addition of bio-protective cultures containing the *Lactobacillus sakei* strain contribute to the acceleration of maturation of preserves and suppression of the process of microbiological damage during refrigerated storage.

Recipes and technology of cold appetizers from herring fillet in jelly preserves were introduced at 'Baltic coast' JSC (St. Petersburg).

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Analysis of safety indicators for poultry products produced in subsidiary farms in penitentiary facilities

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Abstract. The production of poultry, eggs and their processed products is growing rapidly worldwide. Poultry products are in great demand; however, they are a source of a number of risks: physical, chemical, as well as microbiological. Ensuring food safety is currently the main aspect for the global food industry, including for the production of poultry products. The article discusses the safety and quality of poultry products produced in subsidiary farms of correctional facilities of the penal system. Attention is drawn to the risks and threats of sanitary and epidemiological well-being that arise in connection with the intensification of the impact of negative environmental factors. In the subsidiary farms of correctional facilities, the cage-type bird keeping system and the frequency of feeding are regulated depending on the age of the bird. The results of the analysis have shown that poultry and chicken eggs comply with established sanitary standards. Thus, the average heavy metal content in poultry meat was: Hg - 0.0061 mg kg^{-1}, Cd - 0.0233 mg kg^{-1}, As - 0.0501 mg kg^{-1}, Pb - 0.1765 mg kg^{-1}; in the egg: Hg - 0.0072 mg kg^{-1}, Cd - 0.0198 mg kg^{-1}, As - 0.0485 mg kg^{-1}, Pb - 0.1812 mg kg^{-1}. Antibiotics, radionuclides and dioxins were not found in poultry meat. The pesticide content in poultry meat was below the permissible level: by 2.2 times of DTM and its metabolites, and hexachlorocyclohexane (a, b, g - isomers) - by 5 times. Coliforms, Staphylococcus aureus, Proteus, as well as pathogenic salmonella, listeria were not found in poultry meat and eggs. NMAFAnM in meat amounted to 0.5×10^4, in the egg - 1×10^3 CFU g^{-1} incl. In general, poultry products produced in correctional facilities are of high enough quality and can be used to prepare various dishes not only for adults but also for baby food. These estimates allow us to conclude about a fairly high level of quality and safety of this product.

Key words: safety, poultry products, quality, ecotoxicants, penal system.

INTRODUCTION

Food provision is of great importance for the normal functioning of all facilities and bodies of the penal correction system (Moshnenko, 2017). An important role in the implementation of the food self-sufficiency program is played by subsidiary farms in correctional facilities.

The problem of food safety does not lose its relevance at all stages of the development of the state and society, since it is one of the main factors determining the life and health of people. Food safety means not only the absence of danger to human

health when consumed, but also the absence of danger of long-term consequences (carcinogenic, mutagenic and teratogenic effects) (Gul et al., 2016).

The physical health of the population, its labor activity and, ultimately, the pace of economic development of the country directly depend on the quality of food products.

Food products must satisfy the physiological needs of a person in necessary substances and energy, meet the requirements for organoleptic and physicochemical quality indicators, meet the requirements established by regulatory documents for the content of chemical, radioactive, biological substances and their compounds, microorganisms that pose a danger to the health of the present and future generations (Goldfein et al., 2015).

At the present stage of development, the quality and safety of food products are the main factors determining their competitiveness in the market. These indicators are becoming increasingly significant, leaving criteria such as the price of the product and the range of its traditional consumption far behind (Dabakhov et al., 2005).

Over the past decade, there has been a steady increase in poultry meat consumption (Glamoclija et al., 2013). In the implementation of the state policy in the field of healthy nutrition, it is important not only to increase the volume of production of this product, but also to ensure its safety. Poultry farming is the most science-intensive and dynamic industry, which makes a significant contribution to the provision of food security in the penal system. In addition, the expansion of this product area can serve as an impetus for the development of production activities in correctional facilities.

One of the modern directions of intensification of the productivity of poultry meat and egg is the use of modern methods of feeding and keeping poultry (Petracci & Berri, 2017).

The composition of the feed should include all the necessary components and meet the needs of the body of the bird, the effectiveness of meat poultry farming depends on this to a greater extent. However, in connection with the permanently arising environmental problems, fodder used for feeding poultry and, as a result, poultry products are contaminated (Gulieva et al., 2018).

In this regard, the purpose of this article was to study the safety indicators of poultry products produced in subsidiary farms of correctional facilities of the penal system.

MATERIALS AND METHODS

Poultry keeping conditions are an important link in obtaining quality products (Damaziak et al., 2017; Matt et al., 2019). Cage-type bird keeping system. It was established that the content of chickens in the cages is the most rational and effective, as applied to the subsidiary farms of correctional facilities. Its advantage is to increase the useful coefficient of the used area - it is possible to place 2–3 times more chickens on one square meter than with outdoor keeping.

With this type of keeping, it is easy to care for poultry, give food and water, remove litter. Nesting material is not necessary. In addition, the placement of birds in cages creates good veterinary and hygienic conditions, as there is no contact of the bird with the litter. Therefore, chickens are less likely to suffer from invasive and fungal diseases. A plus is the reduction in bird energy consumption for movement. Due to this, feed costs for a production of one unit of egg or meat products are reduced by 10–15%.

The feeding regime was strictly observed during growing poultry for meat and egg. Timely feeding was carried out with a certain frequency, with the aim of forming a strict rhythm of physiological processes and developing conditioned reflexes, which enhance the internal secretion of the digestive organs, that in turn leads to better digestibility of the feed and, as a result, increase the productivity of chickens.

The frequency of feeding was regulated depending on the age of the bird. So, in the first days of life, chickens were given food 5–6 times a day, at the age of one to four weeks - 3–4 times. Adult chickens were fed 2 times a day in the morning and in the evening. The amount of feed is determined taking into account the age and type of productivity of the bird.

An approximate diet for feeding poultry of meat and egg breeds is presented in Table 1.

Table 1. Sample rations for chicken of meat and egg breeds with the combined type of feeding, g per head per day

No.	Type of feed	Winter	Spring	Summer	Autumn
1	Whole grain (2 types)	50g	45g	40g	45g
2	Crushed grain (2–3 types)	50g	55g	60g	55g
3	Seed cake, oil cakes	12g	13g	12g	12g
4	Wheat bran	10g	10g	10g	10g
5	Boiled potatoes	50g	40g	20g	20g
6	Feeding yeast	3g	4g	3g	3g
7	Silage or carrot	40g	20g	-	20g
8	Herbal flour or fresh herbs	10g	10g	50g	30g
9	Meat-bone and fish meal	5g	7g	5g	5g
10	Fresh skimmilk	20g	30g	30g	20g
11	Shell, chalk	4g	5g	4,5g	4g
12	Bone flour	1g	1.5g	1.5g	1g
13	Common salt	0.5g	0.5g	0.5g	0.5g

The research material was samples of chicken meat and eggs, received for testing for microbiological safety indicators in accordance with TRCU 021/11 year, TPCU 034/13 year.

According to the requirements of normative and technical documentation, three carcasses were selected from each batch of birds by random sampling, as the weight of the carcasses was more than 900 g.

To test the safety indicators of chicken eggs from a batch of eggs, which amounted to 3,000 pcs., 300 pieces of eggs were selected, that was 10% of the total volume of the consignment. Packaging units were selected from different places of the batch (top, middle, bottom). Damaged packaging units were not included in the sample. Eggs in damaged packaging units were 100% sorted. To determine each safety indicator, samples of poultry muscle tissue were taken in the amount of 200 grams, and chicken eggs - 12 pieces.

The determination of heavy metals
The determination of lead and cadmium was carried out by atomic absorption spectrometry method, which is based on the mineralization of the product by dry or wet

ashing and determination of the concentration of an element in a mineralized solution by flame atomic absorption.

The determination of arsenic was carried out by the inversion-voltammetric method. The method of inversion-voltammetric measurements is based on the ability of elements to electrochemically precipitate on the indicator electrode from the analyzed solution at a given potential of the limiting diffusion current, and then dissolve in the process of anode polarization at a specific potential characteristic of each element.

The determination of mercury is based on the 'wet' (acidic) mineralization of samples, the reduction of mercury ions contained in the solution to an elemental state under the influence of a chemical reducing agent, the conversion of mercury into the gas phase, and subsequent quantitative determination by flameless atomic- absorption spectrometry.

The determination of pesticides, Cs-137, dioxins

The pesticide content was determined by gas the chromatography method. The method is based on the extraction of pesticides with organic solvents, purification of the extract, followed by the analysis of the resulting solutions on an automatic gas chromatograph with an electron-capture detector to determine the composition and the mass fraction of pesticides.

Measurement of cesium Cs-137 activity was carried out on radiometric devices of scintillation and semiconductor gamma spectrometers.

The amount of dioxins was determined by high resolution chromatography-mass spectrometry. The method is based on the extraction of analytes with organic solvents, sequential purification of the extract using column chromatography on various sorbents and quantitative analysis by high-resolution chromatography-mass spectrometry using surrogate isotope-labeled standards - analogues of the determined compounds introduced into the sample at the sample preparation stage.

The determination of microbiological indicators

The research was carried out by the microbiological method. The following nutrient media were used for the research: Endo, BSA, XLD, RVS-broth, Kessler's medium, Palcam-agar, Frazier, ISM (Wilson-Blair), Vitelline salt agar physiological saline, meat-and-peptone agar, etc.

Statistical processing of the obtained results was carried out by generally accepted methods (assessment of significance by Fisher and Student criteria) using the Microsoft Excel software package.

RESULTS AND DISCUSSION

The facts of the use of growth stimulants and modern compound feed with directionally modified properties, which lead to the rapid accumulation of heavy metals in poultry carcasses, are known from literature sources (Gayeva et al., 2013). Changed environmental conditions for keeping poultry, new technological methods for the production and storage of feed, the intensive use of veterinary preparations and industrial pollution of the environment by various chemicals significantly increased the risk of contamination of poultry meat and poultry products by heavy metals.

The maximum level of concentration of a specific toxic element in animal feed and diets should be considered taking into account the duration of use of contaminated feed products, their processing and storage method. Long-term exposure of heavy carcinogenic elements in small amounts, especially with chronic lack of feed, unbalanced diets for essential nutrients, and keeping animals under poor conditions can also have a devastating effect on the body (Shah et al., 2010).

Of the food safety indicators, the parameters of the content of heavy metals, pesticides, and radionuclides in the muscle tissues of birds are of the greatest importance to human health. These substances, being in meat products, cause food toxicosis, have a carcinogenic and mutagenic effect, and also worsen the technological properties of animal raw materials, making it difficult or impossible to prepare high-quality food products (Oforka et al., 2014).

The most dangerous toxicants to human health are heavy metals. They are classified as thiol poisons that block sulfhydryl groups of proteins and disrupt metabolic processes in the body (at low doses), in large doses they can act as blockers and other functionally active groups of proteins - amine, carboxylic, etc. When the ecotoxicants enter the body, they are unevenly distributed in it. The main organs of excretion (kidneys, liver, lungs, etc.) take the first fall on themselves. If they enter the liver, they can undergo various changes, even with a favorable outcome for the body that contributes to their inactivation and excretion through the intestines and kidneys. If these mechanisms no longer work, the accumulation of heavy metals occurs in the animal's body (Bokoye et al., 2011).

The results of laboratory research have shown that the lead content in chicken meat does not exceed the maximum permissible values. The concentration of this element in the muscle tissue of birds ranges from 0.0788 to 0.3887 mg kg^{-1}, which is safe for humans. In poultry meat grown in poultry farms not related to the facilities of the penal correction system, an insignificant (2%), but an excess of the MAC content of arsenic is recorded. The arsenic concentration in the test samples did not exceed the maximum permissible concentrations (0.1 mg kg^{-1}), and amounted to 0.0118–0.0857 mg kg^{-1} (Table 2).

Table 2. The results of laboratory analysis of the content of heavy metals and arsenic in the muscle tissue of poultry carcasses

Toxicant	Value	MAC, mg kg^{-1}	Actual content, mg kg^{-1}
Mercury	minimum	0.03	0.0004 ± 0.0001
	maximum		0.0189 ± 0.0053
	average		0.0061 ± 0.0014
Cadmium	minimum	0.05	0.0061 ± 0.0007
	maximum		0.0452 ± 0.0053
	average		0.0233 ± 0.0022
Arsenic	minimum	0.1	0.0118 ± 0.0003
	maximum		0.0857 ± 0.0017
	average		0.0501 ± 0.0069
Lead	minimum	0.5	0.0788 ± 0.0119
	maximum		0.3887 ± 0.0561
	average		0.1765 ± 0.0199
LSD$_{0.95}$			

The note: standard values of maximum allowable concentrations (MAC) are given in accordance with SanR&S 42-123-4089-86 'Maximum allowable concentrations of heavy metals and arsenic in food raw materials and food products'.

The research of the cadmium content in chicken meat has shown that this element varies from 0.0061 to 0.0452 mg kg^{-1} and does not exceed acceptable standards.

The mercury content was within allowable concentrations. The maximum mercury value was 0.0189 mg kg^{-1} with a MAC of 0.03 mg kg^{-1}.

A chicken egg, acting as an equally important poultry product, is a valuable food product and is included in the diet of suspects, accused and convicted persons. The digestibility of egg components is 96–98%.

The chemical composition of a chicken egg depends on the breed, age and feeding of the bird. Not only nutrients and elements that have nutritional value can come as part of this product in the human body, but also a variety of pollutants, the source of which is the bird. As a result, the degree of contamination of the chicken egg with heavy metals is directly related to their quantity in the body of chickens.

In our research, we evaluated chicken eggs by the content of heavy metals in it. The results of the content of heavy metals in the chicken egg are shown in Table 3.

Table 3. The results of laboratory analysis of the content of heavy metals and arsenic in chicken eggs

Toxicant	Value	MAC, mg kg^{-1}	Content, mg kg^{-1}
Mercury	minimum	0.02	0.0003 ± 0.0001
	maximum		0.0179 ± 0.0044
	average		0.0072 ± 0.0018
Cadmium	minimum	0.05	0.0053 ± 0.0006
	maximum		0.0332 ± 0.0054
	average		0.0198 ± 0.0029
Arsenic	minimum	0.1	0.0099 ± 0.0004
	maximum		0.0875 ± 0.0033
	average		0.0485 ± 0.0066
Lead	minimum	0.5	0.0778 ± 0.0119
	maximum		0.3988 ± 0.0569
	average		0.1812 ± 0.0269
LSD$_{0.95}$			

The lead content in the chicken egg did not exceed the maximum allowable concentrations. The maximum value of this element was 0.3988 mg kg^{-1}, its average content was 0.1812 mg kg^{-1}.

The lead content in the chicken egg did not exceed the maximum allowable concentrations. The maximum value of this element was 0.3988 mg kg^{-1}, its average content was 0.1812 mg kg^{-1}.

The quantitative content of arsenic in the studied samples varies widely: from 0.0099 to 0.0875 mg kg^{-1}, which does not exceed the maximum allowable concentration and meets the established requirements.

In poultry farms not related to the facilities of the penitentiary system, there is an excess of the cadmium content in the chicken egg by 1.4–8.2 times over the MAC, and the supply of cadmium in the chicken egg above the maximum level is recorded in 30% of the total number of samples studied. In the samples studied by us, the quantitative content corresponds to the established standards, and its concentration is below the MAC by 1.2–9.4 times.

The research results have shown that the mercury content in the egg did not exceed the MAC of 0.02 mg kg^{-1}. In accordance with safety requirements, hormones and some antibiotics should not be detected in eggs and poultry meat. There are quite stringent rules in relation to these preparations in our country regarding their residual amount, both in raw materials and in finished products.

Currently, special additives are used in the feeding process to stimulate the production of eggs and poultry meat. Therefore, quite often the bird receives giant doses of growth stimulants, significantly exceeding its physiological needs. According to experts, the egg and meat of such a bird cannot be considered safe.

Dioxins are a by-product resulting from some production processes (bleaching of pulp using chlorine, production of some pesticides and herbicides, melting). They get into the environment during the operation of waste incineration plants. Moreover, despite the fact that the release, as a rule, takes place in a certain area, the distribution of dioxins has become widespread - they can be found in almost any environment around the world. Most of them are in the soil, sediment, and food (meat, fish, dairy products).

The test results of meat raw materials in accordance with the requirements of SanR&S 2.3.2.1078-01 show that the meat being tested corresponded to all regulatory requirements in terms of safety.

Cesium, as a radionuclide, was not found in poultry meat. In addition, such highly toxic chemically hazardous substances as antibiotics: bacitracin, chloramphenicol and the tetracycline group were not fixed (see Table 4).

Table 4. The results of laboratory analysis of safety indicators for poultry meat

Name of substance		mg kg^{-1} in poultry	
		Allowable level	Content
Antibiotics	Levomycetin (chloramphenicol)	Not allowed	-
	Tetracycline group	Not allowed	-
	Bacitracin	Not allowed	-
Pesticides	DTM and its metabolites	0.1	0.045
	Hexachlorocyclohexane (a, b, g - isomers)	0.1	0.02
Radionuclides	Cesium-137 Bq kg^{-1} (l)	200	-
Dioxins		Not allowed	-
LSD$_{0.95}$			

The pesticide content in poultry meat was by 2.2 times lower than the allowable level of DTM and its metabolites, and hexachlorocyclohexane (a, b, g - isomers) was by 5 times lower, and amounted to 0.045 mg kg^{-1} and 0.002 mg kg^{-1}, respectively.

An important indicator of product safety and quality is the presence and number of microorganisms (Schaffner & Smith-Simpson, 2014; Baltic et al., 2019). Microbiological indicators of meat and poultry eggs met regulatory requirements (Table 5).

Table 5. Microbiological (CFU g^{-1} incl.) and pathogenic indicators (g (cm^3) incl.) poultry meat and eggs

Test indicators	Standards		Content	
	meat	egg	meat	egg
	Microbiological indicators (CFU g^{-1} incl.)			
NMAFAnM	1×10^5	5×10^3	0.5×10^4	1×10^3
Coliforms	not allowed in 0.0001 g in 0.01^{-1} g		not found	not found
Staphylococcus aureus	not allowed		not found	
Proteus				
	Pathogenic (g (cm^3) incl.)			
Salmonella	in 25 g not allowed		not found	
Listeria				
LSD$_{0.95}$				

The determination of the number of mesophilic aerobic and facultative anaerobic microorganisms (NMAFAnM or total microbial number, TMN) refers to the estimation of the size of the group of sanitary-indicative microorganisms. NMAFAnM contains various taxonomic groups of microorganisms - bacteria, yeast, mold fungi. Their total number indicates the sanitary-hygienic condition of the product, the degree of its dissemination by microflora. For the consumer, the NMAFAnM (TMN) indicator characterizes the quality, freshness and safety of food products.

Based on the results obtained, it is clear that NMAFAnM did not exceed the permissible level. Their content in poultry meat was $5\times10^3\times$CFU g^{-1} incl., in the egg – 1×10^3 CFU g^{-1} incl. No pathogens, such as salmonella and listeria, were found.

CONCLUSIONS

Laboratory research has shown that poultry products produced in facilities of the penal system are environmentally friendly. Such meat and poultry egg can be used to prepare various dishes not only for adults, but also for baby food. All this, in turn, indicates that the poultry production technology adopted by farms with the appropriate set of feeds contributes to the production of high-quality, environmentally friendly products.

Thus, poultry meat and egg produced in the subsidiary farms of penitentiary facilities are safe, and therefore can be highly competitive in the consumer market.

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Rowan powder based acidifying additive acidifying additive - an alternative to sourdough in the rye-wheat bread production

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Abstract. Rye is an important part of the cereal food culture in the Russia and Nordic, Baltic and Eastern European countries. Rye bread is often made of whole-grain flour using long-time sourdough. In Russia, rye bread began to be produced less and less often due to the complexity and duration of its technology. Therefore, the development of fast, natural and safe technologies is relevant. The aim of the research was to develop a nutritional acidifying additive based on plant materials (rowan powder, botanical species *Sorbus aucuparia*) for accelerated rye-wheat bread technology. With an increase in the new plant additive dosage above 3.5%, the dough lifting capacity deteriorated. The bread specific volume and the crumb compressibility deteriorated when additive dosage was higher than 3.5%. It all may be due to the acidity suppression of yeast activity in the dough. Taste and smell was also better in bread with new additive due to the rowan powder chemical composition. The optimum dosage of new additive rowan powder in rye-wheat bread formulation was 3.5% by weight of the flour. Usage of new additive with 0.1% of sodium diacetate allowed slowing down moulding. New acidifying with rowan powder allowed to create accelerated rye-wheat bread technology and to get bread with high consumer properties.

Key words: bread making, plant acidifying additive, sodium diacetate, accelerated bakery technologies.

INTRODUCTION

Rye is one of the most important cereal grains in Russia, Northern and Eastern Europe, where is traditionally used for rye bread (Valjakka et al., 2003; Sahlström & Knutsen, 2010; Poutanen et al., 2014; Juodeikiene, 2016). Rye bread is a good source of dietary fibre phenolic compounds, vitamins, and trace elements and minerals (Nilsson et al., 1997; Nyström, 2008; Rakha, 2010; Wrigley & Bushuk, 2010; Poutanen et al., 2014). Expanding research data about the various components of rye grain beneficial to health have increased the interest in rye bread assortment and technology. Rye bread is often made of whole-grain flour using sourdough technology (Poutanen et al., 2014; Juodeikiene, G., 2016).

In Russia, rye and rye-wheat bread production decreases last years and accounts for only 33% of the total assortment, while bread from wheat flour of different varieties accounts for 58.3% (Seregin & Mosolova, 2011). This is due to the complexity of sourdough bread technology. Traditional rye sourdough bread technologies are very time-consuming. The technological process (including fermentation of the starter and dough before cutting) usually lasts for 5–9 h, which is making the organization of the rye bread production too complicated and long (Kosovan, 2008; Poutanen et al., 2014; Juodeikiene, G. 2016; Stępniewska et al., 2019). Therefore, the development of fast, natural and safe technologies is relevant task (Dubrovskaya et al., 2019).

Baking properties of the rye flour are influenced by the functional properties of the main rye components such as starch, proteins and pentosans. The gelling properties of the starch together with the high α-amylase activity represent a critical factor for the rye baking technology. Starch and pentosans are very important components of rye flour. Pentosans play a main role in developing the dough properties at temperature below 45 °C, while starch has an influence on the structure of crumb when temperature exceeds 45 °C (Puchkova et al., 2005; Banu, 2007; Andersson et al., 2009; Buksa et al., 2010). Rye starch gelatinizes at temperatures of 55–70 °C, at which the activity of α-amylase is at a maximum (Gräber, 1999, Puchkova et al., 2005; Arendt et al., 2007, Rosentrater & Evers, 2018). In order to avoid excessive amylolytic breakdown of the starch, the pH of the dough is lowered for making rye soft bread. PH may be lowered by acid modification in a 'sourdough' process, preferably by lactic acid fermentation with species of *Lactobacillus* (Puchkova et al., 2005; Poutanen et al., 2014; Rosentrater & Evers, 2018), and by acidifying additives usage such as organic acid (Puchkova et al., 2005; Arendt et al., 2007; Gagiu et al., 2017; Gioia et al., 2017; Rosentrater & Evers, 2018. Dubrovskaya et al., 2019). Acidifying additives are more convenient to use, therefore, are widely used in Russia and in Europe.

The disadvantage of acidifying additives usage is the small spectrum of acids in their composition and low content of aromatic substances. Most commonly only one or two organic acid are used as acidifying additives in rye bread making. For example, during sourdough fermentation lactic acid, citric acid, acetic acid, pyruvic acid and succinic acid are produced (Valjakka et al., 2003; Ardent, 2007; Poutanen, 2009). That is why the taste and smell of bread made with acidifying additives is most often not pronounced compared to the sourdough bread, the crumb is a little sticky or crumbles; the color of the crusts is not bright enough. Nutritional value is lower than in bread made with sourdough (Corsetti et al., 2000; Poutanen, 2014; Dubrovskaya et al., 2019).

When accelerated technology and acidifying additives are used, bread quickly undergoes microbial spoilage and has short shelf-life. The most pressing problem is moulding and wild yeast development, due to the development of mould and yeast on the surface of the product. Mould - affected bread may contain mycotoxins - substances harmful to the human health (Corsetti et al., 2000; Kurtzman et al., 2010; Dubrovskaya et al., 2019; El Sheikha & Mahmoud, 2019).

The aim of our research was to create a multifunctional plant acidifying additive to increase the organoleptic and physicochemical quality indicators, nutritional value, shelf life and microbiological stability during storage of rye-wheat bread.

MATERIALS AND METHODS

Characteristics of ingredients

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, 2017).

The composition of the developed plant acidifying additive is: powder from the fruits of red-fruited rowan (67.7), dried whey (3.8), fermented rye malt (10.0), enzyme preparation 'Fungamil' (0.5), citric acid (18.0) (Dubrovskaya, 2012).

To enhance the effect of the developed plant acidifying additive with rowan powder (3.5%) and to increase the shelf life of rye-wheat ('Darnitsky') bread, a sodium diacetate in an amount of 0.1–0.3% by weight of the product was used. Its optimal amount was determined by the microbiological parameters of the finished bread and by the duration of the technological process. The control was the acidifying mixture with rowan powder without sodium diacetate.

Bread making procedure

To determine the optimal amount of the developed plant acidifying additive 'Citrasol – 6' in the formulation of rye-wheat bread ('Darnitsky'), test laboratory baking was carried out.

The dough was made from following ingredients (g per 100 g total amount of flour): rye flour (60.0), wheat flour baking first grade (40.0), new acidifying additive (2.5, 3.0, 3.5, 4.0 and 4.5), salt (1.5), and yeast (1.2). Water was added in an amount to ensure the humidity of the dough 48.0–48.5%. 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 60 minutes. Then, dough pieces were shaped into 300 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 baked in an oven SvebaDahlen (Sweden) at the temperature of 210 °C for 18 minutes with steam introduced for 6 seconds. The control was bread made with well-known acidifying additive 'Citrosol', developed by the St. Petersburg branch of State Research Institute of Baking Industry (Kosovan, 2008), which was used in amount of 3 g per 100 g total amount of flour.

Analysis of the biochemical composition of rowan powder

The analysis of the organic acid composition in the rowan powder 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 (State Standard of the Russian Federation, 1996).

Microbial contamination assessment

The number of mesophilic aerobic and facultative anaerobic microorganisms in rowan powder (MAFAM's quantity) was determined according to ICC Standard Method 133, the number of yeast and moulds was determined according to ICC Standard Method 134. The number of *Escherichia coli* group bacteria in 1 gram of the product was determined by plating the product and its dilutions on an agarized selective diagnostic

medium. After incubation at 37 °C, typical and atypical colonies were counted and the ability of bacteria from these colonies to ferment lactose with the formation of gas was determined (Standard of Russian Federation GOST 31747-2012, 2012). *Staphylococcus aureus* was determined according to Russian Standard GOST 31746-2012. Samples of the product and a series of dilutions were inoculated into a selective liquid nutrient medium and incubated at 37 °C for 24–48 h. Then it was inoculated to Petri dishes with Baird-Parker agar. Confirmation of the belonging of typical and atypical colonies to coagulase-positive staphylococci was carried out by studying the ratio of the identified microorganisms to Gram stain, determining the presence of catalase and coagulase in them.

Salmonella genus was determined according to Russian Standard GOST 31659-2012. Bacteria of the genus *Salmonella* may be present in the product in a small amount, along with a large number of other bacteria from the *Enterobacteriacea* family or other families. Therefore, preliminary enrichment was carried out necessary to detect a small number of bacteria of the genus *Salmonella* or sublethally damaged bacteria of the genus *Salmonella*. For this, a weighed mass of 25 g was introduced into buffered peptone water. T hen, they were incubated at a temperature of 37 ± 1 °C for 18 ± 2 h. After the culture, tetrathionate broth was introduced into Mueller-Kaufmann medium and incubated at a temperature of 37 ± 1 °C for 24 ± 3 h. Then was inoculated on xylose-lysine-deoxycholate agar in Petri dishes and incubated at a temperature of 37 ± 1 °C for 24 ± 3 h. Colonies presumably related to bacteria of the genus *Salmonella* obtained on Petri dishes were identified using biochemical tests.

The determination of spore-forming bacteria amount in the flour was carried out by plating a heated sample on meat-peptone agar. For this, 10 g of flour was mixed with 100 cm^3 of sterile water. The thoroughly homogenized mixture was heated in a water bath for 10 minutes at a temperature of 90–94 °C in order to inactivate all vegetative cells. Then a series of ten-fold dilutions were made from the heated suspension. 1 cm^3 of obtained dilutions was inoculated into sterile Petri dishes on meat-peptone agar, which was previously melted and cooled to 40 ± 1 °C and cultivated in a thermostat at 37 ± 1 °C. Grown colonies were counted (Blackburn K. de V, 2008).

The dough assessment

Mass proportion of moisture of the new plant acidifying additive was determined by drying at a temperature of 130 °C during 40 minutes in drier (SHS-1M, Russia). The dough lifting capacity was determined by the rate of floating up of the 10 g of dough with humidity of 45%, shaped in the ball, in a glass of water at a temperature of 32 °C (Puchkova, 2004). 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 M solution of NaOH (Puchkova, 2004).

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 and 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 principle 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 sample 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 the 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: sensory parameters – (shape, surface, crumb colour, condition of crumb (porosity and texture), taste and smell; physic-chemical and physical parameters – moisture was determined by drying at a temperature of 130 °C during 45 minutes in a drier SHS-1M, Russia), acidity was determined by titration, using a 0.1 M solution of NaOH (State Standard of the Russian Federation GOST 5670–96, 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 (Skurikhin & Tutelyan, 2002).

Determination of water-soluble antioxidants content in bread was conducted by amperometric method using device 'Color Yauza-01-AA.' (Russian) according to Russian Standard (State Standard of the Russian Federation, 2010).

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 colour 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 rowan powder on moulding of bread was investigated. Sterile bread slices were contaminated by a pure culture of the mould *Penicillium chrysogenum*. *Penicillium chrysogenum* was used in this study because this type of mould often infects bread (Blackburn, 2008). This strain was isolated from mouldy bread. It was identified and is used as a typical strain of *Penicillium chrysogenum*. Immediately after baking, 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 on sterile Petri dishes. An aqueous suspension of a pure culture of the mould, *Penicillium chrysogenum*, was prepared for the inoculation of bread slices. The biomaterial of *Penicillium chrysogenum* was transferred from a tube

containing a pure culture of mould grown on malt agar to 1 cm^3 of sterile water using 'Tween-80' and 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 of a growth of mould colonies appeared (Dubrovskaya, 2018).

Statistical analysis of the data

When analysing the results of experiments, standard approaches of probability theory and mathematical statistics were used: Duncan's test of two-factor analysis of variance with one repetition (ANOVA), 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

Rowan powder has high titratable acidity (40 degrees or 5.7% in terms of malic acid). That is why the content of different organic acids was established in the rowan powder (Table 1). It was found that rowan powder contained a large amount of organic acids including fatty and phenolic acids. Quinic (2002.68 mg 100 g^{-1} dried weight) and chlorogenic (11.5 mg 100 g^{-1} of dried weight) acids were represented in the greatest amount among the phenolic acids. Organic acids in rowan powder did not match the acids in the sourdough (Arendt et al., 2007; Poutanen, 2009), but it is important that they were represented by a wide range. This allows suggesting the effectiveness of the use of rowan powder as an acidifying additive and as smell and taste improver.

Table 1. Organic acid content in rowan powder

Acid	mg 100 g^{-1} of dried weight
malic	2,941.13 ± 146.15
succinic	66.01 ± 3.33
lactic	12.40 ± 1.10
sorbic	27.83 ± 1.96
2-hydroxypropionic	759.62 ± 36.95
3- hydroxypropionic	4.89 ± 0.26
phosphoric	63.64 ± 3.43
glyceric	29.20 ± 1.46
saccharic	0.16 ± 0.01
malonic	28.87 ± 2.01

Table 2. Microbial indicators

Indicators	Rowan powder	Requirements of TR CU 021/2011
MAFAM, CFU·g^{-1}	(1.2 ± 0.1) ·10^2	≤ 5·10^3
Moulds, CFU·g^{-1}	-	≤ 1·10^2
		The mass of the product (g) in which it is not allowed:
Escherichia coli group	-	1 g
Staphylococcus aureus	-	1 g
Pathogenic, including *Salmonella* genus	-	25 g

The microbiological safety of rowan powder was investigated for compliance with the requirements of the Technical Regulation of the Customs Union 'Food Safety' TR

CU 021/2011. Microbiological indicators of rowan powder fully complied with the requirements of TR CU 021/2011 (Table 2). Therefore, the powder can be safely used in the formulation of the acidifying additive and for bread making.

When the new acidifying additive was used, the acidity of the dough and bread increased (Table 3). This can be due to the high acidity of the rowan powder. At the same time, the dough lifting capacity also improved with increasing additives to 3.5%. This may be due to the fact that the rowan powder contained vitamins and minerals necessary for the nutrition of yeast (Kurtzman et al., 2011; Verheyen et al., 2015), while the acidity is not so high as to suppress yeast activity. With an increase in the dosage of the additive to 4.0 and 4.5%, the dough lifting capacity deteriorated compared to bread with a 3.5% of additive. This can be due to an increase in acidity which inhibits yeast (Zhou et al., 2014).

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

Indicators	Control	Bread with new additive (g on 100 g of flour)				
		2.5	3.0	3.5	4.0	4.5
Dough:						
Acidity, degrees	9.4 ± 0.1[a]	9.0 ± 0.1[b]	10.0 ± 0.1[b]	11.3 ± 0.2[c]	12.3 ± 0.1[d]	12.6 ± 0.1[f]
Lifting capacity, min.	5.0 ± 0.1[a]	5.0 ± 0.1[a]	4.0 ± 0.1[b]	3.0 ± 0.1[c]	4.0 ± 0.1[b]	4.0 ± 0.1[b]
Bread:						
Acidity, degrees	6.0 ± 0.1[a]	5.9 ± 0.1[a]	6.4 ± 0.1[b]	7.2 ± 0.1[c]	7.8 ± 0.1[d]	8.2 ± 0.1[f]
Porosity, %	66.0 ± 0.2[a]	66.0 ± 0.2[a]	66.0 ± 0.2[a]	67.0 ± 0.2[a]	67.0 ± 0.2[a]	66.0 ± 0.2[a]
Specific volume, cm^3 g^{-1}	1.89 ± 0.01[a]	2.03 ± 0.02[b]	1.9 ± 0.02[c]	2.13 ± 0.02[d]	1.98 ± 0.01[f]	1.89 ± 0.02[a]
Compressibility, U	26.0 ± 0.3[a]	26.0 ± 0.1[a]	28.0 ± 0.3[b]	28.0 ± 0.1[b]	27.0 ± 0.2[c]	26.0 ± 0.1[a]

* a–f = Means ± SD within the same line with different lowercase superscript letters denote significantly different among dough types ($p \leq 0.05$).

The bread specific volume and the crumb compressibility increased when the additive dosage increased to 3.5%. With a further increase of the additive dosage to 4.0 and 4.5%, the bread specific volume decreased. This can be associated with a slowdown in yeast activity in the dough due to high acidity (Zhou et al., 2014). Another reason may be sorbic acid presented in the rowan powder. Sorbic acid has detrimental effects on dough, bread and yeast-raised goods characteristics. The baked products may have reduced volume and an irregular cell structure (Gioia et al., 2017). With an increase in dosage of rowan powder, the content of sorbic acid in the dough also increased. This could affect the decreasing of specific volume.

Test baking results clearly demonstrated that optimal amount of new acidifying additive was 3.5% by weight of flour (Table 3). It allowed making bread with intensively dark brown colored crust, developed with thin-walled uniform porosity, not crumbly, with a pronounced harmonious taste and smell (Table 4). A further increase in the plant acidifying additive dosage was impractical because organoleptic and physico-chemical indicators worsen.

Since rowan powder contains organic acids having a preservative effect, the effect of new acidifying additives with rowan powder on the bread microbiological spoilage was investigated.

Yeast and mould were absent on the surface and in the crumb of bread after baking. In the control and in the bread made using 2.5% of the new acidifying additive, $1.1 \cdot 10^3$ and $0.5 \cdot 10^3$ $CFU \cdot g^{-1}$ of spore-forming bacteria were detected, respectively. In samples containing 3.0, 3.5 and 4.0% of the new acidifying additive, spore-forming bacteria were not found. This indicates that the acidifying additive inhibited spore-forming bacteria vital activity. The inhibitory effect was probably caused by the acids in rowan powder. High titratable acidity is known to effectively inhibit *Bacillus* strains (Oscroft et al., 1990; Katina et al., 2002; Lavermicocca, 2016).

Table 4. Bread sensory characteristics

Indicators	Control	Bread with new additive (g on 100 g of flour)				
		2.5	3.0	3.5	4.0	4.5
Crust						
Shape	4.80 ± 0.18^a	4.80 ± 0.13^a	4.81 ± 0.22^a	4.82 ± 0.32^a	4.82 ± 0.32^a	4.82 ± 0.32^a
Surface	4.80 ± 0.18^a	4.80 ± 0.13^a	4.81 ± 0.22^a	4.82 ± 0.32^a	4.82 ± 0.32^a	4.82 ± 0.32^a
Colour	3.20 ± 0.16^a	3.31 ± 0.19^a	3.89 ± 0.04^b	4.90 ± 0.15^c	4.90 ± 0.15^c	4.90 ± 0.15^c
Crumb						
Colour	3.5 ± 0.10^a	3.49 ± 0.38^a	3.64 ± 0.14^a	4.80 ± 0.21^b	4.81 ± 0.27^b	4.62 ± 0.32^b
Odour	2.78 ± 0.18^a	2.82 ± 0.28^a	3.99 ± 0.23^b	4.01 ± 0.06^b	4.00 ± 0.12^b	2.89 ± 0.12^a
Taste	2.75 ± 0.18^a	2.95 ± 0.18^b	3.19 ± 0.19^c	3.19 ± 0.19^c	4.09 ± 0.04^d	4.01 ± 0.04^f
Chewiness	3.19 ± 0.19^a	3.25 ± 0.15^a	3.34 ± 0.29^a	4.01 ± 0.10^b	3.92 ± 0.05^c	3.89 ± 0.14^c
Porosity	4.59 ± 0.23^a	4.63 ± 0.31^a	4.65 ± 0.25^a	4.69 ± 0.38^a	4.60 ± 0.22^a	4.60 ± 0.28^a

* a–f = Means ± SD within the same line with different lowercase superscript letters denote significantly different among dough types ($p \leq 0.05$).

It was found out that in the control bread slices, contaminated by P*enicillium chrysogenum,* the mould colonies growth was observed in 48 ± 2 h, and in samples with 2.5 and 3.0% of the new acidifying additive the mould colonies growth was observed in 56 ± 2 and 60 ± 2 h, respectively. On slices of bread made with 3.5 and 4.0% of acidifying additives mould growth slowed by a 24 h compared to the control. The usage of the rowan powder allowed slowing down the rye-wheat bread mould disease. However, this result is not successful enough. Obviously, organic acids content in new additive are not enough to stop moulding (Sadeghi Mahounack & Shahidi, 2001; Gioia et al., 2017).

Therefore, to enhance the effect of the plant acidifying additive with rowan powder and to increase bread shelf life, sodium diacetate was used in an amount of 0.1–0.3% by weight of the product (Sadeghi Mahounack & Shahidi, 2001). Its optimal amount was determined not only by the microbiological parameters of the bread, but also by the duration of the technological process, because when sodium diacetate was used the shelf life was extended. The control was prepared with a plant acidifying additive with rowan powder without sodium diacetate (Table 5).

It was established (Table 5) that with the addition of 0.1% sodium diacetate, the proofing time increased in 1.3 times compared with the control. The *Penicillium chrysogenum* mould colonies growth on bread slice with new acidifying additive containing 0.1% sodium diacetate was a day later than on the control. With the addition of 0.2 and 0.3% sodium diacetate, the proofing time increased by 1.9 and 3.9 times, respectively. Mould colonies in these samples were not detected during the entire storage period (7 days). The inhibitory effect in this case was probably due to the sodum diacetat action (Sadeghi Mahounack & Shahidi, 2001).

Therefore, it is recommended to use 0.1% sodium diacetate. With this concentration, the proofing time increased slightly, but the rate of mould development slowed down significantly.

Table 5. The effect of sodium diacetate in the new plant acidifying additive on the quality of the dough and bread

Indicators	Sodium diacetate quantity in new acidifying additive, %			
	0	0.1	0.2	0.3
Dough:				
Acidity, degrees N	9.5 ± 0.1^a	10.5 ± 0.1^b	10.9 ± 0.1^c	11.5 ± 0.1^d
Lifting capacity, min.	8.0 ± 0.1^a	9.0 ± 0.1^b	14.0 ± 0.2^c	$35.0 \pm 0.2d$
Duration of proofing, min	35.0 ± 0.4^a	45.0 ± 0.7^b	66.0 ± 0.5^c	138.0 ± 0.1^d
Acidity, degrees N	6.4 ± 0.1^a	7.2 ± 0.1^b	7.6 ± 0.1^c	8.0 ± 0.1^d
Porosity, %	66.0 ± 0.1^a	67.0 ± 0.1^b	69.0 ± 0.1^c	66.0 ± 0.2^a
Specific volume, $cm^3 \, g^{-1}$	1.9 ± 0.1^a	1.9 ± 0.1^a	2.0 ± 0.1^a	1.9 ± 0.1^a
Volatile acids, % of the total acidity	6.25 ± 0.03^a	10.10 ± 0.13^b	13.80 ± 0.02^c	16.90 ± 0.05^d
Quantity of alcohol, % of dried weight	0.50 ± 0.01^a	0.51 ± 0.01^a	0.64 ± 0.01^b	0.42 ± 0.01^c
The storage time before *Penicillium chrysogenum* growth, h	96 ± 1^a	120 ± 1^b	> 7 days	> 7 days

* a–f = Means ± SD within the same line with different lowercase superscript letters denote significantly different among dough types ($p \leq 0.05$).

The bread made with diacetate had the better compressibility then the control bread throughout the entire storage period (Fig. 1). The results confirmed the data obtained by Sadeghi Mahounack & Shahidi (2001), that sodium diacetate allows to inhibit bread staling.

Swelling decreased during storage (Fig. 2). The swelling capacity of the samples with rowan powder was higher than that of the control bread during the storage period. This is probably due to a decrease in the ability of colloidal substances in control bread to absorb water by compacting the structure of starch and proteins during bread aging. Besides the rowan powder has high fiber content with high swelling index. Staling of bread with rowan powder was slower, and it was confirmed by measuring of the compressibility (Fig. 2).
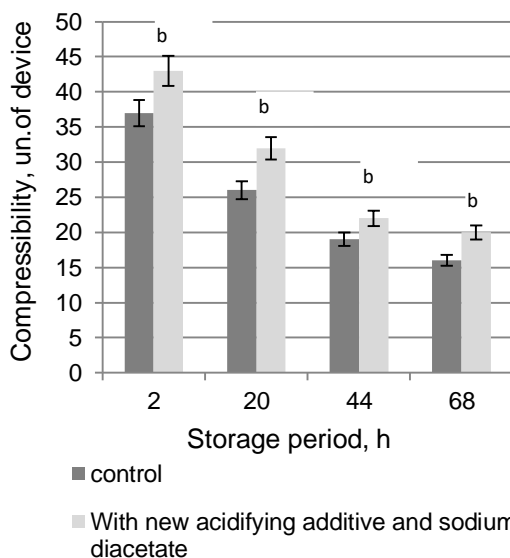

Figure 1. Crush compressibility during bread storage*.

* 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.

The effect of the new acidifying additive with rowan powder (3.5%) on the nutritional value of rye-wheat bread is shown in Table 6.

The addition of rowan powder in the composition of a new plant acidifying additive showed a positive effect on the content of dietary fiber. Its content in relation to control increased by 10%. Therefore, due to the use of rowan powder, we can observe an increase in the daily requirements for these substances for adults.

Rowan powder in the composition of new plant acidifying additive had a positive effect on the content of vitamins and minerals. Vitamins A, E, ascorbic acid and mineral elements (manganese and selenium) were found in the experimental samples, while their

Figure 3. Change in the swelling of the crumb during the storage of bread.

content in the control bread was below the detection limit. Rowan powder had the significant effect on the enrichment of rye - wheat bread with vitamin B2 and iron.
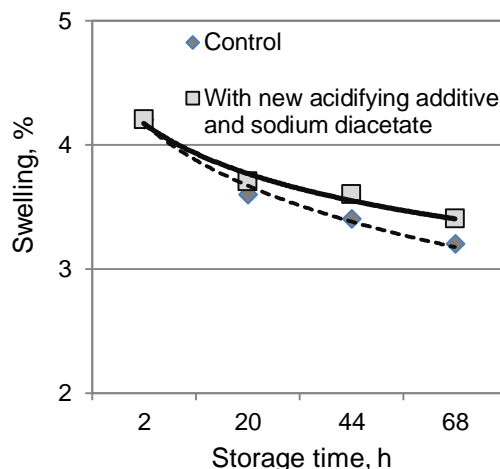
Table 6. Satisfaction of daily requirements or nutrients

| Substance | Consumption norm | Requirements, % | | t* |
		Control	Bread with 3.5% of new additive	
Proteins, g day^{-1}	75	8.1 ± 0.1	8.3 ± 0.1	3.34[a]
Fats, g day^{-1}	83	1.2 ± 0.02	1.2 ± 0.1	0.53
Digestible carbohydrates, g day^{-1}	365	11.8 ± 0.02	11.9 ± 0.2	4.05[a]
Dietary fiber, g day^{-1}	30	19.3 ± 0.1	21.7 ± 0.4	0.46
Vitamins:				
ascorbic acid, mg day^{-1}	90	-	1.0 ± 0.1	
A, mg day^{-1}	900	-	1.1 ± 0.1	
E, mg day^{-1}	15	-	5.5 ± 0.2	
B$_1$, mg day^{-1}	1.5	9.3 ± 0.1	9.3 ± 0.1	
B$_2$, mg day^{-1}	1.8	3.8 ± 0.1	4.0 ± 0.1	2.09
Minerals:				
Sodium, mg day^{-1}	1,300	25.7 ± 0.3	33.6 ± 0.5	19.35
Magnesium mg day^{-1}	400	8.8 ± 0.1	8.9 ± 0.1	0.65
Potassium, mg day^{-1}	2,500	7.3 ± 0.1	7.8 ± 0.2	2.74
Calcium, mg day^{-1}	1,000	2.3 ± 0.06	2.8 ± 0.1	4.55
Iron, mg day^{-1}	14	17.1 ± 0.2	20.7 ± 0.2	16.66
Manganese, mg day^{-1}	2	-	1.8 ± 0.2	
Zinc, mg day^{-1}	12	-	0.083 ± 0.012	

*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).

It is known that the main sources of bio antioxidants for humans are food products based on plant materials. Rowan powder is known to contain antioxidant such as vitamins C and E, selenium and carotenes, etc.

When the total content of water-soluble antioxidants was determined, it was found that in bread prepared used new acidifying additive with rowan powder the content of water-soluble antioxidants was higher than in control by 55.6%.

Thus, studies have shown that the use of a new plant acidifying additive with rowan powder for accelerated technology of rye-wheat bread lead to an improvement in organoleptic and physico-chemical parameters, and antioxidant activity has increased.

CONCLUSIONS

New plant acidifying additive with rowan powder was created to improve the quality, nutritional value and microbiological stability of rye-wheat bread made in accelerated way without sourdough usage. With an increase in the new plant additive dosage above 3.5%, the dough lifting capacity deteriorated due to the suppression of yeast by the acidity. The bread specific volume and the crumb compressibility increased when the additive dosage increased to 3.5% and was worse when additive dosage was higher than 3.5%. This can also be associated with a slowdown in yeast activity in the dough due to high acidity. Taste and smell was also better in bread with new additive due to the rowan powder chemical composition. The optimum dosage of new additive rowan powder in rye-wheat bread formulation was 3.5% by weight of the flour. The bread with 3.5% of new additive had an intensely dark brown crust, developed thin-walled uniform porosity, non-wrinkling crumb, pronounced harmonious taste and smell of new acidifying additive allowed inhibition of spore-forming bacteria vital activity in bread and inhibition the rye-wheat bread mould disease, but it was not satisfactory enough. The sodium diacetate usage in an amount of 0.1% by weight of the product allowed significantly slow down the rate of mould development in bread without deterioration of bread quality. The bread made with diacetate had the better compressibility and swelling capacity then the control bread throughout the entire storage period. Bread with new additive contained more dietary fiber, vitamin B2 and iron. Vitamins A, E, ascorbic acid and mineral elements (manganese and selenium) were also found in bread with new additive, while in the control bread they were below the detection level. New acidifying with rowan powder allowed to create accelerated rye-wheat bread technology and to get bread with high consumer properties.

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Applying beetroot as food ingredient in ice-cream production

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Abstract. The development of new food products with functional ingredients of plant origin is highly promising and relevant direction in food industry. Assortment of products, including ice cream is constantly expanding due to the introduction of various plant ingredients into its composition, enriching the product with health beneficial nutrients. In this study, beetroot (*Beta vulgaris*) was selected as a plant component for ice cream production. The roots of common beets contain many useful inorganic and organic substances, such as carbohydrates, proteins, organic acids, mineral salts, betalaines, vitamins, folic acid and betaine. The influence of different thermal treatment techniques of beetroots on the content of dry substances and organoleptic properties of beetroot puree have been compared and analyzed. The heat exposure technique recommended for beetroots is microwave processing for 9 minutes at a power of 800 watts. This method of heat treatment ensures the culinary readiness of vegetable raw materials while preserving dry substances in it, including betanine. The effect of the beetroot puree dose on the formation of the ice cream quality was studied. The recommended dose of the beetroot puree was established as 20%.

Key words: ice cream, beetroot puree, betanines.

INTRODUCTION

Currently, the food production industry features a trend to produce functional food products, which reduce the risk of diseases and drug loading. The important tasks of food industry science include the development of original technologies for new products that improve the condition of the gastrointestinal tract, support the functioning of the cardiovascular, and immune systems.

The trend of scientific research in this area is clearly traced in meat industry (Bazarnova et al., 2019; Zinina et al., 2019), dairy industry (Zabodalova et al., 2014; Skripleva & Arseneva, 2015; Evstigneeva et al., 2016; Dubrovskii et al., 2019; Hurda et al., 2019), baking industry (Nilova & Malyutenkova, 2018; Dubrovskaja et al., 2019), as well as in cheese making (Iakovchenko & Silantjeva, 2014; Nadtochii et al., 2015; Chechetkina et al., 2016). Designing new food products involves the addition of plant extracts of high nutritional value and dietary fiber (Zinina et al., 2019). To obtain extracts, vegetable raw materials with prominent functional properties are used, for example, chlorella microalgae (Bazarnova et al., 2019), fennel (Dubrovskii et al., 2019), green tea (Evstigneeva et al., 2016). Powders from rowan berries (Dubrovskaja et al., 2019) and sea buckthorn (Nilova & Malyutenkova, 2018) increase the nutritional value of bakery products. Biologically active substances isolated from natural raw materials

are used as functional additives in the preparation of yogurt, for example, curcumin, grape seed oil, hyaluronic acid and chondroitin sulfate (Hurda et al., 2019). β-Carotene, which is included in liposomes to improve technological characteristics, is recommended for dairy formulations (Zabodalova et al., 2014).

Among many food products, ice cream is in great demand. A sweet frozen dessert is made from dairy raw materials with the addition of various ingredients that form the consistency and taste characteristics of the product. Traditional types of ice cream are characterized by high calorie content, due to the high content of sugar and fat, which reduces its physiological value and increases its price. New types of ice cream with therapeutic, prophylactic, functional, and dietary properties and developing technologies are promising (Serova et al., 2016).

Today, there exist types of ice cream that can assist in restoring of the cardiovascular system, slowing the aging process, and reducing the likelihood of inflammatory and oncological diseases (Petash, 2013). For example, an ice cream with low-calorie ingredients and special substances (L-carnitine) was developed for overweight people (Hausmanns & Kovalchuk, 2006). An ice cream recipe is proposed for people suffering from diabetes with amaranth flour (Yakovleva, 2012).

The use of an amino acid complex in ice cream formulas allows to recommend a product to reduce fatigue. The original composition of calcium and magnesium ions with vitamin A in ice cream makes it prophylactic against depression and insomnia (Melnikova et al., 2012).

The types of ice cream have a therapeutic and dietary orientation, when vegetable raw materials are used as fillers. A promising source of plant materials with a unique set of food and biologically active substances are pine nuts kernels and their processed products. Cedar flour as a protein supplement, and cedar oil as a source of essential fatty acids, offer great opportunities for creating dairy products with a given nutritional and biological value (Artyukhova, 2006).

The technology for therapeutic ice cream with stevia extract and lingonberry juice was also developed (Chaika et al., 2009). Ice cream was obtained from goat milk with hawthorn, which had high nutritional, biological value and antioxidant activity (Drevin et al., 2012). There is a method to enrich a frozen dessert with milk and soy proteins, dietary fiber, natural vitamins and minerals (Tikhomirova, 2013).

The formula and technology for the preparation of ice cream with various additives are described: with cereal concentrates (Eremina & Ivanova, 2008), chicory powder and wheat bran (Shambulova et al., 2016), with a microparticulate whey protein and Maxilact, a purified lactase (Stanislavskaya, 2012), with citrus fibers (Gubina, 2013).

The analysis of publications revealed two main trends to obtain new types of ice cream that are more beneficial for health than traditional ones. The first one is the decrease in calorie content, and the second one is the introduction of various functional components in the form of berries, nuts, fruits and vegetables, and the addition of probiotics and prebiotics as well.

Beetroot is a rich source of healthy ingredients for the human body. It contains a significant amount of vitamins and minerals (Prokopets et al., 2014). Beet fiber, interacting with cholesterol, prevents its absorption into the blood (Kurguzova et al., 2012). There are many phenolic compounds in beetroots, mainly free catechins and flavonol glucosides (Sidorenko & Shtonda, 2013). Beetroots are rich in betalaines, which have a lipotropic effect, and are used in the treatment of diseases associated with

impaired fat metabolism (Lechner & Stoner, 2019). Betalaines combine yellow pigments - betaxanthines and pigments with a red and purple color - betacyanins, which are rich in red beets. In addition to their coloring ability, betacianins are wide and diverse biologically active. Their high antioxidant properties contribute to the assimilation of food proteins. Betacianines are actively involved in the formation of choline, which increases the activity of liver cells, and inhibit the proliferation of cancer cells, preventing the onset of malignant tumors (Saenko et al., 2012) The most investigated betacianin is a betanin pigment, which is called the Beetroot Red (E162). There is evidence that betanin is also a hepatoprotector, participating in the neutralization of toxins in the human body (Frank et al., 2005; Lee et al., 2005). Substances responsible for beetroot pigment reduce blood pressure, alleviatespasms and strengthen capillaries; they also inhibit the development of malignant tumors. As beetroot contains macroelements of potassium and magnesium, and trace elementsiron, it is recommended for the prevention and treatment of hypertension, atherosclerosis, other cardiovascular system diseases, as well as for the prevention of iron deficiency anemia (Kurgusova, 2013). Studies on the composition of biologically active substances of Bordeaux 237 confirmed the beetrootto be a source of dietary fiber with antitoxic, antioxidant, radioprotective, hypocholesterolemic and lipid-correcting properties, as well as a source of vitamins C and B9, P-active substances with antioxidant properties (Gorash et al., 2015).

There exist various processed beet root products in food compositions. Beetroot powder was added to wheat flour in pasta production (Grazyna, 2015). Studies show that along with other herbal supplements, beets have a positive effect on the nutritional and energy value of noodles, while giving the product a burgundy color. Beet cryopowder was used as a herbal supplement in the production of oil paste (Podkovko & Rashevskaya, 2015). The authors consider red beetroot to be a potential source of immunomodulation, radioprotection and antioxidant effects. Beet fiber of the 'DIVINKA' company in minced meat product allows to increase the protein content, lower the fat content, increase the yield of finished products by 10-12% on average. Compared to wheat bran, it contains twice as much dietary fiber, as well as pectin and hemicellulose, which positively affect metabolism. It has the unique ability to bind water and fat in ratio 1: 8: 8 (Nikitin et al., 2016).

All of the above determines the prospects of applying beetroot in the production of functional products.

This work was aimed to develop the composition and identify the technological parameters for manufacturing of ice cream using Bordeaux table beet.

MATERIALS AND METHODS

The objects of the study were Bordeaux beetroot (Beta vulgaris), beetroot puree, ice cream mixtures, control and experimental samples of soft and hard ice cream.

Determination of betanin content in beetroot
We used a method based on the extraction of beet betanine in an acidic medium, measuring the optical density of the extracts obtained and comparing the values obtained with the optical density of a standard solution, which is 1% aqueous solution of cobalt sulfate (Bazarnova, 2013).

Experimental samples of beetroots were crushed into a puree using mixer grinder Bosch MSM 2650B. Weighed portions of the beet puree were 1 g each with accuracy of 0.001 g, they were placed into glass beakers with a capacity of 50 cm^3, and the weighed portions were transferred to volumetric flasks with a capacity of 250 cm^3. Then, 10 cm^3 of concentrated hydrochloric acid was poured into each flask, and the volume of the contents of the flask was adjusted to 250 cm^3 with distilled water. The contents of the flasks were thoroughly mixed for 1 minand then filtered through paper filters. The extracts obtained were further used to determine the concentration of betanin.

The optical density of the extracts obtained and a standard solution of cobalt sulfate was determined on photocolorimeter KFK-3-01 at a wavelength of 535 nm using a 10 mm thick cuvette.

The content of betanine was calculated by the formula (1)

$$X = \frac{0.022 \cdot D_1}{4m \cdot D_2} \cdot 10^5,$$ (1)

where X – content of betanine, mg per 100 g; 0.022 – mass of betanine, which in color corresponds to 1 dm^3 of a standard solution, g; D_1 – optical density of the test solution, rel. units; D_2 – optical density of the standard solution, rel. units; m – mass of the sample, g.

Determination of the mass fraction of solids in beets

The method consists of drying a product sample distributed over an absorbing surface under heating (GOST 28561-90).

Metal cups with weighed samples of filter paper weighing 4–5 g were dried together with lids at 100–110 °C for 1 h, cooled for about 20 min in a desiccator, and weighed. A portion of beetroot puree weighing about 5.0000 g was placed in a glass with filter paper and the product was evenly distributed on the filter paper. The product was kept in adrying cabinet at temperature of 105 °C. Periodically, the cups were closed with lids, cooled for about 20 min in a desiccator and weighed. Drying of the samples continued in the given mode, conducting control weighings at time intervals equal to approximately 10% of the total drying time. The change in the sample weight was determined during each of these drying periods, and the test was terminated if the weight change was less than 0.0020 g.

Mass fractionof moisture in the product (W) as a percentage was calculated by the formula (2)

$$W = (M_1 - M_2) \cdot 100/(M_1 - M_3),$$ (2)

where M_1 – mass of the cup with a lid, filter paper and a sample before drying, g; M_2 – mass of the cup with a lid, filter paper and a sample after drying, g; M_3 – mass of the cup with a lid and filter paper, g.

The percentage of mass fraction of solids in the product (DS) was calculated by the formula (3)

$$DS = 100 - W.$$ (3)

Thermal treatment of beetroot

We used the following methods of heat exposure: cooking in boiling water for 1 h; roasting in the oven at temperature of 150 °C for 1.5 h; microwave processing at a power of 800 W for 3–12 min in increments of 3 min. The results to determine the mass fraction

of solids in beetroot puree during various heat treatment techniques were analyzed in statistical program Minitab 19.

Manufacturing of ice cream

The test and control samples of ice cream were manufactured from a mixture of fresh cow milk, cream, skimmed milk powder, granulated sugar, and a stabilizer. The mixture of components was pasteurized at a temperature of 85 °C for 5 min, cooled to 4–6 °C, and kept for 2 h for protein swelling. Then, for the control sample, the ripened milk mixture was processed on the brand freezer Miken MK-25FTB. In the production of the test samples, beetroot puree prepared from beets subjected to microwave processing for 9 min at a power of 800 W was introduced into the ripened milk mixture. The milk-vegetable mixture was thoroughly stirred, and then also placed into the freezer. At the output of the freezer the product was soft ice cream with the temperature from minus 4 to minus 6 °C. Soft ice cream was packed in plastic cups of 150 cm^3 and frozen to a temperature not exceeding minus 18 °C to become hardened ice cream.

Determination of the conditional viscosity of an ice cream mixture

The nominal viscosity of the ice cream mixture was determined on a VZ-246 viscometer. The method is based on determining the expiration time of 100 cm^3 of the mixture through an opening with a diameter of 4 mm.

Evaluating of titratable acidity

The method is based on neutralization of acids in the product with 0.1 mol dm^{-3} sodium hydroxide solution with a phenolphthalein indicator. Five g of the product were placed into a flask of 250 cm^3, then 80 cm^3 of distilled water was added followed by 3 drops of phenolphthalein addition. The mixture was agitated and titrated with alkaline solution until pink color appearance stable for 1 min. Acidity in Turner degrees (°T) was calculated by multiplication of volume (cm^3) of the sodium hydroxide solution used for titration by 20 (Olenev et al., 2004).

Determination of ice cream overrun

The method is based on measuring the masses of a fixed volume of the mixture entering the freezer, and the same volume of the air-saturated mixture (ice cream) leaving the freezer, and calculating the overrun of ice cream (Olenev et al., 2004). A glass of 100 cm^3 was filled to the brim with ice cream mixture and weighed with the result recorded up to 1 g. Similarly, the dried glass was filled with the ice cream leaving the freezer. A glass of ice cream was weighed with a record of the result up to 1 g. The percentage of ice cream overrun (O) was calculated by the formula (4)

$$O = (M_2 - M_3)\ 100/(M_3 - M_1), \qquad (4)$$

where M_2 – mass of a glass filled with a mixture, g; M_3 – mass of a glass filled with ice cream, g; M_1 – mass of glass, g; 100 – conversion factor of the ratio in percent.

The size of air bubbles in ice cream was determined by the microscopic method. An ice cream sample was applied to a calibrated mesh, covered with a coverslip on top and examined under a microscope at 600 magnification. Knowing the price of mesh divisions, the size of air bubbles was determined (Olenev et al., 2004).

Determination of the resistance of ice cream to thaw

The method is based on measuring the duration of the melting of ice cream (Olenev et al., 2004). A sample of soft or hard ice cream (temperature minus 6 or minus 18 °C, respectively) was taken with a special probe in the form of a hollow cylinder with a diameter of 35 mm and a height of 50 mm and placed in a glass with holes along the edgebottom for free draining of the thawed mixture. The resistance of ice cream to thawing is characterized by the accumulation time of 10 cm^3 of the mixture obtained by melting the ice cream in a thermostat at a temperature of 25 °C.

Examination of organoleptic characteristics

Organoleptic evaluation was conducted using 5-point scale using the sensory analysis method (Kantere et al., 2001). The samples were evaluated by a trained panel of 12 members. Twelve panelists (age 22–38 years) qualified for sensory evaluation techniques and regular consumers of products estimated the sensory properties of the samples.

All experiments were performed with at least three replicates; data was processed by methods of mathematical statistics with 95% confidence level. The confidence interval was calculated according to the standard procedure using Student coefficient for confidence level of 0.95.

RESULTS AND DISCUSSION

The choice of filler preparation method

As previously assumed, since a beetroot filler in a puree form is the most appropriate to be addedto the ice cream mixture before freezing, its introduction has no noticeable effect on the process. In this case, to ensure the microbiological safety of ice cream, it is necessary to heat the beetroot puree.

A comparative evaluation of the samples was conducted according to the processing technique. The samples of pureewere obtained from medium size beetroots of one batch with the same mass, which were subjected to various heat treatment techniques.

Heat treatment was performed until the required degree of readiness of beetroot was achieved. After heat treatment beetroots were cooled, and then processed with mixer grinderto obtain a puree consistency. The organoleptic evaluation of the puree samples prepared was conducted according to the developed point scale, where the mark '5' characterizes a well-defined taste and smell inherent in beets without extraneous smacks and odors and a homogeneous puree-like consistency without the presence of solid particles.

Figure 1. Organoleptic characteristics of beetroot puree for various heat treatment techniques.

The results of the organoleptic evaluation of the samples of beetroot puree are shown in Fig. 1.

The two puree samples obtained from beetroots cooked and 9-min microwave processed have a well-pronounced beetroot taste and smell, as well as the best consistency.

Another criterion for choosing a heat treatment method was the mass fraction of betanine in the beetroot puree (Table 1).

The study results revealed the minimal losses of betanine, which were observed in the case of heat treatment of beetroots in a microwave for 3–9 min. A longer treatment led to a noticeable decrease in the content of betanine because of overheating of beetroots and their partial drying. When cooking, part of the coloring

Table 1. The influence of the method of heat treatment on the content of betanin in the beetroot puree

Heat Treatment Technique	Content of Betanin, mg per 100 g
Control	654 ± 42
Cooking	348 ± 40
Roasting	366 ± 71
MV-processing, 3 min	602 ± 42
MV-processing, 6 min	595 ± 53
MV-processing, 9 min	513 ± 70
MV-processing, 12 min	410 ± 62

matter goes into water, as evidenced by the decoction intense color. Exposure to high temperatures during prolonged baking in the oven significantly reduced the content of betanine in red beets.

The results of the analysis of heat treatment technique influence on the mass fraction of solids in the samples are presented in Fig. 2.

The data analysis shows that among the samples studied, the largest amount of solids was lost during cooking (up to 30% compared with the control sample), which is associated with their extraction into water. When roasting and MV-processing, the mass fraction of solids in beetroot puree increases due to moisture evaporation.

The results to determine the mass fraction of solids in beetroot puree during various heat treatment techniques were analyzed in statistical program

Figure 2. Mass fraction of solids in beetroot puree during various heat treatment techniques.

Minitab 19. For the analysis we have chosen a standard level of significance alpha equal to. To compare treatment tecniques variance analysis (ANOVA) was applied. The evaluation of input values quality for the variance analysis showed normal data distribution. The analysis was based on Anderson-Darling test.

The fact that the value of p-probability (0.000) is less of any reasonable alpha-level proves the treatment techniques significantly influence on the content of solid mass fraction. The model obtained is highly valid as it accounts for 99.61% of dispersion. Table 2 outlines average indicators of solid mass fraction in beetroot puree with a standard deviation, as well as confidential intervals, and grouping of treatment methods according to Tukey's honestly significant difference test.

Table 2. Results of statistical analysis in Minitab 19 for the indicator of solid mass fraction in beetroot puree during various heat treatment techniques

Technique	Number of measurements	Average values for solid mass fraction	Standard deviation	Confidential intervals	Grouping
1	5	14.3270	0.1869	14.1337; 14.5203	B
2	5	10.1248	0.2025	9.9315; 10.3181	C
3	5	17.0660	0.0958	16.8727; 17.2593	A
4	5	17.3970	0.2850	17.2040;17.5910	A

To establish statistically significant differences between the four treatment techniques the dispersion analysis of data was performed with Tukey's range test.

The comparison of the treatment techniques and 3 (roasting) and 4 (microwave) revealed there is no statistical difference between these methods, therefore, they belong to the one group, which is A group. Technique 1 (raw beetroot) and technique 2 (cooking) differ from each other and techniques 3 and 4 belonging to groups B and C. Calculated values of probabilities proved the application of treatment techniques 3 and 4 results in equal solid mass fraction in beetroot puree with 98.87% accuracy. The corrected p-probability indicator for the difference between techniques 3 and 4 is 0.086.

As a result of the studies, it was found that the most delicate method of processing beetroot crops is microwave processing for 9 min (power 800 W), at which their culinary readiness is achieved with minimal loss of dry substances, including betanine.

The choice of a rational dose of beetroot filler

Ice cream formulations with various mass fraction of the beetroot puree are developed. In all samples, the mass fraction of fat (MFF) was 3.0%, dry skimmed milk residue (DSMR) − 11.5%, the mass fraction of sucrose (MFS) − 15.5% in accordance with GOST 31457-2012 'Ice cream milk, cream and ice cream. Technical conditions'. The dose of beetroot puree was 15, 20 and 25%. An example formulation with 20% excipient is presented in Table 3.

Table 3. Ice cream recipe per 1,000 g of mixture

Rawmaterials	Mass, g
Whole milk: MFF = 3.2%; DSMR = 8.2%	547.1
Skimmed milk powder: DSMR = 96%	70.8
Cream: W = MFF%; SOMO = 6.12%	35.7
Beetroot puree: MFS = 6.8%; DS = 17.4%	200.0
Sugar: MFS = 100%	141.4
Stabilizer: DS = 96%	5.0
TOTAL	1,000.0

We studied the effect of the filler dose on the physicochemical parameters of the finished ice cream with beetroot puree addition (Table 4).

Table 4. Physico-chemical characteristics of the finished ice cream

Indicators		Control sample	Mass fraction of beetroot puree,%		
			15	20	25
Conventional viscosity of the mixture for ice cream, c		23±1	26 ± 1	32 ± 1	36 ± 1
Ice cream overrun, %		71 ± 2	59 ± 1	46 ± 2	35 ± 1
Resistance tomelting, min	soft ice cream	16 ± 0.5	18 ± 0.5	15 ± 0.4	10 ± 0.5
	hardened ice cream	40 ± 0.5	60 ± 0.4	53 ± 0.5	49 ± 0.5
Air bubbles size, mcm		33 ± 2	38 ± 3	50 ± 2	61 ± 2
Titratable acidity, ° T		20 ± 1	21 ± 1	22 ± 1	24 ± 1

It was noted that an increase in the mass fraction of beetroot puree was accompanied by an increase in the conditional viscosity of the mixture, a decrease in ice cream overrun, an increase in the size of air bubbles, and a decrease in resistance to melting. Neither clear deterioration or improvement was observed.

The decisive criterion in determining the rational dose of beetroot puree was the organoleptic characteristics of the finished product. The results of the organoleptic evaluation of the test samples are presented in Fig. 3.

The ice cream sample with a mass fraction of beetroot puree of 20% received the highest score. Ice cream with a puree mass fraction of 15% is characterized by a less pronounced color, and beetroot taste and smell were practically not felt. An excessively dense consistency was noted in ice cream with 25% puree, beetroot flavor and odor were too pronounced. According to the results of organoleptic studies and physico-chemical indicators, a rational dose of beetroot puree in an ice cream mixture was chosen − 20%.

Figure 3. Organoleptic evaluation of hardened ice cream with beetroot puree.

The studies conducted allow to conclude that the use of Bordeaux beet broadens the horizons in the production of ice cream with original taste characteristics enriching its composition with valuable nutrients.

In literature available there is no research data on application of beetroot products in ice-cream industry.
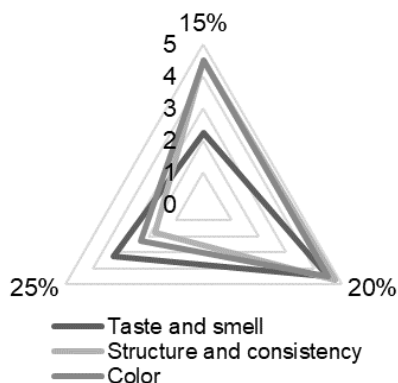
CONCLUSIONS

Based on the studies conducted the following conclusions have been made:

1. Literature data analysis showed that Bordeaux red beet is a biologically valuable product, the addition of which enriches ice cream with the beneficial ingredients beet roots possess.

2. The heat exposure technique recommended for beetroots is microwave processing for 9 minutes at a power of 800 watts. This type of heat treatment ensures the culinary readiness of vegetable raw materials while preserving dry substances in it, including betanine.

3. The study how the filler dose influences on the formation of the ice cream quality determined 20% as a rational dose of beetroot puree.

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The effect of yeast growth stages on the absorption of polyphenols

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Abstract. Colloidal stability of beer is one of the most critical challenges facing the brewing industry. In brewing, stabilization agents are widely used to remove colloids such as proteins and polyphenols. However, the removal efficiency of polyphenols depends on the yeast, which adsorbs these compounds on its surface. The adsorption of polyphenols on the yeast surface is associated with the zeta potential of the cell wall, which correlates with the mannan content in them. The purpose of the study was to investigate the role of yeast strains in adsorption of polyphenols (increasing colloidal stability of beer) and the correlation of this property with the mannan content in the cell wall during the fermentation. Two *S. cerevisiae* strains were used in this study, and the specific growth rate of yeast, the specific rate of change in the polyphenol content and the mannan content were determined. The results of this study showed that the highest mannan content in yeast was after 9 hours (the end of exponential growth phase). Its content was 10.97% by weight of dry matter in the yeast *S. cerevisiae Californian Lager* (M54), and 13.69% in the yeast *S. cerevisiae Belgian Wit* (M21). The desorption of polyphenols was observed during the period from 9 to 24 hours of fermentation, where an increase in the content of polyphenols in the medium was observed. Based on these results, it is necessary to remove the yeast at the end of the exponential growth phase to increase the colloidal stability of the beer.

Key words: colloidal stability, mannan, polyphenols, *S. cerevisiae* yeast.

INTRODUCTION

Beer is a complex alcoholic beverage made from malt, hops, water, and yeast (Werlen et al., 2010). Beer consists of particles: their size ranges from 0.01 to 3 μm, and because of that beer is a complex colloidal system. One of the problems facing the brewing industry is the achievement of colloidal stability of beer and reduction of its turbidity during storage. The turbidity of beer has a different nature. It can be biological turbidity, or non-biological turbidity (colloids). Beer colloids are nitrogen-containing substances, in particular proteins and other colloids such as polysaccharides and polyphenols. Polyphenols and proteins primarily form visible turbidity (haze), but interactions between polypeptides and polysaccharides, or polypeptides and minerals, can also contribute to the haziness of beer. In brewing, to increase colloidal stability, the stabilization agents are used (Mastanjević et al., 2018). The most widely used products today are silica gel for protein stabilization and Polyvinylpolypyrrolidine (PVPP) for

polyphenols stabilization. Most beer polyphenols are derived from malt (70–80%), while about 20–30% are derived from hops (Wannenmacher et al., 2018).

Yeast is an important ingredient in beer production, in addition to its role in the fermentation process, can be a stabilization agent that absorbs polyphenols on its surface. The yeast cell wall carries a negative charge due to the ionization of the carboxyl and phosphodiester groups in the cell wall proteins and phosphor-mannans, respectively; therefore, the yeast cell will attract particles that carry a positive charge, such as polyphenols. In other words, the removal efficiency of polyphenols depends on the yeast, and this phenomenon is associated with the composition of the cell wall and, in particular, with its peripheral layer (Stewart, 2018).

The cell wall of the yeast consists of three layers, and it is mainly composed of β-D-glucans, mannan proteins, and a small amount of chitin, which is mainly located at the bud scars on the surface of mother cells. The middle layer consists primarily of β-glucan, which is responsible for the stiffness and strength of the cell wall. β-D-glucans, which are the first essential polysaccharides of the cell wall in *S. cerevisiae* yeast and other yeasts are divided into two subtypes in accordance with the mode of glucose bonds. They are β-1,3-D-glucose, and β-1,6-D-glucose. These polysaccharides represented 50–60% (by weight) of the total cell wall (Liu et al., 2008). Mannan oligosaccharides (MOS) are the second essential polysaccharides in the cell wall. Most of the mannan oligosaccharides are associated with proteins that form a complex called mannoprotein.

Characteristically, the mannans of *Saccharomyces cerevisiae* consist of long D-mannose chains, (where mannose molecules of 50–200 units bind to each other via α1-6 bonds) with short side chains in α-1-2 and α-1-3 bond. These macromolecules bind to asparagine in the protein. Also, mannan has short oligomannose units with α-1-2 and α-1-3 bonds that are associated with serine and threonine in the protein. Thus, the mannose chains bind to the polypeptide part by two methods. The first method consists of using an O-glycosidic bond between the hydroxyl group of amino acids (serine and threonine) and mannose residues. The second method is through the di-N-acetylchitobiose bridges that are formed by the N-glycosidic bond between the N-acetylglucosamine residue and the amide nitrogen of the asparagine polypeptide chain (N-linked mannoprotein) (Maru et al., 2015).

Mannoprotein, which represents 30–40% (by weight) of the total cell wall (Moreno et al., 2008), is mainly localized in the outer layer of the yeast cell wall and plays an essential role in determining the charge on the surface of the cell wall, in other words, it directly affects the zeta potential of yeast (Lipke & Ovalle, 1998).

For each yeast strain, the adsorption properties depend on the zeta potential of the cell wall, which in turn, correlates mainly with the mannan content in yeast (Lavaisse et al., 2019).

The amount of mannan polysaccharides in *Saccharomyces cerevi*siae varies depending on the characteristics of the yeast strain, their physiological state, as well as the physicochemical conditions of cultivation (Bzducha-Wróbel et al., 2013). In the same context, Lavaisse and others showed that the zeta potential of yeast varies depending on the stages of yeast growth and cultivation conditions (Lavaisse et al., 2019). It is well known that the surface properties of yeast cells determine processes such as adsorption, flocculation, and adhesion to various surfaces. The surface charge, which value can be determined indirectly through the measurement of the zeta potential, is one of these properties.

Afonin and colleagues showed that yeast adsorbs the colloidal particles, which have size ranged from 0.31 to 0.39 µm on their surface, while the phenolic compounds in beer are significantly smaller (from 1 nm to 10 µm). The yeast cell in good physiological condition adsorbs these low molecular weight compounds due to negative surface charge (Afonin et al., 2012). In context, the purpose of this study was to investigate the role of yeast strains in the absorption of polyphenols on its cell wall during wort fermentation and establish the correlation this characteristic with the mannan content in yeast cells at the various stages of growth yeast.

MATERIALS AND METHODS

Strain and growth conditions

Research objects were dry yeast *S. cerevisiae Californian Lager (*M54) (Mangrove Jacks, New Zealand) and *S. cerevisiae Belgian Wit* (M21) (Mangrove Jacks, New Zealand). The inoculum was 3×10^7 cfu mL^{-3}. The flasks with capacity 300 mL were used, and the usable volume was 264 mL.

Spraymalt light barley wort extract (Muntons, England) was used to prepare the medium. 200 g of extract was mixed with 1,500 mL of water in conical flask. Then, the mixture was heated for 20 minutes.

The concentration of dry matter of nutrient medium was determined using refractometer PTR-46 (Shimadzu, Tokyo, Japan). The dry matter content after adding the inoculum was 8.6%. The fermentation process was carried out in incubator (TC-1/80 CPU, Russia) on the principle of a batch culture without forced aeration at temperature 28 °C for 29 hours.

The specific growth rate of yeast, the specific rate of change in the polyphenol and mannan content were determined in this study.

Extraction and estimation of crude mannan:

The yeast biomass was separated from nutrient medium using centrifuge (ULAB, Beijing, China) at 6,000 rpm for 15 min. The supernatant was decanted and the sedimented yeast was washed with distilled water twice and dried according to Huang et al. (2010). Weight of dry cell mass estimated in grams. The mannan were obtained from 2 g of dry yeast extraction with 1% NaOH (50 mL) at 100 °C for 2 hours, cooling and neutralizing to pH 7 with dilute HCl solution.

Deprotienization by the TCA (trichloroacetic acid) method: The concentrated solution of crude mannan oligosaccharides were adjusted to pH 3 with 10% TCA solution overnight. The centrifugation at 5,000 rpm 10 min^{-1} was used to obtain the mannan in the supernatant (Huang et al., 2010). The quantitative estimation of mannan was determined by the phenol-sulfuric acid method using glucose as standard (Maru et al., 2015).

Determination of polyphenols:

The polyphenols were determined according to the EBC method 9.11 using a 'UV 1240' spectrophotometer from 'Shimadzu' at the wavelength of 600 nm (Dvořáková et al., 2007).

The specific rate of change in the polyphenol content in the culture fluid (adsorption, desorption) was calculated using the equation:

$$\rho = \ln(y - y_0)/(t - t_0) \tag{1}$$

where ρ – the specific rate of polyphenols adsorption, h^{-1}; y – concentration of polyphenols at the beginning of measurement (t_0), mg L^{-1}; y – concentration of polyphenols at the end of measurements (t), mg L^{-1}; t-t_0 – the time interval between measurements, h.

The accumulation of yeast biomass was determined by the weight method after drying the suspension of washed yeast to constant weight at 105 °C for 24 h in cabinet dryer ES-4610 (Reaktivsnab, Shymkent, Kazakhstan).

The specific growth rate of yeast was calculated by the equation:

$$\mu = \ln(x - x_0)/(t - t_0) \tag{2}$$

where μ – the specific growth rate, h^{-1}; x_0 – biomass at the moment of the inoculation (t_0), g; x – biomass at the time τ, g; $t - t_0$ – the time interval between measurements, h.

Data Analysis

Data generated were subjected to analysis of variance (ANOVA) using Origin statistical software (version 6.1) at 95% significance. All measurements were made in at least triplicate. Results were reported as means ± standard deviations.

RESULTS AND DISCUSSION

The growth and multiplication of yeast in a batch culture is described by the growth curve (Figs 1, 2). The growth curve of the yeast *Saccharomyces cerevisiae Californian Lager* (M54) (Fig. 1) showed that the lag phase lasted 5 hours, then, after a short phase of increasing growth, a log phase was started and lasted 3 hours. Then from 9 to 25 hours, there was a slow decrease in biomass growth and the cell death phase was observed for the next 4 hours, as evidenced by the decreasing value of the specific growth rate of yeast (Table 1). Fig. 2 shows the growth curve of *Saccharomyces cerevisiae Belgian Wit (*M21) yeast. The lag phase lasted 2 hours, and then the growth acceleration began and lasted 1 hour. The decrease in biomass was started after the log phase, which lasted 5 hours, and the cells entered the stationary phase after 9 hours of cultivation. The stage of the cell death began in the last 6 hours of cultivation as shown at the growth curve and this evidenced by the decreasing value of the specific growth rate of yeast at the last hours of cultivation (Table 1).
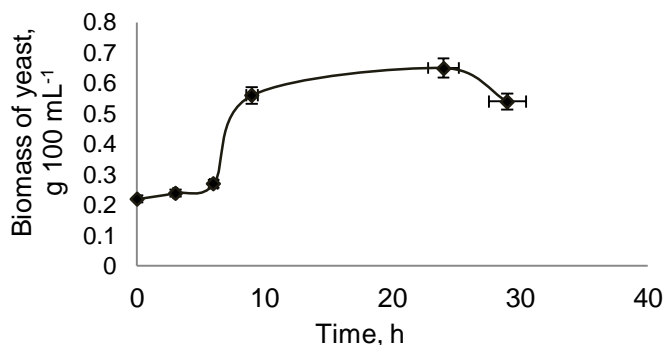

Figure 1. The growth curve of the *Saccharomyces cerevisiae Californian Lager M54* in a batch culture.

Therefore, the log stage of the *Saccharomyces cerevisiae Belgian Wit (*M21), which lasted 5 hours was 1.6 times more compared to the *Saccharomyces cerevisiae Californian Lager* (M54), and in both yeasts, biomass began to decrease after 9 hours of cultivation, while the yeast cells of *Saccharomyces cerevisiae Belgian Wit (*M21) entered the phase of death before other yeasts.
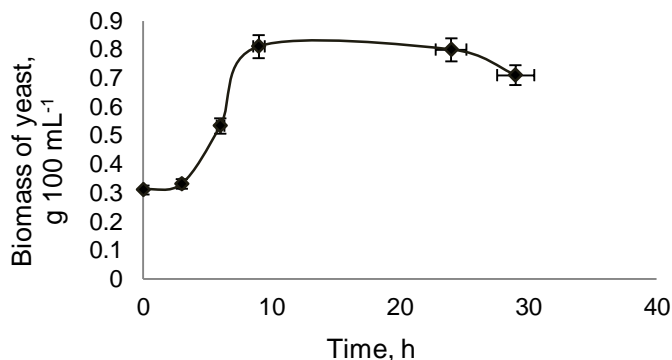

Figure 2. The growth curve of the *Saccharomyces cerevisiae Belgian Wit M21* in a batch culture.

Table 1. The variation of the specific growth rate of yeast in the culture fluid and the content of mannan in yeast during the time of cultivation

Period of the cultivation (h)	*S. cerevisiae Californian Lager* (M54)		*S. cerevisiae Belgian Wit* (M21)	
	Mannan (%*)	Specific growth rate (h^{-1})	Mannan (%*)	Specific growth rate (h^{-1})
0	6.96 ± 0.007	-	6.88 ± 0.006	-
3	7.63 ± 0.006	0.029	9.35 ± 0.008	0.081
6	8.85 ± 0.007	0.034	11.21 ± 0.002	0.090
9	10.97 ± 0.008	0.10	13.69 ± 0.003	0.106
24	10.45 ± 0.007	0.045	13.16 ± 0.002	0.040
29	9.77 ± 0.005	-0.04	12.75 ± 0.004	-0.028

* mg mannan mg^{-1} dry cell weight.

The content of polyphenols in the culture fluid during the time of cultivation was identified (Table 3). The results showed the decrease of their content when the cells were in good physiological activity, and this confirms the ability of yeast to adsorb these colloids on its surface, as Afonin and colleagues showed through his study (Afonin et al., 2012).

To clarify the relationship between the growth phases and polyphenol adsorption, the change in polyphenol content at different time intervals corresponding to a specific yeast growth phase was determined. Also, the mannan content in yeast was determined at the various phases of growth in order to study its effect on the adsorption properties of yeast.

When the growth of yeast slow down, and the cells entered the stationary phase as reported in Table 1, the desorption of polyphenols from the surface of yeast was observed. It can be seen from the increasing of the content of the polyphenols in the medium (the positive value of the Specific Adsorption Rate of Polyphenols) (Table 2, 3).

Determination of mannan in yeast showed that it accumulated in the cells when the cells were in good physiological activity.The highest mannan content in yeast was at the end of the exponential phase of growth (log phase), i.e. after 9 hours of the cultivation. Its content was 10,97 % in the yeast *S. cerevisiae Californian Lager* (M54) and 13,69 % in the yeast *S.cerevisae Belgian Wit* (M21) by the weight of dry matter of yeast. A decrease in the amount of mannan was observed when the yeast entered the stationary phase (after 9 hours). According to these results, the mannan content in yeast cells varies depending on the phases of yeast growth (Moreno et al., 2008).

Table 2. The specific rate of change in the polyphenol content in the culture fluid during the time of cultivation

Time intervals (h)	The specific rate of change in the polyphenol content (h^{-1})	
	S. cerevisiae Californian Lager M54	*S. cerevisiae BelgianWit M21*
0–3	- 0,155	-0.046
3–6	- 0,102	-0.073
6–9	-0,021	-0.045
9–24	0,016	0.023
24–29	0,026	0.030

In both yeast strains, the highest amount of mannan was when the cells were in the log phase. The mannan content in *S. cerevisiae Belgian Wit* (M21) was more than its content in *S. cerevisiae Californian Lager* (M54). At the same time, the adsorption of polyphenols on the surface of this strain was more than the other during this phase (Table 3). When the physiological activity of yeast cells decreased, the content of mannan decreased, and at the same time, the desorption of polyphenols from the surface of yeast started. In other words, the decrease of the physiological activity of cells leads to colloid adsorption reduction and an increase of beer turbidity and this corresponds to the results obtained by Meledina and colleagues (Meledina et al., 2015).

Table 3. The polyphenol content in the culture fluid during the time of cultivation

Period of the cultivation (h)	Content of the polyphenols (mg L^{-3})	
	S. cerevisiae Californian Lager M54	*S. cerevisiae BelgianWit M21*
0	171.60 ± 2.333	168.91 ± 1.003
3	109.33 ± 1.320	156.66 ± 0.333
6	79.83 ± 3.083	117.33 ± 3.333
9	72.86 ± 1.653	102.66 ± 2.583
24	94.93 ± 4.763	109.73 ± 1,063
29	110.06 ± 1.013	119.03 ± 2.403

CONCLUSIONS

Based on the results obtained from the study conducted on two strains, we conclude that the concentration of mannan varies depending on the yeast growth stages and the yeast strains. At the same time, the adsorption of polyphenols depends on the mannan content in the yeast, which was the highest when the yeast was on the exponential phase (log phase). Therefore, in order to increase the colloidal stability of beer, and reduce the consumption of stabilizers in particular PVPP; it is necessary to remove the yeast from

the fermentation tanks at the end of the exponential phase of growth, that is, before the desorption of polyphenols from the surface of the yeast. This conclusion is valid for the yeast *S. cerevisiae Californian Lager* (M54) and *S. cerevisiae Belgian Wit* (M21).

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Biochemical responses of 5 buckwheat (*Fagopirum esculentum* Moench.) cultivars to seed treatment by *Azospirillum brasilense*

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Abstract. Cultivation condition have a large effect on efficiency of buckwheat. Drought, high temperatures and their fluctuations, salinity, oxygen deficit in the soil, ultraviolet radiation, and abnormal nutrient content in the soil are the most common reasons of decreasing productivity of plants. Suboptimal parameters of the cultivation technology can also cause abiotic stress. Plant can decrease its efficiency from 30% to 50% depend on stress conditions. Using bacterial cultures is one of the preventive approaches to overcoming the negative impact of stressors. Microorganisms produce biologically active substances that stimulate plant growth, increase their resistance to abiotic factors. They are growth regulators and long-acting anti-stressants as well. Malondialdehyde (MDA) is formed as a result of the oxidative degradation of polyunsaturated fatty acids. Fructans are polysaccharides that are derived from D-fructose residues found in higher plants, green algae and bacteria. Fructans are involved in the adaptation of plants to the action of abiotic stressors and are valuable nutrients. The effect of *Azospirillum brasilense* pre-sowing treatment of buckwheat seeds on physiological and biochemical processes of MDA and fructan content was researched. Seed treatment with *Azospirillun brasilense* reduced the content of MDA in Ukrayinka and Syn 3/02. Sofia and Olga had a low level of MDA, but seed treatment increase it. Seed treatment increased the efficiency of photosynthesis (Fv / Fm) in Syn 3/02 from 0.58 to 0.72, in other varieties this effect was negligible. All cultivars have a strong relation between MDA and fructan content, that shows their participation in responses on cultivation conditions. Efficiency of photosynthesis in flowering–seed formation stage (BBCH 65–75) was close to maximum in field condition (0.70 ± 0.05) and seed treatment can increase it.

Key words: buckwheat, malondialdehyde (MDA), fructans, fluorescence.

INTRODUCTION

Limiting factor in buckwheat productivity is its demand for soil nutrients.

Seed microbial preparation of buckwheat can improve plant productivity as a result of improved mineral nutrition and synthesis of organic compounds (Bhattacharyya & Jha, 2012; Bano et al., 2013). The concentration of phenolic compounds and antiradical activity in the plant depends on the stage of development. Itsnumber increases as the seeds germinate (Beitane et al., 2018), but then decreases. Additional sources of

antistressants are needed for improvement stress resistance during active vegetation. *Azospirillum* spp. can be associated with buckwheat root system, increase its productivity and increase the amount of antistressants (Tummaramatti et al., 2014; Singh et al., 2015). The accumulation of fructans is the basic strategy of plants to counteract the unfavorable factors. Seed treatment by *Azospirillum brasilense* increases fructan content but the level of sucrose in plants decreases (Bagheri & Jafari, 2012). Genotypes with high fructan accumulation in stress condition can decrease malondialdehyde in plant (He et al., 2015). Seed treatment with microbial preparations can lead to a decrease in MDA concentration in some plant species may have species and cultivar reaction (Taran et al., 2016).

Malondialdehyde is a marker of the oxidative degradation of unsaturated fatty acids. However, the peroxide radical can also interact with neutral fatty acid molecules (Munne-Bosch & Pinto-Marijuan, 2016). Unsaturated fatty acids in membrane phospholipids can be oxidized in this way, but also free unsaturated fatty acids, residues of unsaturated fatty acids (Kumar & Ebel, 2016). The oxidation of unsaturated fatty acids is controlled by enzymes. The fact that the body has a normal physiological level (background) of malondialdehyde (MDA), diene conjugates (DC), other products of lipid peroxidation, indicates that there is a strict control of lipid oxidation by the entire hierarchical system of hierarchical regulation the DNA turn (You & Nam, 2014). The physiological role of oxidation reactions is to regulate the renewal and permeability of biological membrane lipids, the interaction of eicosanoids - mediators (local hormones) or signaling substances that play an important biological role in the organism (Zhang et al., 2016). Such important membrane processes as electron transfer in the respiratory chain, oxidative phosphorylation, methylation, hydroxylation of a number of endogenous and exogenous substrates by enzymatic systems of the endoplasmic reticulum, and, even, cell division, are accompanied by changes of the level of malondialdehyde (Huang et al., 2015). Malondialdehyde can modify proteins in cells and it leads to irreversible changes (Fenaille et al., 2002).

Fructans are polysaccharides that are derived from D-fructose residues found in higher plants, green algae and bacteria (Harding et al., 2017). Fructans are complex sugars that are important for the plant (Pollock et al., 2017). Fructans are distinguished by several main types: inulin, levan, neo-inulin and neo-levan (Le Roy et al., 2008). The synthesis of fructans from Sucrose involves at least two enzymes – *sucrose:sucrose 1-fructosyltransferase (1-SST)* and *fructan:fructan 1-fructosyltransferase (1-FFT)* (Kanabus et al., 1991; Ritsema & Smeekens, 2003). *1-SST* catalyzes the production of a Glc-Fru-Fru trisaccharide that can be extended with Fructose residues in various ways by *1-FFT* (Kanabus et al., 1991). The amount of D-fructose residues depends on species of the plants and condition of vegetation (Hellwege et al., 1998). Degree of polymerization (DP) of fructans increases under stress conditions in response to a stress factor (Quezada et al., 2017). It should be noted that fructans have an extremely important nutritional value for humans. Consuming a balanced content of fructans of different types (oligofructose and short-chain fructo-oligosaccharides) can increase the body's immune responses and improve the body's overall homeostasis (Roberfroid, 2004).

Chlorophyll fluorescence is one of the most popular techniques in plant physiology because of the ease with which the user can gain detailed information on the state of photosystem II (PSII) at a relatively low cost. It has had a major role in understanding

the fundamental mechanisms of photosynthesis, the responses of plants to environmental change, genetic variation, and ecological diversity (Murchie & Lawson, 2013). The method consists in detecting chlorophyll fluorescence which occurs if illuminated by bright light plants will be adapted to the darkness, known as the Kautsky effect (Kautsky & Hirsch, 1931). Light energy is absorbed by chlorophyll molecules in the leaves can be used for one of three processes: the photochemical reaction, the excess energy is dissipated as heat or re-emitted as fluorescence. These three processes are competitive because the effectiveness of one leads to changes in the other two. Thus the change in intensity of chlorophyll fluorescence gives information on efficiency of flow photochemical reactions (Maxwell & Johnson, 2000).

MDA and fructan content are markers of oxidative stress that occur under cultivation conditions. Determining of MDA level and fructan content in plants allows to establish cultivar responses to cultivation conditions in a specific region. Adverse conditions can affect the efficiency of biochemical processes of photosynthesis. Accumulation of antistressants in treated plants may increase resistance of photosynthesis systems to changing environmental conditions in the field. The reactions of different crops to seed treatment with *Azospirillum brasilense* are often described in the literature, but the evaluation of MDA, fructans and the induction of fluorescence of chlorophyll in buckwheat is poorly understood.

MATERIALS AND METHODS

Field studies were conducted in the Educational-Scientific Laboratory Demonstration Collection Field of Crops of the Department of Plant Science (Kyiv, Ukraine; 50º 22´ N, 30° 30´ E). The investigation was performed in field conditions. The soil of the experimental field was grey forest light loam soil with 2.32–3.01% humus content in the arable soil layer, pH_{KOH} − 5.8–6.1, hydrolyzed nitrogen − 62–83 mg, phosphorus − 75–120 mg, and potassium − 42–101 mg per 1 kg of soil. Cropping system is common to the northern forest-steppe of Ukraine.

Climate conditions

Field experiments were conducted in 2017–2018 (2 seasons). Daily average temperature in buckwheat vegetation in 2017 was 17.8 °C (average multi-annual +15.5 °C), but 2018 was hotter (19.4 °C) and dry. Air temperature in the first decade of May (sowing period) has varied over the years (13.6 °C in 2017, 16.9 °C in 2018). Temperature in first half of buckwheat vegetation (May-June) was abnormally in all region (Mazurenko et al., 2020), that could impact on its organogenesis and biochemistry processes. Summary precipitation in vegetation period was 146 mm in 2017 and 245 mm in 2018 (multi-annual 254 mm). Buckwheat fell into arid conditions with moisture deficiency in 2017.

Cultivation and sampling

Cultivar sensitivity to pre-sowing treatment with *Azospirillum brasilense* was established in 2-factore field experiment. First factor was 5 buckwheat (Fagopyrum esculentum Moench.) cultivars. There was Sofia, Olga, Ukrayinka, Antariya, Syn 3/02 varieties observed. Second factor of the cultivation of buckwheat included seed treatment with *Azospirillum brasilense, norm* 1 L t^{-1} *seed (Az. br.)* and control (without

Azospirillum brasilense, Wt). Pre-sowing treatment of seeds with strain of bacteria *Azospirillum brasilense* (1 mL contained 2×10^9 colony-forming units (cfu) bacteria of the genus *Azospirillum*) was performed.

The experiment was established in 4 replications. The size of elementary plots was 36 m^2 (24 m^2 to harvesting). Tillage system included disking after preceding crop harvesting (winter wheat) and ploughing on 18–20 cm in autumn. Disking for moisture saving was carried out in early spring and pre-sowing cultivation to a depth of 3–4 cm was conducted before sowing. $P_{45}K_{30}$ (superphosphate, 18% P; potassium chloride, 60% K) was applied before ploughing in autumn and N_{30} (ammonium nitrate) before sowing. Buckwheat was sown with 15–cm inter-row spacing with rate 400 grains per square meter. Sowing time (1st decade of May) depended on soil temperature (optimum 10–12 °C). Pesticides did not apply. Buckwheat was harvested when 65–75 % seeds was brown.

Leaf samples for MDA and fructan analyses are taken from the middle tier. Samples was weighed (100 mg), homogenized with the addition of phosphate buffer (pH 7.4, 1 mL) and centrifuged for 15 minutes at 15,000 rpm. The required aliquot was selected to determine the MDA and polyfructan content.

Analysis of the level of accumulation of malondialdehyde

Activity of enzymes of the antioxidant protection system in the plants was determined by the level of malondialdehyde (MDA). A lipid peroxidation test is based on the concentration of a colour complex formed as a result of the reaction of malondialdehyde (MDA) with two molecules of thiobarbituric acid (TBA) (Vladimirov & Archakov 1972). Supernatant was obtained after centrifugation. 300 µL of the supernatant mixed with 900 µL of 5% three chloroacetic acid and 300 µL of 0.8% thiobarbituric acid. This mixture was incubated a half an hour (90 °C). Optical density was determined at a wavelength of 532 nm on a SF-26 spectrophotometer.

Fructan content sampling

The content of polyfructans was determined by the ability of ketotsugars to stain in acid with resorcinol. 100 µL of the extract (supernatant) was mixed with 100 µL of 0.1% alcohol solution of resorcinol and 100 µL of concentrated HCl. Mixture boiled 10 minutes (80 °C) for observing purple colour. Optical density was measured on an Eppendorf biofotometr plus at 550 nm.

The concentration of polyfructans was determined according to the calibration graph (calibration reactions with resorcinol fructose solutions; concentration of 0.62, 1.25 2.5 and 5 mg mL^{-1}). Measurements were made in three replications.

Chlorophyll fluorescence sampling

Samples for induction of chlorophyll fluorescence were taken from the middle tier. Leaves were placed in moistened paper and kept without access to light for 15 minutes (dark adaptation) Then, the fixed part of the leaf is irradiated with light of wavelength 470 ± 15 nm. Under the influence of light the chlorophyll fluorescence is excited. Fluorescence signal is isolated with red filter and enters on the photo detector (wavelength 670 nm) which converts it into an electrical signal and amplified. An electrical signal sensor device is displayed and then stored and transferred to a computer for further analysis. The intensity of the induction of chlorophyll fluorescence (IFH) was

determined using a portable fluorometer 'Floratest' (Romanov et al., 2007). Fast and slow phase of chlorophyll fluorescence was determined for 3 min. Several physiological indices on the IFH curve were identified. There are minimal level fluorescence (Fo), maximum fluorescence (Fm), photochemical activity of FS II(Fv/Fo), potential quantum efficiency of photosynthesis (Fv/Fm) and index vitality (Fm/Fst).

Statistical analysis

Statistical analysis of the data was made using program Statistica 6.0 (StatSoft I.N.C.). Probability of the difference between the arithmetic mean of indicators was established using Student's test. The differences are considered to be significant at a value $P \leq 0.05$.

RESULTS AND DISCUSSION

MDA and fructan content in buckwheat

Buckwheat cultivars had different sensitivity to environmental conditions. Level of MDA was different in different cultivars (Fig. 1). MDA level above 0.3 μM g^{-1} of crude mass was formed by cultivars Ukrainka, Antaria and Syn 3/02. It should be noted that bacterial treatment did not have effect on the overall level of MDA in Antaria and Syn 3/02, but MDA level decreased in Ukrainka.

Sofia and Olga accumulated significantly less MDA. Sofia without treatment accumulated the least MDA, but *Azospirillum brasilense* treatment increased it. Olga also had this tendency, but the average MDA level was higher.
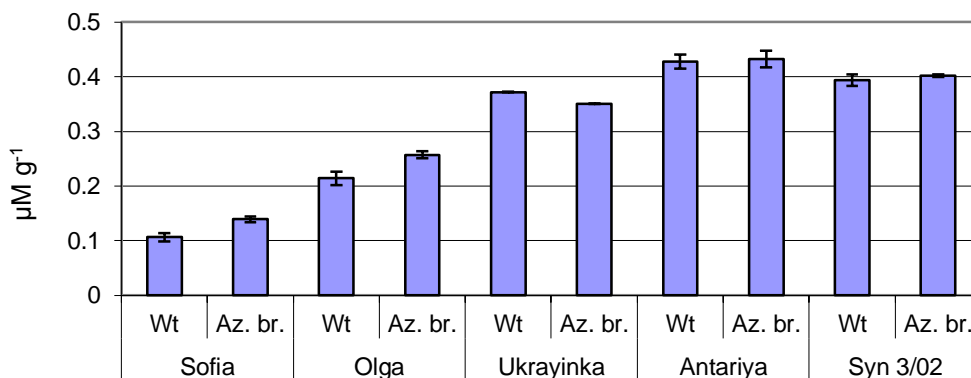

Figure 1. MDA levels a during grow up of buckwheat depending on the variant of the pre-sowing treatment of seeds: Wt – treatment with sterile water; Az. br. – treated with *Azospirillum brasilense*.

Fukami et al. (2018) showed that inoculation with <u>*Azospirillum brasilense*</u> decreases MDA content in roots but increase it in leaves. Tummaramatti et al. (2014) noted that *Azospirillum* spp. have a positive effect on growth processes, which is manifested in the increase of dry and wet weight of plants. However, their resistance to abiotic stress may be reduced with improved nutrition.

Tolerant genotypes under normal conditions have a much lower MDA content than sensitive ones. Certain genotypes do not change the level of MDA in stressful conditions (He et al., 2015).

The content of polyfructans in plants of one species is approximately constant. Changing their concentration may indicate stress or counteract it (Fig. 2). Content of fructans with or without treatment did not make a significant difference in Ukrainian and Olga. Fructan content was reduced by *Azospirillum brasilense* treatment in Syn 3/02 but exceeded the other cultivars. Significant increase in fructan content by treatment was observed in Sofia and Olga.
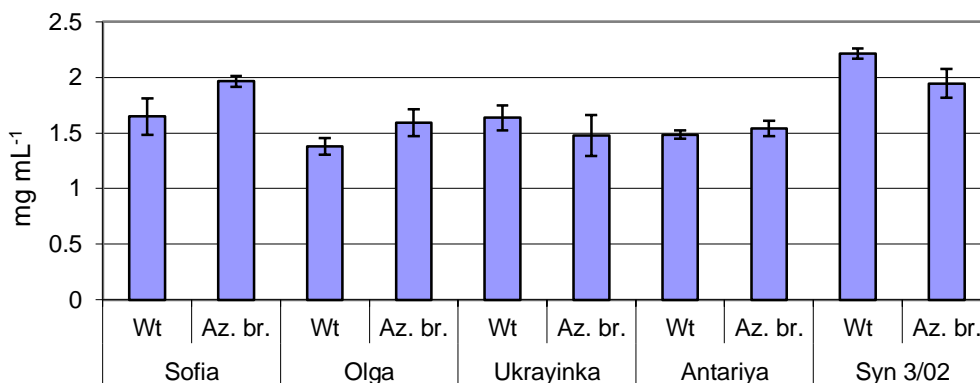

Figure 2. Fructan content of buckwheat depending on the variant of the pre-sowing treatment of seeds: Wt – treatment with sterile water; Az. br. – treated with *Azospirillum brasilense*.

On the other hand, improving nutrition can have a positive effect on the synthesis of metabolites that can counteract stress and bind ROS. The principle of counteracting oxidative stress is based on the properties of fructans react with peroxidases to form fructan radicals and water (Van den Ende & Valluru, 2008; Bolouri-Moghaddam et al., 2010). Fructans can also be a signaling mechanism of stress in plant cells (Van den Ende and El-Esawe, 2014). Content of fructans in sensitive varieties can be significantly reduced, and in resistant it increases. High fructan content does not always characterize genotype tolerance (Nemati et al., 2018).

MDA accumulation has a strong relationship (Table 1) with fructan content in plant. Increasing the fructan content with increasing levels of MDA may indicate adaptive properties of cultivar to environmental conditions. Sensitive to *Azospirillum* spp. cultivars increase the level of polysaccharides have a higher resistance to oxidative stress.

Table 1. Correlation between fructan content and MDA level

Cultivar	Untreated	Treated
Sofia	0.69	0.92
Olga	0.90	0.83
Ukrainka	0.89	0.87
Antariya	0.99	0.87
Syn 3/02	0.94	0.81

All correlation significant at $P \leq 0.01$.

Chlorophyll fluorescence of buckwheat leaves

Efficiency of photosynthesis was dependent on *Azospirillum brasilense* treatment and manifested differently in different cultivars (Table 2).

Table 2. Indicators of photosynthesis activity

Treatment	Cultivar	Fo, r.u.	Fm, r.u.	Fv/Fo	Fv/Fm	Fm/Fst
Without treatment	Sofia	488 ± 41	1,733 ± 134	2.55 ± 0.02	0.70 ± 0.01	3.16 ± 0.09
	Olga	475 ± 12	1,557 ± 89	2.28 ± 0.15	0.68 ± 0.03	3.17 ± 0.12
	Ukrayinka	408 ± 10	1,403 ± 53	2.44 ± 0.06	0.70 ± 0.01	3.13 ± 0.04
	Antariya	427 ± 20	1,733 ± 98	3.06 ± 0.06	0.75 ± 0.01	2.98 ± 0.17
	Syn 3/02	454 ± 46	1,334 ± 26	1.94 ± 0.14	0.58 ± 0.04	3.73 ± 0.11
Azospirillum brasilense	Sofia	439 ± 18	1,387 ± 76	2.16 ± 0.16	0.71 ± 0.02	3.21 ± 0.1
	Olga	446 ± 23	1,573 ± 66	2.53 ± 0.08	0.72 ± 0.01	3.31 ± 0.12
	Ukrayinka	346 ± 32	1,077 ± 128	2.11 ± 0.07	0.69 ± 0.02	2.89 ± 0.17
	Antariya	421 ± 2	1,312 ± 112	2.11 ± 0.16	0.66 ± 0.04	3.08 ± 0.12
	Syn 3/02	336 ± 4	1,205 ± 10	2.59 ± 0.04	0.72 ± 0.01	2.90 ± 0.05

F_o – minimal level fluorescence; Fm – maximum fluorescence; Fv/Fo – photochemical activity of FS II; Fv/Fm – potential quantum efficiency of photosynthesis; Fm/Fst – index vitality; $r.u.$ – relative units.

Minimal level fluorescence (Fo) ranged from 336 to 488 relative units in observed samples. This indicator characterizes the amount of excitation energy that is lost during migration on the pigment matrix. Concentration of clotophyllles that are not bonded to the reaction centers (RCs) are also relevant to this indicator. Treated plants have a lesser energy loss in compare with without treatment. Higher difference between treated and untreated variants was observed in Syn 3/02 (26%) and Ukrayinka (15%). Sofia, Antariya and Olga decreased his Fo parameter, but they did not have significant difference.

Maximum fluorescence level (Fm) shows the fluorescence intensity of chlorophyll at the 'closed' reaction centers of FS II, when all electron acceptor QA (a bound quinone) are restored and unable to receive electrons from the RC. Seed treatment *Azospirillum brasilense* reduced Fm by 20–24% in Sofia, Ukrayinka and Antaria. Other cultivars reduced Fm without significant difference.

Largest difference (indicator Fv/Fo) between treated and untreated variants was observed in Syn 3/02. Increase in photosynthetic activity of photosystem II was observed in cultivar Olga, but it has a lesser improvement. Other cultivars have the highest Fv/Fo for untreated variants and decreased this parameter for *Azospirillum brasilense* treatment.

Lazar (1999) indicated that Fv/Fm reaches 0.82 under optimal conditions. There was Fv/Fm from 0.66 to 0.75 relative units in researched cultivars. It may indicate the effect of stress factor on plants. Improvement of photosynthetic activity of buckwheat plants was observed in Olga (+3%) and Syn 3/02(+9%) for treatment. Antaria had the highest Fv/Fm without treatment, but reduced it in treated variant. Bagheri & Jafari (2012) have indicated that seed treatment by *Azospirillum brasilense* reduces the negative effects in the activity of the photosynthetic apparatus and decreases reduction of Fv/Fm.

Fm/Fst indicator varied quite strongly in Olga and Syn 3/02 in treated and untreated variants. Fm/Fst characterizes the efficiency of the dark phase of photosynthesis, it can be noted that in plants treated with bacteria of the genus *Azospirillum brasilense* although there was an improvement of this process on 2–5%.

CONCLUSIONS

MDA levels and fructan content are important indicators of the relationship of plants to the environment. *Azospirillum* spp. treatment stimulates a lot of processes in plant, but effect on accumulation of antistressants was different in buckwheat cultivars.

According to MDA level, it can be noted that Olga and Sofia have a better oxidative resistance than other cultivars. High fructan content in Sofia indicates high potential for tolerance in different field conditions.

Pre-sowing seed treatment of *Azospirillum brasilense* causes an increase in the efficiency of functioning of the photosynthetic apparatus of buckwheat plants. Features of its action depend on varietal characteristics of plants. Positive influence on all stages of photosynthesis was noted in Olga variety. In other varieties, this effect was not manifested by all the parameters studied. This issue needs further study.

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Enrichment of the low-fat yoghurt with oat β-glucan and EPS-producing *Bifidobacterium bifidum* improves its quality

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Abstract. The addition of β-glucan or EPS-producing bacteria is mainly used to improve the quality and the acceptability of low-fat yoghurt. The purpose of this study was to investigate the effect of adding β-glucan, EPS-producing *Bifidobacterium bifidum*, or both on physical properties, fermentation time, and organoleptic criteria of low-fat yoghurt, additionally to the viability of *L. bulgaricus* and *B. bifidum*. Two types of low-fat yoghurt (1.5% fat) were prepared, with the addition of standard oat β-glucan by 0.15% or without its addition. Each type of yoghurt mixture was inoculated with two kinds of starters: traditional and probiotic (*B. bifidum*) culture. The physicochemical properties, the count of viable bacterial starter culture, and the organoleptic evaluation for all yoghurt types were evaluated after storage 24 h at 4 °C. Moreover, the fermentation time was monitored. The incorporation of both β-glucan and EPS in yoghurt resulted in the highest viscosity (13.7 mPa.s) and WHC (55.94%), besides to the lowest syneresis (28.47%). The acidity and pH of the yoghurt samples were significantly affected ($p > 0.05$) by the β-glucan addition. The yoghurt type *Bifidobacterium* glucan had the shortest fermentation time (215 min), and the maximum viability of both *B. bifidum* (7.63 Log CFU g^{-1}) and *L. bulgaricus* (7.50 Log CFU g^{-1}). The β-glucan had a pronounced effect on the overall acceptability of yoghurt more than the EPS. In conclusion, enriching the low-fat yoghurt with oat β-glucan and EPS-producing *B. bifidum* is the highest effective method for improving the yoghurt's quality and the viability of probiotics.

Key words: acceptability, *B. bifidum*, low-fat, oat β-glucan, quality, viability, yoghurt.

INTRODUCTION

The low-fat yoghurt is one of the yoghurts variety produced from skim milk, which contains milkfat (0.5–2%) (Mbaeyi-Nwaoha & Iwezor-Godwin, 2015). Reducing the fat content in yoghurt performs defects in its viscosity and syneresis, therefore decreasing the quality and the acceptability of low-fat yoghurt (Tamime & Robinson, 1999). Katsiari & Voutsinas (1994) discussed the health disorders caused by excessive dietary

fat intakes such as cardiovascular diseases, obesity, and certain forms of cancer and diabetes. In developed countries, consumers began to look for low-fat yoghurt in the market. However, the consumer's demand the low-fat yoghurt with the same quality of the full-fat yoghurt (Jirdehi et at., 2013). The producers began to assess different additives for improving the quality of low-fat yoghurts such as gelatin, *k*-carrageenan, inulin, pectin, dietary fibres, and other hydrocolloids (Sahan et al., 2008).

Recently, oat β-glucan is considered one of the effective hydrocolloid food additives. The β-glucan possesses different properties such as fat-replacer, stabilizer, and thickener. These properties aid in improving the water-holding capacity, viscosity, and body texture of low-fat yoghurt (Havrlentová et al., 2011; Ibrahim & Selezneva, 2017). Vasiljevic et al. (2007) studied the effect of oat β-glucan on the growth and the metabolic activity of probiotic bacteria in yoghurt; their study was concluded that the addition of β-glucan improves the probiotic viability and stability. The synbiotic effect of β-glucan (prebiotic) on protecting the viability of probiotics was explained by Ladjevardi et al. (2016). The producers and researchers were interested in the β-glucan because it was declared as a functional and bio-active food additive (El Khoury et al., 2012; Aboushanab et al., 2019; Dubrovskii et al., 2019). In 1997, the US Food and Drug Administration (FDA) approved a health claim for the use of oat-based foods for lowering the risk of heart disease and passed a unique ruling that allowed oat bran to be registered as the first cholesterol-reducing food at a dosage of 3 g β-glucan per day, with a recommendation of 0.75 g of β-glucan per serving (Guleria et al., 2015).

Exopolysaccharides (EPS) are either homopolysaccharide polymers or heteropolysaccharides produced by plants, fungi, algae, and bacteria. Among the various EPSs producers are the lactic acid-producing bacteria (LAB) and probiotics (Sanalibaba & Cakmak, 2016). In food industries, the incorporation of *Bifidobacterium* in the process of fermented dairy products has gained special attention because of their ability to produce a huge quantity of EPS. The EPS produced by *Bifidobacterium spp.* are used as viscosifiers, stabilizers, emulsifiers, gelling agents, thickeners, and water-binding agents to modify the rheological properties and texture of fermented milk products. In current years, the EPS produced by *Bifidobacterium* can be considered as natural additives, which are preferred by some consumers over stabilizers of plant or animal origin (Audy et al., 2010; Lal et al., 2019).

The addition of EPS to food products increase their health benefits. Polak-Berecka et al. (2013) discussed the beneficial effects of EPS on human health, such as cholesterol-lowering ability, immunomodulating, antitumoral activities, and prebiotic effect. Moreover, the probiotics, producers of EPS, possess special properties that augment the health benefits of dairy products. These probiotic's properties are anti-pathogenicity, anti-diabetic, anti-inflammatory, anti-obesity, anti-cancer, anti-allergy, and angiogenic activities (Kerry et al., 2018).

The previous researches (Cartasev & Rudic, 2017; Elsanhoty & Ramadan, 2018) didn't determine the most effective food additive for enhancing the properties of low-fat yoghurt and increasing the viability of LAB and probiotic bacteria. Therefore, the objective of this research is to study the effect of oat β-glucan addition or EPS-producing *Bifidobacterium bifidum* utilization or both on the physical properties of low-fat yoghurt, its fermentation time, and its organoleptic criteria. Inspection of the potential effect of oat β-glucan and EPS on the viability of *L. bulgaricus* and *B. bifidum*.

MATERIALS AND METHODS

Materials

Low-fat standardized, homogenized, ultra-pasteurized milk was manufactured by the company 'House in the village' (LLC 'WBD,' Moscow, Russia) according to the Russian standard 'GOST 31450–2013' and was bought from commercial markets in Saint-Petersburg, Russia. The milk ingredients were mentioned on the package as the following: fat 1.5%, protein 3.0%, and carbohydrate 4.7%.

Food grade standard oat β-glucan extract was produced by the company Hangzhou Johncan Mushroom bio-technology Co., Ltd (Hangzhou, China). Its β-glucan content (86%) was indicated on the package.

Two types of lyophilized bacterial starter cultures named 'Yogurtel' were selected for yoghurt preparation. One of them is a traditional yoghurt starter culture *'Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus'* and the other one is a probiotic starter culture containing *'Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Bifidobacterium bifidum'*, both of them were produced by LLC Laktinal Company (Moscow, Russia).

Yogurt preparation

The processing of yoghurt explained by Aboushanab et al. (2018) began with milk mixture preparation, as presented in Fig. 1. Two types of milk mixtures were prepared, one with oat β-glucan addition by 0.15% and the other one without oat β-glucan. The addition of β-glucan must be gradually following by heating up to 60 °C with continuous mixing on a magnetic stirrer to aid the dissolving of β-glucan.

Figure 1. The technology of yoghurt milk preparation.

At the temperature of fermentation (40–42 °C), each type of milk mixture was inoculated by two types of starter cultures with a dose of 0.5 g L^{-1}. The first starter culture was the traditional culture, and the second one was the probiotic culture contains *B. bifidum*. The inoculation process was carried out under a sterile condition of microbiological laminar flow (BAVP-01-Laminar-S-1.2, Lamsystem Company, Russia).

After the inoculation, the milk mixture was appropriately mixed and incubated directly in a thermostable incubator (CLN32, POL-EKO lab, Poland) at temperature 40–42 °C for fermentation. The fermentation process was stopped at a specific pH 4.75, and the fermentation time was determined for each type of yoghurt, followed by sudden cooling at 4 °C.

Determination of yoghurt physical criteria, pH and titratable acidity

All the yoghurt measurements were performed after keeping 24 h at 4 °C. The potential of yoghurt for syneresis was determined by weighing 25 g of yoghurt on a filter paper, let it drain for 2 h at 4 °C and weight the separated whey (Sahan et al., 2008; Kaur & Riar, 2019). The syneresis was expressed by %, using the following equation:

$$Syneresis\ \% = \frac{Weight\ of\ whey\ (g)}{Initial\ sample\ weight\ (g)} \times 100 \qquad (1)$$

The water holding capacity (WHC) of yoghurt was proved by centrifuging 5 g yoghurt at 4,500 rpm at 10 °C for 30 min, preceded by weighting the separated whey (Sahan et al., 2008; Ladjevardi et al., 2016). The WHC was calculated by the subsequent formula:

$$WHC\ \% = [1 - \frac{W_t}{W_i}] \times 100 \qquad (2)$$

where, W_t – weight of separated whey by g; W_i – weight of the initial sample by g.

The viscosity was measured by viscometer (Fungilab V100003 Alpha Series L, Fungilab Inc., USA) with AISI 316 stainless steel spindle R3 by rotation speed 100 rpm and expressed by mPa·s. Measurements were made for 1 min at 15 °C (Mårtensson et al., 2001).

The pH analysis was performed by a digital pH-meter (Titrino plus 848, Metrohn, Swiss) supplied by electrode and thermometer, at 4 °C.

Lactic acid, described as titratable acidity, was estimated by titrating 1 g yoghurt mixed with 9 mL of distilled water and few drops of phenolphthalein (0.10%) as an indicator against 0.1 M NaOH till the appearance of the pink colour (Zainoldin & Baba, 2009; Nikoofar et al., 2013; Skripleva & Arseneva, 2015). The acidity was calculated by the following formula:

$$Acidity\ °T = \frac{10 \times V_{NaOH} \times 90 \times 0.1}{W} \qquad (3)$$

where 10 – dilution factor; V_{NaOH} – the volume of NaOH used to neutralize the lactic acid, 0.1 – molar concentration of NaOH; W – the weight of the initial sample by g, 90 – is the molecular weight of lactic acid.

Determination of culture viability

The viability determination of bacterial culture (*Lactobacillus bulgarigus and Bifidobacterium bifidum*) was initiated by diluting 1 mL of yoghurt samples in sterile peptone water (0.10%) using ten-fold serial dilution method. The samples were cultivated and enumerated from a suitable dilution using a pour plate method on MRS agar medium for *L. bulgarigus* (Ibrahim & Carr, 2006) and on MRS-bile agar medium (contains 0.15% bile salt) for *B. bifidum* (MRS-agar, Oxoid Ltd, Hampshire, UK, and Bile salts, Sigma, Reyde, USA) (Sohrabvandi et al., 2012). Both agar media were autoclaved at 121 °C for 15 min with atmospheric pressure 1 bar (15 psi). All the samples were incubated at 37 °C for 72 h under the aerobic condition of Memmert incubator (IN30, Memmert GmbH + Co., Germany) for *L. bulgarigus* and under the anaerobic condition of CO_2 incubator (MCO-18AC, Sanyo, Panasonic, Japan) for *B. bifidum*. The count number of viable bacteria was calculated in term of CFU g^{-1} of yoghurt, meanwhile studied and analyzed in form of Log CFU g^{-1} (Mortazavian et al., 2007).

Organoleptic evaluation

The acceptance of yoghurts was evaluated by 10 trained panel members from professors and PhD students in the department of food biotechnology, their age varies from 25 till 60 years old. The yoghurts were evaluated for their colour, wheying-off, flavour (aroma and taste), consistency (firmness and texture) and overall acceptability

according to the scoring scale (0–10), where 0 is the lowest quality and 10 is the highest quality. The yoghurt samples were served to panellist cold at 4 °C by random order after explanation of aims and procedure of evaluation (Dinkçi et al., 2015).

Statistical analysis

All the experiments were carried out in triplicate. The mean and the standard deviation ($\pm$ SD) of all the results were calculated using the Origin 61 program. The ANOVA-one-way analysis was performed to the results mean using JASP program, version 0.11.10, by comparing the yoghurt types by paring and with establishing the significant difference at ($p \leq 0.05$). The mean of results and their SD were presented graphically and in tables using Excel 2013 program. As the bacterial counts do not have normal distribution, these data were transformed to logarithmic scale.

RESULTS AND DISCUSSION

Physicochemical properties and fermentation time of yoghurt

The yoghurt properties were described for the 4 types, yoghurt with traditional culture and without β-glucan (TW), yoghurt with *B. bifidum* and without β-glucan (BW), yoghurt with traditional culture and β-glucan (TG), and yoghurt with *B. bifidum* and β-glucan (BG).

The maximum decrease of syneresis ($28.47 \pm 1.03\%$) and the maximum increase of WHC ($55.94 \pm 1.74\%$) were in yoghurt prepared with *B. bifidum* and β-glucan (Fig. 2). The β-glucan addition led to a significantly different ($p < 0.05$) decrease in the syneresis of low-fat yoghurt and to a significantly different ($p < 0.05$) increase in its WHC more than the addition of *B. bifidum* by 7.76% and 7.86% respectively (Fig. 2).

This strong effect of β-glucan proves its ability to bind with the whey of yoghurt and prevent its weeping on the surface (Kaur & Riar, 2019). Cartasev & Rudic (2017) confirmed that the *B. bifidum* is one of the lactic acid-producing exopolysaccharides (EPS) which affect significantly ($p < 0.05$) the syneresis and the WHC of low-fat yoghurt but it is less effective than β-glucan addition, explained by Ladjevardi et al. (2016).

	TW	BW	TG	BG
syneresis	0.46	0.41	0.33	0.28
WHC	0.38	0.43	0.50	0.56

Types of yogurt

Figure 2. The effect of β-glucan and *B. bifidum* addition on the syneresis and the water holding capacity (WHC) of low-fat yoghurt. The columns are the mean of three observations and the black vertical bars are the standard deviations. The different large letters (A, B, C, D) and the different small ones (a, b, c, d) represent the significant ($p < 0.05$) difference in syneresis and WHC between the different types of yoghurt, respectively.

The definition of syneresis means the shrinkage of the gel, which then causes whey separation, and the WHC means the amount of whey bind to the yoghurt curd (Bahrami
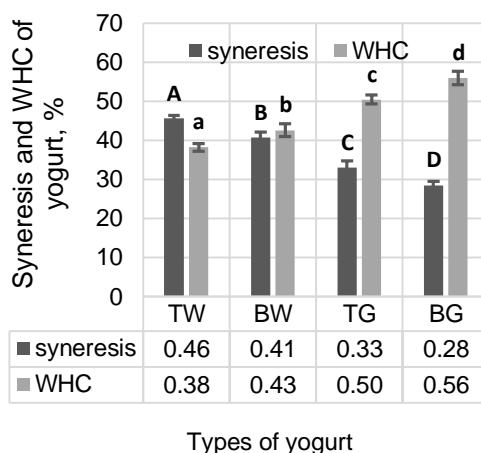
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et al., 2013). These definitions prove the inverse correlation between the results of syneresis and the WHC in yoghurts.

The viscosity of low-fat yoghurt improved with the addition of oat β-glucan and with the presence of *B. bifidum* producing EPS. The addition of both β-glucan and exopolysaccharides to the low- fat yoghurt affected significantly ($p < 0.05$) to its viscosity. The viscosity of low-fat yoghurt increased by 3.8 mPa.s after the β-glucan addition only and by 1.6 mPa.s after the addition of *B. bifidum* producing EPS (Fig. 3, A). This observation agrees with Vasiljevic et al. (2007), who explained the hydrocolloid effect of β-glucan and its interaction with the curd of low-fat yoghurt. Furthermore, the β-glucan has the properties of stabilizer and fat replacer, which aid in increasing the viscosity of yoghurt (Jirdehi et al., 2013). It was reported that the presence of EPS produced by *B. bifidum* helps in enhancing the viscosity of control low-fat yoghurt (TW) (El-Sayed, 2005). The symbiotic relationship between the β-glucan and *B. bifidum* was discussed by Arena et al. (2017). This relationship aids in increasing the production of EPS and in further enhancing the viscosity of low-fat yoghurt.

The enrichment of low-fat yoghurt with oat β-glucan had a significant difference ($p < 0.05$) on its pH and titratable acidity after storage 24 h at 4 °C. The pH and the titratable acidity changed from (4.73 ± 0.012 and 94.3 ± 0.78, respectively) in TW yoghurt to (4.66 ± 0.012 and 102 ± 1.10, respectively) in TG yoghurt (Fig. 3, B, C). In contrast, the presence of ESP produced by *B. bifidum* in low-fat yoghurt affected non-significantly ($p > 0.05$) on its pH and titratable acidity after storage 24 h at 4 °C. The results of Fig. 3, B, C illustrate the changes of the pH and the acidity of low-fat yoghurt from (4.73 ± 0.012 and 94.3 ± 0.78, respectively) in TW yoghurt to (4.71 ± 0.015 and 95.7 ± 1.15, respectively) in BW yoghurt. The BG yoghurt had the highest decrease in pH (4.61 ± 0.015) and the maximum increase in the titratable acidity (105.1 ± 1.11; Fig. 3, B, C).

The increase of the acidity in low-fat yoghurt enriched with β-glucan could be due to the β-glucan effect in increasing the production of acetic and propionic acids during fermentation ((Kaur & Riar, 2019). The same changes of pH and titratable acidity in yoghurt containing β-glucan were observed by Dello Staffolo et al. (2004) compared to the pH and the titratable acidity results of control yoghurt. Ladjevardi et al. (2016) explained the symbiotic effect of prebiotic (β-glucan) in the presence of *Bifidobacterium* had a significant effect ($p < 0.05$) on decreasing the pH and increasing the acidity of low-fat yoghurt. The only presence of EPS-producing *B. bifidum* in low-fat yoghurt had a minimum effect on its pH and acidity (El-Sayed, 2005). The same evidence was indicated by Vinderola et al. (2000), who observed no significant changes in the pH of probiotic yoghurt made with *B. bifidum*.

The addition of β-glucan to low-fat yoghurt affected significantly ($p < 0.05$) on its fermentation time, in contrast to the presence of EPS-producing *B. bifidum*, which had no significant effect ($p > 0.05$). The BG yoghurt had the shortest fermentation time (215 ± 8.89 min; Fig. 3, D). The insertion of β-glucan and EPS together in the medium helped in shortening the fermentation time by 34 min, comparing to TW yoghurt (Fig. 3, D). Singh et al. (2012) explained this shortening by the prebiotic effect of β-glucan in accelerating the initial onset of yoghurt formation. The study of Schmidt et al. (2016) concluded that the presence of EPS-producing *B. bifidum* produced by the lactic acid bacteria in yoghurt doesn't affect its fermentation rate.
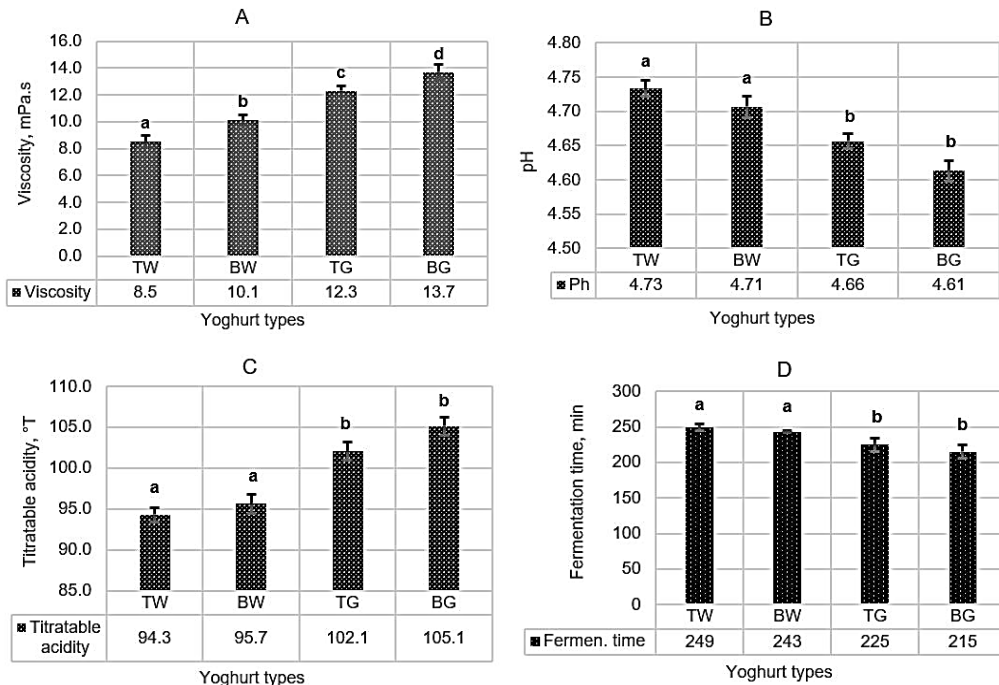
Figure 3. Changes in A. viscosity; B. titratable acidity; C. pH and D. fermentation time of low-fat yoghurt with the addition of the β-glucan and the *B. bifidum* producing EPS. The columns are the mean of three observations and the black vertical bars are the standard deviations. The different letters (a, b, c, d) represent the significance ($p < 0.05$) difference of each character in the different types of yoghurt.

Bacterial viability

As mentioned previously that the oat β-glucan influenced the fermentation time of yoghurt by its shortening, but consequently, it affected the count of viable bacteria (*L. bulgaricus* and *B. bifidum*).

The BG yoghurt had the highest count for the L. bulgaricus (7.50 ± 0.006 Log CFU g^{-1}) and for the B. bifidum (7.63 ± 0.005 Log CFU g^{-1}) comparing to their count in TW and BW yoghurts (Fig. 4). This increment manifests the significant effect ($p < 0.05$) of β-glucan addition on the culture viability. It was explained that the addition of β-glucan protects the Bifidobacterium and the Lactobacillus from the stress conditions such as the low-temperature and the high acidity
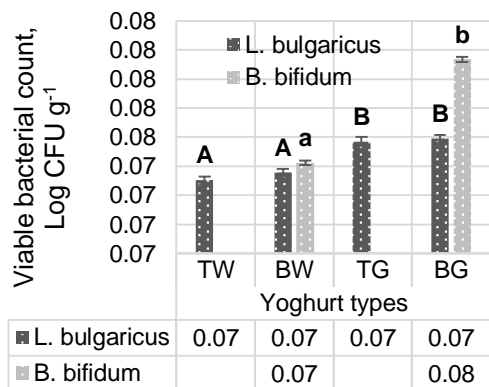

Figure 4. The changes in bacterial culture count with the addition of β-glucan and EPS produced by B. bifidum. The different large letter (A, B) and different small letters (a, b) represent the significant ($p < 0.05$) difference in the viability of L. bulgarigus and B. bifidum respectively, in the different types of yoghurt.

(Rosburg et al., 2010). The β-glucan improved the viability of *B. bifidum* more than the viability of *L. bulgaricus* by 0.12 Log CFU g^{-1} (Fig. 4). The reason for this increment is the synbiotic effect of the prebiotics (β-glucan) on the probiotics, which encourages the growth and the survival of probiotic bacteria (Elsanhoty & Ramadan, 2018).

The production of EPS-producing *B. bifidum* by the *B. bifidum* had no significant difference ($p > 0.05$) on the count of viable *L. bulgaricus*. The previous research works described the restricted effect of EPS on protecting the bacterial starter culture from the harsh external environmental condition during the storage of yoghurt (Kumar Singha, 2012; Cartasev & Rudic, 2017).

Organoleptic characteristics of yoghurt

The ANOVA analysis showed statistically significant effect ($p = 0.0149$) of trial variants. The enrichment of low-fat yoghurt with the β-glucan (TG) or the EPS-producing *B. bifidum* (BW) or both (BG) improved significantly ($p < 0.05$) its wheying-off and its consistency compared to the TW yoghurt. The effect of β-glucan is stronger than the effect of EPS-producing *B. bifidum* on improving the yoghurt wheying-off and consistency (Table 1). Also Elsanhoty & Ramadan, (2018) found in their study, that the enrichment of low-fat yoghurt with β-glucan expressed the greatest changes in texture, appearance, and acceptability of yoghurt, and Han et al. (2016) that the preparation of yoghurt using EPS-producing LAB affects strongly its texture and body firmness.

In our study, the colour and the flavour of low-fat yoghurt were not significantly ($p > 0.05$) affected by the incorporation of β-glucan and EPS-producing *B. bifidum* into the low-fat yoghurt (Table 1). The similar results were reported by Patel et al. (2012), who noticed that polysaccharides and dietary fibres could improve the quality of yoghurt, while not affecting its colour, flavour, and odour.

Table 1. Effect of adding β-glucan and ESP-producing *B. bifidum* on the organoleptic analysis of low-fat yoghurt (mean ± SD)

Yoghurt types	Colour	Wheying-off	Flavour[*]	Consistency[**]	Overall acceptance
TW	8.0[a] ± 0.21	5.0[a] ± 0.40	8.0[a] ± 0.31	4.7[a] ± 0.21	6.4[a] ± 0.21
BW	7.8[a] ± 0.15	6.3[b] ± 0.36	8.1[a] ± 0.23	5.9[b] ± 0.15	7.0[b] ± 0.15
TG	8.0[a] ± 0.20	8.5[c] ± 0.30	8.0[a] ± 0.15	7.9[c] ± 0.21	8.1[c] ± 0.17
BG	8.1[a] ± 0.10	9.2[d] ± 0.25	8.0[a] ± 0.10	9.0[d] ± 0.31	8.6[d] ± 0.15

[*]Flavour includes aroma and taste of yoghurt. [**]Consistency includes firmness and texture of yoghurt. [a, b, c, d] Mean values in the same column with different letters are significantly ($p < 0.05$) different.

The yoghurt type *Bifidobacterium* glucan (BG) had the highest overall acceptance by evaluators. The overall acceptability of yoghurt was significantly ($p < 0.05$) affected by the β-glucan and the EPS-producing *B. bifidum* incorporation and the maximum acceptability (8.6 ± 0.15) was in BG yoghurt.

CONCLUSIONS

The results of the present work confirmed the effect of oat β-glucan and EPS-producing *B. bifidum* on enhancing the quality of low-fat yoghurt and the survival of probiotic bacteria. The incorporation of both β-glucan and EPS in BG low-fat yoghurt

helped to maximize the viscosity and the water-holding capacity, besides to minimize the syneresis defect of yoghurt, compared to the control sample (TW). Fortification of yoghurt with oat β-glucan affected significantly ($p < 0.05$) its titratable acidity and pH after storage 24 h at 4 °C. In contrast, the production of EPS by the *B. bifidum* had no marked influence on the pH and the acidity of low-fat yoghurt. The yoghurt prepared with the oat β-glucan and EPS-producing *B. bifidum* showed the shortest fermentation time, compared to the other types of yoghurt. The survival of *L. bulgaricus* and *B. bifidum* modified considerably in the presence of oat β-glucan. The β-glucan had a prominent effect in increasing the viability of *B. bifidum* more than the viability of *L. bulgaricus*, which proves the synbiotic correlation between the prebiotics (β-glucan) and the probiotics (*B. bifidum*). The involvement of β-glucan into the low-fat yoghurt, which contains EPS-producing *B. bifidum*, boosted its overall acceptability. The addition of β-glucan had a pronounced effect on the wheying-off and the consistency of yoghurt more than the EPS. Therefore, enriching the low-fat yoghurt with oat β-glucan and EPS-producing *Bifidobacterium bifidum* is the highest effective method to improve the physical and the organoleptic properties of yoghurt, to enhance the viability of probiotics.

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Effect of Different Sugar Sources on *P. rhodozyma* Y1654 Growth and Astaxanthin Production

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Abstract. *Phaffia rhodozyma* (also known as *Xanthophyllomyces dendrorhous*) is one of the most promising natural sources of commercial astaxanthin. It has high growth rates, easy cultivation conditions and able to utilize different carbon substrates. This provides an opportunity to further lower production cost by using industrial waste such as molasses. This research therefore evaluates the growth dynamic and astaxanthin production of *P. rhodozyma* Y1654 growing on soy and sugar beet molasses-based media. Liquid growth media based on soy molasses (SM), sugar beet molasses (SBM) and glucose (control) as main sugar source with peptone and yeast extract supplementation were inoculated with 48 h old seed culture (grown in standard glucose media: 2.0% glucose, 1.0% peptone, 0.2% yeast extract) and incubated at 20 °C with stirring speed of 180 rpm for 7 days. Samples were taken daily throughout the study period to assess; cell count, dry cell weight (DCW) and amount of astaxanthin. Soy molasses-based media resulted in the highest biomass yield (7.7 g L^{-1}) followed by SBM (5.8 g L^{-1}). Generally, more than 90% of initial fermentable sugar was consumed at the end of the study. However, about 40% of total sugar in SM was unassimilable by *P. rhodozyma* Y1654. The highest astaxanthin yield was observed in the control media (77 µg g^{-1} of DCW). Cultivation of *P. rhodozyma* Y1654 in SBM resulted in as much as twice (32.8 µg g^{-1} of DCW) the astaxanthin yield of SM (12.4 µg g^{-1} DCW). Molasses-based media are good for growth of *P. rhodozyma* Y1654 but for astaxanthin production, they need further optimization.

Key words: astaxanthin, carotenoids, molasses, *Xanthophyllomyces dendrorhous*, agric-food wastes, byproducts, microbial pigment.

INTRODUCTION

Astaxanthin (3,3'-dihydroxy-β,β'-carotene-4,4'-dione) is a red-orange pigment belonging to the group of oxygenated carotenoids, the xanthophylls. Mainly found in marine organisms, it has been an outstanding colorant with immense importance in the aquaculture feed industry (Fakhri et al., 2018). Over the past two decades, there has been an increased interest in astaxanthin as a potent and promising agent in human health and nutrition due to the biological activities it exhibit, most notably its overwhelming

antioxidant activity (Fang et al., 2019). Its extended structure, with polar regions at each end and a nonpolar middle section, allows for optimal orientation in the lipid bilayer of cell membranes, offering protection against oxidative assaults with its conjugated double bonds (Satoh, 2016). Aside its prominent antioxidant activity, it has been reported to exhibit anti-diabetic, anti-inflammatory, anti-cancer, neuroprotective, cardioprotective and immune-modulative activities. It has also been said to improve skin and ocular health, as well as fertility and reproduction (Fakhri et al., 2018). As a result, it is widely used in the pharmaceutical, nutraceutical and cosmetics industries, in addition to its age-old use as a pigment in aquaculture. It is currently regarded as the second most important carotenoid in the global market, after β-carotene, with a global market size exceeding $ 600 million in 2018 and a projected value in billions by 2026 (Ahuja & Rawat, 2019).

Astaxanthin can be obtained from both natural sources and chemical synthesis, with the latter being the major source representing about 97% of commercially available astaxanthin (Schmidt et al., 2011). The growing demand for products with natural additives due to rising concerns about the harmful and potentially harmful effects of chemical additives coupled with the fact that synthetic astaxanthin has lower biological activity, has led to a wide exploitation of natural sources of astaxanthin with potential for industrialization (Higuera-Ciapara et al., 2006; Stachowiak, 2012). Next to *H. pluvialis,* the red yeast *P. rhodozyma* is one of the most promising sources of natural astaxanthin for the commercial market, capable of biosynthesizing astaxanthin in quantities up to 84% of its total carotenoid composition output (Stachowiak, 2014; Stoklosa et al., 2018). Although astaxanthin yield from *P. rhodozyma* is lower compared to *H. pluvialis*, the former is preferred due to higher growth rates and easier cultivation conditions that might decrease the production time at industrial scale (Amado & Vazquez, 2015). Despite this, natural astaxanthin is unable to compete with synthetic astaxanthin due to its high cost of production. Consequently, many studies have set out to find ways to improve natural astaxanthin production at low cost by searching for hyper-producing strains, optimizing growth conditions and of much interest, finding low-cost media alternatives. There has therefore been heightened interest in the use of low-cost raw materials primarily byproducts of the agro-food industry for the microbial production of astaxanthin. The use of waste/byproducts from the agriculture and food industries is of immense interest because it does not only provide a way to lower production cost of astaxanthin, it as well offers an opportunity to minimize environmental and energetic problems related to the disposal of these wastes (Frengova & Beshkova, 2009). Stimulatingly, *P. rhodozyma* may utilize different carbon substrates: glucose, maltose, sucrose, cellobiose, xylose, arabinose, lactose and many other (Stachowiak, 2014). Due to this ability, *P. rhodozyma* offers the possibility to increase production and lower production cost of natural astaxanthin by the use of new culture medium especially natural complex media based on plant extracts or wastes produced by the agri-food industry (Stoklosa et al., 2018).

To meet the food and nutritional needs of the ever-growing population of the world, the food and agriculture industries have increased their production capacities. This, consequently, has led to the generation of a significant amount of waste which constitute a huge environmental problem in terms of their disposal. Currently, most of these wastes are either incinerated, dumped on landfills, used as animal feed or feedstock for the production of bioenergy (Spalvins & Blumberga, 2019; Tamelová et al., 2018). There is, however, a rising interest in valorization of these wastes into value-added products

including enzymes, single cell oil, enzyme inhibitors, among others (Gruduls et al., 2018; Eveleva et al., 2019; Spalvins & Blumberga, 2019; Vybornova et al., 2019). Prior researches have demonstrated the use of various agricultural feedstock and industrial byproducts for the cultivation of *P. rhodozyma* including sweet sorghum Juice (Stoklosa et al., 2018), corn steep liquor (Urnau et al., 2019), residual pineapple juice (Jirasripongpun et al., 2008), *Yucca fillifera* date juice (Luna-Flores et al., 2010), corn fiber hydrolysate (Nghiem et al., 2009), residual coconut milk (Dominguez-Bocanegra et al., 2007), Jerusalem artichoke (Jiang et al., 2017), hydrolysate of barley straw and sugarcane bagasse (Montanti et al., 2011), mussel processing wastewater (Amado & Vazquez, 2015), cane molasses (Haard, 1988) etc. Nevertheless, little to no information is available regarding exploitation of soy or sugar beet molasses in producing astaxanthin.

In Russia, molasses of soy and sugar beet represent a considerable amount of industrial byproducts generated from the soya processing and sugar industries respectively. Generally, sugar beet molasses contains up to 80% total solids (TS) with > 50% of this representing total sugar (mainly sucrose – 60% of TS; glucose and fructose – < 1% of TS), in addition to nitrogenous compounds (> 2% of TS), minerals and vitamins (Acan et al., 2020; Scoma et al., 2016; Sjölin et al., 2020). On the other hand, soy molasses usually contains not less than 30% (w/w) of total sugar, majority (61%) of which represents carbohydrates. A considerable amount (27% of TS) of this represents raffinose-family oligosaccharides (stachyose – 15% of TS; raffinose – 12% of TS) with just a little over 30% of TS being fermentable sugars (mainly sucrose – 26% of TS; glucose – 4.6% of TS; fructose – 2.9% of TS). Soy molasses as well contains substantial amounts of lipids (15.6% of TS), proteins (6.4% of TS) and some minerals (Romão et al., 2012; Wang et al., 2019). Despite the high sugar content and rich composition of sugar beet and soy molasses, they are primarily used just as animal feed in this part of the world, without any added value. Nevertheless, the use of molasses as an inexpensive fermentation feedstock for production of yeast biomass, biofuels, organic acids, vitamins enzymes, etc. has widely reported. It is of this accord and interest that this study explored soy and sugar beet molasses as cheap carbon sources for the growth of *P. rhodozyma* and production of astaxanthin. In this study, the P. rhodozyma growth and astaxanthin production was evaluated in media of soy or sugar beet molasses supplemented with peptone and yeast extract.

MATERIALS AND METHODS

Microorganism
Lyophilized cells of *Phaffia rhodozyma* Y1654 were purchased from National Bioresource Center, All-Russian Collection of Industrial Microorganisms, Research Institute of Genetics and Selection of Industrial Microorganisms, Moscow, Russian Federation. Cells were revived and grown on solid Yeast Extract–Peptone–Glucose (YPG) media (containing per liter, 20 g glucose, 10 g peptone, 2 g yeast extract, and 9 g agar) at 20 °C. After 3 days of growth, agar plates were then stored at 4 °C for further use.

Feedstock and Chemicals

Soy Molasses (Mass fraction of dry matter – 75%; Mass fraction of sucrose by direct polarization – 60%; Fats – 5% of dry weight; Proteins – 5% of dry weight; pH – 7.7) was provided by Joint-Stock Company 'Agroproduct', Kaliningrad region, Svetly, Russian Federation, Sugar beet molasses (Total solids – 82%; Sucrose – 47%, Reducing sugars – 0.44%; pH – 7.0) was obtained from Krasnodarsky Sugar Plant, Russian Federation, HPLC-grade solvents were supplied by HIMMED Inc., Russia while rest of solvents used in this study were supplied by EKOS-1 Inc., Russia.

Preparation of seed culture

Prior to preparation of seed culture, cells were sub-cultured on fresh solid media. Two loopful of yeast cell from a fresh culture on agar plate was suspended in 5 mL of sterile distilled water. Following aseptic procedures 4 mL of the suspension was transferred into a 250 mL Erlenmeyer flask containing 200 mL of YPG broth (containing per liter, 2.0% glucose, 1.0% peptone, 0.2% yeast extract), and incubated at 20°C and shaking speed of 180 rpm, for 48 h under constant illumination in an incubator equipped with an orbital shaker (Yihder Orbital Shaking incubator LM 570RD).

Inoculation and Cultivation

Fresh sterilized culture media (200 mL) in 500 mL Erlenmeyer flask was inoculated with the seed culture at 10% v/v and incubated in the same conditions as the seed culture for 168 h. All experiments were carried out in triplicates. Three different media based on the source of sugar were used: Yeast Extract–Peptone–Glucose (YPG) medium as control, Soy molasses-based medium (SM) and Sugar beet molasses-based medium (SBM). Each growth medium contained per liter 2.0% sugar, 1.0% peptone, 0.2% yeast extract.

Cell Yield Determination

Cell density was determined by counting with the aid of a Neubauer chamber under a light microscope (Altami Bio 8). For dry cell weight (DCW) determination, 5 mL samples of culture were measured into pre-weighed (W1) 15 mL centrifuge tubes and centrifuged for 10 min at $4,000 \times g$. The supernatant was reserved for measurement of sugar content while the cell pellet was washed twice with 5 mL of distilled water. The biomass after washing was dried together with the container at 50 °C until a constant weight (W2) was obtained when measure. The DCW was quantified as weight difference between W2 and W1.

Determination of Sugar Content

Sugar content was determined using the phenol-sulfuric acid method as described by Nielsen (2010) with some modifications. Molasses were diluted according to the ratio 1:5,000 while for supernatants a ratio ranging from 1:1,000 (on the first day) to 1:50 (on the last day) was used. Absorbance was measured at 487 nm (the Abs_{max} observed in preliminary studies) with a Shimadzu 1800-UV spectrophotometer. The initial sugar concentration of each media was as well measured.

Extraction of Astaxanthin

For the determination of astaxanthin content, 10 mL aliquot of culture was collected and centrifuged at $4,000 \times g$ for 10 min to separate cells. The supernatant was discarded, and cell pellet washed twice with 5 mL of distilled and then stored in the freezer until needed. Cell disruption was carried out as described by Cheng and Yang (2016) with some modifications. The frozen cells were thawed and suspended in 2 mL of DMSO then subject to an ultrasonic bath (Bandelin Sonorex Digitec DT 31 H) at 35 °C for 10 min. Subsequently, 5 mL of petroleum ether and 1 mL of 20% w/v NaCl were added then extracted for 10 min with frequent rigorous shaking. The resultant was then centrifuged at $3,500 \times g$ for 5 min to separate the petroleum ether containing pigment. Petroleum ether was evaporated on a rotary evaporator (Heidolph Hei-VAP) at 35 °C and astaxanthin re-dissolved in 1 mL HPLC-grade acetonitrile and then quantified by HPLC as described as below.

Quantification of Astaxanthin

Extract (100 µL) was manually injected into the HPLC system (Shimadzu LC-20AD HPLC device equipped with a Shimadzu SPD-20AUV/Vis detector). Separation was done on a PerfectSil® Target ODS-3 HD 5µm 150×4.6 mm column with isocratic flow, 95% acetonitrile as the mobile phase at rate of 0.8 mL·min^{-1}. Astaxanthin was detected and measured at 474 nm. Quantification was done using an astaxanthin (synthetic) standard calibration curve.

Statistical Analysis

All experiments were conducted in triplicates. Experimental results are expressed as mean values. Data were analyzed by one-way *ANOVA* or *t-test* at a 95% confidence level. A *P-value* < 0.05 was considered statistically significant. Statistical analyses were done using graphPad Prism 8.

RESULTS AND DISCUSSION

Sugar content of molasses

Prior to being used for the preparation of growth medium, the sugar content of both soy and sugar beet molasses were assessed. It was shown that sugar beet molasses had an average sugar content of 53% per gram, > 1.5 times higher than that of soy molasses (29%) (Table 1). It has been reported sugar beet molasses mostly has no less than 48% of total sugar despite various optimization at the industrial level (Duraisam et al., 2017). A large part (> 97%) of this as reported by Scoma et al. (2016) consist of sucrose, a fermentable disaccharide which is easily converted and utilized by several yeasts including *P. rhodozyma*. The remainder comprises simple sugars (glucose and fructose), however minute concentrations of oligo- and polysaccharides (raffinose and starch) have also been reported (Sjölin et al., 2020). In fact, it is largely due to the high residual sugar that sugar beet molasses is considered as alternative cheap sugar/carbon source for thecultivation and production of

Table 1. Sugar content of Molasses

Description	Average Sugar content (% per gram ± SEM)
Soy Molasses	29.30 ± 0.38
Sugar Beet Molasses	53.60 ± 0.40

SEM = Standard Error of Mean.

microbial biomass as well as their associated products. In many parts of Europe, sugar beet molasses is used as a cheap carbon-rich source for bioethanol production (Akbas & Stark, 2016). On the other, the lower total sugar content of soy molasses (compared to sugar beet molasses) plumbs further as more than 40% of this is reported to comprise of raffinose-family oligosaccharides (Romão et al., 2012). The presence of these complex oligosaccharides limits the application of soy molasses in industrial biotechnology as most of industrial microorganisms including yeasts, lactic acid bacteria, etc. cannot utilize these sugars unless they are pre-hydrolyzed to monomer (Yang et al., 2018). Pre-hydrolysis is nonetheless not attractive as it significantly adds to cost of production.

The low fermentable sugars in soy molasses is, however, augmented by its rich lipid content (an alternative source of carbon which represents up to 15% of total solids), proteins (> 6% of total solids) and other components essential for microbial growth (Romão et al., 2012) – making it as well a suitable inexpensive alternative nutrient source for cultivation of microorganisms to produce value-added compounds.

Sugar consumption by *P. rhodozyma* in different media

P. rhodozyma exhibited fairly the same sugar consumption pattern in all three media, with more than 80% of initial fermentable sugar consumed by the first 24 h (Fig. 1). Nevertheless, not all the sugar in media was consumed at the end of the study; the amount of residual sugar in the control media (YPG) was the lowest followed by SBM with SM having the highest amount of residual sugar. A similar but less rapid sugar consumption pattern was reported by Stoklosa et al. (2018) when *P. rhodozyma* was cultivated using sweet sorghum juice (containing mainly sucrose). The authors also observed that increasing yeast extract concentration in addition to nitrogen availability significantly promoted sugar consumption leading to complete utilization of fermentable sugars by 168 h. Approximately 40–43% of the total sugar in soy molasses was left at the end of the study as residual sugar, unassimilable by the yeast. This most-likely comprise of the complex oligosaccharides (like stachyose and raffinose, which reportedly represent more than 25% of total carbohydrates present in soy molasses (Rodrigues et al., 2017). However, available literature indicates that *P. rhodozyma* is able to metabolism oligosaccharides (Stachowiak, 2012) and even polysaccharides (Schmidt et al., 2011). Notwithstanding this, it is evident that the strain of *P. rhodozyma* used in this study is unable to metabolize some of sugars present in soy molasses. Adjusting the total sugar in SM to compensate for the unassimilable sugar (by increasing the amount of molasses added) did not in any way affect the sugar consumption pattern in soy molasses-based media, just that both the total amount of sugar consumed, and the residual sugar were accordingly increased (Fig. 1, B). Thus, to maximize the utilization of sugar in SM it is necessary to pre-hydrolyze complex sugars. This mostly down using acids or enzymes (Romão et al., 2012). All the same, the results show that *P. rhodozyma* is able to effectively utilize the principal sugar (sucrose) which is present in both molasses. *P. rhodozyma* have long to been reported to produce invertase, which gives it the ability to utilize sucrose (Stoklosa et al., 2019). In the presence sucrose, glucose and fructose, *P. rhodozyma* is reported to rapidly degrade sucrose leading to a lag in monomer assimilation by increasing concentration of both glucose and fructose. Nonetheless, *P. rhodozyma* is known to assimilate and metabolize glucose prior to utilization of any other simple sugar; glucose is considered a priority carbon source for these yeasts and its presence especially at high concentrations repress the utilization of

other sugars (Stachowiak, 2012). This probably accounts for the reason SBM has a slightly higher amount of residual sugar (most of which is suspected to be fructose) when compared to YPG (control).
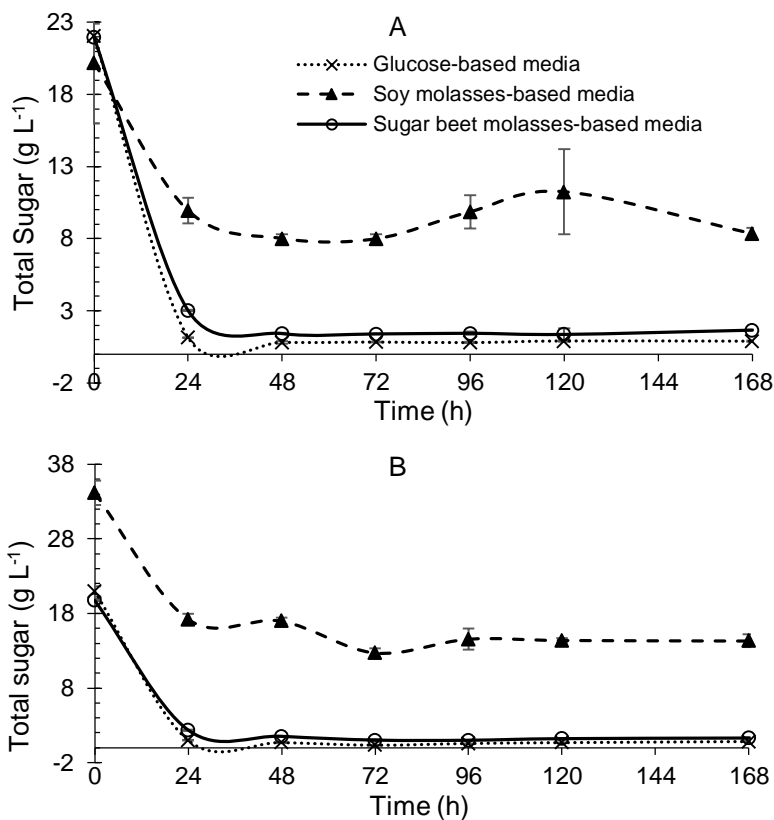

Figure 1. Sugar consumption of different Media (A – when unassimilable sugar was not considered; B – after adjusting sugar concentration in SM).

Effects of different sugar sources on cell growth and biomass production

Given that raw materials for culture media represent from 30 to 70% of bioprocess cost, development of low-cost media, using by-products and residues of agro-industrial origin, for cultivation of microbes have been under intense focus (Villegas-Méndez et al., 2019). As such one of the main objectives of this study was to evaluate how well soy and sugar beet molasses could support the grow of *P. rhodozyma* Y1654. Generally, cells grown on molasses media were somehow larger than those of the control when observed under a microscope. Cell size is an important characteristic that significantly influences nearly all aspects of cellular physiology and thus, a key indicator of overall physiological state of cells. Though under a given condition cells of a given species typically vary less about their mean size, it is well known that extracellular conditions (including nutrient availability) can drastically alter cell size – under nutrient limitation cells tend to have a reduced size and budding yeast produce daughter cells less than 20% of the mother cell size (Turner et al., 2012). In addition, cells grown on molasses media exhibited active budding, even on the last day of the study (Fig. 2).
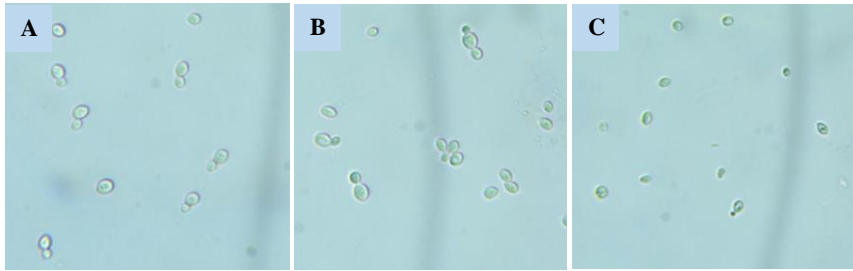
Figure 2. Appearance of cells (400X) at the end of study (A – in soy molasses-based media; B – in sugar beet molasses-based media; C – in glucose based-media).

The presence of digestible nitrogen as well as other essential nutrients like vitamins and minerals in molasses could be one of the major factors accounting for the promotion of cell growth by molasses-based media. Moreover, yeast are known to adjust their growth in response to their nutritional environment, such that depletion of essential nutrients halts cell growth (including proliferation) until nutrients are replenished then cell reenters the cell cycle and begin to proliferate (Gurvich et al., 2017). It could therefore be inferred that the limited budding as seen in the control at the end of the study (Fig. 2, C) is probably as a result of nutrient limitation. In terms of growth profile, a similar growth curve is observed irrespective of the initial sugar source, exponential growth was observed within the first 24 h afterwards only slight changes in cell density was observed (Fig. 3). Nonetheless, the lowest biomass yield was recorded for SM in the first experiment when unassimilable sugar was not considered (Fig. 4, a). Despite this, *ANOVA* analysis of the maximum biomass yield for each media suggested that the difference in biomass among the groups was not statistically significant ($P = 0.167$).

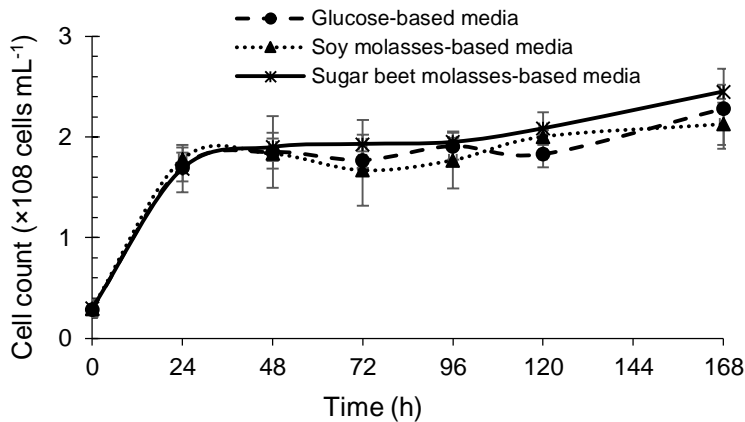**Figure 3.** Kinetic curve of *P. rhodozyma* growth in different media.

However, when the total sugar in SM was adjusted to compensate for the unassimilable sugar, such that the total assimilable sugar in all media where similar, the biomass yield for SM increased significantly ($P = 0.008$) recording the highest biomass yield in the present study (Fig. 4, B). In an earlier study, Jirasripongpun et al. (2007)

similarly recorded increase in biomass production in accordance with increasing molasses concentration in the range of 2–8% v/v. Regardless, SBM always recorded a higher biomass yield than YPG. The highest biomass yield in each case was recorded at the end of 168 h of incubation. Similar observation in biomass accumulation are have been report in other studies, in these studies however, steep rise in biomass (characteristic of the exponential growth phase) is observed after 24 h (Jiang et al., 2017; Stoklosa et al., 2018).
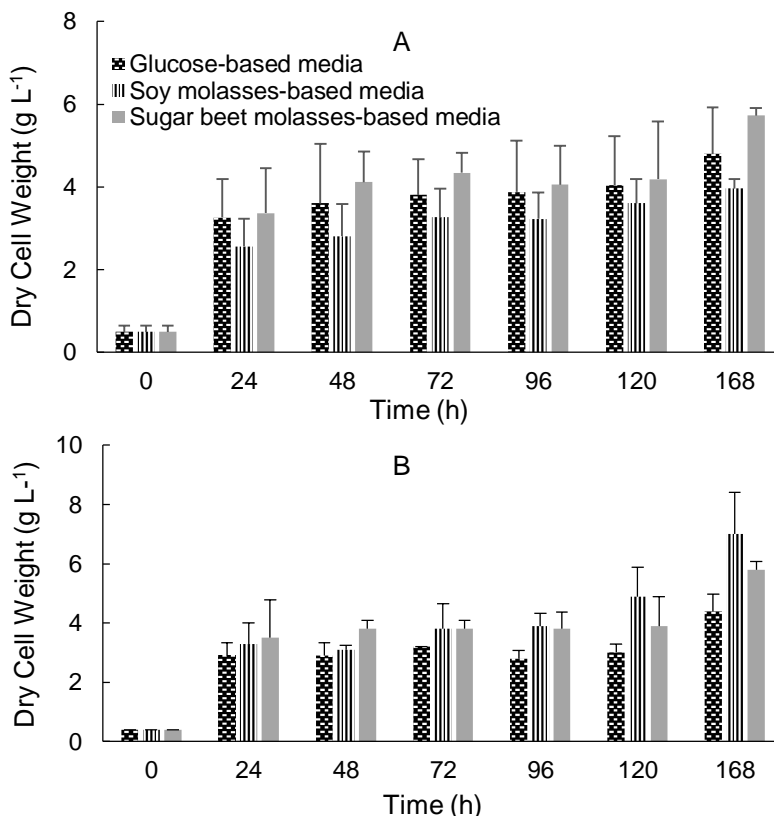
Figure 4. Biomass yield in different media (A – when unassimilable sugar was not considered; B – after compensating for unassimilable sugar in SM).

With regards to specific biomass yield, thus amount of biomass produced (in this case the highest biomass yield for each media) per gram of sugar consumed, SM recorded the highest notwithstanding the fact that in the first experiment the total assimilable sugar in SM lower (just a little over half that in SBM and YPG) while the lowest was observed in YPG (Table 2). When the sugar concentration of SM was increased to compensate for unassimilable sugar a corresponding increase in specific biomass yield was observed. Nitrogen source is one of the indispensable components of a fermentation medium, and the sources as well as concentration affects cell growth and also products produced by these cells (Ni et al., 2007). Although all media were supplemented with peptone and yeast extract, soy molasses is known to contain a substantial amount of nitrogenous substances (up to approximately 9–10% of total solids

(Zhong & Zhao, 2015; Caldeirao et al., 2016)), these nitrogenous sources to some extent might be one of the influencing factors making SM good for biomass production. As observed by Vustin et al. (2004) large amounts of nitrogen in the culture medium or low C/N ratio stimulates active biomass growth. It is therefore plausible assumed that the ratio of C and T is lower in media with molasses media compared to glucose, which is why when cells are grown using media with molasses the biomass content is higher and the cell size is larger. Over the past years, several researchers have evaluated different agro-industrial wastes for cultivation of *P. rhodozyma*. Shake flask cultures in these studies have resulted in varying biomass yields. As much as 4.3 g L^{-1} of biomass was obtained cotton husk hydrolysate, 4.7 g L^{-1} with corncob hydrolysate, and 6.6 g L^{-1} with mesquite pods extract (Villegas-Méndez et al., 2019). Also, 4.9 g L^{-1} biomass yield was obtained with a combination of corn steep liquor, parboiled rice water and glycerol (Urnau et al., 2019). The results of the present study are comparable to those of the above studies. However, much higher biomass yields have also been reported with other agro-industrial byproducts: 17 g L^{-1} with sweet sorghum juice (Stoklosa et al., 2018), 15.6 g L^{-1} with sweet sorghum bagasse hydrolysate (Stoklosa et al., 2019), 12.8 g L^{-1} with barley straw hydrolysate (Montanti et al., 2011). These varying yields in biomass corresponds to the diverse nutritional composition of byproducts from the agrio-food industry. In addition, byproducts from industries come along with different unfavorable compounds, originating from different points during the processing of raw material, these compounds sometimes have growth inhibitory effect on cultivated microbes. In this current study, the chemical composition of molasses was not analyzed to ascertain the presences of possible inhibitors, however as the biomass yield for molasses-based were higher than that of the control it is probable that possible inhibitory compounds were either absent or present in concentration not potent enough to stall growth. Largely, molasses from different sources have demonstrated, in several studies as well as in the present study, to be an excellent and efficient carbon source for large-scale production of various industrial microorganisms. However, the efficiency of use of molasses, like other complex heterogenous industrial waste, for industrial fermentation is highly dependent on intrinsic properties of microbe like nutrient requirements (e.g., flexibility or specificity on carbon source utilization, organic or inorganic nitrogen requirements, mineral and vitamins requirements, etc) (Koutinas et al., 2014; Papizadeh et al., 2020).

Table 2. Summary data for sugar consumption and biomass production

Media	Experiment	Total Sugar (g L^{-1})	Initial Assimilable Sugar (g L^{-1})	Residual Sugar (g L^{-1})	Maximum DCW (g L^{-1})	*P*-value	Specific Biomass Yield (g g^{-1})
YPG	1st	22.063	22.063	0.780	4.800	0.699	0.225
	2nd	20.546	20.546	0.689	4.400		0.222
SM	1st	20.197	12.000	8.130	3.960	**0.008**[*]	0.328
	2nd	34.568	20.000	14.010	7.700		0.375
SBM	1st	21.957	21.957	1.250	5.730	0.797	0.276
	2nd	19.700	19.700	1.194	5.800		0.313

[*]statistically significant at 95% confidence level; 1st – When unassimilable sugar was not considered; 2nd – After adjusting sugar concentration in SM to compensate for unassimilable sugar.

Effect of different sugar sources on astaxanthin production

A typical chromatogram obtained from *P. rhodozyama* extracts is presented in Fig. 5. Seven (7) major peaks were observed, 6 within the span of 20 min and one after 35 min. Since the extraction method used was selective for carotenoids, these major peaks most-likely correspond to various carotenoids or their derivatives. The peak around 9.5–9.8 min was identified to correspond to astaxanthin. As astaxanthin represents more than 80% of total carotenoids in *P. rhodozyma* (Schmidt et al., 2011), it is not surprising that it's corresponding peak is the highest. Though molasses-based media as reported earlier were superior in terms of biomass production, this wasn't the case when astaxanthin production was considered. As reported by Villegas-Méndez et al. (2019), one of the most significant factors affecting the production of carotenoids was the carbon source with a contribution of more than 60%. In the present study, the control (YPG) was supreme in astaxanthin production (reaching a maximum of 0.499 mg L^{-1}) followed by SBM (0.237 mg L^{-1}) then SM (0.059 mg L^{-1}). The maximum accumulation of astaxanthin was recorded on the last day of the experiment (after 168 h) for all media. The accumulation of astaxanthin by cells was time dependent and generally associated with the stationary phase, with a sharp increase after 72 h and reaching a maximum at the end of the study (Fig. 6), typical pattern exhibited by secondary metabolites; this pattern of accumulation of astaxanthin is similar to that reported by de la Fuente et al. (2010) and Amado & Vazquez (2015).

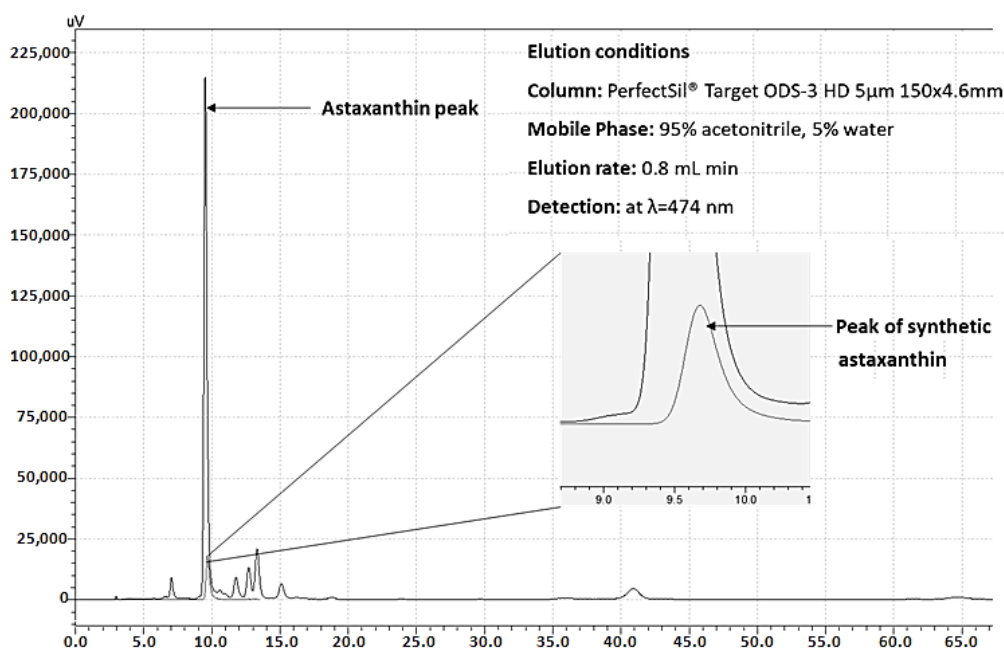**Figure 5.** Typical chromatogram of astaxanthin-containing extracts from *P. rhodozyma.*

Carbon to nitrogen ratio (C/N) is another crucial factor that affect the production of microbial carotenoids. Both high and low nitrogen concentration in media affects carotenogenesis, while in the former excess nitrogen exerts its effect by inhibiting gene expression, the latter impact the output of carotenoids due to nitrogen limitation

(Stoklosa et al., 2019). In *P. rhodozyma* astaxanthin accumulation is reported to be greatly enhanced by higher C/N ratio while lower C/N ratio improves biomass production (Amado & Vazquez, 2015). Pan et al. (2017) reported that increasing C/N ratio up to 76:1 enhanced astaxanthin production, albeit resulting in decreased cellular carotenoid and astaxanthin content. The authors also observed significant alteration in expression of 9 proteins involved in the synthesis of astaxanthin with varying C/N ratio. Generally, C/N ratio < 5 negatively influences the synthesis of carotenoids in *P. rhodozyma* (Vustin et al., 2004; Zhuang et al., 2020). The C/N ratio of the control media was approximately 6:1, however since molasses contain various nitrogenous substance, these in addition to the peptone supplementation increased the total nitrogen in molasses-based hence lowering the C/N ratio and subsequently the production of astaxanthin in this media. Furthermore, at higher carbon and nitrogen loading the oleaginous red yeast *Rhodotorula glutinis* is reported to produce lipids at the expense of astaxanthin. *P. rhodozyma* is known also be oleaginous yeast and can accumulate a large quantity of lipids and as such astaxanthin content is negatively correlated with fatty acid content (Xiao et al., 2015). The presence of alternative carbon sources (like organic acids and lipids) in molasses probably increased the carbon loading in molasse media and led to lipid synthesis at the detriment astaxanthin. It is thus evident from the above that for increased carotenoid production it is imperative to use a proper C/N ratio. Currently, there is a controversy in published literature as to whether accumulation of astaxanthin is growth associated or not, while some authors report that it is growth associated, there are contrary reports that suggest otherwise (Schmidt et al., 2011). In this current study it is difficult to make a conclusion in this regard, while it seems accumulation of astaxanthin is growth-associated considering both maximum astaxanthin was recorded at the same time with maximum biomass of each media, the growth medium that yielded highest biomass (SM) did not correspondingly yield the highest astaxanthin content. Nonetheless, the results observed in this study suggest astaxanthin is associated with the stationary growth phase. In addition, while some researchers like (Stoklosa et al., 2018) have reported that yeast extract supplementation improves both growth and astaxanthin production in *P. rhodozyma*, Ramirez et al. (2000) reported that yeast extract supplementation inhibited astaxanthin synthesis. In the study growth media were supplemented with yeast extract, it is however unclear at this point the effect of yeast extract on growth and astaxanthin production on the strain of *P. rhodozyma* used in this study.
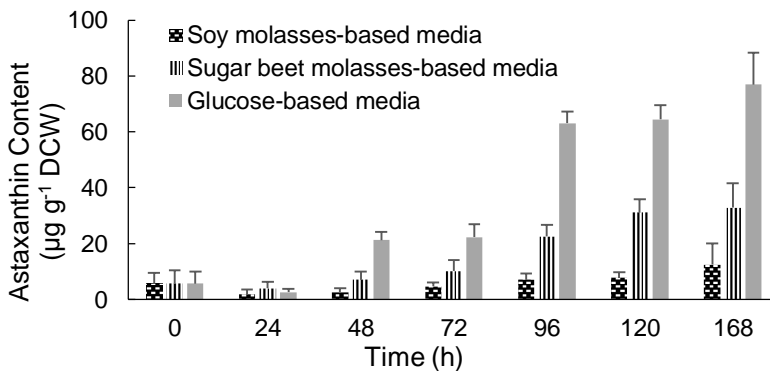

Figure 6. Astaxanthin production in different media.

Increasing the sugar concentration of SM to compensate for unassimilable sugar ultimately led to a decrease in the maximum astaxanthin accumulated by *P. rhodozyma* in SM (from 21.5 µg g^{-1} to 12.4 µg g^{-1} of DCW). Active culture growth during the first 24 h coupled with intense anabolic processes has been reported to inhibits carotenoid synthesis (Xiao et al., 2015). Increasing the sugar concentration in SM resulted in more favorable growth conditions in the initial stages, this increased growth (as evident in the biomass yield) and most likely anabolic process, hence the decrease in astaxanthin synthesis. Molasses come along alone with several compounds some of which hamper carotenoid synthesis. Phenolic for instance have been suggested to inhibit carotenoid synthesis. Results of study by Stoklosa et al. (2019) showed that significant reduction of phenolics by detoxification of sweet sorghum hydrolysate with activated carbon markedly increased biomass and astaxanthin productivity of *P. rhodozyma*. Soy molasses is reputed to be an excellent source of phenolics such as isoflavones while sugar beet molasses likewise is reported to contain substantial amount of phenolic compounds including Gallic acid (Chen et al., 2015; Zhong & Zhao, 2015). The lack of pretreatment of molasses used in the present implies phenolic compounds probably contributed to the low astaxanthin production in molasses-based media. In addition, it has been reported that carotenogenesis, especially with regard to secondary carotenoids like astaxanthin, is associated with stress response (Barredo et al., 2017). Changes in environment of culture media overtime such as depletion of nutrients, accumulation of waste, changes in pH etc., most likely are the corresponding stresses during the stationary phase that promote astaxanthin synthesis. Though changes in pH of media during the course fermentation was not monitored in this present study, it is generally known that pH of media during fermentation changes. While results of studies by Urmau et al. (2019) showed that pH of media increases over the time of fermentation, Stoklosa et al. (2018) generally recorded a lower final pH of media in contrast to pH of media before fermentation in shake flask cultures but a higher final pH in bioreactor experiments. In cultivation of *P. rhodozyma* pH is known to affects cell growth and astaxanthin production differently for each strain. Stoklosa et al. (2018) observed higher astaxanthin was obtained in medium with lower final pH at the end of cultivation. Similarly, higher concentration of astaxanthin was obtained by lowering pH of medium during fermentation (Schewe et al., 2017). These results show that pH is a significant stress that affect astaxanthin production and the lack of pH monitoring and control in the present study represents a major limitation. By and large, like biomass yield, amount of astaxanthin obtained with agric-food wastes is variable. While up to 2.07 mg g^{-1} of astaxanthin was obtained using sorghum bagasse hydrolysate (Stoklosa et al., 2019), a maximum of 293 µg g^{-1} was obtained with mesquite pods extract even after optimization (Villegas-Méndez et al., 2019). Montanti et al. (2011) also obtained astaxanthin yield of 0.36 mg g^{-1} and 0.23 mg g^{-1} with sugarcane bagasse and barley straw hydrolysate respectively. Even though these yields are several folds higher than that obtained in the present study, it is important to note that the cultivation conditions, strain of yeast and media composition (in terms of both concentration and types of compounds) in these studies differ. These parameters contribute significantly to astaxanthin yield. For instance, under the same cultivation conditions and media composition different strains of *P. rhodozyma* bioaccumulate distinct amounts of astaxanthin (Amado & Vazquez, 2015; Montanti et al., 2011). Moreover, astaxanthin yield of the same strain varies on different agro-industrial byproducts (Villegas-Méndez et al., 2019). Furthermore, pH,

temperature and light are crucial factors that affect astaxanthin bioaccumulation. The optimum level these also vary from one strain/mutant to another (Schmidt et al., 2011). Thus, even though the results of the present study show *P. rhodozyma* Y1654 can grow and accumulate astaxanthin in both soy and sugar beet molasses, optimization of the above parameters is needed to improve astaxanthin production in these media. This notwithstanding, at a less glucose concentration (10 g L^{-1}) Villegas-Méndez et al. (2019) reported that *X. dendrorhous* ATCC 24202 cultivated at 20 °C produced significantly higher astaxanthin as compared to the strain used in the present study. Thus, it is highly probably that the strain used in the present study is inferior in terms of astaxanthin production or the cultivation condition where not optimum for astaxanthin production as there is no prior data on optimum condition for increased astaxanthin by *P. rhodozyma* Y1654.

CONCLUSION

The results of this study reveal that soy molasses and sugar beet molasses are good inexpensive sources of sugar for cultivation of *P. rhodozyma* Y1654. However, a significant fraction of sugar present in soy molasses, composed mainly of raffinose and stachyose, was unassimilable by *P. rhodozyma* Y1654 suggesting it lacks pre-requisite enzymes like α-galactosidase. Hence, to improve utilization of sugar in soy molasses it is necessary to pre-hydrolyze complex sugars. For production of astaxanthin by *P. rhodozyma* Y1654, molasses-based media greatly lags behind the standard glucose media, with sugar beet molasses being superior to soy molasses. To achieve comparative astaxanthin yield, molasses-based media needs further optimization.

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Changes in α-amylase activity in honey during the freeze-drying process

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Abstract. Honey is a natural product, which is appreciated for its sweetness, high nutritional value and health benefits all over the world. Despite all benefits, the usage of honey in food industry is limited due to its high viscosity. The use of dried honey could be an alternative to liquid honey, and would allow to use it as an additive in a range of many different food products such as sauces, beverages, yogurts etc. There are many parameters, which are used to determinate the quality of honey. α-amylase (diastase) activity is one of the most important criteria to determine the quality and freshness of honey. The aim of the present study was to investigate and compare α-amylase activity in liquid honey samples and freeze-dried honey samples. Overall, 18 honey samples were dehydrated using a freeze-drying method. Freeze-drying of the samples was carried out at – 50.6 °C and the pressure was 0.036 mbar for 72 hours. α-amylase activity in the honey samples was tested using Amylazyme test tablets. The obtained results showed variability in α-amylase activity after the freeze-drying process. As hydroxymethylfurfural (HMF) is another important quality parameter of honey, the content of HMF was determined in the samples by high performance liquid chromatography. In some samples the concentration of HMF after freeze-drying increased and was higher than it is allowed according to the International Honey Commission (for example, 55.75 mg kg^{-1}).

Key words: α-amylase activity, freeze-drying, honey, HMF.

INTRODUCTION

Honey is a natural food product, which is well-known due to its sweetness, high viscosity, specific flavour and health improving properties (Ramsay et al., 2019). Honey is composed of approximately 200 substances (Geana & Ciucure, 2020). The main constituents of honey are monosaccharides (mainly fructose and glucose). It also contains a wide variety of minor components such as enzymes, amino acids, organic acids, vitamins, phenolic compounds and proteins (Azeredo et al., 2003). The qualitative and quantitative composition of honey depends on many factors such as their floral and geographical origin and climate, and processing (Da Silva et al., 2016).

Honey, as a supersaturated solution, tends to crystallize (Dettori et al., 2018). The crystallization of honey is a natural process. However, the process of crystallization can negatively affect the quality of honey. During the crystallization process, water activity increases and that can lead to yeast growth and unwanted fermentations (Tappi et al.,

2019). The most common way how to prevent the negative effects of crystallization is thermal processing (Ribeiro et al., 2018). Also, thermal processing can be used to transform liquid honey into powder by drying. Powdered honey provides many advantages: extended shelf-life, ease of packing and transporting, ease of use in food industry (Tong et al., 2010; Kılınç & Demir, 2017). Although honey in powder form is an alternative substitute to liquid honey, the production of pure honey powder is a complicated process. The high concentration of fructose and glucose does not allow to easily transform liquid honey in to powdered honey. Honey as a sugar-rich product tends to form lumps or syrup during the drying process (Umesh Hebbar et al., 2008). This problem has been solved by adding different types of carriers to increase the glass transition temperatures of fructose and glucose (Adhikari et al., 2001; Samborska et al., 2015).

Honey powder can be produced by different drying methods (Cui et al., 2008; Nurhadi & Roos, 2016; Sramek et al., 2016; Samborska et al., 2019). Spray drying is the most widely used method to convert liquid honey into powder (Shi et al., 2013). Freeze-drying also can be used as an alternative drying method to obtain honey-rich powder, but as a slow and expensive drying method it is rarely used for production of honey powder (Subramanian et al., 2007). Despite the methods expensiveness, it allows to produce high quality dry food products. During the freeze-drying process water is removed from a frozen product by sublimation (Prosapio & Norton, 2018).

The quality of honey as a food product is very important. The enzymatic activity of honey is one of the indicators to detect its freshness and quality (Kanar & Mazı, 2019). Honey contains various enzymes such as saccharase (invertase), glucose oxidase, catalase, peroxidase and α-amylase, which is the predominant enzyme in honey (Tosi et al., 2008). α-amylase activity and the concentration of hydroxymethylfurfural (HMF) are used together to evaluate the quality of honey. Usually the values of these parameters are used to indicate the intensity of heating during the processing of honey. According to Council of the European Union Directive (Codex Alimentarius, 2001) should not less than 8, expressed as diastase number DN, and the concentration of HMF should not exceed 40 mg kg^{-1}.

The aim of the present study was to investigate and compare α-amylase activity in liquid honey samples and freeze-dried honey samples.

MATERIALS AND METHODS

Honey samples
In this study, eighteen honey samples were used for freeze-drying. Ten honey samples were derived from Latvian beekeepers in 2018 and 2019 from different districts in Latvia. Four honey samples were purchased from a local supermarket in Jelgava, Latvia in 2018. Another four honey samples were purchased in local markets and supermarkets in Estonia, Italy, Hungary and Tajikistan in 2018 and 2019. The origin of honey samples shown in Table 1.

Table 1. Distribution of studied honey samples

Sample	Type of honey	Production year	Country	District
H1	Multifloral	2018	Latvia	Zemgale
H2	Multifloral	2018	Latvia	Zemgale
H3	Multifloral	2018	Latvia	Latgale
H4	Multifloral	2018	Latvia*	Unknown
H5	Multifloral	2018	Latvia*	Unknown
H6	Multifloral	2018	Latvia*	Unknown
H7	Multifloral	2018	Latvia	Vidzeme
H8	Acacia	2018	Tajikistan	Baljuvor
H9	Multifloral	2018	Latvia	Latgale
H10	Multifloral	2018	Latvia*	Unknown
H11	Forest flower	2019	Latvia	Kurzeme
H12	Buckwheat	2018	Latvia	Kurzeme
H13	Buckwheat	2019	Latvia	Zemgale
H14	Linden flower	2019	Latvia	Kurzeme
H15	Multifloral	2018	Latvia	Vidzeme
H16	Chestnut	2019	Italy	Sicily
H17	Multifloral	2019	Hungary*	Unknown
H18	Multifloral	2019	Estonia	Võrumaa

* – blend of European Union and non-European Union honeys.

Determination of pH and free acidity

Determination of pH and free acidity of liquid honey samples was carried out according to International honey standards (Ohe et al., 2000). 10 grams of honey sample were dissolved in 75 mL of carbon dioxide-free water. pH of prepared honey solutions was measured using pH-meter inoLab® pH7110 (WTW, Germany). Free acidity was determined by titrating the prepared honey solutions with 0.1M NaOH to pH 8.30. Free acidity of honey was expressed as milliequivalents acid kg^{-1} honey. It was calculated using an equation:

$$Free\ acidity = V \times 10 \qquad (1)$$

where V – volume of 0.1M NaOH, which was consumed during the analysis, mL.

Determination of fructose and glucose, and hydroxymethylfurfural (HMF)

Sample preparation: 5 grams of liquid honey sample were weighted into a 100 mL beaker and dissolved in 30 mL deionized water. Dissolved material was quantitatively transferred into 50 mL volumetric flask and diluted with deionized water to the mark and inverted multiple times. Prepared sample solutions were centrifuged (Pro-Research, Centurion Scientific Ltd.) for 10 minutes at 10,000 rpm. The content of fructose and glucose was determined by HPLC using an analytical column SUPELCOSIL™ LC-NH$_2$ (4.6 mm×250 mm I.D., particle size 5 µm). Column and detector temperature were set to 30 °C. The mixture of acetonitrile (HPLC grade, Sigma-Aldrich) and water (HPLC grade) was used as a mobile phase. The ratio of acetonitrile and water was 80:20 (v/v). The analysis of the samples was carried out under isocratic conditions. Flow rate was 1 mL min^{-1}. Injection volume of 10 µL was performed using an autosampler SIL-20A. The retention times of obtained peaks of analysed samples were compared to the retention times of fructose (HPLC grade, Fluka) and glucose (HPLC grade, Fluka)

standard solutions. Chromatographic analysis of the samples was performed on Shimadzu LC-20 Prominence liquid chromatograph (Shimadzu USA Manufacturing Inc, Canby, USA) with a Shimadzu RID 10A Refractive Index detector. The obtained concentrations of fructose and glucose were expressed as g 100 g^{-1} dry matter.

The concentration of hydroxymethylfurfural (HMF) in the samples was determined by HPLC using an analytical column PerkinElmer C18 (4.6 mm × 250 mm I.D., particle size 5 µm). Column and detector temperature were set to 25 °C. The mixture of acetonitrile (HPLC grade, Sigma-Aldrich) and water (HPLC grade) was used as a mobile phase. The ratio of acetonitrile and water was 10:90 (v/v). The analysis of the samples was performed under isocratic conditions. Flow rate was 1.3 mL min^{-1}. Injection volume of 10 µL was performed using an autosampler SIL-20A. Detection of HMF was carried out at wavelength of 280 nm. The retention times of peaks were compared to the retention time of HMF (HPLC grade, Sigma-Aldrich) standard solution. Determination was performed on Shimadzu LC-20 Prominence liquid chromatograph (Shimadzu USA Manufacturing Inc, Canby, USA) with a Shimadzu DAD SPD-M20A detector. The obtained concentration of HMF was expressed as mg kg^{-1} dry matter.

Freeze-drying

Two types of formulations were prepared for freeze-drying experiments: 1) 20% aqueous solutions of honey and 2) 20% aqueous solutions of honey with maltodextrin (STAR-DRI® 10 NG, TATE & LYLE). The ratio of honey and maltodextrin was 1:2. All prepared solutions were poured into plastic freezer containers, and the initial solutions thickness were approximately 10 mm. The containers of solutions were frozen to -20 °C within 2 hours. Afterwards the pre-treatment procedure, the freeze-drying process was performed at an absolute pressure of 0.036 mbar. The temperature of ice condenser was set to -50.6 °C. The duration of the drying process was 72 hours. Freeze-drying was carried out using a freeze-dryer ALPHA 1-2 LDplus (MARTIN CHRIST Gefriertrocknungsanlagen GmbH, Germany).

Determination of moisture content

Moisture content of liquid honey and freeze-dried honey samples was determined using a moisture analyzer AND MX-50 (A&D Company, Limited, Japan). One gram of samples was weighted on glass fibre sheets and placed on the sample pan of moisture analyzer. The samples were heated up at a drying temperature of 140 °C. The time of analysis was set to 20 minutes. The software 'WinCT-Moisture' was used to record moisture data.

Determination of α-amylase activity

α-amylase activity was determined in liquid honey and freeze-dried honey samples using Amylazyme HY tablets (Megazyme, Ireland). Determination of α-amylase activity was carried out according to Amylazyme assay procedure. The absorbance of samples was measured using a spectrophotometer 6405 UV/Vis (JENWAY, the U.K.) at wavelength of 590 nm. The enzyme activity was calculated according to the following Eq. (2) and expressed as diastase number (DN):

$$Schade\ Units = 20.0\ \times\ \Delta Abs \qquad (2)$$

where ΔAbs – absorbance of the analysed samples at 590 nm.

Statistical analysis

All experiments were performed in triplicate. The obtained data were expressed as the mean ± standard deviation. The data were processed using MS Office Excel 2016.

RESULTS AND DISCUSSION

Characterization of honey samples

The chemical composition of honey samples was investigated before the drying process. Overall, 18 honey samples were used for freeze-drying experiments. The main characteristic properties of used honey samples represented in Table 2.

Table 2. Chemical composition of honey samples

Sample	α-amylase activity, DN	Moisture, %	Fructose, g 100 g^{-1}	Glucose, g 100 g^{-1}	pH	Free acidity, meq kg^{-1}	HMF, mg kg^{-1}
H1	15.3 ± 0.5	14.8 ± 0.3	34.4 ± 0.3	33.2 ± 0.4	4.12 ± 0.01	23.7 ± 0.3	5.7 ± 0.4
H2	7.6 ± 0.1	12.2 ± 0.4	33.5 ± 0.4	37.7 ± 0.4	4.29 ± 0.01	9.6 ± 0.1	4.7 ± 0.3
H3	18.5 ± 0.3	20.5 ± 0.2	36.4 ± 0.2	31.8 ± 0.2	3.66 ± 0.01	42.0 ± 1.0	41.4 ± 0.5
H4	3.8 ± 0.1	15.2 ± 0.3	39.9 ± 0.4	31.3 ± 0.3	4.59 ± 0.03	10.7 ± 0.3	9.1 ± 0.6
H5	7.2 ± 0.5	18.2 ± 0.6	35.4 ± 0.4	35.1 ± 0.5	3.92 ± 0.01	29.3 ± 0.3	55.8 ± 0.3
H6	3.7 ± 0.1	12.9 ± 0.5	38.7 ± 0.4	30.4 ± 0.5	4.49 ± 0.07	8.8 ± 0.3	11.0 ± 0.2
H7	20.9 ± 0.6	14.1 ± 0.4	34.1 ± 0.4	33.0 ± 0.4	4.34 ± 0.01	22.8 ± 0.3	1.6 ± 0.4
H8	10.6 ± 0.2	14.1 ± 0.5	37.9 ± 0.4	42.5 ± 0.5	3.89 ± 0.01	24.3 ± 0.3	18.7 ± 0.5
H9	25.4 ± 0.8	17.0 ± 0.3	35.8 ± 0.4	37.1 ± 0.5	3.75 ± 0.01	37.7 ± 0.3	21.3 ± 0.4
H10	4.7 ± 0.2	17.2 ± 0.3	40.2 ± 0.5	35.1 ± 0.5	4.53 ± 0.01	5.3 ± 0.3	66.0 ± 0.3
H11	26.1 ± 0.1	15.0 ± 0.3	43.6 ± 0.5	36.5 ± 0.4	4.28 ± 0.01	26.7 ± 0.3	2.4 ± 0.4
H12	27.7 ± 0.2	17.9 ± 0.4	47.7 ± 0.4	37.8 ± 0.4	3.89 ± 0.01	44.6 ± 0.6	8.3 ± 0.3
H13	28.1 ± 0.1	16.5 ± 0.5	47.0 ± 0.4	39.6 ± 0.4	3.88 ± 0.01	41.5 ± 0.5	1.9 ± 0.2
H14	23.7 ± 0.7	15.0 ± 0.3	44.1 ± 0.3	42.7 ± 0.4	3.99 ± 0.04	20.8 ± 0.8	0.8 ± 0.1
H15	28.0 ± 0.1	16.5 ± 0.3	46.3 ± 0.4	37.6 ± 0.3	3.84 ± 0.01	44.5 ± 0.1	8.0 ± 0.1
H16	21.3 ± 0.8	18.3 ± 0.2	45.5 ± 0.4	30.0 ± 0.3	4.62 ± 0.02	36.3 ± 0.3	2.5 ± 0.2
H17	10.5 ± 0.3	14.6 ± 0.4	45.4 ± 0.4	38.4 ± 0.2	3.88 ± 0.01	22.8 ± 0.3	5.3 ± 0.2
H18	22.2 ± 0.6	15.7 ± 0.4	45.2 ± 0.4	40.5 ± 0.3	4.20 ± 0.01	20.2 ± 0.3	0.7 ± 0.1

α-amylase activity and the content of HMF were used to detect the quality of the honey samples. The obtained data of liquid honey samples showed that α-amylase (diastase) activity in 13 of 18 analysed samples were higher than 8. The value of DN in samples H2, H4, H5, H6, H10 was less than 8. These samples, which showed poor α-amylase activity, were blends of European Union and non-European Union honeys, except the sample H2, (Table 1). The differences in α-amylase activity might vary as the enzymatic activity of honey depends on the age of bees, the physical state of the colony, the nectar harvesting period. Also, the profusion of nectar flow can impact the content of enzymes in honey. Large quantity of nectar flow can lead to a lower α-amylase activity in honey (Persano Oddo et al., 1999; Pasias et al., 2017). The content of HMF in the samples ranged from 0.7 to 66.0 mg kg^{-1}. The samples H5 and H10 showed the highest concentrations of HMF (55.8 ± 0.3 mg kg^{-1} and 66.0 ± 0.3 mg kg^{-1}), which were higher than it is allowed in the European Union (Codex Alimentarius, 2001). The

samples H5 and H10 were categorized as low-quality honeys due to their low enzymatic activity and high content of hydroxymethylfurfural.

Freeze-drying of honey

In this study, dehydration of honey was performed by freeze-drying. During the freeze-drying process water was removed from frozen honey solutions by sublimation. This drying technique is gentle and allows to preserve bioactive compounds during the drying process. The dehydration of honey was carried out using two kind of formulations. After 72 hours of freeze-drying the moisture content in the samples decreased (Fig. 1). The highest content of moisture was in the samples, which were prepared as 20 % honey solutions. These samples were not stable and within a few hours rehydrated from the moisture in the atmosphere. Honey is a sugar-rich natural product, which contains low molecular weight sugars such as fructose, glucose and sucrose. The high concentration of these sugars makes it impossible to freeze-dry honey without adding carriers or drying aids (Bhandari et al., 1997). In this case, 20% honey solutions with maltodextrin (MD) were prepared. The ratio of honey and maltodextrin was 1:2. Maltodextrin is a natural polymer with a high molecular weight, increases the glass transition temperature of drying particles and reduces hygroscopicity (Adhikari et al., 2001). The addition of maltodextrin is common practice to obtain dry sugar-rich food products by spry-drying. After freeze-drying the samples, which were prepared as 20 % honey solutions with maltodextrin (MD), did not absorb the moisture from the atmosphere. The results showed that honey in a powder form was obtained by adding maltodextrin (drying aid) to honey. The moisture content in these samples ranged from 4.1 to 6.4 % and did not increase within weeks.
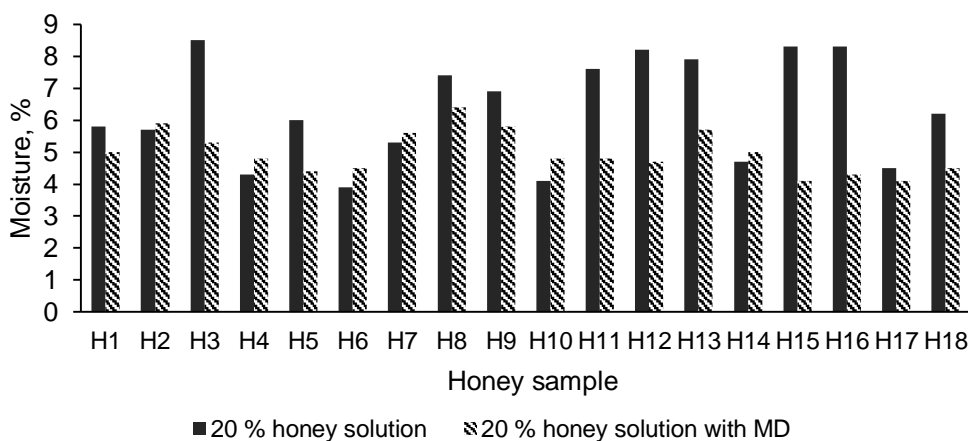

Figure 1. Moisture content of freeze-dried honey samples.

According to study results reported by Sramek and his co-workers (Sramek et al., 2016), they obtained honey powder by the freeze-drying technique. In their study they performed freeze-drying of honey solution in combination with glucose syrup. The final water content of freeze-dried honey powder was 3.1%.

α-amylase activity and the concentration of HMF were determined to detect the quality of obtained freeze-dried honey samples. The obtained results of α-amylase activity were variable (Table 3). There was noted increase of diastase activity in almost all samples after freeze-drying of honey solution. Freeze-drying of honey solutions with maltodextrin (MD) resulted in decrease of α-amylase activity in the most of analysed samples. The obtained results did not clarify the impact of freeze-drying to the enzyme activity. Unfortunately, there is lack of literature data on α-amylase activity changes in honey during the freeze-drying process, which could be used for a comparison of results. The changes of α-amylase (diastase) activity were investigated mainly in studies, where dehydration of honey was performed by spray-drying. In the research, diluted honey solution with Arabic gum was spray-dried. The authors of the research observed reduction of α-amylase activity (Samborska et al., 2017). Sramek and his co-workers stated that diastase (α-amylase) activity as indicator was less suitable and less sensitive to detect the quality of honey during the thermal processing at low temperatures (White Jr. et al., 1964; Sramek et al., 2017).

Table 3. α-amylase activity and HMF concentration in the freeze-dried honey samples

Sample	Freeze-dried honey solution		Freeze-dried honey solution with MD	
	α-amylase activity, DN	HMF, mg kg^{-1}	α-amylase activity, DN	HMF, mg kg^{-1}
H1	16.9 ± 0.2	39.1 ± 0.3	10.7 ± 0.3	48.0 ± 0.4
H2	10.1 ± 0.1	30.0 ± 0.4	8.1 ± 0.4	47.9 ± 0.5
H3	16.6 ± 0.1	32.7 ± 0.2	10.0 ± 0.3	97.0 ± 0.2
H4	4.4 ± 0.4	47.7 ± 0.3	6.3 ± 0.1	54.6 ± 0.1
H5	7.2 ± 0.3	197.2 ± 0.6	6.8 ± 0.7	98.3 ± 0.5
H6	3.9 ± 0.1	58.2 ± 0.5	5.4 ± 0.2	54.1 ± 0.9
H7	21.8 ± 0.4	20.4 ± 0.4	16.9 ± 0.1	40.1 ± 0.2
H8	13.2 ± 0.6	60.0 ± 0.5	10.0 ± 0.4	55.0 ± 0.5
H9	24.2 ± 0.8	55.9 ± 0.3	18.9 ± 0.9	46.0 ± 0.3
H10	5.2 ± 0.2	119.8 ± 0.3	7.7 ± 0.5	75.9 ± 0.5
H11	26.9 ± 0.1	7.3 ± 0.3	22.4 ± 0.4	33.3 ± 0.4
H12	27.6 ± 0.1	53.8 ± 0.4	26.1 ± 0.2	51.1 ± 0.2
H13	28.2 ± 0.1	17.0 ± 0.5	32.7 ± 0.1	36.7 ± 0.1
H14	23.7 ± 0.1	10.0 ± 0.3	16.1 ± 0.9	34.3 ± 0.4
H15	27.7 ± 0.1	66.9 ± 0.3	28.3 ± 0.5	45.8 ± 0.2
H16	22.7 ± 0.4	30.7 ± 0.2	15.1 ± 0.4	40.5 ± 0.3
H17	10.9 ± 0.3	46.8 ± 0.4	8.9 ± 0.3	45.0 ± 0.6
H18	22.7 ± 0.1	9.6 ± 0.4	16.1 ± 0.1	35.1 ± 0.3

The content of HMF is used as an indicator along with α-amylase activity to detected thermal processing of honey. The content of this chemical compound was also evaluated in the freeze-dried samples (Table 3). The highest concentration of HMF was detected in the samples H5 and H10. The formation of hydroxymethylfurfural in honey is unpreventable, as it is mainly composed of fructose and glucose. This heterocyclic organic compound is formed by dehydration of fructose and glucose, which is acid catalysed reaction. Also, pH, low temperature and moisture content are the factors, which catalyses the formation of HMF in honey (Tosi et al., 2004; Stöbener et al., 2019). The content of HMF in the freeze-dried honey samples H1, H2, H3, H7, H13, H14, H16,

H18, which were obtained from diluted honey solutions without maltodextrin, was not higher than it is allowed in the European Union countries. The detected concentration of HMF in the sample after freeze-drying was lower than the initial concentration of HMF. This unusual observation could be caused by high hygroscopicity of the sample. As it was noticed that all samples, which were freeze-dried without adding maltodextrin, were not stable and absorbed the moisture from the atmosphere within a few hours. All other samples showed a very typical increase of HMF after the drying process. The samples H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H12, H15 and H17, which were obtained by drying diluted honey solutions with maltodextrin, contained higher concentration of HMF than it is regulated (Codex Alimentarius, 2001). The observed results showed that concentration of hydroxymethylfurfural in most of the analysed samples after freeze-drying was higher than initial concentration of HMF.

CONCLUSIONS

The present study showed that freeze-drying can be used to obtain dehydrated honey. The samples, which were prepared adding maltodextrin as a drying aid, showed better stability against the moisture in the atmosphere. Unfortunately, α-amylase activity in freeze-dried honey was not suitable tool to detect the enzymatic activity of the samples correctly. The freeze-dried samples, which were obtained without adding maltodextrin, contained HMF in the allowed levels.

Further studies are needed to examine freeze-drying technique as another alternative method of obtaining high quality honey-rich powders. In further studies, there should be improved some freeze-drying conditions to optimise the lyophilization process. The optimisation of freeze-drying would allow to produce high-quality honey-rich powder, that could increase the usage of honey in food industry.

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Development of formulation and technology of non-dairy soy-coconut yogurt

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Abstract. Yogurt provides an organism with probiotics, which can help digestion. However, many people do not consume dairy for a lot of reasons such as lactose intolerance, milk protein allergy, environmental and ethical concerns. The aim of the research was to develop formulation and technology of plant-based yogurt made of coconut and soy milk. The possibility of using the composition of coconut and soy milk was investigated. The effect of using different ratios of coconut and soy milk on rheological and sensory acceptability was studied. Soy milk containing 20, 30, 40 and 50% coconut milk were used in the production of soy yoghurt using commercially available yoghurt starter. The physico-chemical composition, water activity, rheological and organoleptic properties, fatty acid composition and microorganism viability were investigated. Presented production procedure enables the manufacture of a product with satisfactory functional properties and organoleptic properties.

Key words: soy milk, coconut milk, kudzu, lecithin, soy yoghurt, coconut yoghurt.

INTRODUCTION

People do not consume dairy products because of a number of reasons such as lactose intolerance and malabsorption, milk protein allergy, environmental, ethical and religious concerns.

About two-thirds of the world's population is lactose intolerant (Bayless et al., 2017; Storhaug et al., 2017) and varies widely per country. The lactose malabsorption and intolerance are defined by gastrointestinal symptoms: diarrhea, flatulence, nausea, gut distension, cramps, abdominal pain and discomfort, and systemic symptoms such as headache (Nicklas et al., 2009; Storhaug et al., 2017).

Milk protein allergy is a common problem among infants and young children (Skripak et al., 2007). Besides gastrointestinal symptoms milk protein allergy also involves anaphylaxis symptoms such as skin and respiratory tract allergies or other system disorders (Bahna, 2002).

Globally, 9% of population, on average, decides to cut animal products from their diet and follow a vegan diet (Nielsen, 2016). They are standing against animal cruelty and exploitation, environmental safety and for personal health.

Soy milk is the most nutritionally identical to cow's milk. It is a rich source of proteins, which includes all essential amino acids, low in carbohydrates and fats and does not contain cholesterol. Soy milk contains minerals – iron, calcium, potassium, zinc, phosphorus, copper, manganese, and vitamins B-group, E, K (Messina, 2016). Soy proteins have the highest digestibility compared with other proteins of herbal origin and are rich in lysine, threonine, and tryptophan (Degola et al., 2019).

Isoflavones, founded in soybean, have many health benefits such as cardiovascular protective effects, including reduction of cholesterol and menopause symptoms, prevention of osteoporosis and reduction of risk of developing of certain cancers (prostate and breast cancer). They also possess antioxidant properties and have antiviral and hepatoprotective activities (Garcia et al., 2009; Wu & Kang, 2011; Kant & Albrecht Broadway, 2015). Taking into account the foregoing, food manufacturers are encouraged to incorporate more soy components in food formulations (Nadtochii et al., 2015).

Coconut milk has a sweet taste, delicate coconut flavor and creamy texture. It contains potassium, phosphorus, magnesium, manganese, iron, calcium, zink, vitamins C, E and B-group (Kothalawala et al., 2018; Amirah et al., 2019). Coconut milk is a rich source of antioxidants, low in carbohydrates and high in fiber (Alyaqoubiet et al., 2015).

Despite the fact that coconut milk is full of saturated fats, they are medium-chain triglycerides (MCFAs). It means that they can easily be absorbed and used by organism for energy, improve cognitive function and treat from memory loss (Alyaqoubi et al., 2015). MCFAs are quickly oxidized by the liver and thus less obesogenic than low chain fatty acids (LCFAs). Unlike LCTs, MCTs go straight to the liver where they are either used for immediate energy or turned into ketones (an alternate source of energy for the brain) (Schönfeld & Wojtczak, 2016).

Due to lauric acid content, coconut milk possesses antiviral, antibacterial and antioxidant activities. Including coconut milk in a diet can help to reduce the risk of heart disease, stroke and control hunger (Paul et al., 2019). Fats in coconut raise the high-density lipoprotein (HDL) levels while reducing the low-density lipoprotein (LDL) (Ekanayaka et al., 2013).

Other studies confirmed that addition of coconut milk to soy milk improved the sensory characteristics of the yoghurt, helped mask bitter beany flavour (Kolapo & Olubamiwa, 2012).

Yogurt has many advantages. Fermentationis not only one of the oldest ways to preserve food and beverage, but it also improves nutritional and organoleptic quality, physicochemical characteristics of a product, providing an organism with beneficial microorganisms (Bell et al., 2018).

Soy milk fermentation is not an exception. Microorganisms reduce anti-nutritional factors in soy such as proteinase-inhibitors, phytic acid, urease, oxalic acids, oligosaccharides and increase the bioavailability of bioactive components by means of increasing the number of free isoflavones and peptides (Sanjukta et al., 2015). Fermentation can also reduce beany flavor and improve texture (Niyibituronsa et al., 2018).

According to Nielsen's Global Health and Ingredient-Sentiment Survey, 70% of global respondents say they actively make dietary choices to help prevent health diseases and make dietary changes for health reasons (Nielsen, 2016). Therefore, the aim of this study is to investigate the possibility of using the combination of coconut milk and soy milk in the manufacture of yogurt without artificial ingredients for people who does not consume dairy products.

MATERIALS AND METHODS

Soy milk preparation

Soymilk was prepared using blending method. Soybeans were washed and soaked in clean tap water for 12 h. The swollen soybeans were drained and blended with water at a bean-to-water ratio of 1:10 in a blender at low speed for 10 minutes until smooth. The homogenized mass was strained through a double-layered cheesecloth to separate milk from residue.

Yoghurt Manufacture

Soy-coconut yoghurts were formulated to contain: soy milk containing 20% coconut milk (Aroy-D, Thailand); soy milk containing 30% coconut milk; soy milk containing 40% coconut milk; soy milk containing 50% coconut milk. Hereinafter groups are referred as SMCM20, SMCM30, SMCM40 and SMCM50, respectively. The prepared mixtures were pasteurized at 85 ℃ and placed in water bath to cool down to 42 ± 1 °C. The cooled mixtures were inoculated with the starter culture at a rate of 3% (*Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*) and fermented at 42 ± 1 °C for 7 hours. The starter was prepared using soy milk. The yogurt samples were incubated to reach the titratable acidity value equals to 0.585% lactic acid. To avoid the coconut milk to rise on the top and stabilize the mixture kudzu root starch (Mitoku Co.) and lecithin (Cargill Inc., Germany) were used in concentrations 2% and 0.5%, respectively. After incubation, the yoghurt samples were cooled and stored at refrigerator at 5 ± 1 °C until used.

Methods

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

Titratable acidity (TA) was measured by titration of 10 g of sample with 0.1 N NaOH solution and expressed in % lactic acid.

Moulds and yeasts were determined according to GOST 10444.12–12013.

Rheological measurements were carried out using Rheotest 2 type rotating viscosimeter (VEB–MEDINGEN, Germany) with the coaxial cylinder device S_2. The rheological measurements were performed at controlled temperature of 20.0 ± 0.5 °C. The shear stress was estimated as the areas between upward flow curve and downward flow curve.

Apparent viscosity η was calculated using the following formula:

$$\eta = \frac{\tau}{\gamma} \quad (\text{Pa s}) \tag{1}$$

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

The storage time was estimated according to MUK 4.2.1847–04 as described by Dubrovskii et al. (2019). To determine the shelf life of the yogurt, samples were packed in glass containers and stored at 4 ± 2 °C.

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

Amino acid content was analyzed by HPLC method using HPLC system LC-20AD (Schimadzu, Japan) according to ISO 13903:2005.

Amino acid score of essential amino acids was calculated using the following formula:

$$Amino\ acid\ score = \frac{g\ of\ amino\ acid\ in\ 100\ g\ of\ test\ protein}{g\ of\ amino\ acid\ in\ 100\ g\ of\ protein\ FAO/WHO} \times 100, \quad (2)$$

Coefficient of distinction amino acid scores (CDAAS) (Lipatov, 1995), difference of amino-acid score of an essential amino acid and a minimum amino acid score, and biological value (BV) were calculated using the following equations:

$$CDAAS = \frac{\sum_{i=1}^{N}(AAS_i - the\ lowest\ AAS)}{N}, \quad (3)$$

where N is essential amino acids content, and AAS_i is amino acid score of the i-th amino acid (%).

$$\Delta DAAS = C_i - C_{min}, \quad (4)$$

where C_i – score of I – essential amino acid and C_{min} – minimum amino acid score.

$$BV = 100 - CDAAS\ (\%) \quad (5)$$

Mixture stability of control and treatment samples of yogurt was determined by visual observation of the height of the coconut milk layer formed at the top of the test tubes after incubation and during the storage period every 5 days. Produced mixtures were subjected to fermentation in 25 mL test tubes at 45 °C. For each sample there was considered three replicates.

The creaming index (CI) values were obtained from the ratio between the total height of cream layer (CL) and the total height of mixture layer (ML).

$$CI(\%) = \frac{CL}{ML} \times 100 \quad (6)$$

CL and ML were measured directly into a storage test tubes with the help of a graduate scale.

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

Sensory evaluation based of the finished product on flavour, colour, taste, texture and overall acceptance was conducted by the panel of 24 students and staff of Faculty of Food Biotechnology and Engineering (50% of participants were female from 20 to 70 years old and 50% of participants were male from 21 to 70 years old) by using 9 point hedonic scale (9 = like extremely; 5 = neither like nor dislike; 1 = dislike extremely) according to the method as described by Clark et al. (2009). Yogurt samples were placed in cups which were randomly coded with 3-digit number. The panelists were allowed to use spring water and unsalted cracker for palate cleansing between the samples.

Statistical analysis

The significance of differences in results of each test values was determined using *Student's t-test*. Differences were considered significant at the $P < 0.05$ level. All statistical analyses were carried out with the BioStat 6.9 (AnalystSoft Inc.) and one-way analysis of variance (ANOVA). The results were expressed as mean values and standard deviations (SD).

RESULTS AND DISCUSSION

Yogurt samples with different ratios of coconut milk and soy milk were characterized by their thixotropicbehavior. Fig. 1 shows the flow curves for yogurt. Among the four prepared yoghurt samples, the obtained hysteresis loops showed significant difference. Hysteresis loop was assumed to be the difference between the energy required for structural breakdown and samples possibilities of being reconstructed.
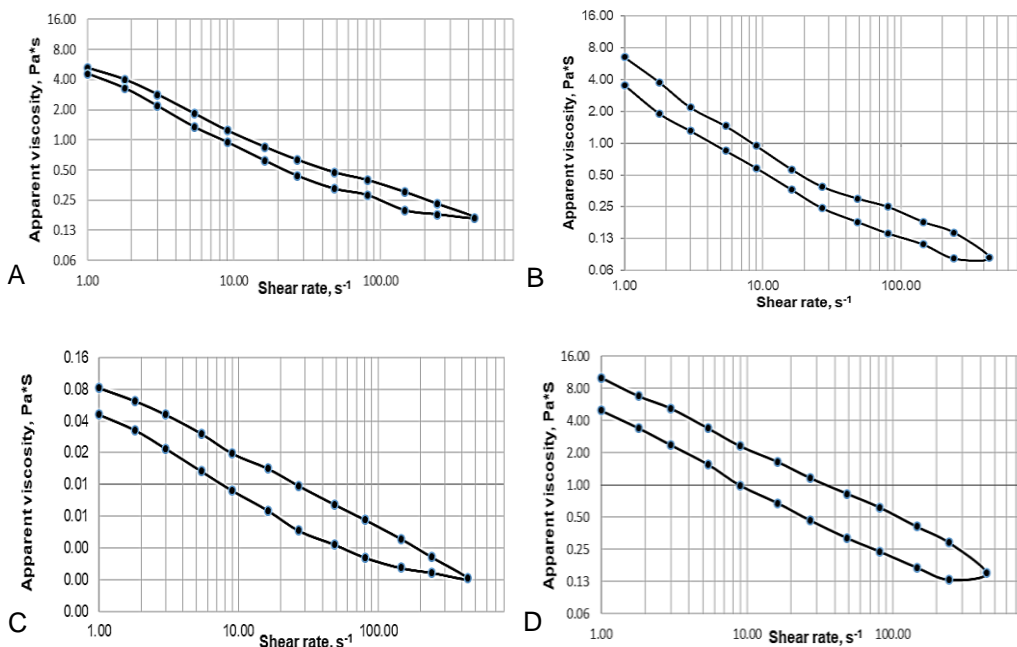
Figure 1. Thixotropy loop of the yoghurt samples: A is yoghurt containing 20% coconut milk and 80% soy milk; B is yoghurt containing 30% coconut milk and 70% soy milk; C is yoghurt containing 40% coconut milk and 60% soy milk; D is yoghurt containing 50% coconut milk and 50% soy milk.

Data presented showed that the thixotropy properties of yogurt samples decreased with the increase in coconut concentration in the mixture. Yaakob et al. (2012) have also found that viscosity of the yogurt is increased with increasing coconut milk concentration.

The hysteresis loop area of the yoghurt sample prepared from with 50% of coconut milk was the largest, while that of the yoghurt sample prepared with 20% of coconut milk was the smallest. The reason of the higher hysteresis area in this sample may be explained by the higher viscosity of the sample containing 50% of coconut milk in contrast to the sample containing 20% of coconut milk. Hence, these samples provided a firmer product because more energy is required to break its structure. The yoghurt sample prepared with 20% of coconut milk tended to have better structural reversibility than that prepared with 50% of coconut milk.

In this work, the indicators that characterize the sustainability of the structure yogurt samples to destruction during mechanical action and its ability to thixotropic recovery, namely, viscosity loss coefficient (VLC), coefficient of mechanical stability (CMS) and indicator of structural recovery (SR) were determined. The data obtained are shown in Table 1.

Analyzing the data presented in Table 1, it can be seen that viscosity loss increased with the increase in coconut milk concentration. The maximum thixotropic recovery showed the sample with 20% of coconut milk.

The data given in Table 1 also show that the decrease in the coconut concentration had no significant influence on the resulting coefficient of mechanical stability of the yoghurt samples, but might have enhanced the indicator of structural recovery of yogurts.

Table 1. Structure stability characteristics of yogurt samples

Coconut milk: soy milk ratio	Indicators		
	VLC(%)	CMS	SR(%)
20:80	9.8 ± 1.2	1.11 ± 0.11	96.7 ± 2.5
30:70	12.5 ± 1.1	1.11 ± 0.11	93.1 ± 2.7
40:60	13.0 ± 1.2	1.16 ± 0.11	86.8 ± 2.7
50:50	23.1 ± 1.2	1.31 ± 0.11	76.9 ± 2.9

Data from the Fig. 2. present the organoleptic characteristics of yogurt samples. It can be seen, that the mean scores for overall acceptance and taste was higher for SMCM30 sample than in the others. Products with higher percent of soy are often less accepted by consumers due to its flavour, identified as bean-likeflavour. Addition of coconut milk in yoghurt formulation improved the flavour evaluation scores. Flavour and texture of SMCM30 and SMCM40 samples presented the same degree of liking by the participants of the organoleptic characteristic's analyses. In terms of colour all yogurt samples were well accepted by the panelists. The result of the sensory evaluation from this study has shown that coconut-soy yoghurt could become a more acceptable to the consumers if an appropriate amount of coconut milk is added to the formula.

Based on the results of the studies of rheological and organoleptic characteristics, for further studies SMCM30 sample was chosen.
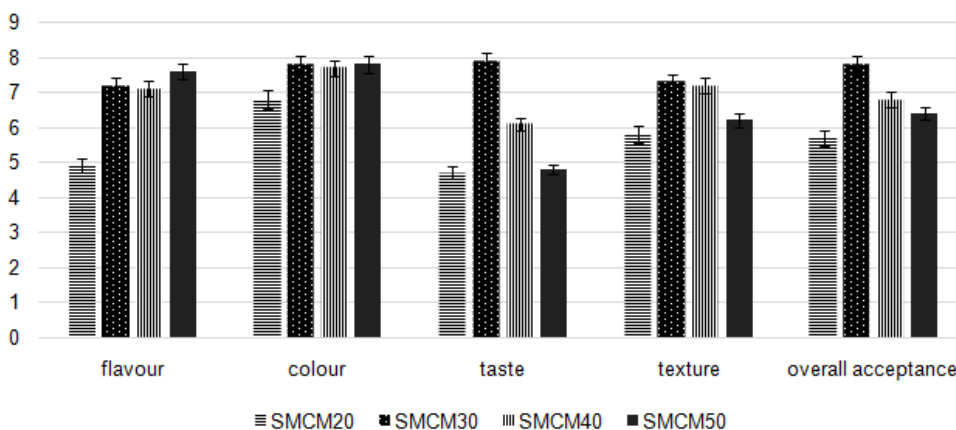

Figure 2. Hedonic scale for flavor, colour, taste, texture and overall acceptability of the samples with different ratios of coconut: soy milk. Values are means ± standard deviation. Data represent significant differences within each sensory attribute between the yogurt samples ($P < 0.05$).

The analyzed samples presented 11 fatty acids, which were identified and quantified (Table 2).

Fatty acids were grouped as follows: short-chain saturated fatty acids (C4-C10, SCFA), medium-chain saturated fatty acids (C12-C15, MCFA), long-chain saturated fatty acids (C16-C24, LCFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). A total of 11 fatty acids comprised of both saturated and unsaturated fatty acids. As shown in Table 2, 91.3% of the fatty acid were saturated, 8.5% were monounsaturated and 0.3% were polyunsaturated.

Table 2. Fatty acid composition of the vegan yogurt

Fatty acid profile	Group	Fatty acid content from total amount of fatty acids (%)
caproic acid (6:0)	SCFA	0.81 ± 0.07
caprilic acid (8:0)	SCFA	8.13 ± 0.62
capric Acid (10:0)	SCFA	6.02 ± 0.45
lauric acid (12:0)	MCFA	46.04 ± 2.96
myristic acid (14:0)	MCFA	18.12 ± 1.13
palmitic acid (16:0)	LCFA	8.92 ± 0.48
stearic (18:0)	LCFA	3.4 ± 0.21
oleic (18:1)	MUFA (omega-9)	5.8 ± 0.31
linoleic acid (18:2)	PUFA (omega-6)	2.6 ± 0.14
linolenic (18:3)	PUFA (omega-3)	0.3 ± 0.03
arachidic (20:0)	LCFA	0.1 ± 0.01

As can be seen from the Table 2, saturated fatty acids were the predominant fatty acids. The level of caprilic acid (8:0), lauric acid (12:0), myristic acid (14:0) and palmitic acid (16:0) in fermented product were the highest among all fatty acids. The proportion of MCFA in yogurt sample was the highest, followed by SCFA, LCFA, MUFA and PUFA. Increasing of SFA in coconut milk doesn't lower its health benefits (Bawalan & Chapman, 2006) cleared that coconut oil is unique as it contains the highest percentage of medium chain fatty acids which are metabolized differently in the human body to other saturated and unsaturated fats or oils. They are similar in structure to the fats in mother's milk that provide babies immunity. There are also similar beneficial effects in adults (Kabara, 2000).

The amino acid composition and Amino acid score of the yogurts are presented in Table 3.

Calculated quality characteristics of protein component of soy-coconut yogurt are presented in Table 4.

As can be seen from the Table 3, the developed yogurt contains all essential and non-essential amino acids and the scores for all essential amino acids, except lysine amino acids, are higher than 100%. The low amount of lysine amino acid in yogurt is a consequence of lack lysine in coconut milk (Sousa & Kopf-Bolanz, 2017). Calculation of amino acid scores (Table 4) showed the high biological value of soy-coconut yogurt protein.

Table 3. Amino acid content and score of the coconut-soy yogurt

Amino acid		FAO/WHO, 2007, g 100 g^{-1} of protein	g 100 g^{-1} of protein	Amino acid score of yogurt (%)
essential amino acids, incuding:	amino acids total	27.7	34.9	133.7
histidine		1.5	2.06 ± 0.15	137.2 ± 9.9
isoleucine		3.0	3.46 ± 0.27	115.4 ± 9.2
leucine		5.9	6.38 ± 0.30	108 ± 4.9
lysine		4.5	4.61 ± 0.34	102 ± 7.5
methionine + cysteine		2.2	1.76 ± 0.29	79.8 ± 13.4
phenylalanine +tyrosine		3.8	7.01 ± 0.26	184 ± 6.67
threonine		2.3	3.54 ± 0.29	154 ± 12.6
tryptophan		0.6	1.26 ± 0.26	210 ± 34.5
valine		3.9	4.42 ± 0.30	112.8 ± 7.5

The data given in Table 5 shows that the viability of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus* decreased in all yogurt treatments at the end of storage periods compared with their counts when fresh. No mould and yeasts were detected in soy-coconut yogurt samples during the first 15 days of refrigerated storage. Analyzing the data, it can be concluded that quality indicators during the shelf life of the samples do not exceed the established parameters for yogurt. The results revealed that considering the reserve ratio according to MUK 4.2.1847–04 the recommended period of storage soy-coconut yogurt is 20 days at 4 ± 2 °C.

Table 4. Quality characteristics of the yogurt

The name of the quality characteristics	The value of the quality characteristics
minimum amino acid score (%)	79.8
ΔDAAS (%)	485.2
CDAAS (%)	53.9
BV (%)	46.1

Table 5. Quality indicators of yogurt

Indicators	Soy-coconut yogurt				
	Storage time, days				
	0	8	15	20	26
titratable acidity, % lactic acid	0.59 ± 0.03	0.62 ± 0.03	0.67 ± 0.02	0.79 ± 0.03	0.81 ± 0.03
Streptococcus thermophilus, CFU mL^{-1}	6.5×10^8	4.6×10^8	2.2×10^8	5.6×10^7	1.5×10^7
Lactobacillus delbrueckii subsp. Bulgaricus	4.8×10^6	2.3×10^6	1.0×10^6	3.6×10^5	2.3×10^5
Yeasts and molds, Log CFU mL^{-1}	-	-	-	0.5 ± 0.1	0.9 ± 0.1

The results of the yogurt stability during the storage period are shown in Fig. 3. Regarding the creaming index data, the lowest CI rates were observed for the formulation of coconut-soy yogurt with addition of kudzu starch and lecithin at the rate of 2% and 0.5%, respectively. Separation of the two phases was almost complete in less

than 8 days in all samples. After 25 days, lower CI was observed for the sample with kudzu starch and lecithin and characterized by high stability.
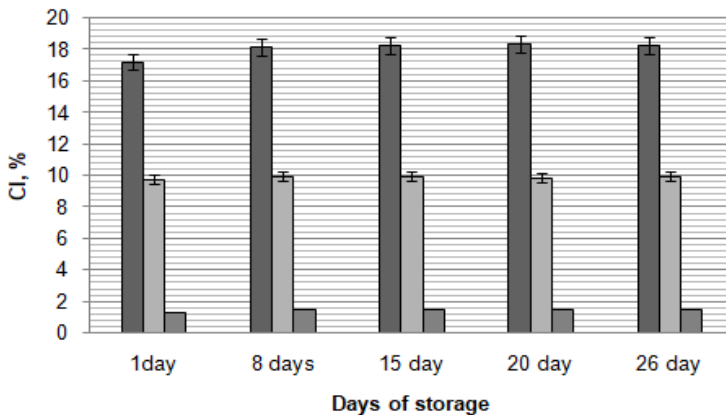

Figure 3. Creaming index versus days: ■ – control sample without kudzu starch and lecithin; ▨ – treatment sample with use of kudzu starch; ▨ – treatment sample with use of kudzu starch and lecithin. The values represent the mean of three determinations ± standard deviation. The differences were significant ($P < 0.05$).

CONCLUSIONS

Plant-based milk alternatives are a rising trend. In general, they can serve as inexpensive substitutes to cow's milk to those who cannot afford cow's milk because of high cost and limited availability or to those who are allergic to cow's milk. The present research efforts were carried out to create healthy plant-based beverage with high overall acceptability as an alternative to bovine milk which is palatable as well as nutritionally adequate to meet the present demands of consumers.

The results of the study showed that it is possible to produce plant-based yogurt to satisfy organoleptic needs of consumers, especially to people who are lactose intolerant or follow the vegan diet. The developed coconut-soy yogurt is a good source of amino and fatty acids. It is rich in lauric acid which is helpful in boosting immune system and maintaining the elasticity of the blood vessels.

The recommended storage period of yogurt without significant changes in viability of microorganisms is 20 days. Kudzu starch in the amount of 2% and lecithin in the amount of 0.5% can be used to improve the textural properties and stability of yogurt during the storage period without affecting the flavour of the final product.

The results of the study showed that blending two types of plant-based milk allows to obtain the product with sufficiently high nutritive and biological values and expand prospective for health food market.

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The usage of a binder system for frozen berries in the manufacture of confectionery

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Abstract. The aim of the research was to create binding systems for confectionery using gelling agents. The possibility of using partially hydrolyzed liquid egg white (egg hydrolyzate) in the binding system of gelling agents (egg hydrolyzate - agar (EG-A), egg hydrolyzate - starch (EG-S)) was determined to obtain the required mechanical characteristics when creating coatings, ornaments or fillers in confectionery with whole berries or pieces of fruit. In this regard, a technology has been developed for the hydrolysis of liquid egg white in the presence of an acidic reagent. The best rheological characteristics of the gelling agent from egg white were obtained under the following hydrolysis conditions: egg white: 1% HCl ratio = 1:2, process temperature - 66°C, duration - 40 minutes, the pH of the egg hydrolyzate was 6.53, the amount of dry solids was 11.78%. The newly created systems with agar (E406, Germany), chemically modified food starch (E1442, Germany) and hydrolyzed egg white (egg hydrolyzate) allow to adjust the properties of the coating for quick-frozen berries used in semi-finished confectionaries or cakes. It is established that the coating for quick-frozen berries, which includes a system consisting of 1% E406 and 0.5% egg hydrolyzate, should be carried out in 2 stages. In the first stage, a 10-minute exposure of the coating, which has a tensile strength of 580 g cm^{-2}, allows to create a strong capsule around the berry, which prevents the processes of destruction from proceeding. The second stage is necessary to obtain a uniform surface coating of the semi-finished mix from the berries. In this case, the tensile strength of the coating should be 480 g cm^{-2}. The system created from the E1442 and egg hydrolyzate gelling agents was also used in 2 stages when making cakes, which were subsequently baked at 180 °C. The content of the binding system in the coating applied to the test substrate was 6.9–7.7%, the effective viscosity of the coating was 120–180 Pa s. The content of the binding system in the coating of the surface of the berries in the second stage was 5.2–6.3% with effective viscosity values of 50–90 Pa s. Semi-finished berry products and ready-made baked cakes, produced with developed binding systems, can be stored at -8 °C for 10 to 12 days. After refrigerated storage, the separated moisture was not observed in the test samples. The absence of the phenomenon of syneresis with the indicated proportions of the introduction of gelling agents in coating systems has been established.

Key words: hydrolysis, egg hydrolyzate, binding systems, jellies, confectionery, refrigerated storage.

INTRODUCTION

To maintain the body's physiological needs (Basu & Lyons, 2014), the use of quick-frozen fruits and berries as an independent dish, decoration or as a part of confectionery is associated with the problem of preserving their physico-chemical properties and sensory indices for a long time.

Preservation of color, prevention of deformation of fruit and berry products as part of confectionery can be considered as a promising direction due to the fact that the existing technologies for using of gelling coatings based on various gelling agents allow to maintain high quality of the products no more than 96 hours, which is a significant problem for manufacturers.

Mainly hydrocolloids are used for obtaining food products of gel-like structure. Under certain conditions, polymer molecules of gelling agents are capable of forming a three-dimensional cross-linked network in solutions. In such a system, water is physically bounded, which results in changing the consistency of the product (DeMars & Ziegler, 2001). The gel is a fixed form of a colloidal solution (sol). For the implementation of the process of transition of the sol to the gel, it is necessary that forces between molecules start to act to ensure the formation of intermolecular bonds. This can be done by various methods: reducing the moisture content (due to evaporation), adding substances that contribute to forming of bonds and cross-linking, changing the temperature and adjusting the pH (Dille et al., 2018). Depending on the structure of the gel molecules, the gelling mechanisms differ significantly.

In the manufacture of gelling coatings for confectionery, a limited number of hydrocolloids is used. It is impossible to use thickeners, because the viscous structure formed by them is not able to stabilize spatially the berries and fruit fragments used for decorations.

The selection of ingredients that stabilize the binding systems in the composition of the coatings applied during the production of confectionery on berry raw materials plays an important role, therefore, it is necessary to know the properties that not only coating should have, but also the gelling agents included in the systems. The properties of thickeners and gelling agents can be changed by modifying them (introducing chemically neutral or ionic substituents into the molecule) to produce, for example, various types of starches (Huang et al., 2017; Lee et al., 2017; Naji-Tabasi & Razavi, 2017). According to the results of the analysis of the properties presented on the market of hydrocolloids, we chose agar-agar (E406) and modified corn starch (E1442). To regulate the strength characteristics of binding systems, (Cregut & Rondags, 2013; Han & Han, 2014; Somboon et al., 2014; Riedel et al., 2015; Rioux et al., 2015; Hayashi et al., 2016) additionally hydrolyzed egg white was used.

Studies of agar in combination with other gelling agents are quite extensive (Santagiuliana et al., 2018). They are essential when creating new foods (Sun et al., 2018) with the necessary properties (Li & Nine, 2016; Ellis et al., 2018). In the manufacture of gelling coatings in the confectionery industry, agar is often used because of its distinctive feature - resistance to temperature (Vao et al., 2016) in an acidic environment. This feature makes it possible to store the agar sol during thermostatting at temperatures higher than the gelling temperature for a sufficiently long time. Thus, the possibility of creating the necessary reserve of coating is used. In addition, agar gels

are characterized by good sensory properties (Kohyama et al., 2016) and high storage stability with little tendency to syneresis.

Depending on the type and extent of hydrolysis of the properties of starches (Włodarczyk-Stasiak et al., 2017) and their influence on the systems they include (Lin et al., 2018), they can vary widely (Astuti et al., 2018). Virtually all starches exhibit thickener's properties, and gelling properties occur when the polymer chains of starch are joined together to form a three-dimensional network when cooled, which leads to a decrease in its viscosity and an increase in elasticity. Starches that have passed through several stages of hydrolysis have the properties of both cross-linked and esterified starches (Włodarczyk-Stasiak et al., 2017).

When choosing the appropriate type of modified starch, it is necessary to be guided by the knowledge of the effect of chemical treatment on the properties of the binding system formed by it, as well as the recommendations of manufacturers. The properties that are most suitable for the manufacture of gelling coatings that withstand heating - cooling, has the starch E1442, which we used in our study.

In the gastrointestinal tract, ether and ester bonds are easily cleave, and the assimilation of modified starches occurs in the same way as the native (Kim et al., 2017).

Egg white, traditionally used in the food industry, has high solubility, foam-and gelling properties (Duan et al., 2018; Li et al., 2018), has adhesive characteristics, increases stability and viscosity of various bio-organic systems. Moreover, the strength characteristics of systems developed with their use are increased with the raise of processing temperature, exposure time and other factors (Raikos et al., 2007). However, the quantitative limits of the introduction of egg white are limited, since it reduces the sensory properties of the finished product (elastic consistency, color dilution). It was of interest to investigate modified egg white in the composition of the coatings, which in its properties is different from liquid egg white. Large reserve of chicken egg white are formed during the production of mayonnaise, since its production uses yolk, and chicken protein here is a by-product. The by-products appearing in the technological process of the main production are raw materials for obtaining new types of full-fledged products and are not waste to be disposed of.

The use of other types of gelling agents and thickeners is also possible, but this is associated either with a decrease in the quality of the product, or with the complication of implementation and control of technological operations, or with the increased cost of the product (Chen et al., 2015). Therefore, the priority in the research presented below was the creation of a new gelling agent with the desired properties from available and cheap raw materials, the usage of binding systems and the research of the possibility of their application in the confectionery products.

MATERIALS AND METHODS

To create the egg hydrolyzate - agar (EG-A) binding system, the best mode of hydrolysis of egg white in hydrochloric acid solutions with concentrations of 0.4%, 0.6, 0.8, 1.0, 1.2 and 1.4% with liquid egg white: HCl solution ratio = 1: 2 and temperatures of 48, 54, 60, 66, 72 ± 0.5 °C for 50 minutes was determined. The egg white hydrolyzed by us is a jelly of white color The pH value was measured by pH-meter 150-MI, 1-12 pH-electrode (Measuring technique, RU). The dry solids content was determined using a laboratory refractometer of the type RL 3 (Poland).

In the recipe of the coating based on the EG-A binding system, consisting of 0.5% EG and 1% E406, starch caramel acid molasses of Novlyansky starch factory (RU) in the amount of 8, 9, 10, 11, 12, 13, 14 % was included. The dry solids content in the molasses was 78%. Adjustment of the pH of the coatings was carried out in the presence of citric acid at a concentration of 0.2%. Water was added in the amount of 46.2–48.3%. The solids content in the formulations remained constant and was regulated by changing the amount of sugar (from 38.1 to 42.8%). The coating formulations are presented in Table 1.

The change in the mechanical characteristics of the EG depending on the concentration of the chemical

Table 1. Coating Formulations with EG-A Binding System

S. No.	Component name	Quantity, g for 100 g of embedding			
		No. of recipe of jellifying embedding			
		1	2*Control	3	4
1	Sugar	42.0	40.0	39.6	38.1
2	Molasses	8.0	10.0	12.0	14.0
3	Agar	1.0	1.0	1.0	1.0
4	Egg white	0.5	0.5	0.5	0.5
5	Citric acid	0.2	0.2	0.2	0.2
6	Water	48.3	48.3	46.7	46.2

reagent, as well as the effect of the content of EG-A on the properties of the coatings, were investigated using a device of the Valent type with umbrella-shaped cap with surface area of 2 cm^2. Mechanical strength of the gel was evaluated as:

$$P = m \cdot S^{-1}, \tag{1}$$

where m – a mass of weight touching the gel surface (stretch), g; S –surface area, to which the pressure is applied, cm^2.

To obtain thermostable, resistant to freeze-defrosting systems, usually use modified starches of various origins (Zhang et al., 2018). Therefore, a coating was developed that combines the properties of thermal stability, the required consistency and durability in the freezing process based on the egg hydrolyzate - starch (EG-S) binding system with 5, 6, 7, 8, 9% E1442 and 0.5% EG recipes. The sugar content in the formulation of the coating ranged from 31.7to 35.7%, citric acid –0.35%, water –58.5%. The coating formulations are presented in Table 2.

Table 2. Coating Formulations with EG-S Binding System

S. No.	Component name	Quantity, g per 100 g of embedding				
		No. of recipe of jellifying embedding				
		1	2	3	4	5
1	Sugar	35.70	34.70	33.70	32.70	31.70
2	Starch	5.00	6.00	7.00	8.00	9.00
3	Egg white	0.50	0.50	0.50	0.50	0.50
4	Citric acid	0.35	0.35	0.35	0.35	0.35
5	Carmin coloring agent	0.05–0.10	0.05–0.10	0.05–0.10	0.05–0.10	0.05–0.10
6	Flavoring agent	0.05–0.10	0.05–0.10	0.05–0.10	0.05–0.10	0.05–0.10
7	Water	58.50	58.50	58.50	58.50	58.50

The effect of the content of the EG-S binding system on the properties of the coating, as well as changes in the properties of the coatings during refrigerated storage, was determined from the values of the effective viscosity coefficient.

Coatings were stored at -8 °C for 12 days. This temperature is used at factories for the refrigerated storage of confectionery or semi-finished products for cakes. Then defrosted and again measured. In the process of research, the force applied to the system varied widely. The mass of the load varied from 8 to 62 g.

Comparison of the effective viscosity values of coatings with different EG-S contents was carried out with a load mass of 36 g. When using a load of smaller mass for coatings with a high content of the binding system, viscosity measurement is difficult because the transit time is quite long and with a larger mass of load for coatings with a low content of the binding system, its transit time is too short, which leads to a significant increase in measurement error.

The essence of the viscosity estimation method of the coating on the rotary viscometer is that the test coating is placed between two brass concentric cylinders of the viscometer – internal and external – and the internal cylinder is rotated. In this case, the internal resistance forces of the material are overcome by external force. The temperature of the investigated coating during the measurements was 20 °C.

The effective viscosity of the coating was calculated for each experiment using the equation: according to the passport for the rotary viscometer PB-8M:

$$\eta_{ef} = \kappa_\lambda \frac{M - M_0}{N}, \qquad Pa \cdot s \tag{2}$$

$$K_\lambda = \frac{Rg}{8\pi^2 \left(\frac{hr_1^2 r_2^2}{r_2^2 - r_1^2} + \frac{r_1^3 r_2^3}{r_2^3 - r_1^3}\right)}, \qquad m^{-1} \cdot s^{-2} \tag{3}$$

where M – the load weight, rotating the cylinder of the viscometer, kg; M_0 the inherent friction of the bearings, which is small and corresponds to the load weight of 0.001 –0.002 kg; N – angular velocity, rad·s^{-1}; κ_λ – the instrument constant for a given experience, m^{-1} s^{-2}; R – the pulley radius, $R = 0.02235$ m; r_1 – radius of the internal cylinder of the viscometer, $r_1 = 0.01605$ m; r_2 – the radius of the outer cylinder of the viscometer, $r_2 = 0.01905$ m; h – the height of the cylindrical part of the rotation body, immersed in the product under investigation, m; g – the gravitational acceleration, $g = 9.81$ m s^{-2}.

The instability of gelling coatings to freezing is manifested in their tendency to syneresis after defrosting, i.e. to the separation of fluid. To assess the tendency to syneresis, frozen coating samples were placed on a funnel with a filter for defrosting, the separated liquid was collected in a graduated test-tube. The amount of separated liquid, related to the mass of the sample, served as a criterion for assessing the tendency of the coating to syneresis after defrosting. The tendency to syneresis was measured after 12 days of storage at -8 °C.

For the manufacture of jelly, frozen fruits and berries grown with the utilization of a plant growth and development stimulator were used (Kremenevskaya et al., 2017). The quick-frozen berries were coated, heat treated (in case of coating with EG-S) and then refrigerated.

The organoleptic estimation of coatings was determined by 7 highly qualified experts using the rating method according to the appearance and condition of the surface, taste and smell, consistency (set 10-point scale), color (5-point scale).

The measurements were performed with five-time repetitions. Statistical processing of the experimental data was performed by the Curve Expert 1.5.0 program at a confidence level of P = 0.95. Statistical processing of the consistency of expert opinions in determining organoleptic indicators was carried out with the STATISTIKA program by calculating the Kendall rank concordance coefficient (W) with subsequent assessment of its significance using the Pearson criterion (Kendall & Stuart, 1973) according to the equation:

$$W = \frac{12S}{m^2(n^3 - n)} \tag{4}$$

where S – the sum of the squares of the differences between the sum of the ranks studied and their arithmetic average; m – the number of experts; n – the number of objects (n = 5).

The consistency of the theoretical and statistical distribution was determined by the Pearson criterion (U) by the expression:

$$U = mwk \tag{5}$$

where $k = a^{-1}$ – the number of degrees of freedom, a –the number of estimated characteristics.

RESULTS AND DISCUSSION

Hydrolysis of liquid egg white

The results of studies related to the determination of the best concentration of the HCl solution during the hydrolysis of liquid egg white were evaluated by the strength (P) of the jelly formed. They are presented in Fig. 1.

As it can be seen from the data presented, the maximum strength of the hydrolysates obtained is observed in the case of the processing of raw materials with a chemical reagent at concentration of 1% in the presented temperature range. The decrease in concentration below 0.3% did not allow to obtain protein jelly, the formation of irregular clots was observed. Increasing the concentration of the reagent above 1.2% led to a significant decrease in the strength of the jelly. In addition, high acid concentrations increase costs.

The selection of temperature regime is associated with the fact that increasing the temperature intensifies the diffusion, distribution and binding

Figure 1. Dependence of the strength of egg hydrolyzed jelly at different concentrations of HCl solutions in the process of hydrolysis at the temperature of 66 °C.
P = 15.4-15.8C + 37.8-19.7, where C –the HCl concentration, where (hereafter): S –Standard deviation, R^2 –correlation coefficient.

of the reagent, the rate of protein hydrolysis increases, therefore, decreases the time of the process, but can lead to undesirable changes in the finished product. In this case, at the temperature of 72 °C, protein coagulation is observed (coagulation of native egg white happens at temperature of 58–60 °C).
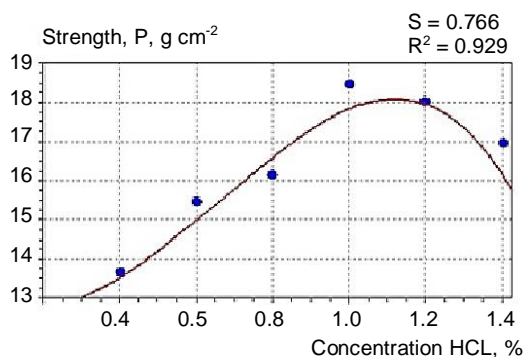
With the optimum HCl concentration and temperatures of 48, 54 and 60 °C, the resulting jelly of white color is less dense, the strength values of which are, respectively, 14.2, 15.8, 16.4 g cm^{-2}. At a temperature of 66 °C, the strength of the jelly reaches 18.4 g cm^{-2}. In connection with the above, the hydrolysis temperature was assumed to be 66°C.

The duration of hydrolyzate exposure is determined by the time required for the formation of strong jellies of white color. Loss of fluidity of a viscous liquid and its transformation into a more or less elastic jelly (broth jell) occurs when bonds form between the side chains of protein macromolecules. These bonds are formed as a result of the interaction of both polar and ionized groups. Protein chains form peculiar spatial three-dimensional frameworks (grids). The best modes allow to carry out the process of rise and gradual ordering in the gelatinizing system of a spatial grid relatively quickly. When conducting research, it was found that the formation of jellies at the reagent concentration of 1%, the process temperature of 66 °C and the raw material and water ratio of 1: 2, begins as early as 15– 20 minutes after the start of the process, and they become stable after an additional 15 minutes away. Therefore, the required time of the process of hydrolysis of egg white under these conditions is 40 minutes. The pH of the hydrolyzate is 7.53, the amount of dry solids is 11.78%.

It is necessary to add that the carrying capacity of the equipment for liquid treatment, the correct management of the process, water consumption for technological needs, the degree of contamination of wastewater depends on the value of the 'raw material: solution' ratio (the so-called liquid coefficient, LC). To increase the carrying capacity of the equipment, liquid processing is advisable to conduct at lower values of the liquid coefficient.

The study of the EG-A binding system

Studies of coatings obtained on the basis of egg hydrolyzate, agar and molasses showed that in order to avoid premature gelation of the finished coating, it is necessary to thermostatize it at a temperature of 66–68 °C before use. The use of the coating should be carried out in the shortest possible time, since the gelling coating based on the proposed system should not be used prematurely because the gel re-formed after melting the coating has extremely low mechanical strength. The effect of the content of the binding system, obtained immediately after production and after reheating on the mechanical strength of the fillings, is presented in Fig. 2.

As it can be seen from the presented data, after repeated heat treatment, the formation of a durable
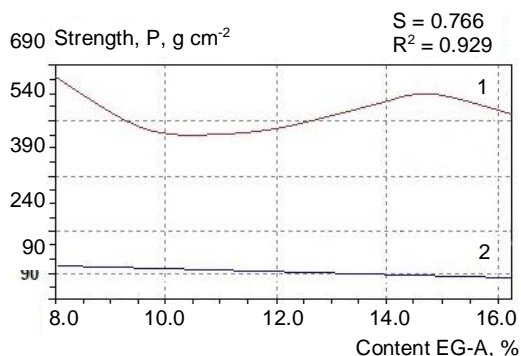

Figure 2. 1 –The effect of the content of the EG-A binding system coating on the mechanical strength; 2 –The effect of repeated heat treatment on the mechanical strength of coatings with different content of the EG-A binding system.
$P_1 = 9701 - 2203C + 173C^2 - 4.44C^3$,
$P_2 = 497 - 94.7C + 7.47C^2 - 0.194C^3$
where (hereafter) C –the content of the binding system in the coating, %.

coating of berry raw materials in the production of confectionery is not possible.

The creation of coatings, the content of bound moisture in which is big enough, it lets reduce the cryoscopic temperature of the product and, accordingly, the temperature of the refrigerated storage. Analysis of the temperature curves showed that the cryoscopic temperature of the coating with EG-A is -8 °C. Traditionally, confectioneries are stored at 4 °C. It was of interest to determine the value of the mechanical strength depending on the content of the binding system of the coating at different temperatures of refrigerated storage.

The research results are presented in Fig. 3.

Comparing the values of the mechanical strength of the coating samples with EG-A after refrigerated storage with the values of samples that have not been subjected to storage, it can be seen that for all coatings, regardless of the percentage of binding systems introduced into them, the following is observed. The values of mechanical strength increase when stored for 12 days under conditions of near cryoscopic temperature of -8 °C and decrease when stored for 5 days under traditional conditions (4 °C).

Figure 3. 1 –Dependence of the mechanical strength values of the coatings on the amount of EG-A before refrigerated storage; 2 – Dependence of the mechanical strength values of the coatings on the amount of EG-A binding system at the storage temperature of -8 °C for 12 days; 3 –Dependence of mechanical strength values on the amount of EG-A binding system at the storage temperature of 4 °C for 5 days.

$$P_1 = 9665 - 2246C + 179C^2 - 4.67C^3$$
$$P_2 = 5287 - 1118C + 87.8C^2 - 2.25C^3$$
$$P_3 = 9701 - 2203C + 173C^2 - 4.44C^3$$

The decrease in mechanical strength during storage is typical for jellies, which contain an agar component, and this is mainly due to a violation of the spatial structure of the gel by reducing the properties of the hydrocolloid. Apparently, at near-cryoscopic temperatures, these processes are slowed down; moreover, by reducing the thermalmotion of the molecules, the gelling agent is able to form a more durable gel. The best strength values of all three coating samples are observed when using 14.5% EG-A.

The presence of two maxima on the dependency diagrams of the jelly's mechanical strength on the content of the binding system in the coating can be explained in the first case by matching the optimum sucrose content, in the second - by the pH value. Such changes in the values reflected in the curves are possible due to the introduction of molasses into the binding system. The monosaccharides that make up the molasses have a lower dehydrating capacity than sucrose, therefore, an increase in the molasses content due to a decrease in the sugar content may lead to a decrease in the gel strength. However, the molasses have an acidic reaction of the medium (pH = 4.0–4.5), and the pH value has a significant effect on the strength and optimum temperature of the jellies (i.e., the temperature at which their strength is maximum). Thus, with an increase in the content of molasses by reducing the amount of sugar, the pH of the medium may decrease so much that the strength of the jelly will increase (Gao et al., 2017). It is
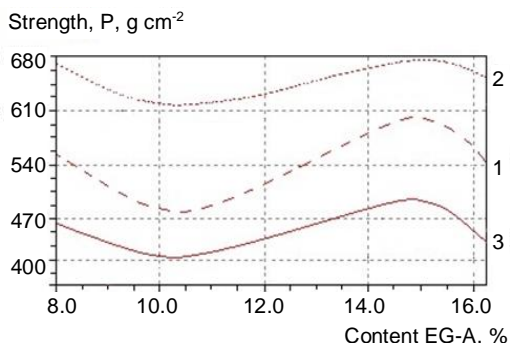
impossible to completely abandon the use of molasses during the creation of the binding system, since in this case saccharification of the coating during the refrigerated storage may occur.

When gelling frozen fruits (raspberries, cherries) and berries (black currants) with freshly prepared EG-A based coatings and their subsequent storage, phenomena that impair the quality of the product were observed to varying degrees, the main features of which were deformation of the film and oxidation of its surface. An organoleptic evaluation showed that after refrigerated storage, the oxidation of the gelled berries is not observed in all samples of black currant. In samples of raspberries, no oxidation of the fruits is observed in the case of their coating of 14.5% EG-A, and with a decrease or increase in the percentage of the binding system, a slight oxidation occurs. When coating the quick-frozen cherries with a content of 14.5% EG-A, there is a minimal oxidation of the fruits; with 9.5 and 15.5% the maximum oxidation of the samples is observed. Deformation of all types of fruits and berries is not observed after storage in the coating with 14.5% of the binding system and is insignificant for samples at 11.5, 13.5% and 15.5%. For cherries, the deformation at 11.5 and 13.5% was also insignificant, and at 15.5% was strongly pronounced. Color changes and the phenomenon of syneresis for all samples were minimal even at 13.5% of the binding system. The area of the uncovered surface of fruits and berries at 9.5 and 11.5% of the system was 20%, at 13.5 and 15.5% –10%.

The thickness of the coating film with EG-A, which forms on the surface of the fruit or berry, ranges from 0.5 to 1.0 mm. Moreover, the formation of a thinner film is characteristic for coatings with lower strength. A sample having a 15.5% of binding system has a crumbly consistency. There are difficulties with filling of raspberries. Raspberries are one of most sensitive fruits and most quickly deteriorating. Also, it has a high water content and due the binding effect may suffer. For sure, the hairy surface will affect the filling as well. The surface of the berry is not fully covered, possibly due to the high surface tension coefficient of the 'hairy' surface of the raspberry. The appearance of the empty surface deteriorates during storage and the surface dries out.

When strictly follow the technology of coating quick-frozen fruits and berries (with an exposure after coating for up to 10 minutes for gelation), it may be obtained a product that does not change its consumption properties when stored at –8°C for 12 days. As the first layer, a coating with 0.5% hydrolyzed egg white, 1.0% agar and 13.0% molasses (strength of 580 g cm^{-2}) was used, for the second layer, a coating with 0.5% hydrolyzed egg white was used, 1.0% agar and 10.0% molasses (strength of 480 g cm^{-2}). The first layer creates a solid capsule around the fruits and berries, preventing the processes of destruction from proceeding, the second layer allows to obtain a uniform coating.

EG-S based binding system study

When creating a system with the addition of hydroxypropyl distarch phosphate, the coating is with a viscous gelled structure, and has thermostable properties being resistant to freeze-defroster cycles. The effect of the EG-S binding system on the viscous properties of the coating is shown in Fig. 4.

For non-Newtonian fluids, viscosity is not a constant, but a function of the force applied to the system. In the process of freezing and refrigerated storage, the viscosity of the coatings slightly increases. This may be due to partial extraction of solutes into the aqueous phase during freezing or with partial drying of the coating during storage. The change in the dependence of the effective viscosity coefficient of the coatings on the amount of EG-S after refrigerated storage is similar. The deviation of values is no more than 5%. The resistance of the coatings to freezing and refrigerated storage was determined indirectly by the value of syneresis. The graph based on the measurement results is presented in Fig. 5.

It can be seen that the introduction of the binding system in the amount of 7.5 and 9.5% prevents the process of syneresis and practically no moisture is released. The use of binding systems in confectionery production allowed to develop technologies of products' coating using quick-frozen fruits and berries as decoration.

Figure 4. Dependence of the effective viscosity (η_{ef}) coefficient of coatings on the amount of EG-S applied before refrigerated storage.
$$\eta = 181 + 45C$$

Fruits and berries covered with EG-S, pre-placed on a test basis, and baked. All coatings used showed thermal stability properties for 10–15 minutes at a temperature of 200–220 °C. The finished products were stored at a temperature of -8 °C for 10 to 12 days. Then determined the condition of the berries. For all types of fruits and berries, the best performance was achieved with the use of 7.5 and 8.5% binding systems - no browning, deformation of the fruit, uncoated surface of the fruit and wetting of the test substrate. The maximum changes were found at 5.5% and 9.5% EG-S. When making a binding system in an amount of more than 8.5%, the viscosity of the coatingincreases and its uniform distribution over the surface of the dough piece, especially fruits, is significantly hampered. In addition, a further increase in the content of starch in the coating adversely affects its taste. When making the system in an amount of less than 5.5%, there is a strong wetting of the dough base and deformation of the fruit during baking. The optimal content of the binding system in the coating: for the top layer - 5.2–6.3% (easily distributed over the surface of fruit, the viscosity is 50–90 Pa s); for the lower layer - 6.9–7.7% (dense enough so that the fruit would
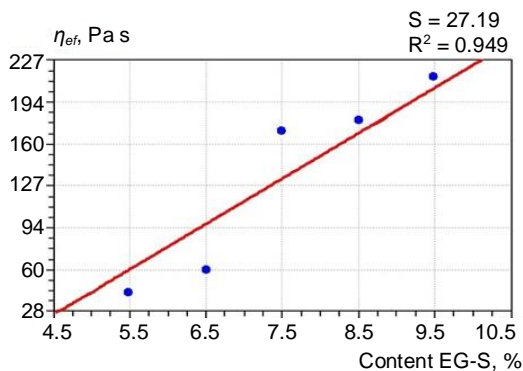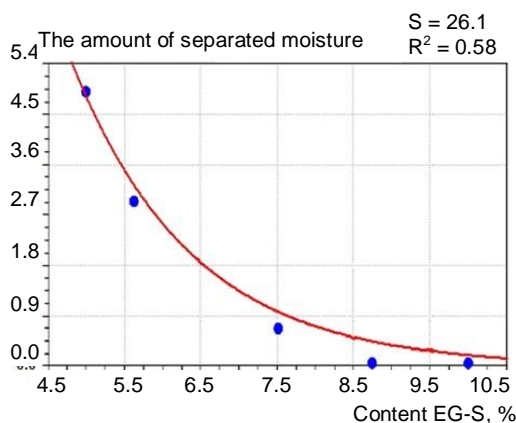

Figure 5. Dependence of the amount of separated moisture (M) of the filling after freezing with a different amount of the binding system.
$$M = 386e^{-0.795C}$$

remain on its surface in a state of indifferent equilibrium, the viscosity is 120–180 Pa s).

Organoleptic studies have shown that the surface of the coatings in all samples before and after refrigerated storage was glossy. The consistency of the samples with a 5.5% binding system had the structure of a thick jelly (berry starch drink) (short droplet type), with 6.5% and higher - a soft jelly. The aroma in all samples was intense, specific to fruits and berries, the taste is sour, pleasant The scoring of the cakes by experts is presented in Table 3.

Statistical studies of the organoleptic evaluation of confectionery by experts showed a strong consistency of expert opinion, since the value of the concordance U was 0.75, which exceeds the threshold value (w > 0.7). Its calculated significance

Table 3. Tasting sheet of the cakes with the EG-S binding system

S.No.	Expert	Taste, smell	Consistency and appearance	Colour
1	1	8	7	4
2	2	8	7	3
3	3	8	7	5
4	4	7	8	3
5	5	7	8	5
6	6	7	8	5
7	7	8	7	4

by the Pearson criterion is 10.5. The critical values of the Pearson correlation at 2 degrees of freedom, 1 and 5% significance levels are, respectively, 9.210 and 5.991, which is less than the calculated value. This suggests that, at a significance level of 1%, the estimated coefficient of concordance can be taken as significant.

The use of binding systems in the production of confectionery has allowed the development of coating technologies for products made using quick-frozen fruit and berry products.

CONCLUSIONS

Research related to obtaining new products from resources, which are usually disposed of or part of them is used in a limited food sector, seems to be relevant. With minimal investment and the simplicity of the production technology, it is possible to obtain ingredients with specified properties, which, in turn, directly change the properties of products produced with their use.

It was established that hydrolysis of liquid egg white at the concentration of 1% HCl solution, the process temperature of 66 °C, the raw material and water ratio of 1: 2 for 40 minutes allows to obtain a product with strength characteristics.

The obtained data of the mechanical strength of various binding systems, which included hydrolyzed egg white, made it possible to develop a coating technology for quick-frozen berries for confectionery. At first, the fruits and berries are immersed in the system with EG-A, characterized by high strength values (570–590 $g\,cm^{-2}$). Then, the gelatinized berries are covered with a new coating layer, which has lower strength indices (470–490 $g\,cm^{-2}$). The first layer creates a solid capsule around the berries, preventing the processes of destruction from proceeding, the second layer allows to obtain a uniform coating.

The use of the technology described above allowed us to obtain a product that did not change its consumption properties when stored at -8 °C for 12 days. Moreover, the condition of the product stored under the proposed modes (-8 °C 12 days) was better than the product stored under the traditional modes (120 hours at 4 ± 2 °C).

In case of using a binding system made of egg white ingredient and modified starch in the coating, the coating with a binding system content of 5.5–6.5%, having an effective viscosity of 45–60 Pa s was used for the upper layer. The EG-S binding systems with such indicator values allow the coating to be easily distributed over the surface of the fruit or berry, including quick-frozen raw materials. For the lower layer, it is better to use EG-S with a binder content of 7.2–7.6%, having a sufficiently thick texture (130–180 Pa s), so that the fruit remains on its surface in a state of indifferent equilibrium. Coatings with EG-S have thermostable properties and can be used for coating berry and fruit raw materials in confectionery developed for 10–15 minutes at a temperature of 200–220 °C. Finished products can be stored at near-cryoscopic temperatures for 10–12 days, while maintaining high consumer properties.

When conducting organoleptic studies by experts, the high quality of the presented samples was revealed. The high convergence of the results suggests the correctness of the statistical study.

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Winter rye (*Secale cereale* L.) antioxidant capacity, total phenolic content and quality indices

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Abstract. Rye (*Secale cereale* L.) grain is an excellent raw material for healthy and tasty foods. Rye products are characterized by their unique composition and properties such as antioxidant capacity and total phenolic. The aim of the study was to assess radical scavenging capacity, total phenolic content, protein, starch content and falling number in rye varieties wholemeal. The trial included population winter rye varieties 'Kaupo', 'Amilo', 'Dankowskie Amber', 'Dankowskie Rubin', 'Inspector' and hybrid rye varieties 'SU Drive', 'SU Mephisto', 'SU Bendix', 'Brasetto', 'Palazzo' grown in Latvia. The antioxidant activity was determined using the DPPH (2.2-diphenyl-1-1-picrylhydrazyl radical) assay and total phenolic content was determined spectrophotometrically according to the Folin-Ciocalteou method. The rye grain antioxidant capacity was estimated as Trolox equivalent, while the total phenolic content was expressed as gallic equivalents (GAE). 'Su Drive' rye variety contained the largest amount of total phenolic (average 208 mg GAE 100 g^{-1} DW), but the lowest – 'Inspector' rye variety 176 mg GAE 100 g^{-1} DW. In general, all rye samples tested in this study demonstrated similar level of antioxidant capacity (from 38.5 to 46.2 mmol Trolox eq. 100 g^{-1}). Statistically higher ($P < 0.05$) total phenols content and falling number had hybrid rye grains, compared to the population rye grains. In the present trial, the differences between hybrid varieties grains protein and *starch content* comparing to population varieties were not observed.

Key words: antioxidant capacity, phenolic, protein, falling number, rye, starch.

INTRODUCTION

Rye grain, like other cereal grains, is important part of human diet. Rye is second to wheat, the most commonly used grain, in the production of bread (Michalska, 2007). The most popular for baking bread is wholemeal rye flour. Whole grain rye, primarily its aleurone layer, germ and bran, has rich sources of phytochemicals including phenolic compounds (Zieliński & Kozlowska, 2000; Žilic, 2016). Thus, whole grains are believed to consume significant health benefits in the prevention of chronic diseases such as cardiovascular disease and diabetes due to the contribution of phenolic compounds (Van Hung, 2016). Among health-promoting phytochemicals found in cereal grain, phenolic

compounds have gained much attention in many scientific research areas due to their strong antioxidant properties. Their concentration in rye grains are at least partly influenced by cultivar (Michalska et al., 2007). Generally, main phenolic compounds in rye grains are phenolic acids, flavonoids, coumarins, proanthocyanidins, stilbenes and lignans (Žilic et al., 2012). The phenolic content of cereal grains is considered to be particularly important for human health, given its antioxidant activity and high cereal consumption among the general population (Mazzoncini et al., 2015). Polyphenols are a group of compounds synthesized exclusively by plants, especially for the protection against UV-radiation and activity of pathogens. About 8,000 plant polyphenol compounds have been identified so far, whereas only some hundred occur in edible plants. They are in fruits, flowers, leaves, roots and stems, whereas external tissues include bigger amounts of these components (Manach et al., 2004). Antioxidant activity is defined as an inhibition of the oxidation of lipids, proteins, DNA or other molecules that occurs by blocking the propagation step in oxidative chain reactions (Augšpole et al., 2018).

The environmental conditions are known to have significant influence on the end-use quality of cereal grains (Augšpole et al., 2018; Linina et al., 2019).

The quality index of rye wholemeal flour is protein and starch content, they are affected by meteorological conditions, fertilizers and varieties (Kunkulberga et al., 2017; Stockmann et al., 2019).

In Latvia, cereal maturation and harvesting can occur during rainfall period, which can often be a reason for a low grain quality and even grain sprouting in ears. The falling number is a quick and easy test used to evaluate the degree of starch hydrolysis in wheat and rye grain samples. That quality index describes the degree of dextrinization of starch caused by amylolytic enzymes, mainly α-amylase. Increased activity of the enzyme is observed during the pre-or post-maturity sprouting of grain (Antonenko et al., 2014; Linina & Ruza, 2015).

The yield and quality of grain is mainly determined by management techniques and environmental conditions; however, it can be influenced by the genetic basis (Zdubel et al., 2009; Stepien et al., 2016; Linina & Ruza 2018; Stockmann et al., 2019).

The aim of the study was to assess radical scavenging capacity, total phenolic content, protein and starch content and also falling number in varieties of rye wholemeal.

MATERIALS AND METHODS

Study fields

Field trials with winter rye population varieties 'Kaupo' (Latvia), 'Inspector' (Germany) 'Amilo', 'Dankowskie Amber', 'Dankowskie Rubin' (all from Poland) and hybrid rye varieties 'Brasetto' (Canada) 'SU Drive', 'SU Mephisto', 'SU Bendix', and 'Palazzo' (all from Germany) were carried out in Priekuli Research Centre, Institute of Agricultural Resources and Economic in 2017/2018. The soil type was sod-podzolic loam, with close to neutral acidity, medium high phosphorus and potassium, organic mater 2.0 g kg^{-1}. Winter rye was sown in 20 September after spring barley. Seeding rate of population winter rye varieties was 500 seeds per m^2, whilst of hybrid rye varieties – 200 seeds per m^2. Trial was arranged randomly in four replications, plot size: 16 m^2. In total, there were 40 trial plots. In autumn NPK 6:26:30 kg ha^{-1} was applied. In spring after resumption of vegetative growth was applied nitrogen N68 kg ha^{-1}, at the shooting

stage was applied N 31 kg ha^{-1}. Winter rye was harvested on 3 August. A sampling procedure for gain quality evaluations was performed according to ICC 101/1 standard for obtaining a mean sample.

Weather conditions

The air temperature in 2018 April was close to long-term average data. May was 5 °C warmer (17 °C), which influenced plant growth and development. Average daily temperature in June was close to long-term average data. Temperature in July (grain filling period) was 20 °C (2 °C warmer compared to long-term average data). Precipitation in April was close to long-term average data. May was dry. In June precipitation was close to long-term average data. The weather in July was very dry. Elevated temperatures and low precipitation significantly influenced plant growth, yield and quality.

Analysis

The rye grains were analysed at the Latvia University of Life Sciences and Technologies in Grain and Seeds Research laboratory and in laboratory of the Department of Chemistry. All winter rye grain samples were ground in a Perten Laboratory Mill 3100 to obtain the wholemeal.

Total phenolic content. For total phenolic extraction 1.0 ± 0.001 g of finely ground rye samples were weighed in to volumetric flasks, 10 mL of extractant, a mixture of methanol, distilled water and hydrochloric acid (79:20:1) was added. The vials were shaken at $+18 \pm 1$ °C for 60 min in the dark, then centrifuged for 10 min at 5,000 rpm. The total phenolic content of the rye extract was analysed spectrometrically according to the Folin-Ciocalteu (Dewanto et al., 2002). Briefly, 2.5 mL of FolinCiocalteu reagent (diluted 10 times with distilled water) was added to 0.5 mL of rye extract. The mixture was then incubated for 3 min, after which 2 mL of sodium carbonate (Na_2CO_3) (7.5 g L^{-1}) was added. The sample was mixed. The control sample contained all the reaction reagents except the extract. After 1 hour of incubation at +20 °C temperature in dark for colour development, absorbance was measured at 765 nm using JENWAY 630 Spectrophotometer. Results were expressed as mg gallic acid equivalents (GAE) 100 g^{-1} dry-matter of rye extract. Analyses were carried out in triplicate for each rye extract sample (Augspole et al., 2017).

Antioxidant capacity. The antioxidant capacity of rye samples was determined according to Afify (2012) with some modifications. Method is based on the radical scavenging ability in reaction with stable 2.2-diphenil-1-picrylhydrazyl (DPPH) free radical. 3.5 mL of DPPH solution (4 mg of DPPH reagent dissolved in 100 mL pure ethanol) was added to 0.5 mL sample extract. A solution was well mixed and stood in dark place at $+20 \pm 1$ °C for 30 min. Absorbance was measured at 517 nm using JENWAY 630 Spectrophotometer. The antioxidant capacity was expressed as TROLOX (6-hydroxy-2.5.7.8-tetramethylcroman-2-carboxylic acid) equivalent antiradical activity mmol Trolox equivalent (TE) 100 g^{-1} DW.

Winter rye wholemeal quality index– *protein content* (%) and *starch content* (%) were analysed by grain analyser Infratec NOVA (Denmark). In Latvia grain analysers are connected in a unified network. Latvian grain network is a complex administrative-technical solution, providing uniform operation of the grain analyzers in the grain trade

companies. Technically it means that every individual grain analyser is regularly controlled and adjusted according to Master Infratec via communication network. Master Infratec is controlled and adjusted according to well-known standard methods.

The *falling number* was determined according to the standard LVS EN ISO 3093:2011. 'Wheat, rye and their flours. Determination of the falling number according to Hagberg-Perten', using Perten Instrument (Sweden) 'Falling number 1500' assessed at using 7 g wholemeal adjusted to a moisture of 15%.

Statistical analysis

Results are presented as means ± standard error. For each trait, mean and the coefficient of variation (V%) were determined. Trial data evaluation was done using one factor analysis of variance by Fisher's criteria and least significant difference ($LSD_{0.05}$). Significant differences for rye grain total phenolic content and antioxidant capacity are marked with different letters in superscript: [A, B, C] and [a, b, c]. Differences of the average antioxidant capacity, total phenolic content and quality index between population and hybrid rye varieties were determined by t-test: Two-sample assuming unequal variances.

RESULTS AND DISCUSSION

The mean of antioxidant capacity (AC), total phenolic content (TPC), protein content (PC), starch content (SC) and falling number (FN) in the rye wholemeal from population and hybrid varieties selected for study are shown in Table 1. The least variation of the starch content (average 61.0%), total phenolic content (average 183.5 mg 100 g^{-1} GAE), protein content (average 10.4%) was noticed in the population varieties, with coefficient of variation (V%) from 0.2 to 2.5%. The greatest variation of the antioxidant capacity and falling number were observed in population cultivars wholemeal: coefficient of variation respectively 6.7% and 6.1%.

Table 1. Winter rye grain average antioxidant capacity, total phenolic, protein content, starch content and falling number

	AC, mmol Trolox eq. $100g^{-1}$	TPC, mg 100 g^{-1} GAE	PC, %	SC, %	FN, s
Population varieties					
Mean ± standard error	42.0 ± 1.3	183.5 ± 2.0	10.4 ± 0.1	61.0 ± 0.1	260.8 ± 7.1
min	38.5	175.9	10.1	60.9	249.0
max	46.2	187.8	10.7	61.2	288.0
V%	6.7	2.5	2.5	0.2	6.1
Hybrid varieties					
Mean ± standard error	42.1 ± 0.8	195.9 ± 4.1	10.0 ± 0.3	61.1 ± 0.1	276.8 ± 2.9
min	39.3	184.9	9.3	60.9	269.0
max	43.8	208.3	10.6	61.3	285.0
V%	4.3	4.7	5.7	5.7	2.3

AC – antioxidant capacity; TPC – total phenolic content; PC – protein content; SC – starch content; FN – falling number; V% – coefficient of variation.

Winter rye wholemeal *total phenolic content* (TPC) significantly varied between hybrid varieties ($P < 0.05$) and also between population varieties ($P < 0.05$) (Fig. 1). The

total phenolic content from hybrid varieties' wholemeal ranged from 185 mg 100 g^{-1} GAE ('SU Mephisto') to 208 mg 100 g^{-1}GAE ('SUDrive'), in population varieties – from 176 mg 100 gAE ('Inspector') to 188 mg 100 gAE ('Kaupo'). Similar results were gained in Poland (Zieliński et al., 2007) where total phenolic content in wholegrain of winter rye varieties was: 216mg 100 g^{-1} GAE ('Amilo'), 237 mg 100 g^{-1} GAE ('Warko') and 218 mg 100 g^{-1} GAE ('Dankowskie Zlote') and also in Serbia: 177 (variety 'DK-R') to 215 mg 100 g^{-1} GAE ('Raša') (Žilić et al., 2011).
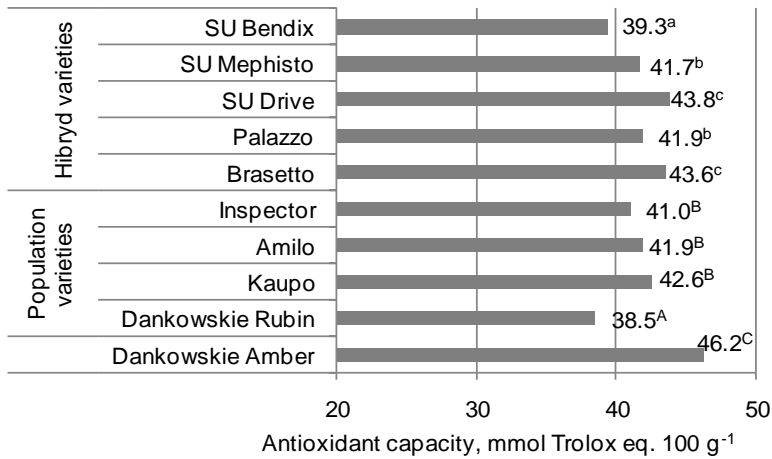

Figure 1. Total phenolic content (mg 100 g^{-1} GAE) in winter rye wholemeal (significantly different means are marked with different letters in superscript: [a, b, c, d, e] – significant difference for rye grain hybrid varieties; [A, B, C] – significant difference for rye grain population varieties).

Winter wheat grains have a higher content of phenols comparing to rye. In the trial Skrajda et al. (2017) in Poland with 24 winter wheat varieties found that antioxidant capacity was from 296 to 359 mg 100 g^{-1} GAE. Total phenolic content and antioxidant capacity depend on the environmental conditions, management techniques and genetic trait of the variety (Zieliński et al., 2007).

In our investigation winter rye wholemeal *antioxidant* capacity (AC) significantly varied between hybrid varieties ($P < 0.05$) and also between population varieties ($P < 0.05$) (Fig. 2). In hybrid varieties the antioxidant capacity ranged from 39.3 mmol Trolox eq. 100g^{-1} ('SU Bendix') to 43.8 mmol Trolox eq. 100g^{-1} ('SU Drive'), in population varieties – from 38.5 mmol Trolox eq. 100g^{-1} ('Dankowskie Rubin') to 46.2 mmol Trolox eq. 100g^{-1} ('Dankowskie Amber'). Grains of hybrid rye varieties had statistically higher total phenolic content ($t_{stat.}2.68 > t_{crit.}1.94$).

Lower results were obtained in Poland (Zieliński et al., 2007) where in winter the antioxidant capacity of rye population varieties was: 14.7 mmol Trolox eq. 100 g^{-1} ('Amilo'), 15.2 mmol Trolox eq. 100g^{-1} ('Warko') and 14.3 mmol Trolox eq. 100 g^{-1} ('Dankowskie Zlote'). In Switzerland trial (Langenkämper et al., 2006) with wheat cultivar 'Titlis' antioxidant capacity in the wholemeal was 15.0 mmol Trolox eq. 100g^{-1}. Augspole et al. (2018) found that the antioxidant capacity of samples could be influenced by lots of factors, such as cultivation, production, storage conditions and test systems.

In another trial with peeled grains of rye was fixed lower total phenolic content (132 mg 100 g^{-1} GAE) and antioxidant capacity 20.0 mmol Trolox eq. 100 g^{-1} (Djordjevics et al., 2011), because whole rye grains contain germ and bran which are rich sources of total phenolic content and antioxidant capacity (Žilic et al., 2011).
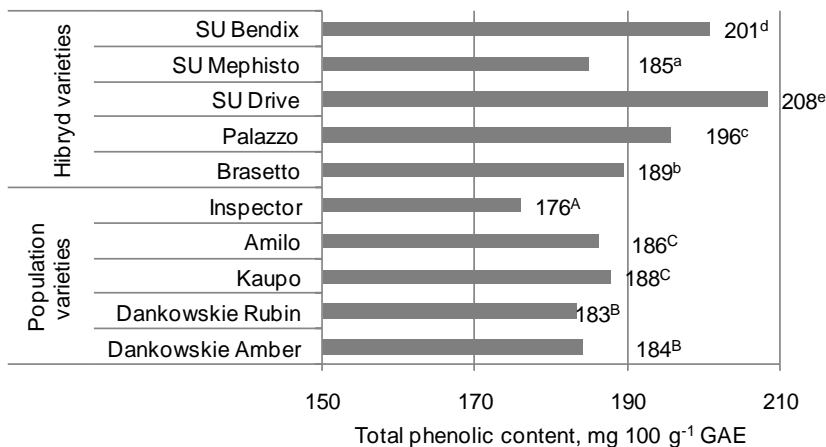

Figure 2. Antioxidant capacity (mmol Trolox eq. 100g^{-1}) in winter rye wholemeal (significantly different means are marked with different letters in superscript: [a, b, c,] – significant difference for rye grain hybrid varieties; [A, B, C] – significant difference for rye grain population varieties).

The protein content, starch content and falling number belong to an important criteria for the quality of rye grain (Linina & Ruza, 2015; Stepien et al., 2016). In our trial grain protein content, starch content and falling number significantly ($P < 0.05$) varied depending on the variety (except starch content for hybrid varieties). The results of analysed index are presented in Table 2. The *protein content* from hybrid varieties ranged from 9.3% ('Palazzo') to 10.6% ('SU Mephisto' and 'SU Bendic'), in population varieties – from 10.1% ('Dankowskie Amber') to 10.7% ('Dankowskie Rubin'). Zielinski et al. (2007) showed that the average content of protein for population varieties were: 'Amilo' 8.7%, 'Dankowske Zlote' 10.3% and 'Warko' 11.6%, similar results were obtained in Estonia (Järvan et al., 2018) respectively 8.4% to 11.9%.

Starch is an important component of rye flour, ranging from 60.9% to 61.3% for both varieties.

In another investigation (Linina et al., 2019), the starch content in grain

Table 2. Winter rye wholemeal protein content, starch content and falling number

	PC, %	SC, %	FN, s
Hybrid varieties			
Brasetto	9.8	60.9	280
Palazzo	9.3	61.0	278
SU Drive	9.7	61.3	272
SU Mephisto	10.6	61.2	269
SU Bendix	10.6	61.1	285
LSD$_{0.05}$	0.04	0.12	ns*
Population varieties			
Dankowskie Amber	10.1	61.0	253
Dankowskie Rubin	10.7	61.2	249
Kaupo	10.2	61.0	252
Amilo	10.6	60.9	288
Inspector	10.6	60.9	262
LSD$_{0.05}$	0.12	0.13	7.14

PC – protein content; SC – starch content; FN – falling number; *ns – not significant.

of hybrid and population varieties of winter rye fluctuated over a larger range: from 58.8% to 64.8%, while in Poland (Zieliński et al., 2007) trial starch content was lower: from 53.3% ('Warko') to 55.7% ('Amilo').

In the present trial, the differences between hybrid varieties grains protein and *starch content* comparing to population varieties were not observed ($t_{stat.} < t_{crit.}$).

Hagberg falling number is the major quality attribute of rye grain. In 2018, the weather during the harvest was dry and sunny, therefore falling number for all winter rye samples was high. Falling number of hybrid varieties ranged from 269 s ('SU Mephisto') to 285 s ('SU Bendic'), in population varieties – from 252 s ('Kaupo') to 288 s ('Amilo'), which considerably exceeds the minimum requirements for grain processing in Latvia (120 s). In our investigation the falling number of grains was significantly ($P < 0.05$) different for population cultivar. Statistically higher falling number was obtained of hybrid rye varieties ($t_{stat.}2.08 > t_{crit.}2.01$).

Linina et al. (2019) in the previous study (2015–2017) discovered, that the falling number in hybrid and population varieties of winter rye was from 130 s to 305 s, in Estonian trial it was 62 s to 289 s (Järvan et al., 2018), while in Poland it was from 316 to 397 s (Michalska et al., 2007). The falling number is influenced mainly by meteorological conditions and varieties (Linina & Ruza, 2015; Kunkulberga et al., 2017).

Winter rye properties demonstrate that the quality of the studied varieties fit the requirements for high–grade rye for food consuption. The varieties 'SU Mephisto', 'SU Bendic', 'Dankowkie Rubin', 'Amilo' and 'Inspector' with higher protein content are more required for the cereal industry for further processing.

CONCLUSIONS

In our investigation total phenolic content, antioxidant capacity, protein, starch content and falling number are determined in winter rye population and hybrid varieties wholemeal.

Protein content and starch content, falling number, total phenolic content and antioxidant capacity in winter rye varied significantly depending on the cultivars. The greatest variations of the antioxidant capacity and falling number were observed in population varieties wholemeal, compared to the hybrid varieties. Statistically higher total phenolic content and falling number was in grain of hybrid rye varieties, compared to the population varieties. Differences between hybrids and population varieties of grain protein and starch content were not observed.

This study showed that whole rye grains, which are widely used in human consumption, contain high levels of total phenol content and show a relatively moderate antioxidant capacity. It can be suggested that in future more and more rye products will be available on market, especially those originated from wholemeal.

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Review article: Current research trends in fruit and vegetables wastes and by-products management-Scope and opportunities in the Estonian context

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Abstract. Globally on an annual scale, considerable amounts of fruit and vegetables wastes (FVW) are generated in the agri-food industrial sector. Costs insured for safe disposal of FVW remains uneconomical and they can pose a serious environmental hazard if left untreated. However, FVW have high potential for reuse, recycle and recovery, which is an indication that there are productive, sustainable and affordable ways of reducing and tackling them at the industrial levels. Recent years have seen progressive innovative research on FVW management strategies, which has been developed with an idea of reducing wastes and fully exploiting its potential. Further, FVW represents a potential source of valuable compounds and bioactive ingredients. Today, there are many proposed innovative approaches for handling the FVW. These include reintroducing sub-standard fruit and vegetables (small sized or misshaped fruit and vegetable) in the market, reusing FVW for soil amendments, composting, or as an animal feed, and much more. In addition, the extracted bioactive compounds from FVW and by-products can find wide applications as a natural additive in food, cosmetics and/or in pharmaceutical applications. Currently, novel cost effective strategies have been developed for effective valorisation of agri-food wastes and by-products. The field of FVW management is still limited, thus leaving a wide gap for new ideas, novelty and applications of more efficient green techniques for complete utilization of agri-food wastes and by-products. Some of the interesting aspects on wastes and by-products management are discussed in relevance to Europe and in Estonia.

Key words: food wastes and loss, circular economy, waste management, bioactive compounds, livestock feed.

INTRODUCTION

Globally agri-food sector generates enormous volumes of food wastes and by-products. The majority of these wastes are disposed as landfillscreating serious negative impact on the environment (FAO, 2011; Wadhwa & Bakshi, 2013; Stenmarck et al., 2016; Sagar et al., 2018). Unsustainable disposal of wastes can also represent high economical cost for many of the food processing industries (Stenmarck et al., 2016). According to FAO (2019), food losses and waste can occur in the food supply chain, starting from harvest up to retail and consumption levels. Food wastes can also be categorised as to unprocessed and processed ones. On a global scale, an average of 30–40% of annual food production is wasted (Laufenberg et al., 2003; Parfitt et al., 2010;

FAO, 2016; Gu et al., 2019; Huang et al., 2019). This adds up to about 1.3 billion tons of waste created every year in the world (FAO, 2011). In the EU alone an annual food waste ranging up to 88 million tonnes are generated, resulting in a cost of around 143 billion euros (EU Fusions 2020). There has been an increased focus on food loss and waste, which is included as a part of 'Sustainable Development Goals' (SDG) of the UN (FAO, 2019). SDG contribute to Zero Hunger goal, aimed to achieve food security and sustainable agriculture systems. Itis witnessed that food wastes generation to be much higher in industrialized countries than in developed ones. In the industrialized world, consumers at the very end of the food supply chain tend to waste much of the food. In developing countries, food loss is much higher, owed to presence of poor processing infrastructure and preservation technologies. However, very little amount of edible portion of food is wasted by consumers (FAO, 2011). For a better understanding, it is a necessity to explain differences between a food waste and the food loss. Food loss represents food mass that is lost during the various stages of production, postharvest and processing along the agri-food supply chain. Lack of appropriate processing infrastructures, handling, transportation and storage facilities as well as the extrinsic climatic conditions can also contribute for the loss (Papargyropoulou et al., 2014; Sagar et al., 2018). On the other side, food wastes occur during final consumption stage, more precisely when a decision to discard the food happens, and this is commonly attributed to the behaviour of retailer and/or consumers.

Back in 2014 and 2015, food waste analysis was undertaken from researcher in Estonia (by SEI Tallinn). The purpose of this analysis was to estimate the amount of food wastes in the country and this was undertaken from the support of Ministry of the Environment of Estonia. There were two separate studies, one which estimated the amount of wastes in Estonian households and catering institutions, such as restaurants, bars and pubs, cafés, canteens, buffets, schools, kindgartens and a hospital (Moora et al., 2015), while the second one included retail and wholesale sector and food industries (Moora et al., 2018). Based on the results, it was estimated that food wastes are created mostly at households (up to 71,000 tons per year) followed by catering services (up to 13,000 tons per year), and then retailers and food processing industries (Moora et al., 2015; Siani et al., 2016).

Fruits and vegetable wastes (FVW)

The production of fruit and vegetables has recently increased significantly due to growing population and changing of eating habits, with more number of people shifting to vegetarian based diets (Schieber et al., 2001; Vilariño et al., 2017, Sagar et al., 2018). The increased production coupled with poor handling of fruits and vegetables and behaviour of retailers and consumers towards wastage have led to huge quantities of loss and waste of vital agri-food commodities (Sagar et al., 2018). FAO (2011) has estimated that fruit and vegetable wastes contribute to nearly 60% of all the food waste. With regard to FVW in Estonia, in an analysis conducted by SEI Tallinn, but this study did not precisely concluded on how much of the wastes came from fruits and/or vegetables (Moora et al., 2018). FVW represents a serious problem for environment as most of them are being disposed of as landfills or directly disposed off to water bodies such as rivers (Wadhwa & Bakshi, 2013). This causes serious environmental pollution, as they are easily perishable due to their high moisture content, which leads to microbiological

contamination (Banerjee et al., 2017; Plazzota et al., 2017, Pham Van et al., 2018; Coman et al., 2019; Bas-Bellver et al., 2020).

WASTE MANAGEMENT STRATEGIES

Recent years have seen progress in the FVW management strategies. There are many sustainable approaches for handling FVW, and some of these include: reusing FVW as an soil amendment or animal feed, extracting of bioactive compounds, and much more (Arvanitoyannis et al., 2008; Prokopov et al., 2014; Plazzotta et al., 2017; Coman et al., 2019; Peng et al., 2019; Salehiyoun et al., 2019). Some of these aspects are discussed in the preceding text.

In Estonia, the utilization of FVW is under consideration in various institutions of higher learning, government as well as the involved R & D sector. The necessity of identifying the existing research gaps on use of FVW in Estonia, and filling these gaps with new and innovative ideas have been identified. The quest for new ideas and improvement of current methods will contribute to creating of sustainable food/feed production systems and the further utilization of FVW in various industries. The vegetal waste and by-product are currently analysed for their composition, bioactive compounds and bioactive properties. Novel technologies of FVW utilization and applications is being explored and improved. Under the prestigious ERA Chair for Food (By-) Products Valorisation Technologies at the Estonian University of Life Sciences, active research activities is being undertaken aimed towards minimizing agri-food wastes and efficiently utilize by-products in order to support zero waste concept and circular economy of the region.

In the preceding text, we have summarized some of the important aspects relevant to trends in waste management. The present article is structured as follows. First part includes methodology used for writing this review article, and second part presents the methods of prevention of food waste (including fruits and vegetable waste) generation and management of food surplus. In the sections three to eight, current research trends of fruit and vegetable waste management are discussed with the special focus on scope and opportunities in Estonia. As there is a serious lack of data and information's available in the database or published research articles on fruit and vegetable waste management in Estonia, the discussions on current use of FVW in Estonia is mostly based on author's opinions and point of view. Further, in the chapter nine, use of fruit and vegetable waste in Estonia is summarized, followed by the conclusion part.

Nevertheless, the main objectives and aim of writing this review is to focus on the current fruit and vegetable waste management strategies on the global scale and to evaluate if these ideas can be extended with regard to Estonia.

Methodology

The methodology for writing this review used a descriptive approach. Accordingly, only those published articles that can find potential scope and practical applicability in Estonian context were shortlisted and selected. For identification of the research articles, a detailed search was undertaken by using popular electronic database such as Scopus (https://www.scopus.com/) and PubMed (https://www.ncbi.nlm.nih.gov/pubmed/). Additional search included websites of EU commission and Google databases. The keywords used were: Food wastes and loss; fruit and vegetables wastes and by-products,

Circular economy; Waste management; Bioactive compounds from wastes and by-products; Food and livestock feed, EU status, Statistical data and Estonia. The short listed references were scanned for additional relevant information's. No time limitation were applied for shortlisting of the references.

Marketing of malformed fruits and sub-standard fruits and food rescue programs

In the present global scenario, significant amounts of agri-food wastes provide negative environmental, economical and social impacts. Hence, every country world over are focusing on waste prevention, management and valorization strategies for achieving sustainable development. In the waste hierarchy, it is vital to distinguish waste prevention from waste management as well as food surplus and food wastes. Waste prevention mainly represents those actions taken against generation of wastes, while waste managements are related with the waste that has already been generated in the supply chain. On the other note, food surplus is related to the oversupply of foods (Papargyropoulou et al., 2014).

This chapter discusses certain food waste management (fruits and vegetables) options (both globally and in Estonia) with the special focus on retail sectors. Retail sectors contribute to generation of food wastes by disposing overstocked and oversupplied commodities. These might be damaged or spoilt ones, having a damaged package, nearing the expiry date or those which do not fit the set quality standards (Cicatiello et al., 2019). Approximately one third of fruits and vegetables never make it to the grocery shelves or the supermarkets, as they do not meet the quality standard criteria concerning weight, size, appearance and shape (FAO, 2018). Majority of the supermarket chains are not interested to buy such products, which do not meet the quality specifications as per the consumer's needs (Mena, et al., 2011; Plazzotta et al., 2017). These malformed fruits and vegetables are referred to as sub-standard. Re-introducing these type of products into the market is one of the new initiatives that has begun or is rather being under consideration globally. Even though these products have flaws in the appearance, they do not lack in quality and value (Economist, 2018). Their unusual look (malformed) does not affect the taste, nor compromise the health benefits they impart. Several companies in Europe and United States have begun providing these malformed fruit and vegetables to retail customers with minimal costs (Slate, 2015; Economist, 2018). However, there is no available information on the initiatives of introducing substandard fruits or vegetables in the supermarket chains in Estonia. In addition to this initiative, there are many organizations world-over, which are directly linking farmers/ producers with the community and relevant industries. There are also food rescue programs wherein retailers work in hand with charity organizations to donate food that is close to best before dates (Plazzotta et al., 2017; Hecht et al., 2019). Further, phone applications have been created to reduce/reuse restaurant, bakery, fruit and vegetable market based products, which are either sold at lower costs or are given for free (O'Sullivan, 2016; Hajjdiab et al., 2018). According to Siani (2016), contributions coming from food industries in Estonia are infrequent, as they require additional work and labour, leading to high economical costs. In certain cases, these food donation and contributions can also involve food safety issues, which can result in legal matters. In addition, most of the industries do not have available storage place for food, which needs to be donated, and they cannot afford to provide them.

In the 'Sustainable Development Forum' from Tallinn, Estonia, several waste management strategies were considered. One of the strategies was raising of consumer awareness as it was witnessed that majority of the consumers had trouble distinguishing labels such as 'best before' and 'use by' which resulted in disposing of food which were still good for consumption. Consumer's habits regarding separating of the waste are discussed, as the separation of waste is still not reaching the satisfactory levels in Estonia comparing to other EU countries. The solution to this could be the enhanced/improved waste collection system, which is yet to be developed. Increasing of food prices is also being questioned! This strategy was opined to make people more reluctant to disposing of food. However, as food insecurity is still present in Estonia, this suggestion has been discarded. Introducing the idea of informal food sharing was considered as well, based on the concepts developed in Germany and Finland (Saarniit, 2016).

Fruit and vegetable waste (FVW) as soil amendments

Soil represents an important natural resource and consists of the minerals (inorganic part), remains of plants and animals, and living organism that reside in it (organic part). Soil pollution is present world over, with common pollutants such as toxic compounds and chemicals; pathogens, radioactive materials, etc. (Mench et al., 2003; Mareddy, 2017). Even though soil contamination represents a significant problem, nowadays there are many effective soil remediation strategies. Soil amendment based practices are low-cost and environmentally safe. These can be efficiently utilized to enhance overall soil quality in terms of its structure and biochemical function, as they enhance soil fertility and promote site condition stabilization (Hartley et al., 2009; Beesley et al., 2014; Maiti, 2019). Today, many countries use fruit and vegetable waste as soil amendments. Composts and charcoal has been utilized in the agri-culture for a long period of time (Banerjee et al., 2017). Their utilization offers various benefits in regards to improvement of soil health (Burgos et al., 2010). However, FVW have high biological instability which leads to growth of microbiological contaminants (Ajila et al., 2012) and its long term effect is questionable which is why periodic re-applications are recommended (Madejón et al., 2009a; Madejón et al., 2009b).

In Estonia, according to Statistics Estonia, average yields of crops have been increasing annually, leading to a higher quantity of fruit and vegetable waste (Statistics Estonia, 2020). The utilization of FVW as soil amendments is still very rare. However, it is widely believed that this will not only reduce the amount of wastes, but it would enhance organic matter and nutrient content of soil, avoid certain negative impact associated with use of inorganic fertilizers and much more (Sharma & Chetani, 2017). The shift to utilization of fruit and vegetable waste is highly encouraged.

Fruit and vegetable flour
Fruit and vegetable flour as heavy metal adsorbents

Food industries generate large amounts of residues during the processing of fruits and vegetables (Ayala-Zavala et al., 2010; Sousa et al., 2011; Ferreira et al., 2015). Despite creating major environmental issues, FVW residues contain large amounts of high-valued bioactive compounds, which have high potential for re-use and recyclability (Sousa et al., 2011; Banerjee et al., 2017; Coman et al., 2019). Raw fruits and vegetables, are quickly subjected to degradation and pathogen contamination due to their high moisture content which makes their utilization restricted (Carle & Schieber 2006;

Plazzota et al., 2017). However, processing of fruits and vegetables residues into the flour can prolong their availability for different kinds of utilization (Ferreira et al., 2015). This makes processing of fruit and vegetable waste into the flour a very attractive option (Laufenberg et al., 2003; Ayala-Zavala et al., 2010; Ferreira et al., 2015). Fruit and vegetable wastes have been processed to flour for different purposes: for food application, pharmaceutical applications and adsorbing pollutants such as heavy metals from water and ground (Njikam & Schiewer, 2012; Roberta et al., 2014; Plazzotta et al., 2017; Santana et al., 2017).

There have been multiple studies, examining the efficacy of the utilization of fruit and vegetable wastes for adsorption of heavy metals. FVW that have been used include: Orange wastes (Pérez Marin et al., 2007), olive oil wastes (Doyurum & Çelik, 2006), banana pith (Low et al., 1995), mandarin peels (Pavan et al., 2006), orange juice and peel wastes (Azouaou et al., 2010, Lasheen et al., 2012), citrus peels (Njikam & Schiewer, 2012), hazelnut shell (Cimino et al., 2000), maize barn (Hasan et al., 2008), etc. It has been established that FVW are efficient heavy metals adsorbents due to their fibrous and porous structure which entraps pollutants (Plazzota et al., 2017). The adsorption is attributed to the specific interaction of pollutants with cellulose, pectin, hemicellulose and lignin obtained from FVW (Hashem et al., 2007; Azouaou & Mokaddem, 2008). The functional carboxylic and the alcoholic hydroxyl groups in cellulose, pectin, hemicelluloses and lignin havean ability to bind heavy metals (Ghimire et al., 2003). In addition sulphate, phosophate and amino groups contained in proteins, carbohydrates and phenolic compounds of FVW contribute to adsorption of heavy metals too (Meena et al., 2005). During the studies conducted to evaluate the potential of utilization of FVW as heavy metal absorbents, besides the efficiency, particular attention was focused on environmental and economic aspects of their utilization. The abundancy and accessibility of FVW has also been accounted (Cimino et al., 2000; Pavan et al., 2006), as well as the influence of several factors that affect adsorption. These factors included contact time, adsorbent mass, initial concentration of heavy metals, initial pH of the solution, particle size of the adsorbent, temperature, etc. (Pavan et al., 2006, Azouaou, 2008).

Fruit and vegetable flour as an ingredient for the formulation of functional food

Fruit and vegetable flour can be used as value added ingredient for the formulation of novel *functional* food products. Fruit and vegetable residues, wastes and by-products represent a rich source of nutritious compounds such as polyphenols, dietary fibres, carotenoids, and vitamins, which are beneficial for human health (Figuerola et al., 2005; Makris et al., 2007; Ajila & Prasada Rao, 2013). They contribute to prevention and treatment of human diseases (Roberta et al., 2014; Heidor et al., 2019; Javed et al., 2019). Several studies have been conducted to evaluate the functional properties, microbiological stability, proximate composition and antioxidant capacity of flour processed from different types of fruit and vegetable wastes. For example: Ferreira et al. (2015) has evaluated functional properties of flour obtained from residues generated during beverage manufacture. The beverage was produced from several fruits and vegetables: orange, watermelon, passion fruit, lettuce, taro, carrot, courgette, mint, spinach, cucumber and rocket. The fruit and vegetable residue flour was used for making of cereal bars and biscuits. Results showed that the obtained flour was suitable for production of functional food. It showed high dietary fibre, mineral and protein content

as well as water and oil holding capacity (Udenigwe & Aluko, 2012). In addition, Černiauskiene et al. (2014) have evaluated pumpkin flour as a source of food enrichment in dietary fiber. Bakery based foods prepared from pumpkin flour manifested high content of vitamins, minerals, and dietary fiber (Noor Aziah & Komathi, 2009). Futher on, Juarez-Garcia et al. (2006) used unripe banana fruit for developing of banana flour. Banana flour has been used for producing bread and it was evaluated based on its chemical composition, rate of starch available starch, resistant starch content and digestion *in vitro*. As a comparison, control product based on wheat flour was used. Banana flour had significantly higher protein, dietary fibre and starch content than control bread. It had low glycaemic index, which made it suitable for people with special low caloric requirement use.

These are just few of the many studies that have been conducted with the aim of investigating the potential of processing fruit and vegetable residues into the functional flour. All of these studies have showed that using FVW flour for development of functional food and products is a sustainable low-cost method, which is beneficial for environment and human health. However, the major issue related to utilization of fruit and vegetable flour is that fruit and vegetable waste has high water content. Therefore, for the production of the FVW flour, fruit and vegetable waste needs to undergo the process of drying which can be highly time-consuming and can affect FVW composition and content (Plazzotta et al., 2017).

The scope of using FVW flour for potential food and pharmaceutical applications, as well as for removal of heavy metals from water and ground, is gradually increasing world over. Estonia, being a small country produces enormous amounts of wastes and by-products from industries linked with apple, potato, beetroot, carrot, pumpkin, sea buckthorn, and other production. These wastes and by-products, which are rich in bioactive compounds (e.g. polyphenols, dietary fiber, natural pigments, etc.) can be processed into value added flour and can be fortified with bakery-based products or can be used for developing other novel food and feeds.

Fruit and vegetable waste in food industry

FVW is widely used in food industry. Today, there are many new functional food products containing fruit and vegetable waste on the market and their production is increasing every day (Kowalska et al., 2017; Donno et al., 2018; Majerska et al., 2019). FVW have been commonly used as natural food additives. It was observed that artificial additives, which have been used in the food industries up until recently, can be very harmful for human health, especially when in high doses. This is why the interest for natural food additives has increased (Schilderman et al., 1995; Wadhwa & Bakshi 2013; Quintin et al., 2019). Certain extracts from FVW, such as anthocyanin from eggplant, betalains from beetroot, anthocyanins from banana bract, lycopene from tomato by-products have been used as potential natural food colorants in the modern food industry (Pazmiño-Durán et al., 2001; Rizk et al., 2014; Gengatharan, et al., 2015; Faustino et al., 2019). In addition, agro-food by-products, have been used as preservatives, anti-oxidants, texturizing agents, emulsifiers, bulking agents, firming agents, etc. (Faustino et al., 2019).

FVW has been also utilized in the production of juices (Laufenberg et al., 2003), jellies (Madhav & Pushpalatha, 2006), and jams (Singh et al., 2009). They are also added in the production of herbal and fruit teas (Wadhwa & Bakshi, 2013; Majerska et al.,

2019), and in making of alcoholic beverages. For example, apple pomace has been used for the production of cider and beer (Benvenutti et al., 2019; Way et al., 2019; Gultip & Jokshi, 2000). Black currant, bilberry, chokeberry and grape pomace have been valorised for making of wine, while apple and grape pomace showed potential as additives for improvement of its taste too (Majerska et al., 2019). The potential of certain fruit and vegetable waste, such as orange peel and apple pomace have been evaluated for production of brandy (Gultip & Jokshi, 2000). In addition, wastes from pineapple, banana and certain vegetable waste have been utilized for vinegar production (Fatima & Mishra, 2015; Aye, 2016; Chakraborty et al., 2018). Some of the FVW has proven to positively affect dairy products (de Souza de Azevedo et al., 2018; Majerska et al., 2019). Saraç & Dogan (2016) established that incorporation of dietary fibre extracted from stone pear, celery root, celery leaves, spinach and oranges into the production of butter, made butter easily spreadable, more firm, and it extended its shell life. Besides showing positive effects on dairy products, FVW can be used as their replacement for production of food products for lactose intolerant people. The incidence of lactose intolerance has grown in recent years. Certain fruit wastes are reported to be probiotic food cariers due to their high content of vitamins, minerals, antioxidants and the absence of dairy allergens (Majerska et al., 2019). For example, Vodnar et al. (2019) did a study of using bioactive compounds from agro-industrial waste for production of probiotic juices. In addition, Ouhrabková et al. (2010) did a study on utilization of vegetable raw materials for producing probiotic dairy-like food. The study showed that obtained products containing raw vegetable material could be used as a replacement to cow's milk.

FVW is successfully utilized for improvement of meat products too. For example, Fernández- López et al. (2008), conducted a study based on adding fibre obtained from orange to dry fermented sausage. The fibre did not affect flavour, it promoted the growth of necessary microflora andit decreased the levels of nitrite. It also extended its shell-life (O'Shea et al., 2012). Viuda-Martos et al. (2010) from their established that adding of orange fibre reduced the growth of pathogens in the sausage and retarded the oxidation, extending its use (O'Shea et al., 2012). Apple and fresh plum pomace have also been successfully added to meat products, increasing its antioxidant capacity, nutritional value and extending its shelf-life (Henriquez et al., 2010; O'Shea et al., 2012; Lorenzo et al., 2017). In the study conducted by Sánchez-Alonso (2007), addition of grape pomace dietary fibre to fish, enhanced its health and sensory properties, improved its water retention capacity and retarded lipid oxidation (Sánchez-Alonso et al., 2007).

Overall, from the available literature, it is clear that there is a huge variety of successful ways in which fruit and vegetable wastes, by products and their extracts can be incorporated into food for human consumption. FVW improves food's nutritional quality, sensory characteristics and enhances its health properties as well. In Estonia, fruit and vegetable wastes and by products are gradually being considered as ingredients in food industries. Currently there are ongoing studies evaluating the potential of FVW for development of value added products and functional food. Fruit and vegetable wastes included in the studies are mostly apple, beetroot, potato, and carrot wastes since these fruits and vegetables are readily available and abundant in the country.

Extraction of bioactive compounds from fruit and vegetable waste (FVW)
FVW represents a highly underexploited resource of valuable bioactive compounds and phytonutrients such as dietary fibers, polyphenols, pigments, enzymes, sugar

derivatives, vitamins, minerals and oils (Sagar et al., 2018; Coman et al., 2019). These compounds and nutrients can be used in a wide range of different industries. Some of them have proven health effects, which include antimicrobial, anticancer, antiviral, anti-mutagenic, antioxidant and cardio protective activities (Dilas et al., 2009; Banerjee et al., 2017; Vodnar et al., 2017; Sagar et al., 2018). In addition, they have proven useful in food, cosmetics, pharmaceutical, paper and textile industries (Laufenberg et al., 2003; Galankis, 2012; Banerjee et al., 2017; Sagar et al., 2018). The process of extraction is being constantly improved by the application of novel technologies (Herrero et al., 2010; Sagar et al., 2018). These novel technologies and their modifications are introduced into the methodology with the aim of maximizing the yield and rate of the target compounds, clearing the compounds from toxic substances and impurities, providing grade nature of the final product and avoiding any functionality loss during processing (Galanakis, 2012).

Dietary fibres

Dietary fibre represent biomolecules, resistant todigestion by gastrointestinal enzymes. They include cellulose, hemicellulose, pectic substances, beta glucans, resistant dextrin, inulin, gums, chitosan oligosaccharides, lignin, etc. (Mudgil, 2017). Based on their water solubility, dietary fibre can be soluble and insoluble (Dhingra et al., 2012). FVW dietary fibre are largely used in various industries. They manifest wide range of health promoting activities. They contribute to prevention and treatment of diabetes, cardiovascular diseases, obesity and pancreatic, colon and colorectal cancer (Ferguson, 2005; Figuerola et al., 2005; Nawirska & Kwasniewska, 2005; Evans et al., 2019; Koulouris et al., 2019; Villanueva-Suárez et al., 2019). Recently there has been a lot of interest in FVW lignocellulosic biomass and bioactive polysaccharides and their renewable sources. This could be due to newly found approaches for valorisation of cellulose, hemicelluloses, pectin and lignin. The utilization of cellulose has extended to biomedical section. Nowadays, it is often utilized for medical diagnostics and cancer screenings (Ratajczak & Stobiecka, 2019). In addition, it has also been used as pharmaceutical excipient (Domínguez-Robleset al., 2019), filler in solid oral dosage formulations (Credou & Berthelot, 2014; Banerjee et al., 2017), and for drug delivery, in wound dressings, tissue engineering scaffolds and bio-imaging (Du et al., 2019; Fu et al., 2019). It is also frequently used in the food industries, and for the extraction of phenolic compounds from grape pomace (Drider et al., 1994; Meyer et al., 1998; Sagar et al., 2018). For production of cellulose, following fruit and vegetable waste is often used: citrus peels (Güzel & Akpınar, 2019), banana waste (Elanthikkal et al., 2010), potato peel (dos Santos et al., 2012; Sagar et al., 2018), mandarin peel (Hiasa et al., 2014), etc.

Pectin, successfully extracted from a varied source of FVW has been widely used in food industry. Apart from that, it is often utilized in pharmaceutics, mostly as thickening agent and for production of products preventing diarrhoea (Rabbani et al., 2001; Liu et al., 2003; Banerjee et al., 2017). It also used for biomedical applications: for drug and gene delivery, tissue engineering and wound healing (Munarin et al., 2012). Resistant starch, obtained from fruit waste such as banana peel, jackfruit seeds, durian waste and mango kernel, like hemicelluloses, can only be digested in colon and its fermentation results in many health benefits too (Fuentes-Zaragoza, et al., 2010; Banerjee et al., 2017; Ho & Wong, 2019).

Proteins

FVW represents a good source of proteins as well. Their content is especially high in seeds (Banerjee et al., 2017). Apart from fruit and vegetable seeds, pea straws (Wadhwa & Bakshi, 2013), potato peels, avocado waste; (Chitturi et al., 2013) cabbage leaves; carrot pomace, apple pomace, citrus peel, green pea peels, mango peel, pineapple peel, tomato solid waste, pea pods, orange peel, cauliflower leave, and others have a good amount of proteins too (Mamma & Christakopoulos, 2014; Bakshi et al., 2016; Sharma et al., 2016; Sagar et al., 2018). Certain fruit and vegetable waste have more than 20% of crude protein, which is why they are used as ruminant feed. These include, cabbage leaves, bottle gourd pulp, potato vines, radish leaves sugar beet leaves and snow peas (Bakshi et al., 2016). Apart from contributing to nutritional value of animal feed, certain proteins exhibit potential health beneficial activities, and this the reason they are extracted from FVW. For example, kiwifruit seed has been used for the extraction of actinidin. This enzyme is responsible for digestion of proteins in small intestines. Due to its probiotic activities, it is commonly added to dairy products (Puglisi et al., 2012, Boland, 2013).

Enzymes

Enzymes represent biomolecules, which contribute to various industries. For example, amylases is abundantly used in food industries for the production of fruit juices, cheese, chocolate cakes and syrups (Laufenberg et al., 2009; Toumi et al., 2016; Sagar et al., 2018). It is used in pharmaceuticals, brewing, and textile industries (Saini et al., 2017). The most common FVW used for production of amylases are: bananapeel (Oshoma et al., 2019), orange peel (Uygut & Tanyildizi, 2018), potato peels (Pereira et al., 2017), date wastes (Acoureneet al., 2014; Sagar et al., 2018), rice bran, wheat bran (Almanaa et al., 2019), and mango kernels (Kumar et al., 2013; Sagar et al., 2018). Invertase is used for the production of invert sugar, artificial sweeteners, chocolates, lactic acid, glycerol, candies and confectionary (Sagar et al., 2018; Veana et al., 2018; Mashetty & Biradar, 2019). The production of this enzyme using fruit and vegetable waste, also requires the presence of sucrose, lactose and fructose (Sagar et al., 2018). FVW that has been so far used is pineapple peel (Oyedeji et al., 2017); pomegranate peel (Uma et al., 2012); papaya peel (Chelliappan & Madhanasundareswari, 2013). Apart from food industry, it is used for production of pharmaceutical products and extension of shell life of products (Kumar & Kesavapillai, 2012; Panda et al., 2016). Pectinase is commonly used for making of wine and fruit juices (New et al., 2018; Nighojkar et al., 2019). It is also applied for extraction of pigments (Munde et al., 2017) and essential oils from FVW (Sagar et al., 2018; Castilho et al., 2000). Additionally, it plays a role in production of good quality paper (Ahlawat et al., 2008; Rebello et al., 2017), for fermentation of coffee and tea and for treatment of pectic waste (Kashyap et al., 2001; Rebello et al., 2017). Citrus waste peel (Ahmed et al., 2016); banana peel (Sethi et al., 2016); mango peel (Kuvvet et al., 2019); sugarcane bagasse (Biz et al., 2016.), pineapple stem (Kavuthodi & Sebastian, 2018), have been used for the production of pectinases. An example of using FVW for production of enzymes is given in Table 1.

Table 1. Production of enzymes using fruit and vegetable waste (FVW)

Enzymes	Purpose	FVW used for enzyme production
Amylases	Food industries for the production of fruit juices, cheese, chocolate cakes and syrups (Laufenberg et al., 2009; Toumi et al., 2016; Saini et al., 2017); pharmaceutical, brewing and textile industries (Saini et al., 2017).	Banana peel (Oshoma et al., 2019); orange peel; (Uygut & Tanyildizi, 2018); potato peels (Pereira et al., 2017); date waste (Acoureneet al., 2014; Sagar et al., 2018), rice bran, wheat bran (Almanaa et al., 2019); mango kernels (Kumar et al., 2013; Sagar et al., 2018)
Invertase	Production of invert sugar, artificial sweeteners, chocolates, lactic acid, glycerol, candies and confectionary (Sagar et al., 2018; Veana et al., 2018; Mashetty & Biradar, 2019), production of pharmaceutical products and extension of shell life of products (Kumar & Kesavapillai, 2012; Panda et al., 2016; Sagar et al., 2018).	Pineapple peel (Oyedeji et al., 2017); pomegranate peel (Uma et al., 2012); papaya peel (Chelliappan & Madhanasundareswari, 2013)
Pectinase	Production of wine and fruit juices (New et al., 2018; Nighojkar et al., 2019); for extraction of pigments (Munde et al., 2017) and essential oils from fruit and vegetable wastes (Castilho et al., 2000; Sagar et al., 2018); production of good quality paper (Ahlawat et al., 2008; Rebello et al., 2017), for fermentation of coffee and tea and for treatment of pectic waste (Kashyap et al., 2001; Rebello et al., 2017).	Citrus waste peel (Ahmed et al., 2016); banana peel (Sethi et al., 2016); mango peel (Kuvvet et al., 2019); sugarcane bagasse (Biz et al., 2016.), pineapple stem (Kavuthodi & Sebastian, 2018)
Cellulases	Textile industry, for production of detergents, food industry (Imran et al., 2016); industrial biontechnology (Bajaj & Mahjan, 2019)	Potato peel (Taher et al., 2019); orange waste (Srivastava et al., 2017); Pineapple peel (Oyedeji & Ojekunle, 2018); cucumber peel, banana peel (Viswanath et al., 2018);
Xylanases	Paper, biofuel industry, food industry, textile industry; as supplement to animal feed (Kumar et al., 2018; Singh, 2019),	Banana peel (Zehra et al., 2020); passion fruit peel (Martins et al., 2018); cassava peels (Olanbiwoninu & Odunfa, 2016);orange peel (Silva et al., 2018)

Lipids

FVW are also rich in lipids or the oils. For example: the orange peels (Boukroufa et al., 2015), fennel waste (Cautela et al., 2019); apple and pear seed (Yukui, 2009), mango seed (Yadav et al., 2017), apricot seeds (Stryjecka et al., 2019), tomato seeds (Giuffrè et al., 2017), lemon peel (Ciriminna et al., 2017), represent a good source of oil. Oil extracted from FVW exhibits antioxidant, anti-cancer, anti-inflammatory, antimicrobial and immunomodulatory activities (Pérez et al., 2011; Bhalla et al., 2013; Ayoub et al., 2017; Geraci et al., 2017; Moosavy et al., 2017; Irshad et al., 2019). In addition, it can manifest anti-obesity properties and hypolipidemic effects as well (Yang et al., 2018). It used as an ingredient of skin healing creams, due to its moisturizing properties (Mandawgade & Patravale, 2008; Banerjee et al., 2017), and in pharmacology

as a reagents for producing products that contribute to good digestion (Njoroge et al., 2005; Wadhwa & Bakshi, 2013).

Polypenols

Today there is a great interest in polyphenols and their induction in the everyday diet and products. They manifest many important biological health properties such as, scavenging free radicals, prevention of oxidation reactions in foodand prevention of oxidative stress (Popa et al., 2008; Ignat et al., 2011; Deng et al., 2012; Sagar et al., 2018). FVW represent a valuable source of polyphenols. They exibit anti-cancer, anti-viral, anti-bacterial, anti-inflammatory, and anti-oxidative effects (Colomer et al., 2017; de Albuquerque et al., 2019; Fratianni et al., 2019; Musarra-Pizzo et al., 2019). Their use is also considered for prevention of neurodegenerative diseases (Renaud & Martinoli, 2019). In Table 2, phenolic compounds of certain fruit and vegetable wastes can be seen.

Table 2. Phenolic compounds present in selected fruit and vegetable wastes

Commodity	Type of waste	Phenolic compounds	Reference
Apple	Pomace	Hydroxycinnamates, phloretin glycosides, quercetin glycosides, catechins, procyanidins	Foo & Lu, 1999; Lommen et al., 2000; Schieber et al., 2001; Sagar et al., 2018
Apple	pulp	Protocatechuic acid (+)-Catechin, Chlorogenic acid (−)-Epicatechinp-Coumaric acid, Phloridzin	Veberic et al., 2005
Apple	peel	Ferulic acid, caffeic acid, p-coumaric acid	Leontowicz et al., 2007; Saini et al., 2019
Banana	peel	Hydroxycinnamic acids: Ferulic acid; Ferulic acid-hexoside; Sinapic acid; p-cumaric acid methylester Flavonols: Rutin, Quercetin -7-rutinoside; Quercetin -3-rutinoside; Kaempferol-3-rutinosid; Kaempferol-7-rutinoside; Isorhamnetin-3- rutinoside; Myricetin-3-rutinoside; Laricitrin-3-rutinoside; Syringetin-3-rutinoside Flavan-3-ol monomers: (+)-Catechin, Epicatechin, Gallocatechin	Rebello et al., 2014; Waghmare & Kurhade, 2014; Passo Tsamo et al., 2015b; Vu et al., 2018
Bilberry	leaves	Caffeic acid, myricetin-3-O-galactoside	Teleszko & Wojdyło, 2015; Sagar et al., 2018
Blueberry	peel	Epicatechin, catechin, cyanidin 3-glucoside, gallic acid Chlorogenic acid	Deng et al., 2012; Saini et al., 2019
Beetroot	peel	tryptophane, p-coumaric and ferulic acids, cyclodopa glucoside derivatives	Kujala et al., 2001; Deo et al., 2018
Carrot	pomace	Carotene (α and β), chlorogenic acid, dicaffeoylquinic acids	Schieber et al., 2001; Zhang & Hamauzu, 2004; Wadhwa & Bakshi, 2013; Sagar et al., 2018

Table 2 (continued)

Chokeberry	leaves	(−) epicatechin, neochlorogenic acid, chlorogenic acid, quercetin-3-O-rutinoside, quercetin-3-O-robinobioside, quercetin-3-O-galactoside	Teleszko & Wojdyło, 2015; Sagar et al., 2018
Citrus fruits	peel	Naringin, hesperidin, neohesperidin, diosmin, luteolin, sinensetin, rutin, kaempferol, quercetin, Caffeic acid, chlorogenic acid, ferulic acid, sinapic acid, p-coumaric acid β-carotene, zeaxanthin, lutein, β-cryptoxanthin, Neochrome, lutein, β-cryptoxanthin, β-citraurin, luteoxanthin, cryptochrome, ξ-carotene	Coll et al., 1998; Agócs et al., 2007; Wang et al., 2008; Matharu et al., 2016; Sagar et al., 2018; Saini et al., 2019
Cucumber	peel	Chlorophyll, Pheophytin, Phellandrene, Caryophellene	Zeyada et al., 2008
Cranberry	leaves	(+) Catechin, procyanidin B1, (−) epicatechin, myricetin-3-xylopiranoside, quercetin-3-O-galactoside, dimethoxymyricetin-hexoside, methoxyquercetin-pentoside	Teleszko & Wojdyło, 2018; Sagar et al., 2018
Garlic	husk	Ferulic acid, gallic acid, hydroxybenzoic acid, caffeic acid, p-coumaric acid, Di-Ferulic acid, chlorogenic acid, Caffeic acid-O-glucoside, Coumaroylquinic acid, Coumaric acid-O-glucoside, Caffeoylputrescine	Kallel et al., 2014
Grapes	seed	Procyanidins Epicatechin, gallic acid, chlorogenic acid, catechin, cyanidin 3-gluco- side, homogentisic acid, protocatechuic acid, -p-hydroxybenzoic acid	Deng et al., 2012; Saini et al., 2019
Grapes	Pomace	Quercetin, Gallic acid, Protocatechuic acid, Luteolin, (+)-Catechin, (-)-Epicatechin, Vanillic acid, Kaempferol, Syringic acid, p-Coumaric acid, Ellagic acid	Sanhueza et al., 2017
Guava	peel	Catechin, Epicatechin, Kaempferol, Quercetin Myricetin, Rutin, Hydrate, Naringin, Apigenin	Marina & Noriham, 2014
Eggplant	peels	Delphinidin-3-rutinoside-5-glucoside, cyanidin-3-rutinoside, delphi- nidin-3-rutinoside, malvidin-3-rutinoside-5-glucoside, petunidin- 3-rutinoside	Ferarsa et al., 2018; Saini et al., 2019
Kiwifruit	peel	Gallic acid, Protocatechuic acid, Catechin, P-hydroxybenzoic acid, Chlorogenic acid, Vanillic acid, Caffeic acid, L-epicatechin, Syringic acid, coumaric acid, Rutin, Phlorizin, Quercetin, Kaempferol	Wang et al., 2018
Mango	Seed kernel	Gallates, gallotannins, gallic acid, ellagic acid	Arogba, 2000; Schieber et al., 2001; Sagar et al., 2018
Mango	peel	Catechin, Epicatechin, Kaempferol, Quercetin Myricetin, Rutin, Hydrate, Naringin, Apigenin gallic and ellagic acids	Wadhwa & Bakshi., 2013; Marina & Noriham, 2014

Table 2 (continued)

Quince	leave	(+) Catechin, procyanidin B1, procyanidin B2, procyanidin C1, 4-O-caffeoylquinic acid, kaempferol-3-O-rutinoside, kaempferol-3-O-glucoside, quercetin-3-O-galactoside, quercetin-3-O-rutinoside	Benzarti et al., 2015; Teleszko & Wojdyło, 2015
Olive	leaves	Oleuropein, apigenin 7-glucoside, rutin, vanillin, vanillic acid, Caffeic acid, luteolin 7-*O*-glucoside, luteolin 4'-*O*-glucoside, hydroxytyrosel	Ryan et al., 2002
Olive	peel	Luteolin- 7-glucoside, rutin, olueropein, nuzenhide, dimethyl oleuropein	Ryan et al., 2002
Onion	skin	Quercetin 3,40-O-diglucoside and quercetin 40-O-monoglucoside	Zeyada et al., 2008
Papaya	Seeds	Chlorogenic acid, Caffeic acid Ferulic acid, p-Hydroxybenzoic acid, p-Coumaric acid, Myricetin, Quercetin, Kaempferol-3-O-glycoside, Kaempferol, Quercetin 3-O-glycoside	Castro-Vargas et al., 2019
Pomegranate	Seeds, Peel	Anthocyanins, ellagic acid, gallic acid, punicalin, punicalagin	Akhtar et al., 2015; Deo et al., 2018; Zivkovic et al., 2018; Saini et al., 2019
potato	peel	Chlorogenic, gallic, protocatechic and caffeic acids, p- Hydroxybenzoic, Vanillic acid chloregenic acid isomer II	Zeyada et al., 2008
Red beet	peel	ltryptophane, p-coumaric and ferulic acids, cyclodopa glucoside derivatives	Kujala et al., 2001,
Tomato	peel	Cis-lycopene, Beta carotene, Trans-lycopene, Lutein Ascorbic acid, Quercetin. Kaempferal	Zeyada et al., 2008

Organic acids

With regard to organic acids, citric and lactic acids are the most known representatives. They are abundantly used in food, cosmetic and pharmaceutical industries. Citric acid can be produced using kiwifruit peel (Hang et al., 1987); apple pomace (Dhillon et al., 2011; Sagar et al., 2018); cacao pod husk (Vriesmann et al., 2012): pineapple waste (Imandi et al., 2008) via use of fungi *Aspergillus niger* and yeast such as *Yarrowia lipolytica* (Imandi et al., 2008; Sagar et al., 2018). Lactic acid, however, is produced using *Lactobacillus casei, Lactobacillus delbrueckii, and Lactobacillus plantarum* along with apple peel (Gullon et al., 2008); mango waste (Jawad et al., 2013); wheat bran, wheat straw, bread waste, (Ghaffar et al., 2014; Panesar & Kaur, 2015), potato peel, green peas, cassava residues and sweet corn as the substrates (Ray et al., 2008; Mudaliyar et al., 2012; Sagar et al., 2018).

Pigments

Today, there are many known pigments that exibit health properties. One of them is lycopene, a carotenoid, extracted fromtomato pomace (Knoblich, et al., 2005). Lycopene has antioxidant properties and is known for its contribution to treating cancer, especially prostate, but also lung, colon and breast cancer (Lin et al., 2011b; Grabowska et al., 2019; Jiang et al., 2019a; Jiang et al., 2019b; Kim et al., 2019; Sen, 2019). It also

prevents cardiovascular diseases and some chronic conditions (Costa-Rodrigues et al., 2018; Cheng et al., 2019; Grabowska et al., 2019). Betalains from beet root peel also showanti-cancer, anti-inflammatory, anti-bacterial activities, and prevent cardiovascular diseases (Lechner & Stoner, 2019; Rahimi et al., 2019a; Rahimi et al., 2019b; Vijaya & Thangaraj, 2019).

Extraction methods

In Fig. 1, some of the common conventional and non-conventional extraction methods used are depicted. Bioactive compounds can be extracted via various methods: such as soxhlet extraction, maceration, hydro-distillation, solvent extraction, etc (Khoddami et al., 2014; Sagar et al., 2018). Novel technologies include greener methods such as supercritical fluid extraction, ultrasonication, micro-wave assisted extraction, pressurized liquid extraction, pulse electric field and ionic liquid extraction. Even though, the extraction of bioactive compounds is a promising FVW utilization strategy, there is still a need for improvement of novel technologies to obtain high yields of bioactive compounds. In Estonia, under the ERA Chair in Valortech at EMU, there are ongoing studies related to optimization of extraction processes and identification of valuable bioactive compounds from local FVW. Besides, works are being undertaken towards development of livestock feed, value added products, etc.
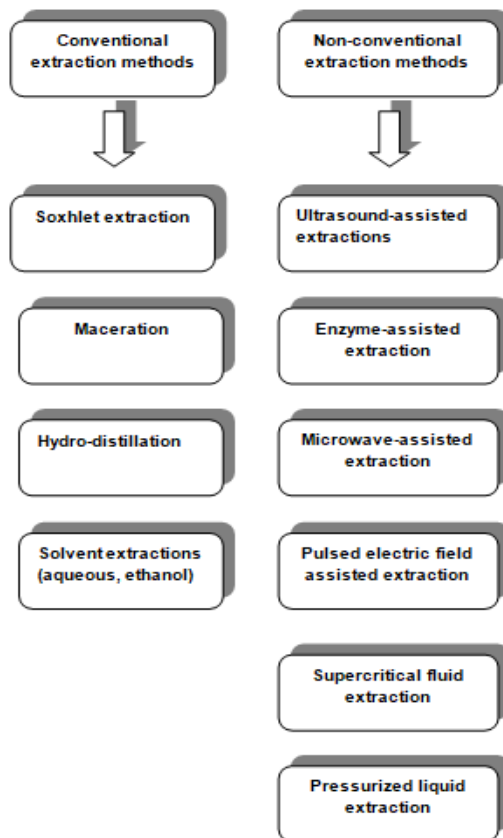

Figure 1. Conventional and Non-conventional extraction methods.

Energy recovery

Providing of a clean and renewable energy source is essential to meet the present day global sustainability challenge. With increasing of population, followed by an increased demand of energy and shortage of fossil fuels, new strategies for energy production are necessary. Waste material shows potential for production of biogas and bio-alcohols via thermochemical, physico-chemical and biochemical strategies (Kothari et al., 2010; Plazzotta et al., 2017; Velebil et al., 2019). Utilizing large quantities of wastes for production of energy is also vital from an environmental point of view as it represents a solution to their disposal in landfills (Sheets et al., 2015). Recently, biodegradable waste has become an important part in production of biogas, bioenergy and biofuel (Spalvins & Blumberga, 2017; Velebil et al., 2019). The concept of bio-

refineries is largely being considered. Bio-refineries represents facilities where fuels, power, heat and value added chemicals are generated using biomass as feedstock. Bio-refineries are created with the aim of contributing to circular economy and reducing the utilization of fossil fuels, which negatively affect environment. In a study conducted by Cristóbal et al. (2018), techno/economic and profitability analysis of the bio-refinery was estimated by calculating the cost – (total capital investment and costs of manufacturing) as well as revenues and profitability. Biomass was evaluated for their utilization in bio-refineries for four agricultural food products which included studies on extractions from potato (phenolic acids and glycoalkaloids), tomato (lycopene and β-carotene), olive (phenolic compounds, fatty acids methyl ester and squalene) and orange waste (essential oils, pectin and phenolics). The results showed that value-added product market price to be important in to estimate the profitability of the bio refinery, and different FVW to have a varied potential with regard to generation of bio-energy.

In addition, several other FVW wastes have been investigated for their suitability for energy recovery and its utilization has been evaluated from economic and environmental point of view. For example, Dubrovskis & Plume (2017) conducted a study to evaluate pumpkin, marrow and apple wastes for production of biogas. The study concluded that all of the wastes are suitable for biomethane production. However, there are certain issues that still need to be overcome concerning the use of FVW for energy recovery (Jiang et al., 2012; Plazzotta et al., 2017). Some of these issues are FVWs high content of volatile solids which can readily get hydrolysed, leading to low pH levels and inhibition of anaerobic digestion (Plazzotta et al., 2017). In addition, the organic fraction of fruit and vegetables wastes include 75% sugars and hemicelluloses, 9% cellulose and 5% lignin (Jiang et al., 2012). Even though, these compounds can contribute to large production of energy, their preferable and balanced mixture needs to be met so anaerobic digestion can be successfully performed (Jiang et al., 2012). It is recommended that fruit and vegetable waste be co-digested organic wastes as well. There have been several studies with successful application of co-digestion (Jiang et al., 2012; Shen et al., 2013). Lin et al. (2011a) reported co-digestion of FVW with food waste. The result showed that co- digestion of FVW and FW, with their proper ratio, improved the stability of anaerobic digestion and achieved higher biogas production (Lin et al., 2011a). However, utilization of the waste for energy recovery has faced several additional challenges. Anaerobic digestion based technologies are significantly less efficient comparing to current fossil fuel strategies. It is quite clear that a lot more research needs to be done so the anaerobic digestion of waste reaches its full potential. It is also important for people to understand the necessity of the utilization of renewable energy resources, as fossil fuel reserves are running out and their negative impact is threatening the environment. Nonetheless, utilization of waste for energy production has excellent potential and it needs to be further encouraged. With future advancements, waste to energy technologies can be completely viable, economically sustainable and the solution to current environmental problems.

In Estonia, valorisation of FVW and by-products remains highly underutilized especially in the production of energy via anaerobic digestion. However, renewable energy strategies are largely being studied and hence FVW can be applied for biogas production at appropriate levels. To the authors knowledge, there are several studies and projects that are ongoing at the local universities in Estonia for the development of biogas production using agricultural wastes. Nevertheless, as opined previously, the

number of scientific publications are still lagging behind comparing to publications in other country such as France, Germany, Spain, Sweden, or Denmark (Luna del Risco, 2011).

Animal/livestock Feed

With the future increase of population, it is expected that an increased demand of food and especially animal products will follow. This will result in a huge demand in animal feed as well. However, animal feed production is already today facing certain challenges, especially in developing countries (Wadhwa & Bakshi, 2013; Bakshi et al., 2016). FVW represents an excellent source of nutrients, and they can be an solution to meet the ever increasing demands for livestock feeds (Mahgoub et al., 2018; Valdez-Arjona & Ramírez-Mella, 2019). Utilization of FVW for animal feed production would not only enhance food security (Bakshi et al., 2016), but also can contribute to mitigation of environmental pollution related to current waste disposal methods (landfilling). In addition, it would reduce the cost of feeding which is economically beneficial for farmers (Wadhwa & Bakshi, 2013). It could consequently decrease the competition between human and animal nutrition (Mirzaei-Aghsaghali & Maheri-Sis, 2008).

FVW can be incorporated into animal feed either as main feed ingredients such as citrus pulps; as dietary supplements with an aim to achieve a particular function like tomato pulp; or as antioxidants or ingredients with more purpose – like olive pomace (Kasapidou et al., 2015). There are various examples of successful incorporation of FVW onto animal feed. For example, some of the fresh forms of FVW can be fed to ruminants ad libitum. These include: baby corn husk, cabbage waste, pea vines, PP (empty pea pods), cull snow peas, and SC husk due to their high protein content (Bakshi et al., 2016). Further, banana peel is considered to add nutritional value to livestock feed, too. It is a good source of minerals, proteins and fibre (Hassan et al., 2018). Bottle gourd peel, pea husk, potato, eggplant and pumpkin peel have been established as promising feed resources (Hossain et al., 2015). Cabbage waste has been added to feed of ruminants, rabbits and poultry due to its high protein content (Bakshi et al., 2016). Cull carrots are commonly used as ruminants feed, while carrot flakes and dehydrated carrots are often given to horses as treats (Bakshi et al., 2016). In addition, Alzawqari et al. (2016) conducted a study on evaluating adding of sweet orange peel and lemongrass leaves to poultry. Even though the addition of this waste had very little effect on the chicken, it improved blood metabolites and antioxidant status (Alzawqari et al., 2016). Dried citrus peel has been proven to be a good cereal substitute to feed the dairy cattle, lactating ewes and gestating sows-without altering milk yield or its composition (O'Sullivan et al., 2003; Assis et al., 2004). Rapeseed pomacehas been successfully added to diet of lactating cows, too, and its inclusion increased the fatty acid content of milk (Musayeva et al., 2016). However, even though fruit and vegetable waste provide a number of benefits when used for production of animal feed, there are certain disadvantages that need to be taken into account as well. Due to their high moisture and microbial loads, FVW is more quickly to spoil, making them often subject to time consuming processes such as drying and ensiling (Kasapidou et al., 2015; Bakshi et al., 2016). Drying process can often result in severe decrease of valuable bioactive compounds and changes to FVW characteristics due to exposure to high temperatures in a long period of time (Kampuse et al., 2018). This is why several factors need to be considered prior to FVW being subjected to it. Furthermore, FVW composition depends on their physical form (peels,

seeds, stems, stones, pulp etc.), the processing technology of the raw material, storage, and handling conditions. It is necessary for FVW composition to be analysed prior to incorporation into animal feed (Kasapidou et al., 2015). Still, feeding animals with agriculture waste and by-products is considered an efficient way to upgrade low quality materials into high quality foods (Elferink et al., 2008). Nonetheless, they remain an underexploited source for the dietary supplementation of farm animals, which is why there is a need for further detailed research on FVW's function, bioavailability and efficacy as feed additives. In Estonia, utilization of FVW for development of livestock feed and thereby their waste management strategies is yet to be utilized to satisfactory levels. The potential of FVW and by-products in production of livestock feed in Estonia is gradually being recognized, however there are certain legal requirements that needs to be considered and overcome. Nonetheless, in the industrial sector in Estonia, post processing, certain fruits and vegetable wastes are readily available in adequate quantities that are not only affordable but suitable to be incorporated in animal diets. These can provide the necessary nutrients required for production of livestock feed. Potato, pumpkin, rapeseed cake, apple pomace wastes specifically stands out as rich source of vital nutrients.
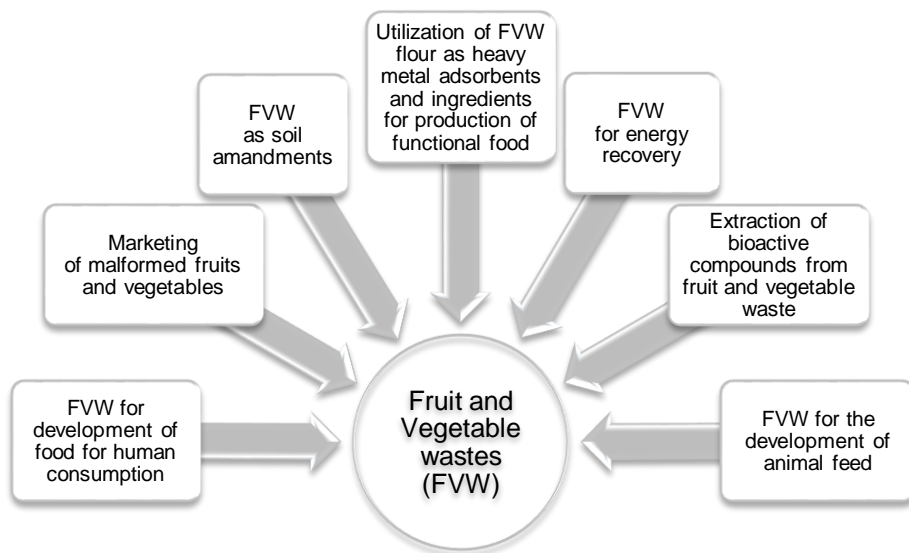
Figure 2. Current fruit and vegetable waste management strategies.

Trends in FVW management in Estonia

In Fig. 2, current FVW management strategies that can be employed in Estonia is depicted. Unfortunately, there is not a lot of data about the management of FVW in Estonia. This is because the data on the waste generation in Estonia lacks in availability and consistency (ETC/RWM, 2007) (Moora, 2009). However, it is known that bio-degradable waste, and among it, fruit and vegetable waste as well, represents the fraction of municipal waste which causes the most number of issues (Blonskaja, et al., 2014). This is attributed to current inefficient processes of sorting, collection and treatment of

bio-degradable waste which are neither economically nor environmentally effective (Blonskaja et al., 2014).

Overall, the Baltic countries are struggling to keep up with other EU countries when it comes to strategies implemented by Circular Economy (Grigoryan & Borodavkina, 2017). For example, the recycling is not nearly as applied in Baltics as it is in other parts of EU (Grigoryan & Borodavkina, 2017). In addition, according to Eurostat data, only 14% of the household waste was recycled in Estonia (Horbach, 2015). However, compared to the other Baltic countries, in Estonia only 16% of waste is landfilled and 64% incinerated (Grigoryan & Borodavkina, 2017). With adoption of The Landfill Directive 1999/31/EC member States, including Estonia, have been compelled to develop new methods of waste management. The Landfill Directive, 1999/31/EC, implemented in 2001, focuses on reducing and preventing disposal of bio-waste in landfills. It encourages the member states to utilize the waste management strategies that support Circular Economy. According to Directive (EU) 2018/850, which is amending Directive 1999/31/EC, by 2030, all member states are required to prevent all the waste, which can be recovered or recycled from going to landfill. By 2035, it is expected that all the member states reduce the amount of municipal wastes disposed of in landfills to 10% or less.

With the aim of estimating amount, quality and energy potential of biodegradable waste, the data about biodegradable waste produced in Estonia from 2002 to 2012 is reported (Blonskaja et al., 2014). In this study, the collected data was from all of the 15 Estonian counties, but the study concentrated on Tallinn and Harju County as this is where most of the biodegradable waste are generated. The major portion of the biodegradable wastes came from the kitchen which comprised of FVW, prepared foods, cooked and uncooked meats and fish, cheese, egg wastes, bread, coffee grinds, tea bags, etc. (Blonskaja et al., 2014). This study established that biodegradable wastes remains underutilized and that it can represent to be good resource for composting and production of renewable energy production, in Estonia.

Regarding the utilization of bio-wastes for composting and production of biochar, it has been estimated that in EU countries, only one third of the bio-waste is used, regardless of the existing compost markets (Meyer-Kohlstock, et al., 2015). There has been a study conducted by Arcadis Belgium nv and Eunomia, in 2010 with the aim of estimating bio/waste potentials and their future developments in all 28 member states of EU (Meyer-Kohlstock et al., 2015). This study also provided data on bio-waste utilization for compost and biogas in 2008 (Meyer-Kohlstock et al., 2015).

Incineration of biodegradable wastes such as FVW has been considered to be one of the successful applications of waste management in Estonia, contributing to reduction of biodegradable waste disposed of in landfills. Incineration has been widely applied and it has been reported that the amount of burned waste and produced electricity and heat energy has been largely increasing in Estonia (SEI, 2017). However, according to Frans Timmermans, recycling is much preferable to incineration (Grigoryan & Borodavkina, 2017). It was estimated that Estonia to hold high potential for the production of biogas. However, this is still not applied to the levels on which it could be (Luna del Risco, 2011). Even though the incorporation of fruit and vegetable waste to animal feed and food seems like a promising strategy for reducing waste of fruit and vegetables, it faces legal challenges. According to Regulation (EC) No 178/2002 of the European Parliament and of the Council, food for human consumption and animal feed are not allowed to contain residues or any potential contaminants (NSW, 2017).

CONCLUSION

Recent years have seen progress in the research of fruit and vegetable waste management strategies. Fruit and vegetable waste (FVW) and by-products show high potential for re-use, recycle and recovery in Estonia. As it can be concluded from this article, there are many implemented strategies presently available aimed towards tackling the fruit and vegetable wastes generated in the agri-food supply chain. However, their complete potential to produce value added compounds and products remains in the infancy stage, especially in the Estonian context. Some of these strategies include utilization of fruit and vegetable wastes as organic soil amendments; their valorization as FVW flour, as heavy metals adsorbents, ingredient for production of functional food for humans and livestock feed; energy recovery or bio-refineries, and much more. In addition, FVW represent a highly unexploited source of bioactive compounds. The recovery of these bioactive compounds through various extraction techniques allows their utilization in food, cosmetics and pharmaceutical industries. Extraction technique are constantly being improved for achieving higher yield and rate of compounds as well as for clearing the compounds from toxic substances and impurities, providing grade nature of the final product and avoiding any functionality loss during processing. However, Estonia is still not fully using the opportunities offered by a circular economy. There is not much information about FVW management, but it is evident that majority of the bio-wastes and by-products of food industries remains underutilized. Some of the underutilized resources that remains underexplored in Estonia include wastes and by-products generated from pumpkin, potatoes, berries, apple, mixture obtained from processing industries, rapeseed husk, and much more. Further, in majority of the instances, bio-waste is mainly incinerated in Estonia. However, there are many novel strategies of using bio waste such as composting, producing biogas or biofuel, bioethanol, etc. which need to be further considered and applied.

Nonetheless, all of the mentioned, globally used FVW management strategies face certain disadvantages and restrictions. An extensive research is required for further standardization, improvement, and development of novel cost effective sustainable strategies for reducing of agri-food wastes in Estonia. In conclusion, FVW management is highly limited, leaving the gap for new ideas, and novel efficient green techniques, which will allow optimal utilization of FVW and by-products in Estonia.

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The influence of milk quality and composition on goat milk suitability for cheese production

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Abstract. The goat milk production sector is growing in Latvia, therefore detailed studies are required to examine goat milk suitability for cheese production. There is still not enough information about the chemical composition and quality of goat milk, and its connection with milk renneting properties. The objective of this study was to analyse the impact of milk composition and quality on goat milk renneting properties. Fat, protein, lactose, urea content, somatic cell count and freezing point were measured by infrared spectroscopy. The curd firmness was analysed by Texture Analyser TA.HD.plus (Stable Micro Systems, UK). In total, 34 samples, including bulk milk samples ($n = 3$) were analysed. The analysed breeds included the Latvian Native ($n = 9$), Saanen ($n = 14$) and milking crosses (closer to Anglo Nubian) ($n = 8$). The samples were arranged according to the lactation, somatic cell count and breeds. Obtained fat content varied from 1.72 to 4.67%, and the protein content – from 2.93 to 4.57% in individual goat milk samples. The highest fat to protein ratio was established in the Saanen breed goat milk (0.96), but the lowest – in milking crosses' milk (0.80). The highest somatic cell count was determined in the second lactation goat milk (1421 thous mL^{-1}) and in milking crosses' goat milk (1027 thous mL^{-1}). The somatic cell count influences curd firmness in cheese, and the highest fat to protein ratio was established in the first group samples with lower somatic cell count.

Key words: goat milk, somatic cell count, renneting properties.

INTRODUCTION

Worldwide, goat milk is known as a healthy product, less allergic, with high concentration of bioactive components and excellent digestibility (Albenzio et al., 2012; Jorge et al., 2018). The composition and quality of goat milk has high importance on cheese yield, composition and sensory properties. Contrary to cow milk, the limit of somatic cell count in goat milk has not been established. The breed, parity, stage of lactation, monthly and seasonal variations have major impact on the somatic cell count in goat milk (Sánchez-Macías et al., 2013). These factors should be taken into account when establishing the somatic cell count limits in goat milk. Also, the effect of somatic cell count in goat milk on milk properties, cheese quality, lipolysis and proteolysis is not clear (Sandrucci et al., 2018). Somatic cells contain lysosomal enzymes and elastase which may cause proteolysis and lipolysis in milk as well in cheese. Sánchez-Macías et

al. (2013) have reviewed the somatic cell count in goat milk and concluded that it is difficult to determine if somatic cells are responsible for changes in goat milk and cheese quality.

The main characteristics of Latvian goats' milk yield and somatic cell count are summarised in Table 1.

Table 1. Milk yield and somatic cell count (SCC) in different goat breeds in Latvia (Agricultural Data Centre Republic of Latvia, 2019)

| Year | Parameter | Breed | | | All goats |
		Latvian Native	Saanen	Milking crosses	
2014	Milk yield, kg	496	614	463	508
	SCC, thous mL^{-1}	1,087	1,316	686	1,202
2015	Milk yield, kg	491	615	505	519
	SCC, thous mL^{-1}	1,011	1,215	908	1,161
2016	Milk yield, kg	499	633	515	539
	SCC, thous mL^{-1}	1,014	1,017	988	978
2017	Milk yield, kg	494	583	532	516
	SCC, thous mL^{-1}	907	1,069	452	1,012
2018	Milk yield, kg	549	552	492	553
	SCC, thous mL^{-1}	966	867	824	804

Saanen breed is the most productive breed in Latvia according to the data of Agricultural Data Centre (2019), but lower somatic cell count is characteristic of milking crosses. The effects of goat breed, the number of lactation, as well as the season on milk renneting properties have not been studied in Latvia before; therefore, the objective of this study was to analyse the impact of milk composition and quality on goat milk renneting properties.

MATERIALS AND METHODS

The study was carried out in August 2019 in one of the largest goat farms in Latvia – the limited liability company Līcīši. Thirty one individual goat milk samples were tested for chemical composition and quality, and fat to protein ratio and curd firmness were measured. Animals were from the second to fourth lactation and represented breeds as followed: the Latvian Native ($n = 9$), Saanen ($n = 14$) and milking crosses (closer to Anglo Nubian) ($n = 8$). Bulk milk samples ($n = 3$) were studied to establish the average results of goat milk chemical composition and its influence on curd firmness. Samples were divided in 3 groups according to the somatic cell count: 1 = less than 500 thous mL^{-1}, 2 = from 500 to 2,000 thous mL^{-1} and 3 = more than 2,000 thous mL^{-1}. Samples were also arranged according to the lactation (second, third and fourth lactation) and breed (Latvian Native, Saanen and milking crosses). Individual milk samples were taken during morning milking, cooled immediately and stored in refrigerator at 4 °C within 24 hours after collection.

Fat, protein, lactose and urea content was analysed by MilkoScan FT 6000 (Foss, Denmark) and somatic cell count – by Fossomatic FC (Foss, Denmark), but freezing point was detected by MilcoScan Mars^{TM} (Foss, Denmark).

The microbial origin rennet (CHY-MAX 1000 IMCU mL^{-1}, Chr. Hansen, Denmark) was used for determining curd firmness (in Newton's). Rennet was diluted 1:100 (v/v), and 1.0 mL of dilution was added to 50 ml of milk. Milk was heated up to 35 °C then rennet was added and samples were further kept in incubator at 35 °C for 30 minutes. Curd firmness was analysed with TA.HD.plus Texture Analyser (Stable Micro Systems, UK) using compression method for determination of curd firmness (technical data: disc A/BE – d45, test speed 1.0 mm s^{-1}, distance in the depth of curd sample 8 mm) (Petrovska et al., 2017).

Statistical analyses were performed using analysis of variance (ANOVA) and mean comparisons of parameters were carried out by *Descriptive statistics*, *t-test, Shapiro.test, Bartlet.test*. Differences were considered statistically significant with a confidence interval of *P* < 0.05.

RESULTS AND DISCUSSION

The chemical composition of goat milk varies according to season, breed, feed, lactation, animal body condition, etc. (Piliena & Jonkus, 2011; García et al., 2014; Leitner et al., 2016; Marcinkoniene & Ciprovica, 2019). Vacca et al. (2018b) have established that Saanen goat breed is the most productive, but this breed milk has showed insufficient results for cheese production. Data on milk composition and quality are summarised in Table 2.

Table 2. Milk composition and quality indices by goat breeds and lactation

Parameter	Breed			Lactation		
	Latvian Native (*n* = 9)	Saanen (*n* = 14)	Milking crosses (*n* = 8)	Second (*n* = 13)	Third (*n* = 13)	Fourth (*n* = 5)
Fat content, %	2.97 ± 0.71[a]	3.30 ± 0.98[b]	2.82 ± 0.21[a]	2.95 ± 0.50[A]	3.12 ± 0.91[AB]	3.31 ± 1.07[B]
Protein content, %	3.27 ± 0.38[a]	3.42 ± 0.46[a]	3.55 ± 0.38[a]	3.49 ± 0.34[A]	3.31 ± 0.40[A]	3.44 ± 0.66[A]
Lactose content, %	4.48 ± 0.27[b]	4.54 ± 0.29[b]	4.37 ± 0.17[b]	4.41 ± 0.19[B]	4.51 ± 0.31[B]	4.59 ± 0.27[B]
Urea content, mg dl^{-1}	46.79 ± 7.64[b]	38.00 ± 6.61[c]	40.41 ± 6.69[c]	41.33 ± 6.39[B]	43.02 ± 7.39[B]	35.98 ± 10.63[C]
SCC, thous mL^{-1}	1,421 ± 1,587[a]	1,100 ± 879[b]	1,559 ± 1,988[a]	1,421 ± 1,587[A]	1376 ± 1,252[A]	1,027 ± 748[B]
Freezing point, °C	-0.486 ± -0.029[b]	-0.500 ±-0.023[b]	-0.499 ±-0.027[b]	-0.495 ±-0.028[B]	-0.492 ±-0.027[B]	-0.508 ± -0.011[B]

Results indicated with the same letter in the lines do not differ significantly (*P* > 0.05).

The mean milk fat content was lower than the protein content, also the analysed milk samples were characterised by reverse ratio of milk fat/protein. Sandrucci et al. (2018) have established that milk fat/protein ratio is significantly influenced by the month of kidding, stage of lactation, herd size, parity and milk production volume. Higher fat and lactose content was established in the Saanen breed goat milk (3.30% and 4.54% respectively), while protein content was higher in milking crosses' milk (3.55%). Estonian researchers have found seasonal differences in fat and protein content in goat

milk, where the average fat and protein content was 3.72% and 3.50% in July–September (Tatar et al., 2015). The differences in freezing point have not been observed.

Fat content is rising with lactation and in the fourth lactation it was 3.31%. Also Piliena & Jonkus (2012) have established that the highest fat content in goat milk is in the fourth lactation. Similar characteristics were found in lactose, protein content and freezing point data. Urea content was higher than the range of 28–32 mg dl^{-1} suggested by Brun-Bellut et al. (1984). High urea content could be an indicator of unbalanced feeding or low nitrogen utilisation (Sadrucci et al., 2018).

During the study, the rut started and also grazing season was close to the end, it could be an explanation for the obtained results in comparison to other research data.

Somatic cell count is higher in goat milk (Leitner et al., 2016; Marcinkoniene & Ciprovica, 2019) than in cow milk (Leitner et al., 2016) and somatic cell count data are summarized in Table 3.

Table 3. Somatic cell count in the analysed milk samples

Group	Number of samples	SCC, thous mL^{-1}	Min SCC, thous mL^{-1}	Max SCC, thous mL^{-1}
1	9	< 500	54	480
2	15	500–2,000	527	1,907
3	9	> 2,000	2,034	19,988

In Latvia, milk is still used in cheese production without analysing the somatic cells count and producers therefore cannot ensure adequate cheese quality. They mainly choose to produce soft and acid-milk cheeses that are less demanding than hard and semi-hard cheeses. Similar situation was also observed in the Czech Republic where Michlová et al. (2016) established the same after studying the Saanen goats' milk quality. Some authors (Bagnicka et al., 2011) have indicated that somatic cell count increases with increasing of number of lactation. Jimenez-Granado et al. (2014) noted that the influence of the number of lactation on the somatic cell count depends on the health status of the udder. Our results coincided with this statement, but also the low number of analysed samples should be taken into account. In Latvia, the average somatic cell count was 804 thous mL^{-1} according to milk monitoring results in 2018 (Agricultural Data Centre of the Republic of Latvia, 2019).

Table 4. Goat milk composition by the somatic cell count

Components	Group		
	1 (*n* = 9)	2 (*n* = 15)	3 (*n* = 9)
Fat content, %	3.11 ± 0.36[a]	3.08 ± 0.54[a]	2.64 ± 0.25[b]
Protein content, %	3.24 ± 0.30[a]	3.38 ± 0.32[a]	3.31 ± 0.38[a]
Lactose content, %	4.52 ± 0.14[b]	4.57 ± 0.24[b]	4.35 ± 0.15[c]
Urea content, mg/dl	43.47 ± 8.29[d]	38.97 ± 8.24[d]	38.86 ± 10.83[d]
Freezing point, °C	-0.513 ± -0.008[e]	-0.497 ± -0.021[e]	-0.473 ± -0.022[f]

Results indicated with the same letter in the lines do not differ significantly ($P > 0.05$).

The highest fat content was established in the first group (3.11%), but the lowest – in the third group (2.64%) or in the samples with the highest somatic cell count. Urea content and freezing point were lower in the second and third group samples, but lactose content – in the third group samples (4.35%). No significant differences in protein content and freezing point were found among the groups (Table 4).

Many researchers (Cecchinato et al., 2011; Bittante et al., 2012; Barrón-Bravo et al., 2013; Malchiodi et al., 2014; Leitner et al., 2016) have established that milk firming time and coagulum strength significantly influence the somatic cell count and, correspondingly, cheese quality and yield. High influence on cheese yield and quality is also exerted by coagulation time and curd firmness (Kübarsepp et al., 2005). Fat to protein ratio is another important aspect in cheese production.

The impact of breed on milk quality and renneting properties is summarised in Table 5.

Table 5. Milk composition and curd firmness by goat breeds

Parameter	Breed		
	Latvian Native ($n = 9$)	Saanen ($n = 14$)	Milking crosses ($n = 8$)
Fat to protein ratio	0.92 ± 0.26^a	0.96 ± 0.25^a	0.80 ± 0.12^b
SCC, thous mL^{-1}	$1,514 \pm 1,260^b$	$1,100 \pm 879^c$	$1,559 \pm 1,988^b$
Curd firmness, N	1.57 ± 0.77^c	1.76 ± 0.59^d	1.59 ± 0.87^c

Results indicated with the same letter in the lines do not differ significantly ($P > 0.05$).

The highest fat to protein ratio (0.96) was established in Saanen goat milk and lowest – in milking crosses' milk (0.80). The Latvian Native breed milk showed an average fat to protein ratio (0.92) and curd firmness (1.57 N). The highest curd firmness was in the Saanen breed goat milk (1.76 N) and the lowest – in milking crosses. Saanen goats are very productive in terms of milk yield and for these reasons usually also present higher fat and protein contents compared with the low-producing breeds (Scheepers et al., 2010; Michlová et al., 2016). Saanen breed is an excellent dairy breed but it showed less technological aptitude for cheese production (Pazzola et al., 2018). Crossbreeding and genetic development is an important factor in understanding renneting properties and cheese outcome (Malchiodi et al., 2014). Vacca et al. (2018a) have established that chemical composition, renneting properties and cheese outcome vary among the breeds.

Table 6. The study of milk suitability for cheese production by lactation

Parameters	Lactation		
	Second ($n = 13$)	Third ($n = 13$)	Fourth ($n = 5$)
Fat to protein ratio	0.86 ± 0.20^a	0.94 ± 0.25^b	0.96 ± 0.25^b
SCC, thous mL^{-1}	$1,421 \pm 1587^c$	$1,376 \pm 1252^c$	$1,027 \pm 748^c$
Curd firmness, N	1.84 ± 0.74^d	1.55 ± 0.63^e	1.47 ± 0.81^e

Results indicated with the same letter in the lines do not differ significantly ($P > 0.05$).

Fat to protein ratio in goat milk increases with lactation. There are no significant differences in the somatic cell count in milk. Higher curd firmness results were established in the second lactation goat milk (1.84 N) which could be explained by a higher protein content in the analysed goat milk (Table 6). It was noticed that curd firmness decreases with increasing number of the lactation which could be explained with the differences in chemical composition and higher fat to protein ratio in goat milk. Piliena & Jonkus (2012) have established that milk composition changes with increasing number of lactation. Higher fat content was established in the fifth and older lactation goats and significantly higher protein content was found in the first lactation goats' milk

(Piliena & Jonkus, 2012). Vacca et al. (2018a) have established that the rennet coagulation time becomes shorter at the end of lactation making the coagulum softer.

Table 7. Milk renneting properties by somatic cell count

Parameter	Group		
	1 ($n = 9$)	2 ($n = 15$)	3 ($n = 7$)
Fat to protein ratio	0.97 ± 0.09^a	0.92 ± 0.18^a	0.79 ± 0.212^b
SCC, thous mL^{-1}	248 ± 192^a	$1,095 \pm 425^b$	$3,262 \pm 1,387^c$
Curd firmness, N	1.82 ± 0.54^b	1.93 ± 0.25^b	2.18 ± 0.50^b

Results indicated with the same letter in the lines do not differ significantly ($P > 0.05$).

Analysing milk renneting properties, significant differences were not found among samples with different somatic cell count. Significant differences were found in the somatic cell count among the groups ($P > 0.05$). Fat to protein ratio showed close values and significant differences were observed in the third group samples (0.79). Bagnicka et al. (2011) could not approve their hypothesis that there exists a correlation between lactose content and somatic cell count in goat milk. In our study, milk samples with highest cell count lactose had the lowest somatic and vice versa. It clearly shows that a more detailed study is necessary to understand the limitation of somatic cell count in healthy goats' milk.

Results of chemical composition and quality indices in bulk milk samples are shown in Table 8.

Bulk milk showed the highest curd firmness (2.18 N) and fat to protein ratio (0.98). Bulk milk samples were collected from all farm animals ($n = 154$), therefore results significantly differ from individual goat milk data. Analysing milk suitability for cheese production, it is important to evaluate bulk milk to understand the variations in milk chemical composition from different aspects.

Table 8. Bulk milk composition

Components	Average
Fat content, %	3.48 ± 0.23
Protein content, %	3.54 ± 0.27
Lactose content, %	4.30 ± 0.06
Freezing point, °C	-0.473 ± -0.005
Fat to protein ratio	0.98 ± 0.03
Curd firmness, N	2.18 ± 0.14

CONCLUSIONS

1. The goat breed, number of lactation and season has an impact on the somatic cell count in goat milk.

2. The study results showed a tendency that somatic cell count has impact on curd firmness, also the number of lactation and goat breed significantly influence milk composition.

3. Further studies are necessary to understand the effect of genetics on goat milk quality (especially somatic cell count), curd firmness and cheese outcome.

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Low-fat high-protein fermented milk product with oat extract as a nature stabilizer

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Abstract. Nowadays, the use of plant components in terms of their pronounced functional properties is of high relevance. Oat extract contains gums, β-glucans, vitamins (A, B1, B5, B9, PP, H), minerals (Fe, I, K, Si, Mn, Cu, Mo, etc.) and essential amino acids. It has been proven that a long-term use of β-glucans showed the reduction of the risk of cardiovascular disease and diabetes and the regulation of cholesterol and blood sugar. B-glucans also have immunoprotective, anti-inflammatory, antimicrobial, prebiotic effects and improve intestinal motility. The aim of study was to develop the technology of low-fat high-protein fermented milk product with functional characteristics. Oat extract was used as a natural stabilizer and a source of β-glucans. Maceration technique was used for the extraction. The recommended extraction parameters were established and physicochemical characteristics of the extract were studied. The recommended doses of oat extract when introduced into milk and the optimal heat treatment conditions of the milk-oat mixture were determined. The influence of temperature on the gel-forming properties of oat extract was investigated. The effect of oat extract on rheological behavior, water-holding ability and shelf life of the finished product was studied. Regular consumption of lactic acid microorganisms has a positive effect on the digestive system and metabolism. Based on the organoleptic characteristics and physicochemical changes during the fermentation process in comparison with the control sample (without oat extract), the recommended starter culture combinations (*Lactobacillus acidophilus*, *Lactococcus lactis subsp*, *Lactobacillus bulgaricus*) were proposed.

Key words: β-glucans, oat extract, fermented milk product.

INTRODUCTION

In the modern world, one of the main causes of death is cardiovascular disease. According to the WHO data, people of various socio–economic classes, age categories and gender are affected by this group of diseases.

Risk factors for cardiovascular disease can be represented as uncorrectable (which cannot be changed) and correctable. The first group includes gender, age, and heredity. It is proved that men are more likely to suffer from cardiovascular diseases and for the first time, symptoms are detected at a younger age than women. However, with age, the risk of developing cardiovascular disease increases in both sexes. People with predisposing heredity are most susceptible to cardiovascular disease. This fact cannot be changed, and in this case, it is necessary to develop an individual program of preventive

measures. Separately, diabetes can be identified as the cause of cardiovascular disease. Correctable factors include mainly dyslepidermia (a violation of the level of cholesterol and or triglycerides in the blood plasma), hypertension (high systolic pressure syndrome), smoking, decreased physical activity, overweight, psychological stress, etc. these factors are determined by a person's lifestyle, his social adaptability, and the level of organization of informing the population about the need for disease prevention at the state level (Krulev, 2008; Oganov & Maslennikova, 2017).

From the point of view of the food industry, the use of functional nutrition can be positioned as a way to implement the WHO health policy 'Health 2020' and considered as a means of reducing the risk of CVD.

The aim of this work was to study the effect of oat extract on consumer and technological properties of a dairy product with desired properties.

Fermented dairy products are popular among the population of different countries in view of their unique organoleptic characteristics and the positive effect exerted on the state of the body, in particular, on the organs of the gastrointestinal tract. As a rule, it is typical for functional nutrition to use probiotic strains of microorganisms as a starter culture (*Streptococcus thermophilus*). Probiotics affect not only the intestinal microflora, but also the musculoskeletal system, the normalization of mineral–ion metabolism and the absorption of vitamins (Winkler et al., 2005; Katharina et al., 2007; Scholz-Ahrens, 2007; Kolsoom et al., 2013).

Mass consumption of fermented milk products is the main reason why the developed product was fermented.

In order to optimize resource conservation in this work, skim milk was used as the basis for a dairy product.

Oat extract was chosen as a functional ingredient, which was also a natural stabilizer and a source of β-glucans and gum.

Whey protein concentrate was used to increase the biological value of the product.

Oat extract is a homogeneous viscous liquid, light brown in color with a gray tint, obtained by aqueous extraction (maceration). In view of the fact, that mainly polysaccharide components (β-glucans and arabinoxylans) pass into the aqueous phase during extraction, during heat treatment (85 °C) followed by cooling, gel formation is observed, the strength of which depends on the dry matter content in the extract. Repeated studies prove the content of β-glucans in oat extract (Sangwan et al., 2014).

β-glucans or 1,3:1,4- β-D glucans are specific water-soluble high molecular weight polymers of glucose with glycosidic bonds, which help to lower cholesterol (Kusmiati & Dhewantara, 2016), triglycerides and glucose in the blood, contribute to weight loss, have oncoprotective (Yoona, 2013), antimicrobial, immunomodulatory properties (Wood, 2007; Novak, 2008).

Such physiological effects are explained by the fact that when it enters the gastrointestinal tract, the viscosity of the natural environment of the colon and its contents increases at the time of digestion of food. The resulting mucus makes it difficult to assimilate nutrients, mainly carbohydrates, cholesterol, bile acids and their derivatives (Khoury, 2011; Nwachukwu et al., 2015).

The whey protein concentrate has valuable functional characteristics, high nutritional value, in comparison with casein, which is explained by a high content of sulfur-containing amino acids and essential amino acids (isoleucine, tryptophan, lysine,

tyrosine). Using WPC gives certain technological properties to the product, in view of the good hydrophilic and lipophilic properties (Ertaş et al., 2015).

MATERIALS AND METHODS

Skimmed milk powder (manufactured by Bob's Red Mill, U.S.A.) was used as a raw material. Recovery was carried out based on the protein content of 3% reduced skim milk. For this, the required amount of milk powder was hydrated with filtered tap water at a temperature of 40 ± 2 °C with continuous stirring until the dry residue was completely dissolved on an IKA EUROSTAR 20 digital mixer (1,000 rpm) and left for 2 hours to swell the protein component. The reconstituted milk was filtered through a fabric filter.

Milk preparation
Whey protein concentrate is added to the restored skim milk $t = 37 \pm 2$ °C to a protein content of 6% with constant stirring until it is completely dissolved.

In order to prevent protein coagulation during heat treatment, a mixture of citric and pyrophosphoric salts was used in an amount of 0.6 g per 1,000 g of the mixture (0.03%).

The milk protein mixture was subjected to heat treatment at a temperature of 76 ± 2 °C with a holding time of 15 min, followed by cooling and storage on demand at a temperature of 4 ± 2 °C.

Preparation of oat extract
To obtain oat extract, oat bran was used in accordance with GOST 21149-93. Extraction was carried out by maceration at temperatures of 20, 30 and 40 °C. The hydraulic module was determined empirically in the range of 1:1–1:10. The end time of the extraction was determined by the dry matter content, which was determined by the arbitration method.

Before extraction, oatmeal was preliminarily subjected to autoclaving at a temperature of 121 ± 2 °C for 10 min in order to prevent side enzymatic processes caused by the presence of extraneous microflora (yeast, mold, etc.)

Preparation of milk-oat mix
At the temperature of 37 ± 2 °C, oat extract was added to the prepared milk protein mixture in an amount of 20%. The determination of the amount applied depended on the organoleptic and thixotropic properties of the finished product.

The milk-oat mixture was pasteurized at $t = 87 \pm 2$ °C with a holding time of 30 seconds. The pasteurization mode is due to the ability of the oat extract to gel and the final structural and mechanical properties of the finished product.

The percentage of introduced starter culture was determined empirically in relation to the process of acid accumulation over time in the range of 3–5% in increments of 1%.

METHODS

Method for the determination of solids in oat extract
The determination of solids was carried out by drying to constant weight at a temperature of 105 ± 2 °C in an oven with forced air circulation according to GOST 3626-73.

Method for the determination of β-glucans

The content of β-glucans in oat extract was determined in accordance with GOST R 57513-2017. The method is based on the enzymatic hydrolysis of β-glucan using lichenase and β-glucanosidase enzymes to gluco-oligosaccharides and glucose, respectively. Hydrolyzed β-glucan is determined by the colorimetric method according to the degree of staining of glucose molecules with a glucose oxidase reagent at a wavelength of 510 nm compared to a control sample of glucose.

Method for determination of protein in oat extract

Protein determination in oat extract was carried out using a Shimadzu UV-1800 spectrophotometer at a wavelength of 280 nm. The method is based on the ability of proteins to absorb light in the ultraviolet region due to the presence of aromatic amino groups (mainly tyrosine and tryptophan) according to OFS.1.2.3.0012.15.

Method for determination of active and titratable acidity

pH values were measured using a pH-410 pH meter with a combined glass electrode (Research and Production Association TECHNOKOM, Russia).

Acidity was determined by titration in accordance with the AOAC method 947.05 (AOAC, 2007).

Method for determining water retention capacity

The water holding capacity was determined by centrifugation at 1,000 rpm. 10 mL of the fermented product was subjected to centrifugal treatment for 30 minutes. The amount of serum excreted was evaluated every 5 minutes. A fermented milk-protein mixture without oat extract was taken as a control sample.

Method for determining thixotropic properties

Thixotropic properties were measured using a Rheotest 2 rotational viscometer (RHEOTEST, Germany). The measurement was carried out after ripening after cooling to 4 ± 2 °C with a shear rate range from 1.00 to 437.4 min^{-1}. To achieve a homogeneous consistency, the samples were subjected to 10-fold mixing.

The ability to recover was estimated as the ratio of the initial effective viscosity of the experimental and control samples at the same selected velocity gradient to the effective viscosity after the relaxation time (15 min).

The ability to recover was estimated as a percentage by the formula:

$$B = \frac{n_p \cdot 100}{n_H} \qquad (1)$$

where B – recovery ability, n_p – effective viscosity after relaxation for 15 minutes at a given speed gradien, $n_н$ – effective viscosity at the initial time with a given velocity gradient.

For this, the sample was sheared in a rotating ring, the readings of the device are taken every 15 seconds for 2 minutes. Then the sample is left alone for 15 min to restore coagulation bonds, after which the value of the restored structure was recorded.

The mechanical stability coefficient was calculated by the formula:

$$K = \frac{n_H}{n_p} \qquad (2)$$

where K – mechanical stability coefficient, n_p – effective viscosity after relaxation for 15 minutes at a given speed gradien, $n_н$ – effective viscosity at the initial time with a given velocity gradient.

The coefficient of viscosity loss was calculated by the formula:

$$\Pi = \frac{(n_н - n_p) \cdot 100}{n_н} \qquad (3)$$

where Π – viscosity loss coefficient, n_p – effective viscosity after relaxation for 15 minutes at a given speed gradien, $n_н$ – effective viscosity at the initial time with a given velocity gradient.

The method of determining the number of microorganisms

Streptococcus thermophilus were calculated according to GOST 33951-2016.

Method for assessing organoleptic properties

Assessment was carried out in accordance with GOST ISO 4121-2016 and GOST ISO 6658-2016.

The hedonic scale used for the assessment is summarized in Table 1.

Organoleptic indicators (taste, smell, texture, appearance, color) were evaluated by a group of tasters from 28 people of different age groups and in equal percentage by gender on a hedonic scale with a neutral desirability level of '0', 4 positive and 4 negative levels.

For the reliability of the sensory evaluation, the tasters rinsed the mouth with clean water after testing each sample to remove the residual taste and paused for 1–2 minutes.

Table 1. Hedonic scale for assessing the quality of products

Desirability Levels
1. Very desirable (+4)
2. Highly desirable (+3)
3. Middling (+2)
4. Unwanted (+1)
5. Neutral (0)
6. Slightly desirable (-1)
7. Middling (-2)
8. Highly Unwanted (-3)
9. Very unwanted (-4)

RESULTS

The solids content in the oat extract, depending on the hydromodule and the temperature of extraction (Table 2).

When using a hydraulic module 1:1–1:3, it is difficult to separate the extract due to the strong swelling of the bran and its increased viscosity. At an extraction temperature of 30 and 40 °C, enzymatic processes begin and the extract acquires an acidic taste and is unsuitable for further use due to the negative effect on the organoleptic properties of the finished product. When using hydraulic modules 1:6–1:10, the finished product acquired a watery consistency. The choice between extracts with a 1:5 and 1:4 hydromodule was based on the content of β-glucans, which were determined using a Shimadzu UV-1800 spectrophotometer.

The content of β-glucans in the oat extract with hydromodules 1:4 and 1:5 are shown in Table 3.

Table 2. The solids content in the oat extract with a hydromodules of 1:1–1:10

Hydromodules	The temperature of extraction, (°C)	Solids content, (%)
1:10	20	1.233 ± 0.006
	30	1.542 ± 0.032
	40	1.611 ± 0.012
1:9	20	2.354 ± 0.054
	30	2.442 ± 0.035
	40	2.645 ± 0.162
1:8	20	3.676 ± 0.055
	30	3.783 ± 0.024
	40	3.995 ± 0.087
1:7	20	4.132 ± 0.034
	30	4.787 ± 0.034
	40	4.945 ± 0.032
1:6	20	5.236 ± 0.034
	30	6.696 ± 0.052
	40	7.035 ± 0.062
1:5	20	8.312 ± 0.023
	30	8.967 ± 0.024
	40	9.225 ± 0.045
1:4	20	9.882 ± 0.081
	30	10.407 ± 0.022
	40	11.071 ± 0.065
1:3	20	12.277 ± 0.084
	30	13.132 ± 0.142
	40	13.797 ± 0.344
1:2	20	15.235 ± 0.141
	30	16.056 ± 0.062
	40	17.324 ± 0.044
1:1	20	17.565 ± 0.032
	30	17.832 ± 0.252
	40	18.024 ± 0.041

Table 3. The content of β-glucans in the studied samples

Hydromodules	Extraction temperature, (°C)	The content of β-glucans, (%)
1:4	20	4.74 ± 0.02
	30	5.02 ± 0.01
1:5	20	3.72 ± 0.03
	30	4.63 ± 0.01

According to the results of the experiment, an extract with a 1:4 hydromodule obtained at a temperature of 20 °C was used for further studies.

The protein content in the oat extract with a 1:4 hydraulic module is 1.02 ± 0.02%
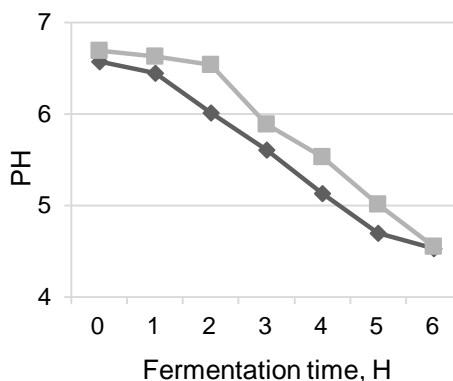

Figure 1. Changes in pH during fermentation: ■ – control sample, ◆ – sample with oat extract.

The dependence of the active and titratable acidity during the fermentation time is presented in Figs 1, 2.

The fermentation process was carried out until all samples reached pH = 4.55 and the titratable acidity was not lower than 65 °T. The control and prototypes reach the set values after 6 hours. In this case, the indications of active acidity do not differ significantly, therefore, oat extract does not inhibit the growth of starter microflora. It is worth noting that after 3 hours of fermentation, the consistency of the prototype changes. Compared to the control sample, it has a stronger casein gel, while the control remains liquid.

The percentage of introduced starter culture varied from 3 to 5% of the total weight of the fermented mixture. The leaven was previously activated in order to achieve a uniform distribution of the culture of microorganisms in the samples, as well as to reduce the time of adaptation of microorganisms to fermentation conditions. In all cases, the process of acid accumulation in the first 2 hours of fermentation proceeds slowly, which is probably due to the increased solids content in the samples. Changes in pH and titratable acidity are noticeable only 3 hours after the start of fermentation. In this case, the following relationship can be established: an increase in the concentration of starter culture for every 1% reduces the time of ripening by about 1 hour in both the control and the experimental sample. However, the presence of oat extract is not an inhibitory factor for the starter culture, but rather contributes to a more intensive fermentation process. The ripening time is not more than 6 hours subject to temperature conditions (Hurda, 2019). The percentage of leaven introduced was 3%.
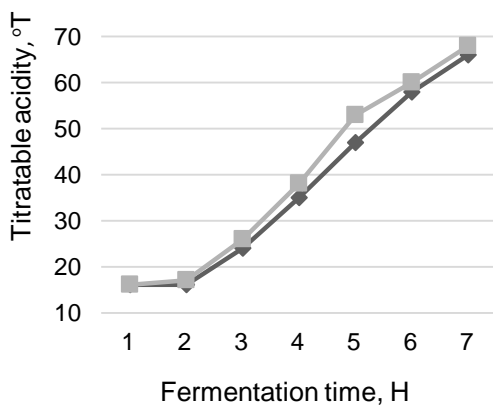
Water retention studies are shown in Fig. 3.

Figure 2. Changes in titratable acidity during fermentation: ◆ – sample with oat extract; ■ – control sample.
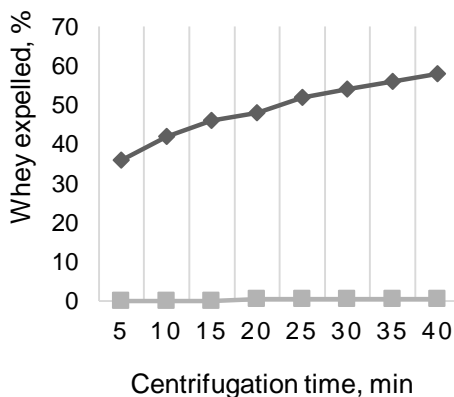
Figure 3. Water holding capacities of yogurt samples: ■ – sample with oat extract; ◆ – control sample.

In the experimental sample, the serum practically does not separate. After 30 minutes of centrifugation, the amount of serum released was 0.5 mL. The control sample was centrifuged until the amount of serum released stopped changing.

The thixotropic properties of the product characterize its ability to restore the structure after mechanical action and, accordingly, model the behavior of the fermented milk product when it is moved along production lines after the fermentation process. In production conditions, it is more profitable to use the reservoir method, but not every product is able to restore the coagulation structure after packaging. The use of stabilizers in this case of β-glucans increases the degree of structural restoration and improves the structural and mechanical characteristics of yogurt (Jingyuan et al., 2013).

Thixotropic properties are shown in Figs 4 and 5.
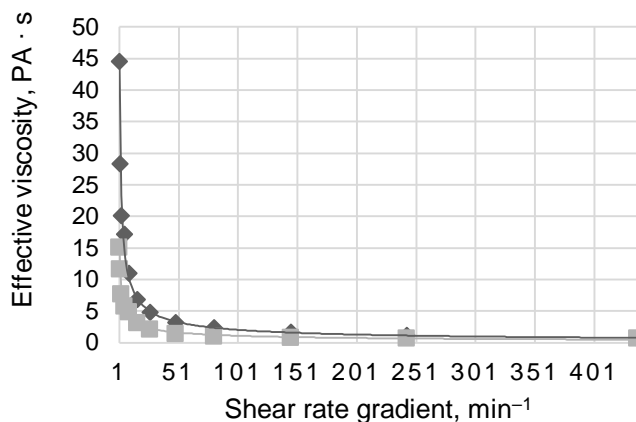
Figure 4. Dependence of the apparent viscosity of samples with oat extract on the shear rate at 4 °C.
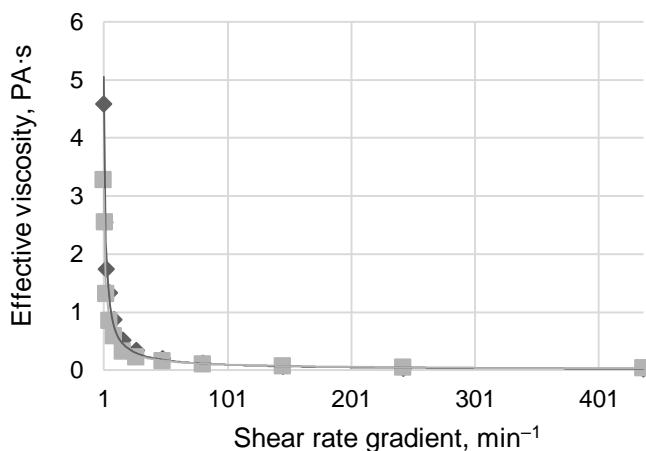
Figure 5. Dependence of the apparent viscosity of control samples on the shear rate at 4 °C.

Despite the fact that the area between the ascending and descending branches of the hesteresis loop in the control sample is smaller, the effective viscosity of the prototype is much higher.

Thixotropic characteristics are shown in Table 4.

Table 4. Thixotropic characteristics of the control and experimental samples

Significative	Value	
	development type	control sample
The ability of the coagulation structure to recover, (%)	90.44	85
Mechanical stability coefficient	1.11	1.18
Viscosity loss coefficient	9.56	15

The effective viscosity of the sample decreases over time with the same velocity gradient. Various parameters influence rheological properties: mass fraction of fat, solids content, properties of starter culture (viscous and inviscid strains), and the presence of stabilizers.

In this work, we used the starter culture produced by Danisco (France), which contains viscous strains and exopolysaccharide-producing strains, which affects the thixotropic and water-holding properties.

The hedonic scale is shown in Fig. 6.
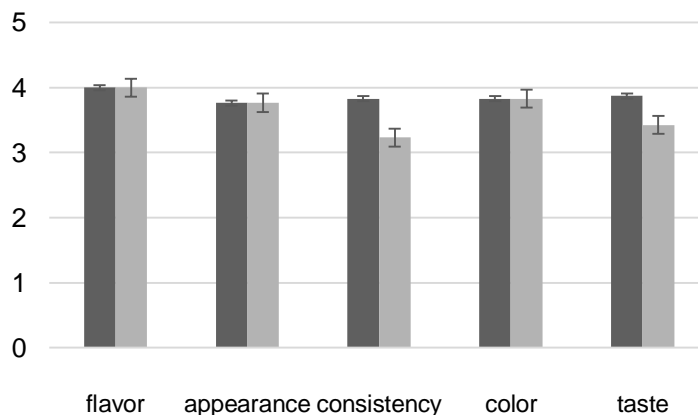
Figure 6. Hedonic scale for appearance and overall acceptability of the samples: ☐ – control sample. ■ – samples with oat extract.

The taste of the finished product was evaluated by a group of tasters as pleasant, sour-milk, with a slight aftertaste of oatmeal. There are no extraneous unpleasant tastes. By consistency, the finished product is homogeneous without impurities, the clot is elastic despite the fact that the product is low-fat, does not spread, during testing, the volume of the product in the container is not broken. After mixing, no separation of serum is observed, but the product acquires a stretching and viscous consistency, which does not cause negative opinions about it. The color is light beige.

Recommended technological scheme for producing fermented milk with oat extract (Fig. 9).

CONCLUSION

The effect of oat extract on consumer and technological properties of fermented skim milk product was investigated. The data obtained confirm that the use of the extract at a concentration of 20% does not adversely affect the organoleptic properties of the finished product, and also increases its rheological properties. Oat extract does not affect the development of microorganisms in the starter culture during fermentation. The extract has the greatest influence on the water-holding ability.

Raw material acceptance	
Oat bran	In accordance with the current regulatory and technical documentation of the country
Skimmed milk	
Water	
Microbial starter culture Streptococcus thermophilus	
Whey protein concentrate	

Preparation of oat extract	
Oat bran heat treatment	121 ± 2 °C, 10 min, P = 1 atm.
Oat bran maceration	Hydro module 1:4, t = 20 °C, t = 6 h
Oat extract filtration	Membrane filters

Normalization
By mass fraction of protein, by adding WPC
The introduction of oat extract in an amount of 20% by weight of the normalized mixture, taking into account losses

Heated to homogenization temperature
T = 60–65 °C

Homogenization
P = 10–20 MPa, T = 60–65 °C

Pasteurization
T = 87 ± 2 °C, 30 sec.

Cooling
T = 38–42 °C

Introducing a starter culture
Direct starter culture or activated starter culture in an amount of 5% by weight of the fermented mixture

Fermentation
pH = 4.55

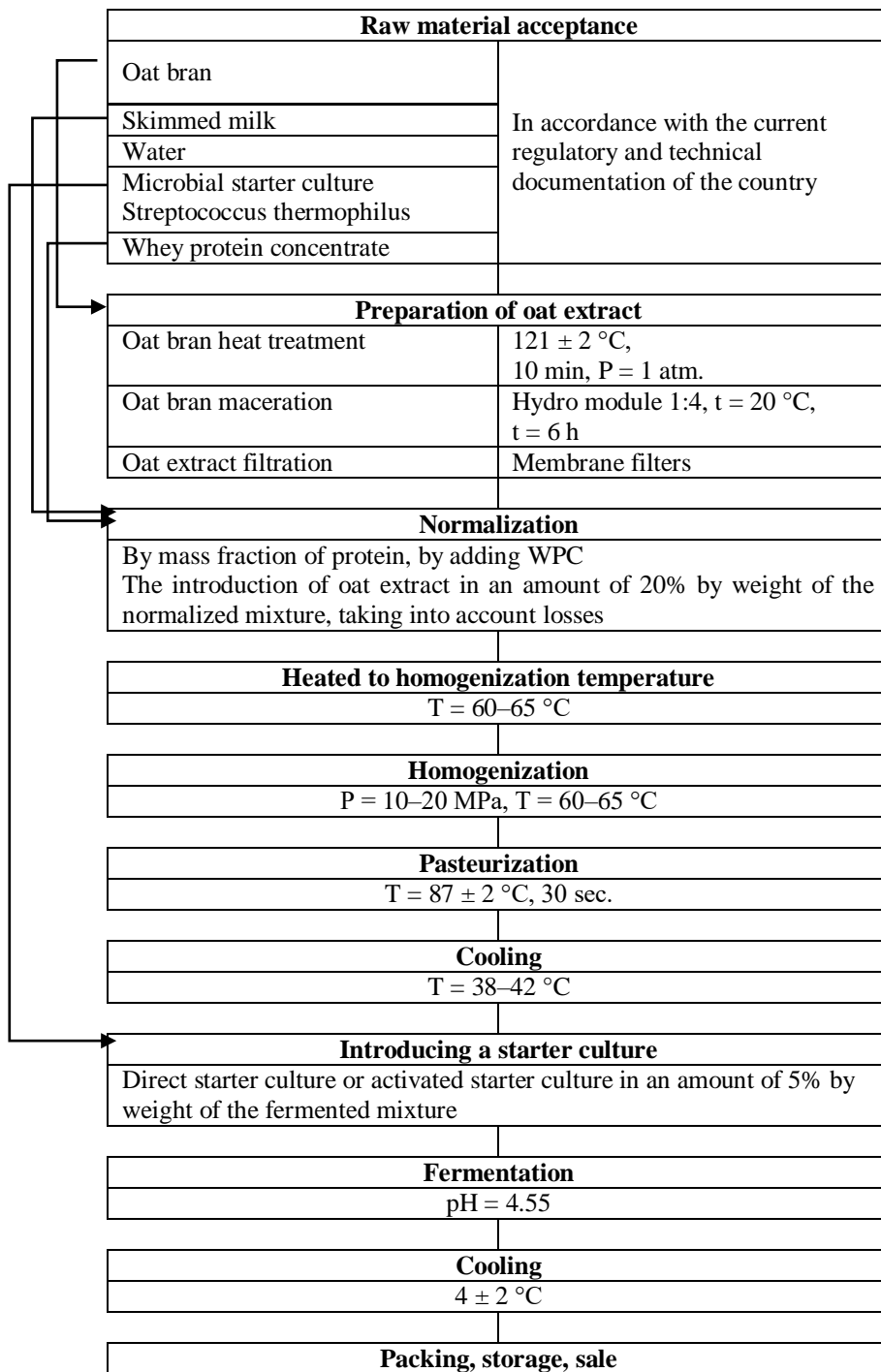**Cooling**
4 ± 2 °C

Packing, storage, sale

Figure 9. Technological scheme for producing fermented milk with oat extract.

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Rheological and physical–chemical properties of yogurt with oat–chia seeds composites

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Abstract. Currently chia seeds (*Salvia hispanica L.*) are considered as a filler of functional food. However, ground chia seeds have a low viscosity and cohesion properties that are limited its applications. Based on previous data oat-chia seeds composites in different proportions as filler for yogurt have been tested. The investigation of water–holding capacity of samples allowed to select the yogurt with filler in the ratio of 1:1 (oat bran:chia seed) in the amount of 3% and 5% as the most close to the control sample without any filler. The rheological characteristics of yogurt samples were investigated and their thixotropic and viscoelastic properties were identified depending on the amount of filler in the product. The yogurt without any filler had the less thixotropic properties in compare with yogurt with oat-chia seeds composites. The structure recovery of yogurt with 3% and 5% filler was close to 100% and greater than 100% respectively. Based on the data of G' and G" moduli was possible to ascertain the yogurt with filler has more viscoelastic properties compared with yogurt without filler. Yogurt with 5% filler exceeds yogurt without filler in biological value according to the content of essential amino acids and polyunsaturated fatty acids.

Key words: yogurt; *Salvia hispanica L.*, oat–chia seeds composites, rheological properties, thixotropic properties, viscoelastic properties, water–holding capacity.

INTRODUCTION

Yogurt is one of the most popular fermented foods in many countries (Nakasaki et al., 2008). Yogurt is a cultured dairy product that is widely consumed as a healthful and nutritious food and for its sensory properties (Innocente et al., 2016). Yogurt is most commonly produced from cow's milk by slow lactic fermentation of milk lactose under controlled temperature. Starter microflora of yogurt consists of a symbiotic culture of the bacteria *Lactobacillus delbruikii ssp. bulgaricus* and *Streptococcus thermophilus* (Marshall, 1993).

The popularity of yogurt is due to its suitability for combination with various fillings, which provides wide taste properties of the final product. In addition, yogurts, like other milk–based drinks, are a convenient form for creating functional foods–by

enrichment with specialized additives or microbiological synthesis in the product itself (Suchkova et al., 2014; Zabodalova et al., 2014). However, natural fillers effect on the consistency of yogurt, often causing syneresis during storage, so various structure stabilizers are widely used in yogurt production, especially stirred yogurt, to prevent syneresis (Kumar & Mishra, 2004). Polysaccharides are widely used for these purposes due to the ability to retain water and to form hydrogels (Gu et al., 2016). Polysaccharides can be used in dairy products to modify the rheological properties (Sanchez et al., 2000). Furthermore, polysaccharides of vegetable sources are becoming increasingly popular due to the increasing number of vegetarians (Karim & Bhat, 2008). Some polysaccharides from plants could serve as therapeutic agents, excipients, thickeners, stabilizers, emulsifiers, encapsulants, coating agents and texture modifiers due to their considerable availability, diverse functionality, non–cytotoxicity and ease of modification (Amal & Ahmad, 2014; Rohart & Michona, 2014; Pang et al., 2016).

Many authors consider chia seeds as raw material with high gelling properties. Chia seed (*Salvia hispanica L.*) polysaccharide is extracted from the chia seed coat Gu et al., 2016). The processed products of chia seed possess excellent water–holding capacity and good stabilizing properties, which are very important for the yogurt production (Segura–Campos et al., 2014). Besides chia seeds are important raw material for functional food due to its health promoting properties (Fernandez et al., 2008). Chia seeds contain of protein (15–25%), fats (30–33%), carbohydrates (26–41%), dietary fiber (18–30%), and ash (4–5%), also a high amount of vitamins, minerals, and antioxidants. However, there are some limitations in the use of chia seeds in food composition due to their small size and hard seed coat, high oil content and cohesiveness (Ixtaina et al., 2008). The research of authors demonstrated the ability to use chia seeds in combination with oat bran (Yakindra et al., 2015).

The oat–chia seeds composites could optimize the original oat bran quality by the nutritional value of chia seeds. Besides the nutritional aspects the oat–chia seeds composites could improve the water–holding capacity, viscoelastic properties of the individual components (oat bran and chia seeds). The physical and chemical properties of oat–chia seeds composites could be valuable for developing a new functional food having desirable texture and improved nutritional value for consumers health (Yakindra et al., 2015).

Thus, the purpose of this research was to explore the possibility of using the oat-chia seeds composites in the recipe of yogurt, in particular, to improve the physical-chemical and rheological properties of yogurt with filler.

MATERIALS AND METHODS

Preparation of oat–chia seeds composites

The oat–chia seeds composites were created by a feasible dry blending procedure. The chia seeds (*Salvia hispanica L.*) were supplied by 'Adowel Inversora S.A.', Eastern Republic of Uruguay. The jet–cooking oat bran (brand '*Mistral*', Russian Federation) was purchased in the trade network. The chia seeds and oat bran were separately ground by industrial grinder Bulava–1, Russian Federation (the specifications: the weight – 20 kg, the capacity–up to 90 kg h^{-1}; the dimensions of the receiving hole: 60×40 mm; the hopper capacity–up to 6 liters; the voltage–220 V; the power consumption–1,800 W; the rotor speed – 3,000 rpm; the dimensions: 550×350×1,000 mm. Ground oat bran and chia

seeds were compiled in the following proportions of 1:0; 9:1; 4:1; 1:1; 0:1 and mixed by N–50 Hobart mixer (Canada) for 1 min. The oat–chia seeds composites were ground again by industrial grinder Bulava–1 for 40 s to obtain the desired outlet fractions size less than 1.0 mm.

Yogurt processing

Partially skimmed raw cow's milk (fat content $1.5 \pm 0.5\%$) in an amount of three liters was submitted to heat treatment at 90 °C for 15 min, cooled to 40 °C and inoculated with 0.2 U per kg yogurt culture containing *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* (CBL–1, MARINO, Italy). The yogurt was produced with using the handicraft fermentation equipment (Milk & Cheese M & C100, Modena, Italy) at 41 °C for 4–5 h until a pH value of samples equal 4.24 ± 0.06 was reached (Tamine & Robinson, 2007). After that the yogurt was subsequently cooled to 4 ± 2 °C and used for preparing of the batch of samples. The experiment was included 11 samples: 1–yogurt without filler (control) and 10–yogurt with oat–chia seeds composites in different proportions. All samples were prepared in triplicate.

Preparation of yogurt with oat–chia seeds composites

The oat–chia composites (filler) in the five proportions of 1:0; 9:1; 4:1; 1:1; 0:1 in the amount of 3 and 5% were added in the yogurt to obtain the samples with the filler in the volume of 200 mL. First the samples were stirred manually, then using a Magnetic Stirrer (Ulab us–1550A) at 50 rpm, 20 °C for 10 min. Finally, the yogurt samples were packaged in a volume of 200 mL and stored at 5 ± 1 °C for 21 days. All samples were prepared in triplicate.

Study of organoleptic properties of yogurt with oat–chia seeds composites

The yogurt samples were evaluated by trained panel of 12 members. Twelve panelists (age 22–38 years) familiar with sensory evaluation techniques estimated the sensory properties of the yogurt samples.

Measurement of water–holding capacity of yogurt samples

The study of water–holding capacity of yogurt samples were evaluated using the SIGMA 4–16S at $1,590 \times$ g for 10 min at 20 °C according to the method described by Nadtochii & Koryagina, 2014. This research was conducted as follows: 10 mL test sample was placed into the measuring tube and centrifuged for 30 min, noting the precipitated serum volume every 5 min by stop the centrifuge every 5 minutes and run the test on the same samples. The water–holding capacity of the samples was evaluated by determining the quantity of the separated serum (%) in the process of centrifugation, considering the fact, that the whole sample volume (10 mL) is 100%. Three samples were analyzed per each batch of yogurt.

Post– acidification analyses of yogurt samples

Post– acidification analyses of the yogurt samples were conducted on 0, 7, 14 and 21 days of storage. The pH of the yogurt samples was measured at 20 °C using the digital pH meter (pH 301, Hanna Instruments, Inc., RI, USA) and were carried out in triplicate.

Investigation of rheological properties of yogurt samples

The yogurts samples after being stirred were cooled to 10 °C, which corresponds to the storage temperature of yogurt before the delivery to the consumer. Then the samples of required volume were loaded to the rheometer (RN 4.1, RHEOTEST Medingen GmbH, Germany) with using of coaxial cylinders particularly 4 cm diameter parallel stainless cylinder 3.8 cm outer diameter (housing) and 3.5 cm inner diameter cylinder. All rheological measurements were carried out at 10 °C using circulation system within ± 0.1 °C. The steady shear viscosity of the yogurt samples was measured as a function of shear rates from 0.1 to 10 s^{-1} (in the forward direction). To assess the ability of yogurt to recover of the structure after mechanical impact the samples were left at rest for 15 minutes and then again they were subjected to mechanical stress rates from 10 to 0.1 s^{-1} (in reverse). The frequency sweep test was performed to obtain storage modulus (G$^{'}$) and loss modulus (G'') at frequencies ranging from 1 to 10 rad × s^{-1}. The strain of 0.5%, which was within the linear viscoelastic range, was used for the dynamic experiments. To find the numerical value of the yield point of the samples we used the most popular equations, such as Bingham's equation (Bingham, 1922); Caisson equation (Mills, 1959); Hershel–Bulkley equation (Hershel & Bulkley,1926). The yield point of yogurt was studied by following equations (1–3):

$$\text{Bingham: } \sigma = \sigma_\gamma + \eta_\rho \dot{\gamma} \tag{1}$$

$$\text{Caisson: } \sigma^{1/2} = \sigma_\gamma^{1/2} + (\eta_\rho \dot{\gamma})^{1/2} \tag{2}$$

$$\text{Hershel–Bulkley: } \sigma = \sigma_\gamma + K\dot{\gamma}^n \tag{3}$$

Investigation of biological value of yogurt samples

The biological value of the protein component was evaluated by the generally accepted method of calculation of the essential amino acids scores (FAO, 2007). Besides the indicators of biological value of protein component was investigated such as biological value of protein component (BV, %) and coefficient of differences of amino–acid scores (CDAAS, %) (Nadtochii et al., 2015). This indicator (in days) was calculated by the Eqs 4 and 6:

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

$$CDAAS = \frac{\sum \Delta DAAS}{n} \tag{4}$$

DAAS – difference of amino–acid score of an essential amino acid and a minimum amino acid score was calculated by the Eq. 5, where n – amount of essential amino acids equal 9.

$$\Delta DAAS = Ci - Cmin \tag{5}$$

Ci – score of i – essential amino acid, %; Cmin–minimum amino–acid score, %.

$$BV = 100 - CDAAS, \% \tag{6}$$

The evaluation of the biological value of the lipid component was produced according to FAO, 2008.

Statistical analysis

All experiments were performed with at least three replicates. Data was processed by methods of mathematical statistics at theoretical frequency 0.95. Data was expressed as mean ± standard mean error.

RESULTS AND DISCUSSION

Organoleptic properties of yogurt with oat–chia seeds composites

The yogurt samples with fillers in particularly with oat bran and chia seeds in the proportions of 1:0; 9:1; 4:1; 1:1; 0:1 were investigated (Yakindra et al., 2015). The yogurt without fillers was used as a control sample. Percentage of filler (3 and 5%) was defined as the most commonly used in the formulation of products with different fillers (Nadtochii & Koryagina, 2014). Fig. 1 shows the changes in color and appearance of yogurt samples with different fillers.

The yogurt with filler based on oat bran (1:0) had cream color tone, pronounced odor and taste of oat bran. However, the consistency changes were observed during storage: oat bran settled down to the bottom of a container. Filler based on chia seed (0:1) provided the product gray–cream color tone and excessively gelled consistency, taste and odor of the seeds were neutral, and filler accumulated on the top part of the container during storage, showing a tendency of sample phase separation. The yogurt with fillers based on the priority of oat bran (9:1 and 4:1) showed mostly similar organoleptic properties to the samples with filler based on oat bran. Yogurt with filler based on the same amount of oat bran and chia seed (1:1) demonstrated the absence of exfoliation of consistency and most harmonious organoleptic properties in comparison with the other samples of yogurt with fillers. Moreover, the organoleptic properties of the yogurt samples were most pronounced at adding 5% filler compared with 3% of fillers. The control sample had a dense, homogeneous consistency, without separation of serum on the surface, and white color with cream shade, clean fermented flavor.
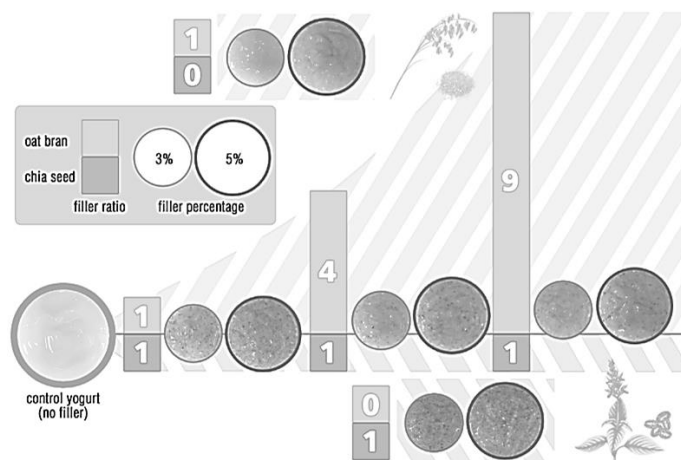

Figure 1. The organoleptic assessment of the yogurt samples with oat – chia composites.

Water–holding capacity of yogurt samples

The ability of the ingredients to retain moisture as the important property in the development of new types of food products, in particular yoghurt is evaluated (Tamime & Robinson, 1999; Lucey, 2002). Water–holding capacity of yogurt without any filler and with fillers in the amount of 3 and 5% are shown in Fig. 2. It should be noted, that the control sample showed more regular release of serum for 30 minutes due to the mechanical action (linear characteristic curves were noted). During the first 15 minutes of the mechanical action yogurt with the filler demonstrated more intensive separation of serum in comparison with the further exposure after 20–30 minutes of centrifugation.

Stirring duration in the production of yogurt with a filler is on average about 20 minutes, so the experiment results about 15–20 minutes of mechanical processing are the most important for technical applications. Fig. 2, a shows the water–holding capacity of yogurt with the addition of 3 and 5% filler on the basis of oat bran or chia seeds (1:0 and 0:1) in comparison with the control sample. The yogurt with chia seed possesses the highest water–holding capacity in compared to the control sample and the sample of yogurt with oat bran filler (Fig. 2, a). In addition, more amount of chia seed filler in yogurt demonstrate the higher level of the water–holding capacity. On the contrary the greater the amount of oat bran filler, the lower the water–holding capacity of the yogurt sample. Fig. 2, b demonstrates the more intensive serum separation of the yogurt sample with oat–chia seeds filler in the proportion of 9:1 and 4:1 in the amount of 3 and 5% in comparison with control samples during of mechanical processing. The oat bran as a part of the filler reduce the water holding capacity of yogurt with the filler (Fig. 2, b). The yogurt with oat–chia filler in proportion of 1:1 in the amount of 3 and 5% is closer to the control sample compared to other samples. In addition, the influence of the amount of filler in this case is not substantial. As a result of studying the samples water–holding capacity, the yogurt with 3–5% oat-chia seeds composites in the ratio of 1:1 is closer to the control samples and this filler proportion was selected for further research.
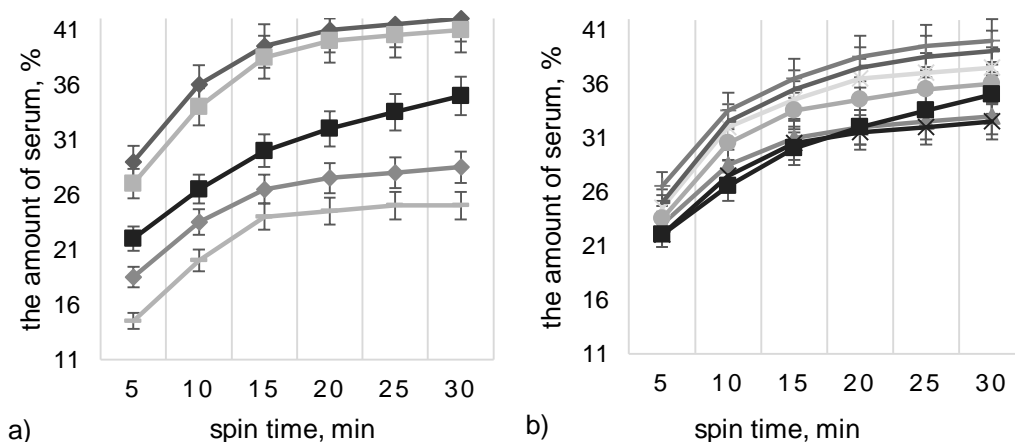

Figure 2. Water–holding capacity of yogurt with fillers: a) ▬ – 5% oat bran; ● – 5% chia seed; ▬■▬ – control; – 3% chia seed; ◆ – 3% oat bran; b) ✳ – 5% 1:1; ● – 5% 4:1; ▬ – 5% 9:1; ▬■▬ – control; ✱ – 3% 1:1; – 3% 4:1; ▬+▬ –3% 9:1.

Post– acidification analyses of yogurt samples

Post– acidification is important quality property for dairy products during storage. Research of differences in the initial pH values and the intensity of pH changes of yogurt samples during storage have been carried out (Fig. 3).

The initial pH of yogurt without any filler was 4.24 ± 0.06 that the lower than in other yogurt with 3 and 5% filler with differences 0.04 and 0.14 respectively. The comparably same decrease in pH level of samples on the 7th day of refrigerated storage (0.01–0.03) was noted. However, the reduction in pH of the yogurt with filler was more intensive compared to the yogurt without filler up to the 14th day of storage, especially for the yogurt with 5% filler. In addition, the intensity of changes in pH of yogurt

Figure 3. pH changes during storage: ─✦─ –control; ─■─ –3%; ─▲─ – 5%.

with filler was less than in control sample during the period from the 14th to the 21st day of storage.

The final pH of the samples was relatively the same, indicating the difference reduce in initial pH of samples. The pH level of yogurt with 5% filler on the 14th day of storage corresponds to the pH of control sample on the 7th day of storage. However, the changes in pH in the yogurt with 5% filler were 2 times higher compared to the control sample during the whole storage period. Obviously, this is due to the fact that the polysaccharides are a substrate for various starter cultures. The yogurt clot with filler based on polysaccharides could activate a further increase of the yogurt starter microflora during storage.

Rheological properties of yogurt samples

Rheological tests are widely used in the food industry for the evaluation of technological and consumer properties of food products (Edvards et al., 2001; Keentok et al., 2002). Analysis of the rheological properties of different food products show that non–Newtonian viscosity properties, availability of yield strength and thixotropy (Haque et al., 2001). Yogurt refers to the group of pseudoplastic liquids with the manifestation of thixotropic properties (Tamime & Robinson, 1999). Complex of the rheological research of the samples have allowed to characterize their resistance to mechanical impact and the ability to restore the structure after the specified time. The rheological properties of yogurt samples were obtained by characteristic curves: the apparent viscosity on the shear rate in the forward direction (in the destruction of the structure) and in the reverse direction (after keeping the samples at rest for 15 minutes); and complex dynamic viscosity, the elastic (storage) modulus (G') and the viscous (loss) modulus (G") at different frequencies. The values of the yield point for the yogurt samples were calculated.

The yogurt samples demonstrated a nonlinear reducing dependence of the apparent viscosity with the increasing shear rate (Fig. 4). The lack of the linear dependence of the viscosity on the shear rate proves non–Newtonian viscosity properties of yogurt samples
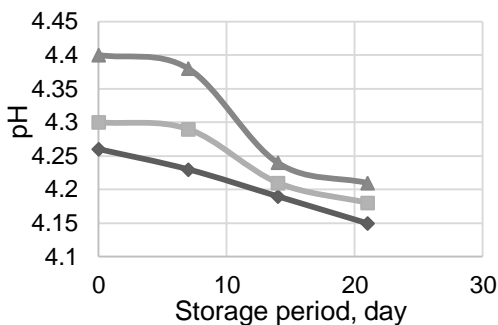
(Malkin & Isayev, 2006). Obviously, yogurt with the filler showed much greater indices of viscosity at initial shear rates in comparison to the control sample. Moreover, when the smaller the shear rate on the samples than the more substantial the difference in the apparent viscosity of the samples. However, the apparent viscosity of the samples was not significantly different at high shear rates (up to 10 s^{-1}). Thus, all samples were demonstrated a decrease in apparent viscosity at the initial stage of research, that confirms the possibility to flow at low shear rate. This is due to the low strength of molecular linkages, that is typical for traditional yoghurt (Gabriele et al., 2001).
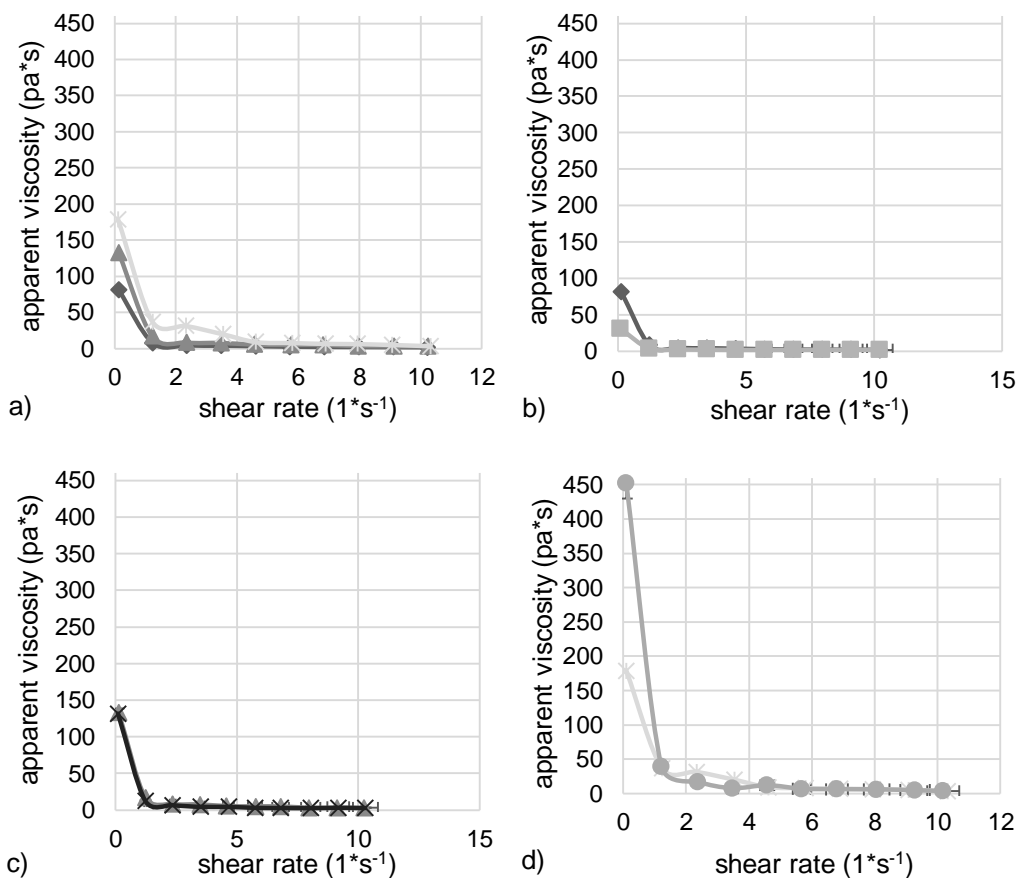
Figure 4. Apparent viscosity yogurt samples versus shear rate (1 s^{-1}): a) — control forward; — 3% forward; — 5% forward; b) — control forward; — control back; c) — 3% forward; — 3% back; d) — 5% forward; — 5% back.

The study of the apparent viscosity of yogurt samples in the reverse direction (at a shear rate of 10 to 0.1 s^{-1}) showed that samples with the filler have the most expressed thixotropic properties. The structure of yogurt sample without filler have been partially restored after holding at rest that is typical for fermented dairy products (Haque et al., 2001). Yogurt sample with 3% filler showed practically 100% restore of the structure. Structure recovering of yogurt with 5% filler had an untypical character for dairy

fermented product at low shear rates: the apparent viscosity of yogurt 'in reverse' had higher values in comparison with the viscosity of yoghurt 'in the forward direction'.

Obviously, this was due to the intensification of the intermolecular linkages at interaction of the yogurt with the filler that appears under mechanical action (in gentle conditions) and prolonged holding at rest (for 5 minutes). Such high thixotropic characteristic of yogurt with a filler is typical for foods, which include natural polymeric substances such as oat bran and chia seeds (Yildis & Kokini, 2001).

The differences between the highest and the lowest viscosity values allows to evaluate of yogurt sample structure. The structuring food materials are usually measured by value of order $n \cdot 10^{5-6}$ (Kokini & Plutchok, 1987; Gallegos et al., 1999). Differences between the highest and the lowest viscosity values of yogurt samples were not large to characterize them as structuring food materials (Table 1).

The yield point indicators are important in terms of practical using of food products (Malkin et al., 2004). The data in Table 2 allows to estimate yield point of yogurt samples. The observed values of yield point of the yogurt samples were different considering different calculation equations.

Obviously, the yield point value correlates with the share of filler in the product and increases with the amount of filler in the yogurt. Based on the average value of the indicator the

Table 1. The difference between the highest and the lowest value of the viscosity of the samples

Values	Yogurt samples		
	without filler	with 3% filler	with 5% filler
η_{max}/ η_{min}, Pa*	43.6	43.6	47.5

Table 2. The yield point of the yogurt samples

Equation	The yield point of yogurt, Pa*		
	without filler	with 3% filler	with 5% filler
Bingham	9.6	19.5	49.1
Caisson	8.6	10.9	31.5
Hershel–Bulkley	8.6	12.6	37.8
The average values	8.9	14.3	39.5

highest rate of yield point was marked for yoghurt with 5% filler and was equal 39.5 Pa which exceeded the indicator for yogurt with 3% filler and yogurt without filler in particularly on 25.2 Pa and 30.6 Pa respectively.

To simulate the slow stirring of the product in a reservoir large volume the rheology of yoghurt samples was studied (at the constant strain of 0.5%) with varying frequency. Fig. 5 reflects the dependence of the moduli: G' (an elastic (storage) modulus), G" (a viscous (loss) modulus) and tan(δ) at frequencies ranging from 1 to 10 rad s^{-1}. All samples exhibited the higher level of modulus G' compared with the modulus G", that is typical for a yogurt (Malkin & Isayev, 2006). The elastic modulus G' of all samples showed the larger dependence on the frequency than the viscous modulus G". There was a direct correlation of the modulus G' and increased amounts of filler in the composition of yogurt.

The highest values of the modulus G' were observed in yogurt with 5% of the filler. The intensity of the modulus G' in this sample was increased with higher frequency that exceeded to the others. This can be explained by the polysaccharide's properties of the yogurt filler. The lowest value of the moduli G' and G" were in the control sample.

The meaning of tan(δ) is the ratio of loss modulus G" to storage modulus G' (George et al., 2014). In this research the ratio of the energy lost to the energy stored in the yoghurt samples by tan(δ) was investigated (Fig. 5, b). The tan(δ) values demonstrated the different values for samples, particularly all samples showed the reduction of the

studied parameter at the beginning (at frequency from 1 to 5 rad s⁻¹), but then it is further nearly unchanged (at frequency from 5 to 10 rad s⁻¹). Yoghurts with filler have a higher tan(δ) value compared to control sample. But there are no significant differences in tan(δ) dependence to the amount of filler in the yogurt, that is confirmed on Fig. 5, a.

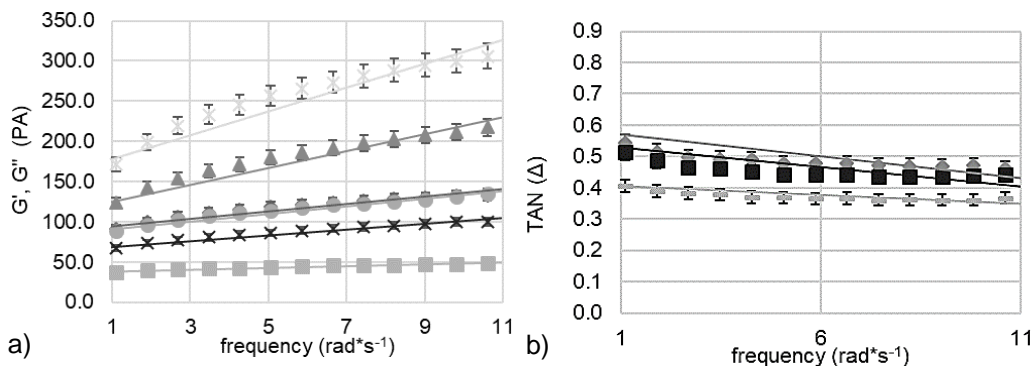Thus, the yogurt with filler had the higher elastic and viscous properties compared with yogurt without filler.

Figure 5. Values of G', G" and tan (δ) versus frequency (rad*s⁻¹): a) ◆ – G' control; ▬ – G" control; ▲ – G' 3%; × – G" 3%; ＊ – G' 5%; ● – G" 5%; b): ▬ – tan(δ) control; ◆ – tan(δ) 3%; ■ –tan(δ) 5%.

Biological value of yogurt samples

Table 3 data shows the difference in the biological value of the protein component of the yogurt samples. Tryptophan was the limiting amino acid in the yogurt without filler, because its amino acid score was equal 95%. The yoghurt samples with filler did not contain any limiting amino acids. The amino acid score of tryptophan in the yogurt with 3% and 5% filler was 104% and 110% respectively.

Table 3. The biological value of the protein component of the yogurt samples

Essential amino acids	Content of amino acid, g / 100 g protein				Amino acid score, %		
	FAO/ WHO*, 2007	yogurt without filler	yogurt with 3% filler	yogurt with 5% filler	yogurt without filler	yogurt with 3% filler	yogurt with 5% filler
Histidine	1.50	2.48	2.49	2.49	165	166	166
Isoleucine	3.00	5.46	5.40	5.40	182	180	180
Leucine	5.90	10.09	10.03	9.97	171	170	169
Lysine	4.50	8.96	8.87	8.76	199	197	195
Methionine+ Cysteine	2.20	3.87	3.92	3.96	176	178	180
Phenylalanine+ Tyrosine	3.80	10.49	10.45	10.45	276	275	275
Threonine	2.30	4.12	4.09	4.09	179	178	178
Tryptophan	0.60	0.57	0.62	0.66	**95**	104	110
Valine	3.90	8.27	8.19	8.15	212	210	209

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The coefficient of differences of amino acid score of the samples (CDAAS) for the yoghurt without filler and yogurt with 3% and 5% filler was equal 89%, 80% and 75% respectively. The biological value of the protein component of the samples (BV) was equal 11%, 20% and 25% respectively for the yogurt without filler, yogurt with 3% and 5% filler. The biological value of the protein component of the samples was increased with filler content increasing. The biological value of yogurt samples with 3% and 5% filler was more over 9% and 14% in compared to yogurt without filler. The biological value of the lipid component of the samples was evaluated (Table 4) according to FAO, 2007. The samples total fat included of 20–35% E[*]; saturated fatty acids (SFA): 10% E; total polyunsaturated fatty acids (PUFA): 6–11% E; n–6 PUFA: 2.5–9% E; n–3 PUFA: 0.5–2% E (*% E–percent of energy).

Table 4. The biological value of the lipid component of the samples

Samples	Fat content, %	Content of fatty acids, g per 100 g lipids				
		SFA	MUFA	PUFA	n–3	n–6
Yogurt without filler	1.55	64.52	27.48	2.84	0.84	2.00
Yogurt with 3% filler	2.07	50.23	23.36	21.21	13.62	7.60
Yogurt with 5% filler	2.42	44.13	21.59	29.07	19.08	9.99

Yogurt without filler contained more SFA, less MUFA, in the minor–PUFA, more than 2/3 of which are omega–6 acids. Fatty acid composition of the filler was represented in the greater degree by PUFA–58.17%, the significantly lesser–MUFA and SFA respectively as 20.66% and 14.86%. Filler introduction in yogurt allowed to optimize the product fatty acid composition by reducing of the SFA content and increasing of the PUFA content.

CONCLUSIONS

The feasibility of using the filler on the basis of oat bran and chia seeds (1:1) in the composition of yogurt formulations is shown. Yogurt with 3–5% of the filler in the recommended proportion (1:1) is most close to yoghurt without filler in the water–holding properties compared to other filler proportions. Study of the rheological properties of the samples showed that the yogurt with the filler has the higher values of effective viscosity and resiliency structure compared to yogurt without filler. Moreover, yoghurt with the filler demonstrates higher values of the elasticity modulus and the elastic modulus compared to yogurt without filler. The developed yoghurt formulation allowed to enhance the biological value of the protein and lipid composition. Adding filler effects, the change of active acidity of the product at the initial stage of its storage. Obviously, the presence of polysaccharide in the filler composition activate yoghurt starter microflora. In a further study the effect of the filler on various types of lactic acid bacteria should be explored. It is also necessary to study the process of collaborative fermentation of the dairy–plant base with the addition of up to 5% filler in the mixture. Perhaps the presence of polysaccharides in the filler will have a positive impact on the enrichment of fermented milk product with bifidoflora.

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The possibility of using microwaves to obtain extracts from berry press residues and jelly products with bioactive characteristics

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Abstract. The paper explores the possibility of development a microwave technology for obtaining water extracts from berry press residues (wild bilberries and cranberries as the objects) and jelly products based on them, which will allow using the waste of freshly squeezed juices in business, such as restaurants and catering services. The antioxidant activity (DPPH and FRAP methods), content of phenolic compounds, flavonoids, anthocyanins, ascorbic acid were determined in berries and in berry press residues. The antioxidant activity of bilberry press residues was due to anthocyanins, and the activity of cranberry press residues was due to flavonoids. Using the microwave oven (magnetron power 800 W, frequency 2,450 MHz), water extracts were obtained in the ratio: for cranberries 1.5:10, for bilberries 1:10. The antioxidant activity of extracts depended on the type of berries and was greater in extracts from bilberry press residues. Extracts of bilberries and cranberries and their compositions (sugar-free and sugar-added) with gelatin as a gelling agent were used to produce the jelly products. Combining bilberry and cranberry extracts (70:30) with gelatin makes it possible to obtain jelly products without sugar. Heating of the ready recipe mixture after preliminary swelling of gelatin and without swelling of gelatin was carried out in the microwave oven. The antioxidant activity of jelly products was higher when using bilberry extracts than cranberries. An increase in the antioxidant activity of the extracts led to a slowdown in structure formation, but increased the plasticity of the products.

Key words: bilberries, cranberries, berry press residues, extracts, microwaves, jelly products, antioxidant activity, deformation.

INTRODUCTION

Berries, as sources of biologically active compounds (BAC) that come in an easily digestible form, play a huge role in human nutrition. Compared to other fruits, they contain more phenolic compounds, flavonoids and anthocyanins, which give them a higher antioxidant activity (AOA). Wild berries contain more biologically active compounds compared to their cultivated analogues (Häkkinen et al., 1999; Rupasova et al., 2013; Ruiz-Torralba et al., 2018). Regular consumption of fresh berries and berry juices helps prevent lipid peroxidation, oxidative stress, and reduces the risk of

cardiovascular and oncological diseases (Williams & Hord, 2005; Caillet et al., 2011; Kivimäki et al., 2012; McKay et al., 2015; Cásedas et al., 2018).

The trend of processing fruits and berries in juice production has brought to attention ways to utilize the remaining berry press residue: a valuable product, in which BAC are even more concentrated (Pertuzatti et al., 2012; Aaby et al., 2013; Barakova et al., 2016; Bamba et al., 2018; Klavins et al., 2018). Berry press residues that display high antioxidant and/or antimicrobial properties, can be dried and then used as additives in food products (Nilova et al., 2015; Dubrovskaya et al., 2017; Lorenzo et al., 2018; Nilova & Malyutenkova, 2018; Tian et al., 2018). Seeds that are also contained in press residues, can be used as the source of oils or waxes (Klavins et al., 2016; Klavins et al., 2019).

Extracts from fruits and berry press residues are most often obtained from raw products, since grinding and drying (as in production of powders), leads to the loss of BAC (Klavins et al., 2018; Michalska et al., 2018; Nemzer et al., 2018). Various methods and solvents are used for extraction of BAC. The maximum extraction of biologically active compounds is possible when using acidified methanol, water-ethanol or water-methanol solutions (Vulić et al., 2011; Klavins et al., 2017; Tian et al., 2017; Bamba et al., 2018; Tian et al., 2018) it further intensifies when combined with high pressure processing, irradiation, dense phase carbon dioxide, ultrasonic processing, pulsed electric field, membrane processing technologies, cold plasma, and hydrothermodynamic cavitation (Li et al., 2017; Cvetanović et al., 2018; Khan et al., 2018; Nowacka et al., 2018). Such methods help to decrease processing time and temperature, improve processing efficiency and minimize nutritional losses. However, when using water as the extracting agent, they become less effective (Klavins et al., 2017; Albuquerque et al., 2018).

Water extracts do not require further processing and can be directly used in food production. Microwave extraction is more effective when water environment is used (Albuquerque et al., 2018), even though it can be carried out without a solvent, due to cyclic heating under pressure, which ensures destruction of pathogenic microflora (Michel et al., 2011; Kretova et al., 2018). Microwave extraction allows extracting both free and bound phenolic compounds, which increases their bioavailability in the human body (Dibanda et al., 2020). Temperature and duration of exposure are important factors in terms of BAC preservation. Aaby et al. (2013) have shown that heating the berry press residues diluted in water up to 100 °C for 4 minutes increases the yield of BAC, especially anthocyanins, while heating it to the temperature above 100 °C decreases the yield. At the magnetron power of 600 W, the duration of water extraction can reach 6 minutes, however, the temperature of the extract should not exceed 85 °C (Wei et al., 2018).

Confectionery products, including jelly cups, and other jelly sweets, are very popular in Russia. Traditionally, they were produced on the basis of extracts made from whole fruits or berries, but in recent years, jelly products have been produced from gelatin with the use of artificial food colors, flavors and acids, which reduces their nutritional value. The use of extracts from fruit or berry press residues makes it possible both to enrich jelly products with natural antioxidants (Rasidek et al., 2016), and to stop using artificial food additives.

The purpose of the work is to study the composition of BAC and the antioxidant activity of berry press residues remaining after squeezing juice from them for use in microwave technology as raw materials for water extracts and jelly products based on them with bioactive characteristics.

MATERIALS AND METHODS

Samples of berries and berry press residues

For the purposes of this research, we used Vaccínium genus berries – bilberries and cranberries – collected in the Leningrad Region, Russia, and frozen to minus 18 °C. Before the studies, berries were thawed to the room temperature, and then the juice was squeezed out of them by the method of pressing. Studies were carried out in the whole berries and in the berry press residues.

Extraction with the use of microwave energy

Water extracts were obtained from raw pressed berries in a microwave oven 'Bork', Bork Elektronik GmbH, at the power of 800 W, and the frequency of 2450 MHz, with the range of exposure modes from 144 to 800 W. The microwave power was checked in accordance with IEC 60705:2006 'Household microwave ovens - Methods for measuring performance'. For the extraction used water with a temperature of 20 ± 1 °C. Water extract was obtained in the ratio (hereinafter water ratio): 1:10 and 1.5:10 (berry press residue: water). At the same time, the extract was not allowed to boil, and the process was controlled by the temperature by Infrared Thermometer 'Ouest GM270', China, and the extract volume.

Technology of jelly production

For production of jelly products, we used water extracts of bilberries, cranberries and their mixtures both without sugar and with addition of sugar 2.5 wt%, as well as a building agent (gelatin) 3 wt% (Grade P-11, produced by LLC Russian Grocery Company, Russia). As a control, a water-based jelly product was used and gelatin was added at the same concentration.

Production of jelly products was carried out in two methods. When the first method was applied, all components were mixed together and left to sit for 40 minutes, then mixed again, heated at 800 W in a microwave oven for 1 minute, cooled to the room temperature, and placed in a refrigerator 4 ± 2 °C until solidified. When the second method was used, gelatin was not allowed any time to swell. As the control sample, we used jelly products, produced from similar components according to the traditional technology, which involves giving the gelatin mixture time to swell, boiling and molding the finished product while cooling (Golunova, 2003).

Research Methods

Extractive solids of water extracts of berry press residues were determined by IRF-454 refractometer manufactured by Biomer LLC, Russia.

The determination of ascorbic acid (AA) was made by titrimetric method with a solution of 2,6-dichlorophenolindophenolate sodium. Extraction of ascorbic acid from the raw material was carried out with 2% hydrochloric acid.

Total phenols assay by Folin-Ciocalteau reagent. Ethanol extracts of berries and berry press residues with Folin-Ciocalteau reagent incubated at room temperature in the dark for 30 min. The optical density was measured on a SHIMADZU 1240 spectrophotometer ('SHIMADZU', Japan) at a wavelength of 735 nm. The results are expressed in mg of gallic acid (Rogozhin & Rogozhina, 2015).

The total content of flavonoids was determined spectrophotometrically by reaction with aluminum chloride. The extraction of flavonoids was made using 60% ethyl alcohol. The optical density was measured after 30 minutes, using the SHIMADZU 1240 spectrophotometer ('SHIMADZU', Japan) at a wavelength of 420 nm. The obtained results were denoted in mg of rutin (Rogozhin & Rogozhina, 2015).

The total content of anthocyanins in terms of cyanidin was determined by pH-differential spectrophotometry at pH 1.0 and 4.5 of the samples, at wavelengths of 510 and 700 nm, using SHIMADZU 1240 spectrophotometer ('SHIMADZU', Japan). To prepare the anthocyanin extract, 3 g of berries or raw berry press residues were mixed with 16 mL of reagent (0.1 N hydrochloric acid and 80% ethanol solution (15 : 85 by volume), homogenized and centrifuged for 10 minutes at the speed of 3,000 rpm. (Nowacka et al., 2018).

Methods for assessing the antioxidant activity

Determination of AOA was carried out by two methods: by their reaction to the DPPH-radical, by FRAP method.

A determination of AOA was carried out by use of the Glavind method (Rogozhin & Rogozhina, 2015). Berries, berry press residues and jelly products were ground and extracted with 50% ethanol solution. A total of 0.2 mL of the extract was added to 2 mL of DPPH solution. The resulting solution was incubated in the dark for five minutes, after which the absorbance levels of the resulting solution were measured at a wavelength of 517 nm using a SHIMADZU 1240 spectrophotometer ('SHIMADZU', Japan). The AOA was determined according to the calibration curve and expressed in terms of ascorbic acid (AC).

Determination of the antioxidant activity (chelating ability) using the FRAP method (Rogozhin & Rogozhina, 2015). This method is based on the ability of ferric chloride (III) to oxidize antioxidants. During the process, ferric chloride (III) is reduced to ferric chloride (II), the amount of which is determined by the color intensity when o-phenanthroline is added to it. Berries, raw press residues and jelly products were crushed and extracted with the use of the 50% ethanol. 0.2 mL of the 25 mM solution of o-phenanthroline, 2.4 mL of the 96% ethanol and 0.2 mL of the 12.3 mM FeCl3 solution (added drop by drop) were added to the extract. After stirring, the mixture was kept in a dark place for 10 minutes. The reaction was stopped by adding 1 mL of the 0.4 M HCl solution. The control sample consisted of the original products to which 0.2 mL of the 25 mM o-phenanthroline solution, 2.6 mL of the 96% ethanol, 0.2 mL of the 12.3 mM FeCl3 solution, and 1 mL of the 0.4 M HCl solution were added. The light absorption of the extract was measured against the solution of the 96% ethanol with the use of SHIMADZU 1240 spectrophotometer at the wavelength of 505 nm. The specific amount of extract light absorption was subtracted from the amount of light absorption of the control sample. The antioxidant activity was determined according to the calibration curve and expressed in terms of AC.

The strength of the 'Bloom strength' jelly products was determined with the use of 'ST-2 Structometer', manufactured by Quality Laboratory LLC, Russia. This method is based on measuring penetration force by the Bloom indenter, when it penetrates the prepared jelly sample to the depth of 4 mM (at the penetration speed of 1.0 mM s^{-1}, and the touch force of 7 g).

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 2010. All the results were expressed as means ± standard deviation and the statistical significance was assessed by Student's t test. To establish statistically significant differences between the values of the experimental samples compared to the control in the group, analysis of variance was used (ANOVA). Significant differences were considered when p-value < 0.05.

RESULTS AND DISCUSSION

Bilberries and cranberries collected in the Leningrad Region had a typical biochemical composition comparable with the already published data (Caillet et al., 2011; Aaby et al., 2013). Test samples of whole berries contained total phenolic compounds, total flavonoids, total anthocyanins (which prevailed in bilberries) and vitamin C (which prevailed in cranberries) (Table 1).

Table 1. Composition of biologically active substances, mg 100 g^{-1}, in berries and in berry press residues, ± standard deviation (Student's t-test)

Indicators	Bilberry		Cranberry	
	whole berry	berry press residues	whole berry	berry press residues
Total phenolic compounds	588.9 ± 22.6	682.4 ± 20.9	452.5 ± 18.0	551.6 ± 21.0
Total flavonoids	465.0 ± 18.4	510.2 ± 20.5	358.5 ± 18.5[a]	469.1 ± 20.4
Total anthocyanins	313.0 ± 8.8	514.8 ± 8.5	175.9 ± 9.0[a]	201.7 ± 9.2
Vitamin C	18.34 ± 0.62	6.88 ± 0.53[a,b]	21.20 ± 0.56	7.50 ± 0.39[a,b]

The differences are not statistically significant: [a] – between replicates of experiments; [b] – between raw berry press residues; ($p < 0.05$).

After squeezing the juice, most of these BAC remain in the raw berry press residues. The exception was vitamin C – its amount could decrease either due to the mechanical destruction of cells during squeezing of the juice, or its contact with atmospheric oxygen. It is known that grinding, as well as thermal processing reduces the content of vitamin C during extraction by the rate of 7%–54%. However, mechanical destruction also stimulates the yield of flavonoids and anthocyanins, which generally raises the AOA of the product (Nowacka et al., 2018). Despite the differences in Vitamin C content in whole bilberries and cranberries, its content in raw berry press residues did not show any statistically significant differences. High content of antioxidants of the phenolic type in berries and press residues caused their high antioxidant properties. Both the antiradical activity (DPPH test) and the chelating ability (FPAP test) showed statistically significantly different results in berries and raw press residues (Fig. 1) with significant predominance in bilberries. Only FPAP values did not differ significantly in bilberries and cranberries.

Dependence of bilberries and cranberries AOA on the content of total phenolic compounds, total flavonoids and total anthocyanins is confirmed by the high relation (R^2) between these indicators (Table 2).
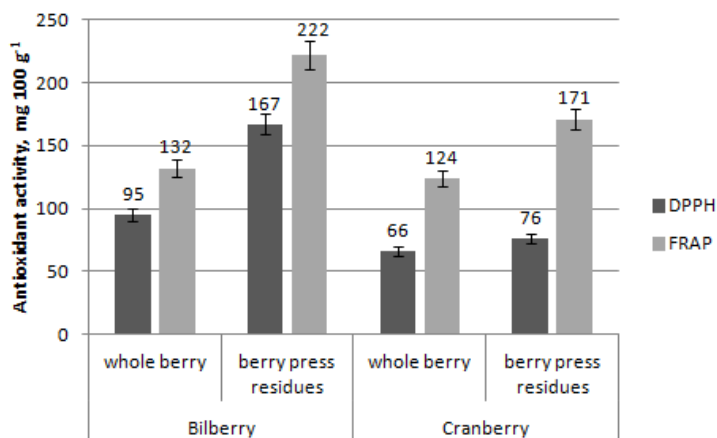
Figure 1. Antioxidant activity (DPPH and FPAP) in bilberries and cranberries, and their respective raw press residues, (mg AC 100 g⁻¹).

Bilberries display AOA due to the high content of anthocyanins (Aaby et al., 2013, Wang et al., 2014, Colak et al., 2016, Tian et al., 2017). Compared to whole bilberries the content of anthocyanins in raw press residues increases from 53% to 75%, i.e. by 22%. Therefore, relation (R^2) between anthocyanins, and DPPH and FPAP tests of bilberries, was 0.998 and 0.991, respectively.

A similar dependence of anthocyanins transition; and their effect on AOA were proved by other authors (Wang et al., 2014, Colak et al., 2016), who experimented both with whole berries from different regions and with different fractions of berries obtained by extraction. Flavonoids displayed lower relation (R^2), since other compounds could also participate in the formation of AOA of bilberries: for example, hydroxycinnamic and hydroxybenzoic acids, and their content could exceed flavonoids (Häkkinen et al., 1999; Aaby et al., 2013).

Table 2. Relation (R^2) between the content of total phenolic compounds, total flavonoids, total anthocyanins and antioxidant activity (DPPH and FPAP)

	AOA	Total phenolic compounds	Total flavonoids	Total anthocyanins	p-value
Bilberry	DPPH	0.939	0.769	0.998	< 0.05
	FRAP	0.953	0.803	0.991	< 0.05
Cranberry	DPPH	0.947	0.973	0.874	< 0.05
	FRAP	0.956	0.979	0.888	< 0.05

In cranberries, anthocyanins displayed a less significant role in the formation of AOA. Relation (R^2) were lower than those of phenolic compounds and flavonoids, and amounted to 0.874 for DPPH and 0.888 for FPAP. Feng et al. (2016) did not find a close correlation between the AOA and the content of anthocyanin in red-colored berries (gooseberries (*Ribes procumbens*), strawberries (*Rubus idaeus*), elderberries (*Sambucus williamsii*) and red currants (*Ribes rubrum*)). Flavonoids (R^2 – 0.973 and 0.979) displayed a greater effect, due to their predominance in the composition of cranberry phenolic compounds from 79% in whole berries, to 85% in raw press residues. The

predominance of flavonoids in phenolic compounds has been shown by several authors (Häkkinen et al., 1999; Caillet et al., 2011), but their content may vary depending on the forms of berry cultivation. Optimization of extraction processes from raw press residues can increase the yield of anthocyanins not only in cranberries, but also in other Vaccinium genus berries (Klavins et al., 2018). Regardless of the predominance of phenolic antioxidants in certain berries or raw press residues, on the whole they display a significant effect on their AOA. Therefore, in further studies, DPPH and FPAP tests were used as indicators for evaluating the effectiveness of the extraction conditions.

Water extraction of bilberries and cranberries press residues was carried out in a microwave oven at different capacities. The extracts were not allowed to boil, and were controlled by their temperature and the change of volume. An increase in the duration and power of microwave led to increase in the temperature of the extracts, which did not reach 100 °C. The maximum heating temperature of the extracts reached 95 °C with a microwave power of 800 W for the duration of 180 s (Fig. 2). But under these conditions, we observed a decrease in the extracts' volume by 20%, due to evaporation of the liquid.
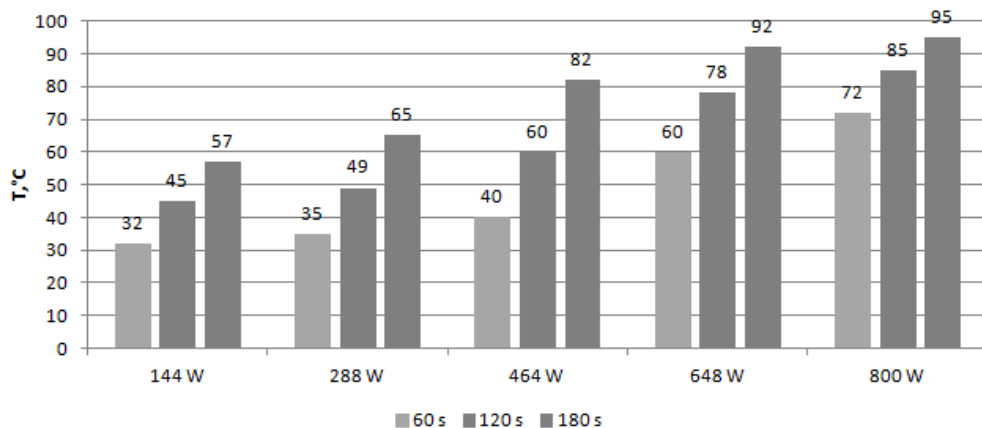
Figure 2. The effect of the microwave exposure conditions (power – W, and duration – s) on the temperature of the extracts.

Reducing the duration of the microwave exposure to 120 s reduced the extract heating by 10 °C, but did not ensure lack of evaporation: as a result the volume of the extract was 6% less, than the initial one. Only when the microwave exposure duration was shortened to 60 s, the extract volume did not change, and its temperature rose to 72 °C. The evaporation of the extract began when it reached the temperature above 76 °C, which happened at the microwave power of 648 W after the duration of 120 s, or at the microwave power of 464 W, after the duration of 180 s.

For further studies, we used the microwave power of 800 W, determining its effect on the yield of the extractive solids and the AOA of the extracts with a water ratio of 1:10, depending on the duration of exposure. Extracts that reduced their volume due to the liquid evaporation, were brought back to their original volume by diluting them with distilled water.

An increase in the duration of the microwave exposure led to increase in the concentration of solids in the extracts. Their sensory properties improved, but their AOA

values decreased. Concentration of the solids during extraction did not show any dependence on the type of berry press residues used, and increased when the duration of the microwave exposure was lengthened (Fig. 3). Thus the microwave exposure for the duration of 180 seconds in comparison to 60 seconds increased concentration of the extractive solids by 12.5%.

Figure 3. The effect of the duration of the microwave on the concentration of extractive solids and the antioxidant activity (DPPH test and FRAP test) of extracts from berry press residues.
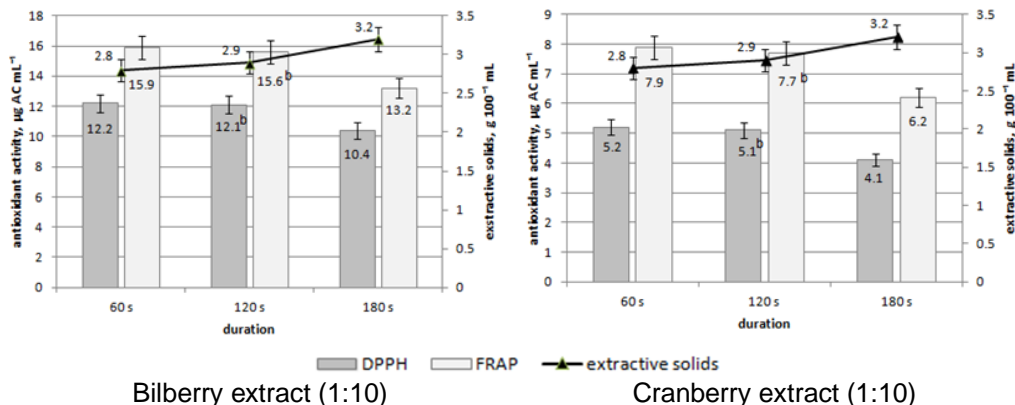
The increase in the duration of the extraction process led to a more pronounced taste, which may have happened due to the transition of acids and sugars into the extract. The bilberry press residues extract had a pronounced bilberry taste after 60 s, while the cranberry press residues extract showed a neutral taste without any pronounced characteristics. The sweet and sour taste, characteristic of cranberries, appeared only after the microwave exposure duration of 180 s.

The AOA of the extracts depended on the type of the used press residues and was higher in the bilberry extracts. In bilberry extracts, obtained by the microwave exposure for the duration of 60 s, DPPH and FRAP values were higher by 2.3 and 2.0 times, respectively, than those of cranberry extracts. The increase in the duration of the microwave exposure led to a decrease in the DPPH and FRAP values in the extracts. When bilberry extracts were exposed for 180 s, their DPPH and FRAP values decreased by 15% and 17%, respectively, compared with the microwave exposure for the duration of 60 s.; similar values decreased in cranberry extracts by 11% and 12% respectively. The increase of the microwave exposure duration from 60 s to 120 s stimulated increase of the concentration of solids in the extract and reduced its AOA, but the results did not show any statistically significant differences. Thus, to obtain extracts from berry press residues, one can use the microwave exposure of 800 W for the duration of 60 s.

To increase the antioxidant and sensory properties of the cranberry press residue extracts, it was decided to change the water ratio to 1.5:10, which led to an increase in the AOA of their DPPH and FRAP tests by 1.5 and 1.3 times respectively (Fig. 4). The taste of the extract became more pronounced, displaying the characteristic acidity.

Extracts from cranberry press residues (water ratio 1.5:10) brought their antioxidant activity values closer to the extracts from bilberry press residues (water ratio 1:10), although they were less by 1.5 and 1.6 times.
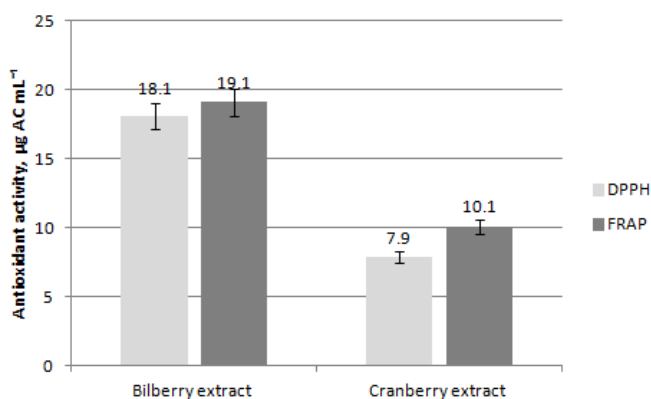
Figure 4. Antioxidant activity, µg AC mL^{-1}, of berry extracts (water ratio 1.5:10).

Staroszczyk et al. (2020) have shown that differences in the AOA of extracts from blueberry and rowanberry press residues differ by 1.5 to 2 times depending on the determination method. The change of the water ratio in bilberry press residues extracts led to an increase in their DPPH and FRAPS values by 1.5 and 1.2 times respectively. The taste of the extracts became more pronounced, but there was no change in the color, which remained dark purple. When using mixed extracts from bilberry and cranberry press residues, it is better to use extracts that have similar antioxidant properties.

Water extracts from bilberry press residues (water ratio 1:10) and cranberries (water ratio 1.5:10) were used alone or in the mixed form to produce jelly products. When mixing the extracts, the optimal sweet and sour taste was achieved in order to exclude sugar from the recipe. It was found out, that extracts from bilberry and cranberry press residues, taken at a ratio of 70:30, display the optimal sensory properties.

For the production of jelly products, the prepared recipe based on extracts from berry press residues after gelatin swelling and without preliminary swelling of gelatin was heated in a microwave oven, after which it was poured into molds. The structure of the product was formed after the mixture was cooled to room temperature and held in a refrigerator with a temperature of 4 ± 2 °C. Product composition and technology influenced the duration of product structure formation. Using traditional technology (control), the formation of the product structure took place after 50–60 minutes. exposure in the refrigerator. Microwave technology with preliminary swelling of gelatin increased the duration of clot formation by 25–30%, with a maximum duration of 75–80 min was in jelly products based on extract from bilberries. The absence of gelatin swelling before microwave treatment slowed down the formation of the product structure by more than 2 times, which amounted to 140–160 min.

Jelly products were distinguished by their consistency from dense for samples using traditional technology to plasticity for products obtained by microwave technology. The strength of jelly products depended not only on the technology, but also on the type of extract and the presence of sugar in the formulation (Table 3). Jelly products obtained by traditional technology were denser in structure. Bloom strength values decreased by 12–23% when using extracts from berry press residues. The formation of the gelatin-based product structure and berry press residues extracts is associated with protein-polyphenol interaction and crosslinking (Wu et al., 2013, Gómez-Mascaraco et al., 2019).

Table 3. Bloom strength of jelly products, g, $\pm$ standard deviation (Student's t-test)

Jelly product	Technology					
	traditional		microwave with gelatin swelling		microwave without gelatin swelling	
	without sugar	with sugar	without sugar	with sugar	without sugar	with sugar
Control	106 ± 2	101 ± 2	100 ± 3	93 ± 2	71 ± 3^b	67 ± 3^b
Bilberries extracts	82 ± 2	76 ± 2^c	63 ± 3	58 ± 2	$47 \pm 3^{a,c}$	$42 \pm 3^{a,c}$
Cranberries extracts	93 ± 2	88 ± 2	83 ± 2	78 ± 2	60 ± 3^b	$55 \pm 4^{a,b,c}$
Bilberries & cranberries extracts	86 ± 3	78 ± 2^c	71 ± 2	65 ± 2	$52 \pm 4^{a,b,c}$	$48 \pm 3^{a,b,c}$

The differences are not statistically significant: [a] – between replicates of experiments; [b] – between jelly products without sugar and with sugar; [c] – between jelly products depending on the extracts from berry press residues used ($p < 0.05$).

The physical properties of such a product depend on the amount and composition of the polyphenols. Choi et al. (2018) claim that high concentrations of phenolic compounds due to their molecular mobility and branched structure, leads to a plasticizing effect. However, at high concentrations of phenolic compounds, molecular mobility increased due to grafting/branching reactions resulting in plasticizing effect.

The predominance of anthocyanins in the extract from bilberry press residues led to the formation of a more plasticity product with the lowest Bloom strength values (Fig. 5).
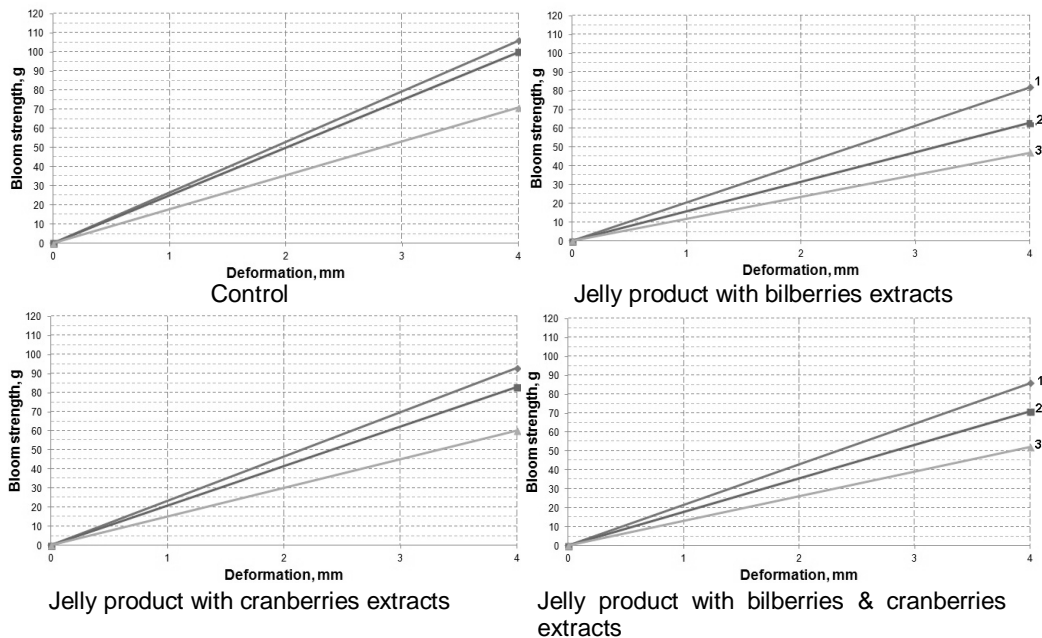

Figure 5. Load force change schedules depending on the depth of implementation of Blume indenter for sugar-free jelly products depending on the technology used (by 'ST-2 Structometer'): 1 – traditional; 2 – microwave with gelatin swelling; 3 – microwave without gelatin swelling.

In jelly products from the composition of extracts and in extracts from cranberry press residues Bloom strength values increased. Many authors point to the influence of anthocyanins in forming the structure of the product with gelatin. Extracts from berry extracts or red cabbage containing anthocyanins contribute to the formation of elastic gelatin films with increased extensibility, which increases with the content of anthocyanins (Nilsuwan et al., 2018; Uranga et al., 2018; Kan et al., 2019; Staroszczyk et al., 2020). For example, films with blueberry extract are 13% more extensible than those with rowanberry extract (Staroszczyk et al., 2020). Yong et al. (2019) have shown on the example of films based on chitosan with sweet potato extract that the formation of structure occurs through the interaction of amino groups of proteins and hydroxyl groups of anthocyanins. The absence of anthocyanins in green tea extract or extract with epigallocatechin gallate form strong gelatine films with reduced stretch (Wu et al., 2013).

The same trend continued with microwave technology, although the Bloom strength values decreased by 6–15%, especially with extracts from berry press residues extracts. The absence of gelatin swelling operation in microwave technology further reduced the Bloom strength values by 25–28%, but there were no statistically significant differences. Regardless of the production technology of jelly products, the introduction of sugar in their composition allowed to produce more stretchy products. Bloom strength values were reduced with the use of the technology: microwave by 6–9%; microwave with gelatin swelling by 6–11%, for traditional by 5–8%.

The use of sugar has not changed the trend of antocyanins influence on the plasticity properties and Bloom strength of jelly products with extracts from berry press residues was aligned the following way: bilberries< bilberries & cranberries< cranberries.

AOA of berry extracts formed them in jelly products with slight losses, which depended on the technology use (Table 4).

Table 4. Antioxidant activity, μg AC g^{-1}, sugar-free jelly products, tandard deviation (Student's t-test)

Jelly product	Technology					
	traditional		microwave with gelatin swelling		microwave without gelatin swelling	
	DPPH	FRAP	DPPH	FRAP	DPPH	FRAP
Control	1.2 ± 0.4 [a,b]	1.4 ± 0.4 [a,b]	1.3 ± 0.3 [a,b]	1.5 ± 0.4 [a,b]	1.2 ± 0.5 [a,b]	1.4 ± 0.5 [a,b]
Bilberries extracts	9.2 ± 0.4 [c]	12.8 ± 0.6	10.8 ± 0.3 [b]	14.6 ± 0.8	10.9 ± 0.4 [b]	14.8 ± 0.6 [b, c]
Cranberries extracts	6.2 ± 0.3	8.5 ± 0.7 [b]	7.0 ± 0.3 [b]	9.8 ± 0.7 [b]	7.3 ± 0.4 [a,b]	10.1 ± 0.7 [b]
Bilberries & cranberries extracts	8.6 ± 0.3 [c]	11.0 ± 0.6	9.8 ± 0.4 [b]	13.0 ± 0.5 [b]	9.5 ± 0.4 [b]	13.8 ± 0.6 [b, c]

The differences are not statistically significant: [a] – between replicates of experiments; [b] – between jelly products by different technologies; [c] – between jelly products depending on the extracts from berry press residues used ($p < 0.05$).

The highest DPPH and FRAP values were jelly products obtained by microwave technology. Compared to traditional technology, these values were 13–17% and 14–18% higher, respectively, for DPPH and FRAP, due to lower temperature effects in microwave technology. The increase in temperature above 100°C promotes the loss of

phenolic compounds, and especially anthocyanins (Michalska et al., 2018, Nemzer et al., 2018).

Preliminary swelling of gelatin or its absence in the formulation did not significantly affect the AOA of jelly products. DPPH and FRAP values had no statistically significant differences. AOA of jelly products were formed not only by extracts from berry press residues, but also by gelatin, as evidenced by the DPPH and FRAP values of jelly products without the use of extracts (control). AOA of jelly products, depending on the used extracts from berry press residues extracts, was aligned the following way: bilberry > bilberry and cranberry > cranberry.

CONCLUSIONS

Bilberries and cranberries and berry press residues from them after squeezing juice contain a complex of phenolic antioxidants, which determine their AOA. In bilberry press residues, the AOA is associated with a predominance of anthocyanins, which confirmed the close relation (R^2) with DPPH and FPAP tests, which were 0.998 and 0.991 respectively. AOA of cranberry press residues is associated with flavonoid predominance: R^2 for DPPH and FPAP is 0.973 and 0.979 respectively.

Microwave technology can be used to obtain water extracts from berry press residues. By increasing the microwave power processing time is reduced and the amount of antioxidants in the extract is increased. If it is necessary to use extracts in food technology in order to form the necessary sensory properties, it is necessary to regulate the ratio of berry press residues and water. The ratio of the berry press residues and water to obtain extracts was experimentally found: 1:10 for the extract from bilberry press residues, and from cranberry press residues – 1.5:10. Mixing the extracts with a pronounced sweetish (bilberry) and sour (cranberry) taste can allow their use without sugar in the production of jelly products.

Water extracts from berry press residues were used to produce gelatin-based jelly products. More plasticity jelly products based on extracts are obtained by microwave exposure of the prepared recipe mixture with preliminary swelling of gelatin or with the absence of this operation. The clot formation time is up to 80 min. at a temperature of $4 \pm 2\,°C$. Absence of pre-swelling gelatin increases time to 140–160 minutes. Manufactured jelly products have greater AOA than products by traditional technology. Microwave technology will allow using of press residues after obtaining freshly squeezed juices for production jelly products without sugar and with AOA in such food business, as restaurants and catering services.

Further research should be aimed at transforming microwave technology developed for catering services to industry, for which it is necessary to determine the conditions of microwave exposure (power and duration) depending on the technical characteristics of industrial microwave ovens and the volume of products manufactured per load.

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Antioxidant content of dark colored berries

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Abstract. Blackberries (*Rubus caesius*), elderberries (*Sambucus nigra L)*, highbush blueberries (*Vaccinium corymbosum* L) and black currants (*Ribes nigrum*) were selected for research on their content of phenolic compounds, including anthocyanins and comparing their content in these berries. Samples were also assayed for ascorbic acid and soluble solid content. The unifying mark of these fruits is purple-black color, which indicates high content of anthocyanins. Analyses of these fruits showed that they contain high biological activity components that justify the uses of these fruits.

The research was done at the Department of Chemistry, Latvia University of Life Sciences and Technologies, year 2019. Berry samples were bought at the supermarket (highbush blueberries) or collected from garden in Jelgava, Latvia (blackberries, elderberries and black currants). All berries were at full maturity. The content of ascorbic acid, total phenols, anthocyanins, flavonoids as well as soluble solids was determined.

Descending order of ascorbic acid content in berries is: black currants > blackberries > elderberries > highbush blueberries. Elderberries had the highest content of anthocyanins, total phenols and flavonoids on average 161.5, 537.9 and 112.6 mg 100 g^{-1} FW, respectively. The content of soluble solids changes from 5.83 Brix (elderberries) to 13.67 Brix (black currants).

Key words: berries, bioactive compounds, phenolic compounds.

INTRODUCTION

Free radicals and other reactive species are capable to cause oxidation and biomolecular damages when oxidative processes exceed the antioxidative protection of the organism. Fruit, especially small berries, contain a wide variety of antioxidant compounds such as phenolics, mostly flavonoids and anthocyanins, that may help protect cellular systems from oxidative damage. Thanks to these compounds berries possess antioxidant, anticancer, antiinflammatory, and antineurodegenerative biological properties (Prior et al., 1998; Liu, 2003; Milivojevic et al., 2011).

Blackberries (*Rubus caesius*), elderberries (*Sambucus nigra L)*, highbush blueberries (*Vaccinium corymbosum* L) and black currants (*Ribes nigrum*) were selected for research on their content of phenolic compounds, including anthocyanins and comparing their content in these berries. The unifying mark of these fruits is purple-black color, which indicates high content of anthocyanins.

Anthocyanins classification is based on the number and position of hydroxyl and carboxyl groups in the flavinium nucleus. The most common anthocyanins found in

plants, including berries, are pelargonidin, cyanidin, delphinidin, penonidin, petunidin and malvidin. Recent studies demonstrate that anthocyanin extracts display a wide range of biological activities, including antioxidant, antimicrobial, anti-inflammatory and anticarcinogenic activities; maintaining eye health and vision and neuroprotective effects (Ramos, 2008; Seeram, 2008).

Blackberries are popular not only in Europe, but also in the southern part of United States. Several reports have demonstrated that the most antioxidant capacity was due to these phenolics and ascorbic acid in blackberries (Pantelidis et al., 2007; Wang & Lin, 2000). Blackberry fruits are abundant in flavonoids, colorants, and organic acids. The blackberry residues exhibited a high amount of malic acid (5,706.37 mg 100 g^{-1} dry bases -db), phenols (4,016.43 mg GAE 100 g^{-1} db), and content of anthocyanins (364.53 mg 100 g^{-1} db) (Zafra-Rojas et al., 2018). These berries contain also phenolic compounds as anthocyanins, flavonols, chlorogenic acid, and procyanidins, which can have beneficial effects on human health (Moure et al., 2001, Siriwoham & Wrolstad, 2004).

Mertz and co-authors reported that ellagitannins and cyanidin-3-glucoside are major phenolic compounds in blackberries. The anthocyanins (cyanidin-3-rutinoside and cyanidin-3-malonyl glucoside), flavonols (quercetin and kaempferol glycosides) and flavan-3-ol (epicatechin) were also identified in blackberries. Hydroxycinnamic acids are minor compounds, and they are found as ferulic, caffeic and *p*-coumaric acid esters (Mertz et al., 2007).

The purple-black fruits of elderberries (*Sambucus* spp. L.) are one of the richest sources of anthocyanins and phenolic compounds among berries and have strong antioxidant capacity (Duymus et al., 2014). Elderberries are widely used in Europe for the productions of healthy food and beverages as well as medicinal products. These fruits have been used for generations in traditional herbal medicine as a remedy for colds, sinusitis, and herpes. Biological value of the fruit is in high level of vitamins, minerals, pectins, colour, cellulose, dietetic fibres, sugars, organic acids and low energetic value (Cejpek et al., 2009). Although berries are often recommended for fresh use, care should be taken when picking fresh elderberries. As fresh elderberries contain cyanogening glycosides, they are slightly toxic and can cause vomiting. The mild toxicity is destroyed by cooking (Vulic et al., 2008). Analysing infusions of elderberry fruits and flowers have shown, that the infusions prepared from elderflowers contained more abundant phenolic compounds than the elderberry infusions. The TPC (total phenolic) of these infusions ranged from 19.81 to 23.90 mg of gallic acid equivalents/g dry weight of sample (GAE/g DW) for elderberries and from 15.23 to 35.57 mg GAE/g DW for elderflowers, whereas the TFC (flavonoid) ranged from 2.60 to 4.49 mg of rutin equivalents/g dry weight of sample (RUTE/g DW) in elderberry infusions and from 5.27 to 13.19 mg RUTE/g DW in elderflower infusions (Viapiana & Wesolowski, 2017).

Blueberry fruits are rich in phenolic acids, flavonols and anthocyanins (Wang & Lin, 2000; Moyer et al., 2002). The content of phenolic compounds in berry fruits is affected by genetic differences among species and within the same species and maturity at harvest.

Ribera et al. (2010) reported that in mature blueberry fruits the content of antioxidant, anthocyanins and total phenolic increases. By contrast, Rodarte et al. (2008) found that phenolic compounds, flavonols and hydroxycinnamic acids concentration and antioxidant activity in highbush blueberry fruits decreased during ripening.

Many of the antioxidant characteristics associated with berries can be attributed to the anthocyanin content. Four anthocyanins: delphinidin-3-rutinoside, cyanidin-3-rutinoside, delphinidin-3-glucoside and cyanidin-3-glucoside make up 98% of blackcurrant anthocyanins, the remaining 2% comprise 11 other anthocyanins including petunidin and malvinidin glycosides. Other polyphenols in blackcurrant are quercetin, myricetin, kaempferol and isorhamnetin (Karjalainen et al., 2009).

Black currants compared to other berries, for example, strawberries and raspberries contain high levels of polyphenols (500–1,342 mg 100 g^{-1} of fresh weight) especially anthocyanins, phenolic acid derivatives (both hydroxybenzoic and hydroxycinnamic acids), flavonols (glycosides of myricetin, quercetin, kaempferol and isorhamnetin) as well as proanthocyanidins. These compounds have health promoting properties. Both myricetin and quercetin as described have neuroprotective activity. Furthermore, quercetin and isorhamnetin reduce blood pressure and improve blood flow evoking a potential protective function against development of vascular type of dementia (Vagiri et al., 2012).

Vitamin C is needed for the formation of blood vessels, bones and connective tissue, promoting absorption of iron and as an antioxidant (Hancock & Viola, 2005).

Black currant are considered to be a rich source of vitamin C. Vitamin C content in black currants in some examples ranging from 130–200 mg 100 mL^{-1} of juice to over 350–450 mg 100 mL^{-1} of juice (Mattila et al., 2011). Black currants are good sources of antioxidants like polyphenols, flavonols and vitamin C which make them a desirable addition to functional foods.

Several studies have been conducted in Latvia and Estonia analysing the chemical composition of berries (Albert et al., 2010; Soots et al., 2017; Karlsons et al., 2018; Klavins et al., 2019).

The aim of research was to analyse content of antioxidants in dark coloured berries – blackberries, elderberries, highbush blueberries and black currants.

MATERIALS AND METHODS

The research was done at the Department of Chemistry, Latvia University of Life Sciences and Technologies, year 2019. Berry samples were bought at the supermarket (highbush blueberries) or collected from garden in Jelgava, Latvia (blackberries, elderberries and black currants). 500 g of each fruit species were taken for analysis. All berries were at full maturity. Approximately 100 g of randomly selected fruits were purified and finely homogenized for 3 min. For determination of chemical parameters, three repetitions for each fruit species were performed.

Chemicals and spectral measurements
All the reagents used were with the analytical grade from Sigma Aldrich, Germany. UV spectrophotometer UV-1800 (Shimadzu Corporation, Japan) was used for the absorbance measurements.

Analytical methods
The contents of soluble solids were determined by refractometer, Refractometer A. KRÜSS Optronic Digital Handheld Refractometer Dr301-95, calibrated at 20 °C with

distilled water and expressed as ºBrix. Analysis were performed in six replicates and expressed as mean values ± standard deviation.

The content of ascorbic acid was analysed according to Moor et al. (2005), using iodometric titration based on an oxidation-reduction reaction between ascorbic acid and iodine. The endpoint was determined using 1% starch suspension till blue-black colour, which does not disappear during 30 seconds.

For extraction of total phenols, flavonoids and anthocyanins 1.0 ± 0.001 g of finely ground fruit samples was weighed into volumetric flasks, 10 mL of extractant (methanol, distilled water and hydrochloric acid 79:20:1 v/v/v or acidified methanol with 0.1% HCl) was added, shaked at 20 °C for 30 min in the dark and then centrifuged for 10 min at 5,000 rpm.

The content of total phenols was determined quantitatively using 2.5 mL of Folin Ciocalteu reagent, 0.5 mL of extract and 2 mL of 7.5% sodium carbonate (Singleton et al., 1999). The absorbance was measured at wave length 765 nm. The results were expressed as mg gallic acid equivalents (GAE) 100 g^{-1} FW of fruits.

For determination of anthocyanins the light absorption of acidified methanol extract was detected with a spectrophotometer at 530 nm.

Determination of flavonoids were performed using aluminum chloride colorimetric method with quercetin as standard (Kim et al., 2003). To 2 mL of H_2O was added 0.50 mL of extract and 0.15 mL of 5% $NaNO_2$. After 5 min 0.15 mL of 10% $AlCl_3$ solution and after 10 min 1 mL of 1M NaOH was added. The absorbance was measured after 15 min at 415 nm using 6705 UV/VIS YENWAY Spectrophotometer.

Statistical analysis

Analyses were conducted in three replicates and each one was measured for three repetitions. Data were expressed as mean of triplicates assay ± standard deviation; for mathematical data processing the value of $p < 0.05$ was regarded as statistically significant. One-way analysis of variance (ANOVA) was used to determine the significance of differences.

RESULTS AND DISCUSSION

Berries are rich in vitamins, organic acids, minerals and antioxidants, high in fibres, therefore berries are among the healthiest products. Daily use of these products improves health and very often, dark coloured berries are of particular interest due to the best dietary sources of different bioactive compounds, such as antioxidants. The bioactive compounds in berries contain mainly phenolic compounds (phenolic acids, flavonoids, such as anthocyanins and flavonols, and tannins) and ascorbic acid (Skrovankova et al., 2015). Statistics shows that only 37% of the Baltic population consumes fresh fruits and berries on a daily basis. Our study of content of bioactive compounds in dark colour berries shows that nutritionists should also pay attention to less commonly grown crops – elderberry and blackberry.

The obtained results (Fig. 1) showed that content of ascorbic acid in black currants ranged from 40.25 to 44.71 mg 100 g^{-1}, while it ranged from 10.14 to 14.95 mg 100 g^{-1} for elderberries and from 16.11 to 18.03 mg 100 g^{-1} or from 7.43 to 9.98 mg 100 g^{-1} for blackberries and highbush blueberries respectively. Our findings are similar to results reported in literature (Schulz & Chim, 2019).
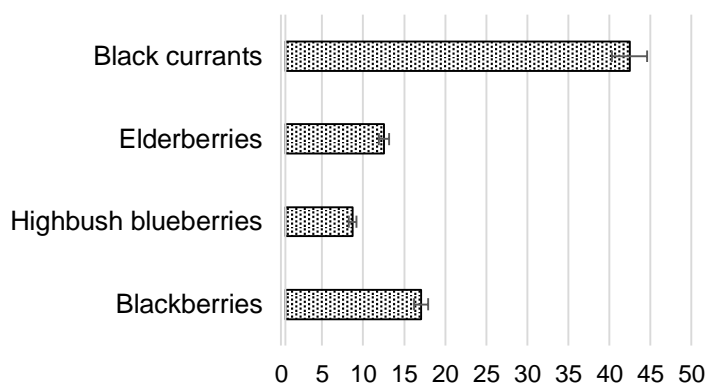
Figure 1. The content of ascorbic acid mg 100 g^{-1} in dark coloured berries.

Therefore, black currants had the highest ascorbic acid content comparing with three other analysed samples. Significant differences ($p < 0.05$) were found in the ascorbic acid content comparing black currants and other analysed samples. We can conclude, that with an average content ranging from 8.71 to 12.54 mg 100 g^{-1} FW, the vitamin C level is not high enough to classify blueberries and elderberries as a rich source of vitamin C.

Content of bioactive compounds – anthocyanins, total phenols and flavonoids – in different berries is shown in Table 1.

Table 1. Content of bioactive compounds in berries

	Anthocyanins*	Total phenols**	Flavonoids***
Blackberries	76.83 ± 2.53	342.48 ± 5.58	58.77 ± 2.72
Highbush blueberries	28.35 ± 0.59	228.63 ± 5.59	44.16 ± 1.58
Elderberries	161.51 ± 0.07	537.96 ± 9.53	112.65 ± 0.44
Black currants	137.88 ± 2.08	513.54 ± 15.97	73.26 ± 2.37

*mg 100 g^{-1} FW; **mg GAE 100g^{-1} FW; ***mg QE 100g^{-1} FW.

Total phenolic content is known to influence antioxidant and other health-related bioactivities (Milivojevic et al., 2011). In this study the elderberries had the highest amount of total phenols ranged from 528.43 to 547.49 mg GAE 100 g^{-1} FW, confirming that elderberries are an important source of health-related compounds. Significantly lower amounts of total phenols were observed in highbush blueberries and blackberries (228.63 and 342.48 mg GAE 100 g^{-1} FW, respectively). Significant differences were not found in the total phenolic when comparing between elderberries and black currants fruits.

Higher phenols content confirm reports of Mikulic-Petkovsekaet al. (2016) and Lee & Finn (2007), but are lower then results mentioned by Pedro Silva et al. (2017), Coklar & Akbulut (2017) and Ferreira et al. (2020).

Data obtained by Acosta-Montoya et al. (2010) corroborate low phenols content in highbush blueberries and blackberries.

Quantitatively elderberries were richest in anthocyanins and flavonoids (161.51 mg 100 g^{-1} and 112.65 mg QE 100g^{-1} FW respectively). The very high anthocyanins content of elderberries, 2-fold greater than blackberries and 6-fold greater

than blueberries, showed it's potential as an important source of bioactive compounds and possible use for humans health. These results are significantly lower than that mentioned in the literature (Lee & Finn, 2007; Silva et al., 2017; Ferreira et al., 2020). Differences between the present results and those reported in the literature may be associated with different environmental and growing conditions, as well as the use of different solvents used for extraction.

From the results showed in Table 1 it can be seen that the highbush blueberries exhibited the lowest concentrations of anthocyanins (average 28.35 mg 100 g^{-1} FW) compared to blackberries, black currants and elderberries.

Berries are unique food due to source of vitamins, enzymes, minerals, carbohydrates, pectins and other valuable substances. Flavonoids are biologically active substances, which have not historically been classified as nutrients, however, have recently been increasingly mentioned in terms of healthy nutrition to maintain health. These compounds could be found in almost all fruits and vegetables, moreover dark coloured berries are a good source of flavonoids in the diet.

Descending order of flavonoids content in analysed berries is: elderberries > blackberries > black currants > highbush blueberries. Overall, we can conclude that elderberry is rich in all known antioxidants and recommended for people's health.

The total soluble solids (TSS) content, measured as °Brix, represents one of the main quality parameters of berries and it also allows monitoring the stage of fruit maturation (Salvador et al., 2015). The TSS of berries was evaluated using a digital hand held digital refractometer and the obtained results are shown in Fig. 2. By studying, significant differences ($p < 0.05$) were found between the samples regarding the content of total soluble solids. The black currants had the highest mean Brix value (average 13.67), whereas blackberries the lowest (4.93).
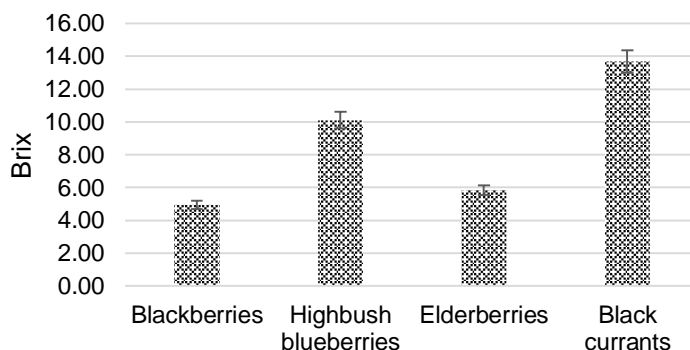
Figure 2. The content of soluble solids in dark coloured berries.

Studies showed that the soluble solids content of the elderberries was lower than those of reported by Salvador et al., 2015 or Ferreira et al., 2020. These differences could be due to different stage of maturity and different climatic conditions were the plants were grown. There are reports (Moggia et al., 2016; Schulza et al., 2019) indicating higher content of TSS of blacberries – about 8 Brix, but our results are similar to results found in Acosta-Montay et al. (2010) research.

CONCLUSIONS

Berries are a good source of vitamins, minerals and antioxidants in our diet. The results of the study confirmed that the dark colour berries are rich in antioxidants because of the high content of phenols, flavonoids and anthocyanins. The analysis of obtained results showed that the elderberries are the most valuable in this respect, since they contained the most of these biologically active compounds in significant amount. If you need to increase the amount of ascorbic acid in your diet, black currants are recommended.

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Radiation use efficiency by tomato transplants grown under extended photoperiod

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Abstract. The study focused on the effect of an extended photoperiod on the radiation use efficiency (*RUE*) by the tomato transplants *(Solanum lycopersicum* L.*)* in the pre–reproductive period. In two consecutive series of experiments, the photoperiod was 16 and 22 hours. The photon irradiance at the plant tops was maintained at low, medium and high levels: 100, 170 and 240 μmol m^{-2} s^{-1}, respectively. The plants were grown under two lighting systems with different light quality. The difference was 7% higher blue flux share in Spectrum II. The use of an extended photoperiod, especially in combination with high irradiance level, resulted in the plant leaf chlorosis. When varying the radiation dose components, the deviation from the reciprocity law was recorded. By the analysis results, the chlorophyll degradation was a response to the extended photoperiod rather than the radiation dose. Without additional blue flux, under a regular photoperiod, *RUE* reduced by 8% at the high irradiance level. Under extended photoperiod, the shift from the low to high irradiance level reduced *RUE* by 20–37%, with bigger reduction values being observed at higher irradiance levels. Seven percent addition of blue flux made it possible to increase *RUE* by 5–8% at the same and lower irradiance levels and under the regular photoperiod. With the extended photoperiod under these conditions, *RUE* decreased by 8–21%. The study results verify a great influence of an extended photoperiod on *RUE*, while the degree of influence depends on other parameters of light environment – light quality and irradiance level.

Key words: photoperiod, irradiance, light quality, doze, biometry, radiation use efficiency, chlorophyll, chlorosis.

INTRODUCTION

The greatest global challenge existing beyond national boundaries is to maintain a balance between the growing production and environmental sustainability. The way to achieve such a balance is the maximal use of energy and material resourcesin the production of goods without compromising the agroecosystems. The photosynthetically active radiation (PAR) is an essential environmental factor when growing plants. The radiation affects them in many ways – from the variation in their productivity to such

reasonably subtle implications as the influence on their developmental stability (Rakutko et al., 2018).

Numerous studies focused on plant cultivation under the artificial irradiation with different photoperiods (Sysoeva & Markovskaya, 2008). A promising trend is the use of an extended photoperiod, up to continuous lighting, in order to maximize the plant productivity and to minimize the costs of indoor plant lighting (Adams & Langton, 2005). In this case, the energy saving is achieved by the extended service life of light sources as there is no transient mode in the on/off switching (Ohyama & Kozai, 1998; Sysoeva et al., 2010).

Various plant species respond to an extended photoperiod differently. Tomato demonstrates the accelerated development in the early ontogenesis stages with the subsequent slowdown (Demers & Gosselin, 2002). For this reason, the low–intensity continuous lighting with alternating air temperature is used for growing tomato transplants (Ohyama et al., 2005).

There is evidence, however, of the negative effects of this method, the photo–damage of leaves, in particular. The possible causes are hyper–accumulation of starch, continuous photo–oxidative pressure, continuous light signalling, a mismatch between the frequency of internal (circadian) biorhythms and the external light / dark cycle (circadian asynchrony), and suppressed light–dependent chlorophyll deficiency (Velez-Ramirez et al., 2011; Shibaeva & Titov, 2017).

The study aimed to explore the effect of an extended photoperiod on the radiation use efficiency (RUE) by tomato transplants in the pre–reproductive period.

MATERIALS AND METHODS

The study object was tomato transplants (*Solanum lycopersicum* L.) of Blagovest F_1 variety. The seeds were sown in a tray filled with the mix of peat and soil substrate in the proportion of 1:2. After the second true leaf had appeared, the seedlings were pricked out into containers of $1,000 \text{ cm}^3$ each and placed in the room with an artificial climate, where they were grown to 39 days after emergence.

The air temperature was automatically maintained at $+21 \pm 1.0 \,°C$ with the humidity of 65–70%. The air velocity in the plant growing zone was $0.2–0.3 \text{ m s}^{-1}$. Two lighting systems were used in the experiment. The light sources were OSRAM L58W/840 LUMILUX Cool White and OSRAM L58W/77 FLUORA fluorescent lamps. The first lighting system (reference) had only fluorescent lamps with the overall spectral ratio blue:green:red = 32 %:34 %:34 % (Spectrum I). The second lighting system had the same number and type of fluorescent lamps as the first lighting system but the LEDs with 440 nm wavelength were added. They redistributed the energy in PAR range towards the shorter wavelengths. The spectral ratio of the second lighting system was blue:green:red = 39 %:31 %:30 % (Spectrum II). Thus, the difference in the light quality was rather a small increase (7%) in the blue band in Spectrum II.

The radiation use efficiency (*RUE*) was evaluated by the amount of dry matter $(g \text{ mol}^{-1})$ synthesised in the plant leaves under the influence of the radiation dose. It was calculated by the formula

$$RUE = SLW/H. \qquad (1)$$

The specific leaf weight (*SLW*), $g \text{ m}^{-2}$, was calculated by the formula

$$SLW = M_L \cdot v_L / S_L, \tag{2}$$

where S_L is the leaf area, m^2; M_L is the wet fresh leaf mass, g; v_L is the leaf dry matter content, rel. units.

The radiation dose H, mol m^{-2}, is the energy generated by the light sources for the entire growing period T, day, calculated as

$$H = DLI \cdot T. \tag{3}$$

The energy generated by light sources per day (DLI, day light integral, mol m^{-2} day^{-1}) was calculated by the formula

$$DLI = 0.0036 \cdot E \cdot PP, \tag{4}$$

where E is the photon irradiance created by the light sources, μmol m^{-2} s^{-1}; PP – photoperiod, h.

Two consecutive series of experiments with different photoperiods – 16 hours (regular) and 22 hours (extended) were conducted. The photon irradiance of tomato transplant tops was maintained at low, medium and high levels: 100, 170 and 240 μmol m^{-2} s^{-1}, respectively. The transplants were watered and fertilized as required.

The chlorophyll content was determined by CCM–200 meter (Opti–Science, USA) in relative units. The leaf dry matter content was determined by drying the leaves to the constant mass at a temperature of +105 °C. The experiments had three replications, with the mean values being calculated per six transplants per replication. The data were processed with *Statistica* 7.0 and *Excel 2003* software packages. Statistical differences were analyzed using one–way analysis of variance (*ANOVA*). The least significant difference (*LSD*) at the 0.95 level ($p \leq 0.05$) was used to compare the mean values by Fisher's test.

RESULTS AND DISCUSSION

The application of extended photoperiod could increase the growth and yields of plants. At the same time, it leads to leaf chlorosis and necrosis. In our experiment with the extended photoperiod, the leaf variegation was observed already on the sixth day at all irradiance levels and both spectra.

Fig. 1 shows the typical tomato transplants grown under different irradiation levels. Fig. 2 shows the tomato leaves under different photoperiods at the end of the experiment. In our experiment, the difference in leaf appearance under different spectra was not recorded.

Figure 1. Tomato transplants grown under different photoperiods (16 h – left and 22 h right) and different irradiation levels (100, 170, and 240 μmol m^{-2} s^{-1}).

The experiment demonstrated that the longer photoperiod resulted in the significant decrease in the chlorophyll content and the development of heavy inter–vein chlorosis in tomato leaves. Table 1 presents the resulting experimental data.

DLI is the initial energy factor affecting the plants. It is not influences by the spectrum type and increases with the increasing irradiance and photoperiod. In the experiment, its values were almost the same, with the differences being 2.2%, in the combination of 240 μmol m^{-2} s^{-1} irradiance and 16–h photoperiod compared to the combination of 170 μmol m^{-2} s^{-1} irradiance and 22–h photoperiod (13.8 and 13.5 mol m^{-2} day^{-1}, respectively, as shown in bold in Table 1). This gives reason to expect compliance with the reciprocity law, according to which the plant response under these irradiation conditions should be the same.

However, the analysis of the data in the rest of Table 1 showed the significant deviations of some indicators from this law under such a variation range of light environment parameters. The above comments are equally applicable to the next parameter – the radiation dose *H*. Numerically, it is defined as the number of moles of photons of the radiation flux generated by the light sources and incident on a surface unit, including the plant leaves. From the photometric point of view, only this particular flux part is useful; the rest should be regarded as waste.
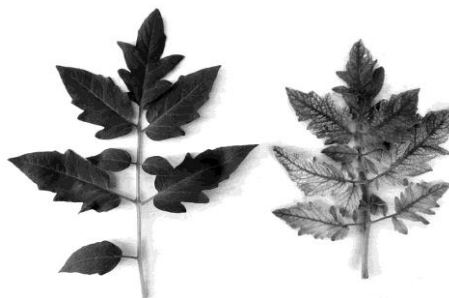
Figure 2. Tomato leaves under the16 h (left) and 22 h (right) photoperiod.

The decrease of leaf chlorophyll content, observed under the extended photoperiod, resulted in the reduced radiation absorption that was a protective response to the excess flux energy. Under the higher irradiance, the chlorophyll content at *PP* = 16 h increased under both spectra. However, its increment rate was lower under the radiation with a bigger share of blue flux (Spectrum II).

DLI values recommended for commercial tomato cultivation are known to be 20–30 mol m^{-2} day^{-1} (Moe et al., 2006). Under our conditions, these values in the variants with *PP* = 22 h under photon irradiance of 100 and 170 μmol m^{-2} s^{-1}, with *DLI* being 7.9 and 13.5 mol m^{-2} day^{-1}, respectively, were even lower than in the variant with *PP* = 16 h and photon irradiance of 240 μmol m^{-2} s^{-1} and *DLI* 13.8 mol m^{-2} day^{-1}, in which the leaf chlorosis was not observed. This suggests that chlorophyll degradation in this case was a response to an extended photoperiod rather than to *DLI* .

The leaf surface area S_L determines the amount of captured flux emitted by the light sources and its further use in photosynthesis. The studies show that an increase in the leaf surface area is more important than an increase in the chlorophyll content (Solhaug, 1991). Moreover, under higher photon irradiance level, the photosynthetic activity may be higher under a lower level of chlorophyll content (Leverenz, 1987). The experiment demonstrated significant (several times) reduction in the leaf surface area of transplants grown under the extended photoperiod. The blue radiation (Spectrum II) reduced the area further.

Table 1. Lighting regimes and values of tomato plant growth indicators under the different light quality, photoperiods, and irradiance at the end of the experiment

Indicator	Spectrum Type	PP, h	Irradiance E, umol·m^{-2}·s^{-1}		
			100	170	240
Daily lighting integral DLI, mol m^{-2} day^{-1}	I, II	16	5.8	9.8	**13.8**
		22	7.9	**13.5**	19.0
Radiation dose H, Mol m^{-2}	I, II	16	224.6	381.9	539.1
		22	308.9	525.1	741.3
Chlorophyll Content Index, rel.units	I	16	24.3 ± 1.8[a]	43.7 ± 3.5[b]	56.8 ± 3.8[c]
		22	4.1 ± 0.3[d]	4.6 ± 0.4[e]	4.9 ± 0.4[e]
	II	16	22.3 ± 1.9[a]	43.4 ± 3.5[b]	48.0 ± 4.5[f]
		22	3.9 ± 0.3[d]	4.9 ± 0.4[e]	5.0 ± 0.4[e]
Leaf area S_L, cm^2	I	16	981 ± 24[a]	1123 ± 25[b]	1029 ± 25[c]
		22	164 ± 3[d]	221 ± 5[e]	229 ± 5[e]
	II	16	926 ± 23[f]	1018 ± 21[c]	993 ± 19[a]
		22	121 ± 3[g]	184 ± 4[h]	199 ± 4[i]
Leaf fresh mass M_L, g plant^{-1}	I	16	29.77 ± 1.21[a]	46.7 ± 1.84[b]	47.77 ± 1.92[b]
		22	4.78 ± 0.22[c]	9.21 ± 0.38[d]	11.13 ± 0.41[e]
	II	16	29.85 ± 1.28[a]	45.83 ± 1.94[b]	48.18 ± 1.95[b]
		22	3.97 ± 0.24[f]	8.34 ± 0.41[d]	10.76 ± 0.42[e]
Leaf dry matter content v_L, %	I	16	9.6 ± 0.6[a]	11.9 ± 0.7[b]	13.8 ± 0.8[c]
		22	11.0 ± 0.8[b]	11.5 ± 0.9[b]	12.4 ± 1.0[b]
	II	16	9.5 ± 0.6[a]	11.9 ± 0.7[b]	12.6 ± 0.8[b]
		22	11.2 ± 0.8[b]	12.5 ± 0.8[b]	14.1 ± 0.9[c]
Specific leaf weight (SLW), g m^{-2}	I	16	29.1 ± 1.8[a]	49.5 ± 3.2[b]	64.1 ± 3.7[c]
		22	32.1 ± 2.1[a]	47.9 ± 2.7[b]	60.3 ± 3.2[c]
	II	16	30.6 ± 1.7[a]	53.6 ± 2.3[d]	61.1 ± 3.3[c]
		22	36.7 ± 2.2[a]	56.7 ± 2.1[d]	76.2 ± 3.4[e]
RUE, g mol^{-1}	I	16	0.130 ± 0.008[a]	0.130 ± 0.009[a]	0.119 ± 0.007[b]
		22	0.104 ± 0.005[c]	0.091 ± 0.005[d]	0.081 ± 0.004[e]
	II	16	0.136 ± 0.006[a]	0.140 ± 0.007[a]	0.113 ± 0.005[b]
		22	0.119 ± 0.005[b]	0.108 ± 0.003[c]	0.103 ± 0.003[c]

Means followed by the same letter do not differ significantly at $\alpha = 0.05$.

The similar pattern was observed for the fresh mass of leaves M_L. The lengthening of the photoperiod is reported to affect not only the accumulation of total biomass, but also its distribution over the plant organs. In tomato plants, the starch accumulation in the leaves is observed that can lead to lower photosynthesis rate (Doraiset al., 1996). In the experiment, the fresh mass increased with higher irradiance for both spectra. In absolute values, under the extended photoperiod, the wet mass yield was significantly lower.

The dry matter content v_L negatively correlates with the relative leaf growth rate and positively – with the leaf age. Leaves with high dry matter content are harder and less subject to physical damage. According to available data, bigger dry mass of plants under the extended photoperiod can be associated either with an increase in the photosynthetic plant area, or with the improved photosynthetic efficiency per leaf surface unit owing to higher chlorophyll content (Langton et al., 2003). However, the maximum chlorophyll content is not always correlated with the maximum fresh mass (Lefsrud et al., 2006).

In the experiment, a monotonic increase in the dry matter content was observed under the increasing photon irradiance with the higher share of blue (Spectrum II). At the same time, under the extended photoperiod, the dry matter content was higher at all irradiance levels. Under Spectrum I, the dry matter content for an extended photoperiod was lower than that under Spectrum II with higher irradiance levels.

The ratio of leaf area to dry leaf mass, i.e. specific leaf weight, *SLW*, is widely used in environmental studies. In the experiment, *SLW* increased with the growing irradiance for both spectra and photoperiods. Blue radiation additionally increased this index. Under Spectrum II, *SLW* values under $PP = 16$ h were smaller than those under $PP = 22$ h at any irradiance levels. Under Spectrum I, *SLW* value was smaller at higher irradiance levels when $PP = 22$ h than when $PP = 16$ h. That means the extended photoperiod contributed to an increase in *SLW* for both types of spectrum and any irradiation level, with the exception of medium and high levels with Spectrum I, where *SLW* values reduced.

Fig. 3 shows the deviation of ΔRUE, %, depending on the irradiance level for different light quality and photoperiods. ΔRUE calculation basis was the experimental conditions with the regular photoperiod ($PP = 16$ h), medium irradiance ($E = 170$ µmol m^{-2}·s^{-1}, and no additional blue flux (Spectrum I).
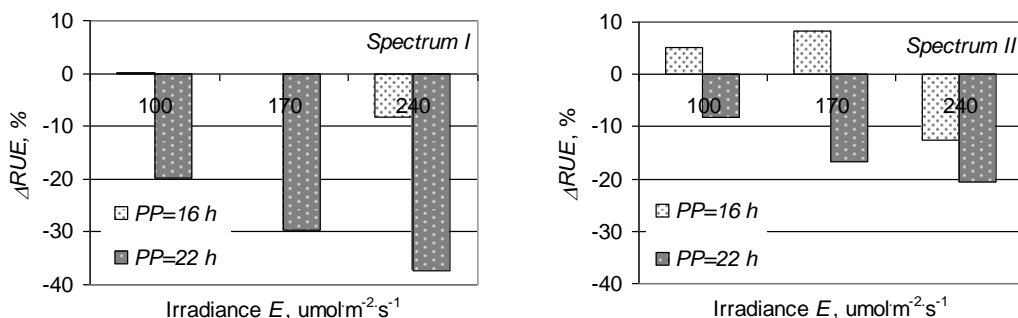
Figure 3. Deviation in *RUE* under varied light environment parameters.

Without additional blue flux, under a regular photoperiod, *RUE* reduced by 8% at high irradiance levels. With the extended photoperiod, under these conditions, the change in the irradiance level reduced *RUE* by 20–37%, with bigger reduction values being observed at higher irradiance levels. Seven percent addition of blue flux made it possible to increase *RUE* by 5–8% at the same and lower irradiance levels and under $PP = 16$ h. Under $PP = 22$ h and these conditions, *RUE* decreased by 8–21%.

CONCLUSIONS

In a series of experiments, tomato transplants in the pre–reproductive period were exposed to PAR with different photoperiods, photon irradiance, and light quality. An extended photoperiod was found to result in the development of leaf chlorosis. When varying the radiation dose components, the deviation from the reciprocity law was recorded. The analysis showed that the chlorophyll degradation was a response to the extended photoperiod rather than to the radiation dose. The chlorophyll content at

PP = 16 h increased with higher irradiance, while its increment rate decreased under radiation with a bigger share of blue flux.

The study results verify a great influence of an extended photoperiod on *RUE*, while the degree of influence depends on other parameters of light environment – light quality and irradiance level.

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Development of metabolic engineering approaches to regulate the content of total phenolics, antiradical activity and organic acids in callus cultures of the highbush blueberry (*Vaccinium corymbosum* L.)

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Abstract. Blueberry (*Vaccinium corymbosum* L.) is increasingly cultivated to produce high quality berries for consumption and potential applications in medicine, nutrition and as industrial precursors. Seasonal availability sets limitations on chemical compound isolation from cultivated plants. Biotechnological solutions, such as tissue cultures and metabolic engineering, can provide sufficient amounts of plant material with reasonably high metabolite levels, which may be adjusted by different strategies. Here, we describe our approach to modifying total phenolic content (TPC), antiradical activity (ARA) and amounts of selected organic acids in *in vitro* cultures of two varieties of *V. corymbosum* by varying the growth media. TPC, ARA and acid levels were determined in mature leaves of field-grown plants and in stable callus cultures derived from leaves of varieties 'Bluecrop' and 'Duke' grown on Murashige-Skoog (MS) and Woody plant (WP) media supplemented with varying concentrations and combinations of different plant growth hormones. TPC varied from 83 mg g^{-1} dry weight (DW) to 142 mg g^{-1} DW in leaves of 'Bluecrop' and 'Duke', respectively, and correlated with their ARA with 'Duke' at the lead. For callus cultures the highest ARA, as well as the highest TPC of 94 mg g^{-1} DW was observed in 'Bluecrop' grown on WP medium with 2,4-dichlorophenoxyacetic acid (2,4-D). High level of quinic acid was found in the mature leaves of all tested varieties, while callus cultures exhibited relative increase in amounts of malic, succinic and citric acids instead. Oxalic acid was found only in callus cultures.

Key words: blueberry, *Vaccinium corymbosum*, total phenolic content, antiradical activity, organic acids, callus cultures.

INTRODUCTION

Genus *Vaccinium* comprises over 450 species of woody, perennial plants that are commonly growing in cool temperate regions of both hemispheres. Several *Vaccinium* species are cultivated to produce high value berries, while others are harvested in the wild, particularly in Northern European countries. Genus *Vaccinium* includes a few

cultivated and many wild berry species, such as blueberry (*Vaccinium corymbosum*, *V. angustifolium*), bilberry (*V. myrtillus*), cranberry (*V. macrocarpon*, *V. oxycoccus*) and lingonberry (*V. vitis-idaea*), which are recognized for their high levels of bioactive compounds (Skrovankova et al., 2015; Karppinen et al., 2016a).

Blueberry (*Vaccinium corymbosum* L.) is emerging as a high quality, nutritious berry crop plant in Northern European countries. The cultivation is increasing also in Latvia, and in 2018 reached approximately 280 ha (Karlsons & Osvalde, 2019). However, seasonal production of blueberries is not able to meet demand for consistent supply of high value bioactive compounds that have wide area of applications in nutrition, pharmaceuticals and cosmetics; therefore, biotechnological solutions are desirable. Along with solving issues of seasonal supply, they also have the potential for more sustainable production of selected compounds with less environmental impact and better control over potential contaminating factors. In addition to field crop, fruit and berry cell cultures are increasingly viewed as source of valuable ingredients for food production (Nordlund et al., 2018). Cell cultures derived from *Vaccinium* species are potentially a rich source of valuable phenolic compounds, including proanthocyanidins (Suvanto et al., 2017). Formation of callus cultures rich in anthocyanin content has also been observed in *V. corymbosum* (Ostrolucka et al., 2004). *Vaccinium* species are reported as abundant source of carotenoids, such as lutein (Karppinen et al., 2016a; Karppinen et al., 2016b), anthocyanins (Routray & Orsat, 2011) and phenolic compounds, such as, resveratrol (Rimando et al., 2004). However, industrial potential of blueberry is not met by the production levels of blueberry crop. Furthermore, industrial use requires year-round availability and uniformity only achievable through biotechnological solutions, such as blueberry *in vitro* cultures. For these solutions to become economically viable, the levels of target compounds need to be increased. Even though the berries are the most important source of bioactive compounds for food consumption, for biotechnological production also other plant tissues can be used, although much less information on bioactive compounds is available. For instance, leaves of several *Vaccinium* species were demonstrated to contain substantial amounts of the three major phenolic compounds: chlorogenic acid (polyphenol), quercetin (flavonol) and arbutin (glycosylated hydroquinone) (Stefanescu et al., 2019). Adult leaves of twenty-seven *Vaccinium* cultivars collected in three geographic regions and over three seasons of the year were shown to exhibit substantial amounts of total phenolic and flavonoid compounds and total antioxidant capacity (Páscoa et al., 2019). Higher total phenolic content (TPC) has been found in blueberry cvs. 'Duke', 'Bluecrop' and 'Nui' leaves than fruits (Fotirić Akšić et al., 2019). Flavonoids and organic acids are recognized as compounds that contribute greatly to quality and taste of blueberry fruits and have potential human health benefits. Citric, quinic, and shikimic acids have been found to be the main organic acids in fruits of different diploid and tetraploid blueberry species and cultivars (Wang et al., 2019), while another study also reported low levels of malic, succinic, and tartaric acids in cv. 'Bluecrop' (Forney et al., 2012); however, the relative amounts in different varieties and species were quite variable. Diploid and tetraploid *V. corymbosum* accessions including varieties 'Bluecrop' and 'Duke' exhibited high concentration of citric acid, but much lower concentrations of shikimic and quinic acids, compared to the other diploid species (Wang et al., 2019).

Metabolic engineering, although not a trivial task, is routinely performed in microorganisms. In plants, due to complex genomes and overlapping metabolic pathways, metabolic engineering is far from routine, although recent advances in genome editing (Zhang et al., 2019) have significantly advanced breeding for certain compounds, e.g., gamma-aminobutyric acid content in tomato (Li et al., 2018). However, heritable changes in metabolite content can only be achieved through genetic engineering and genome editing, which substantially hinders commercialization of the crops due to genetically modified organisms (GMO) regulatory issues, exemplified by the Golden Rice and Golden Rice 2 cases (Giuliano, 2017). Here, we describe our approach to modifying TPC, antiradical activity (ARA) and amount of certain organic acids in *in vitro* callus cultures of two varieties of highbush blueberry *V. corymbosum*. By varying the culture media and combinations of growth regulators, we could achieve TPC in leaf-derived calluses up to 93.89 mg g^{-1} DW. We also observed that media composition substantially affected antiradical activity and yield of organic acids. Results showed that *V. corymbosum* callus cultures may be engineered to achieve substantial amounts of high value compounds with potential health benefiting activities.

MATERIALS AND METHODS

Plant material

Commercial blueberry (*Vaccinium corymbosum* L.) cultivars (cv.) 'Bluecrop' and 'Duke' were used to derive callus cultures. Commercial cv. 'Chandler' and 'Patriot' were used in selected experiments for comparison. Leaf material of cv. 'Bluecrop', 'Chandler', 'Duke' and 'Patriot' were collected in September 2019 from field-grown plants, freeze-dried and stored in dark until extraction.

Callus cultures

V.corymbosum cv. 'Bluecrop' and 'Duke' stem cuttings and leaves were used for callus induction. Plant material was thoroughly washed with clean water and sterilized for 10 min in 15% household bleach (< 5% chlorine-based agent) solution at room temperature with constant gentle stirring followed by brief sterilization with 70% ethanol and washing with sterile water. Sterilized plant material was then placed on agarized 100% Musharige Skoog (MS) (Murashige & Skoog, 1962) or Woody plant (WP) (McCown & Lloyd, 1981) media (all from Duchefa) supplemented with 0.5 g L^{-1} 2-(*N*-morpholino)ethanesulfonic acid (MES), 30.0 g L^{-1} sucrose and 2,4-dichlorophenoxyacetic acid (2,4-D), 6-benzylaminopurine (BAP), 1-naphthaleneacetic acid (NAA), kinetin (KIN) and thidiazuron (TDZ) in concentrations ranging from 0.1 to 5.0 mg L^{-1} and in different combinations with the final pH 5.7 ± 0.2. Composition of growth regulators was changed to assess their potential to boost production of biologically active compounds in callus cultures. Plates were sealed with transparent parafilm and kept in versatile environmental chamber (SANYO) with photoperiod of 16 h of light (~8,000 lm) and 8 h of dark, at 25 °C, temperature on the plate surface was ~28 °C After 35 days of incubation varying degree of callus formation was observed on all tested media and plant growth hormone mixtures. Calluses were divided and transferred to fresh media every 20–30 days according to growth and quality of the callus.

Preparation of extracts for biochemical analyses

Freeze-dried and powdered plant material and callus culture biomass was extracted using 70% ethanol solution. Ratio of 1:100 (mass of freeze-dried plant /callus material : solvent volume) was used for extraction. The mixture was heated at 50 °C for 15 min followed by 18 h extraction at room temperature (22–24 °C) with continuous mixing. Dry extract weight was determined after evaporation of the solvent.

Total phenolic content assay

Total phenolic content (TPC) was determined using Folin–Ciocalteu assay adjusted for microplates with gallic acid as the standard (Singleton & Rossi, 1965; Slinkard & Singleton, 1977). Briefly, dilutions of extracts (25 µl) in 75 µl water were incubated with 25 µl 1N Folin-Ciocalteu reagent for 6 min at room temperature (22–24 °C). 100 µl 7% sodium carbonate was added to the reaction. The absorbance was measured at 760 nm using a microplate reader (TECAN Infinite 200PRO) after incubation of 90 min at room temperature in the dark. All samples were analysed in duplicates, with three technical replicates for plant leaves, and three biological replicates for calluses. Results were expressed as mg of gallic acid equivalent (GAE) per g of dry weight (DW).

DPPH Assay

Anti-radical activity was determined using the 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay modified for microplates (Brand-Williams et al., 1995). Dilutions of extracts (20 µl) were mixed with 150 µM DPPH in 96% ethanol and incubated for 60 min at room temperature in the dark. Absorbance at 517 nm was measured using microplate reader (TECAN Infinite 200PRO). 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) was used as the standard and the results were expressed as µM Trolox equivalent antioxidant capacity (µM TEAC). In addition, the inhibitory concentration of the extracts needed to inhibit 50% of the DPPH radicals was determined (IC50). All samples were analysed in triplicates, with three technical replicates for plant leaves, and three biological replicates for calluses; for each sample eight different dilutions were assayed.

High performance liquid chromatography – mass spectrometry (HPLC-MS) analyses of organic and phenolic acids

Methanol and acetonitrile (LC-MS grade, from Fisher Chemical), formic acid ($\geq$ 98%, from TCI Chemicals) were used for HPLC-MS. Maleic acid ($\geq$ 98.0%) was supplied by Supelco. Primary reference standard of chlorogenic acid and analytical standards of oxalic, succinic, malic, tartaric, citric, quinic, p-coumaric, o-coumaric, m-coumaric, shikimic and caffeic acid were purchased from Sigma-Aldrich (St. Louis, USA).

An external standard calibration was used for quantitative determination of acids in extracts of callus cultures and blueberries leaves. Stock solutions of oxalic, succinic, maleic, malic, tartaric, citric, quinic and shikimic acid standards at a concentration of 1.0 mg mL^{-1} were prepared by dissolving in water; solutions of p-coumaric, o-coumaric, m-coumaric, shikimic, caffeic and chlorogenic acid were prepared by dissolving in methanol. All stock solutions were stored at 4 °C temperature. Working solutions of mixtures of all the standards were divided into 2 parts (A and B) and were prepared immediately before analyses by diluting the stock solution with mobile phase. Seven

working solutions of part A (oxalic, succinic, maleic, malic, tartaric, citric and quinic acid) were prepared ranged from 0.05–100 μg mL^{-1} and six working solutions of part B (p-coumaric, o-coumaric, m-coumaric, shikimic, caffeic and chlorogenic acid) were prepared ranged from 0.01–50 μg mL^{-1}. The standard solution at each concentration was analysed in triplicate. All calibration curves were constructed by plotting the average peak area against concentration. Regression equations and linearity of correlation coefficient (R^2) were calculated using Microsoft Excel 2016, $p < 0.001$. Limit of detection (LOD) and limit of quantification (LOQ) were defined as values three times to noise and 10 times to noise, respectively.

HPLC-MS identification and quantification analyses of organic and phenolic acids were carried out using an Agilent 1290 Infinity series system (Agilent Technologies, Germany) coupled to an Agilent 6230 TOF LC/MS (Agilent Technologies, Germany) with electrospray ionisation (ESI). Two different methods (A and B) were used for analyses of organic and phenolic acids.

In A method, chromatographic separation of oxalic, succinic, maleic, malic, tartaric, citric and quinic acid was performed at 55 °C using a Phenomenex Rezex ROA–Organic Acid H+ (8%), 4.6×150 mm column. The elution was carried out under an isocratic elution at a flow rate of 0.3 mL min^{-1}. Mobile phase consisted of aqueous 0.1% formic acid. The injection volume was 2 μL. The mass spectrometry operating conditions were as follows: negative ionisation mode, gas temperature 285 °C, nitrogen flow rate 10 L min^{-1}, nebulizer pressure 40 psi, capillary voltage 3,500 V and applied fragmentor 75 V. The full scan mass range was set to 50–1,000 m z^{-1}. Internal reference masses 112.9856 m z^{-1} and 1033.9881 m z^{-1} (G1969-85001 ES-TOF Reference Mass Solution Kit, Agilent Technologies & Supelco) were used. Spectrum extraction and peak detection were performed with MassHunter 7.00 Software (Agilent).

In B method, chromatographic separation of p-coumaric, o-coumaric, m-coumaric, shikimic, caffeic and chlorogenic acid was performed at 30 °C using an Agilent Zorbax SB-C18, 4.6×250 mm, 5 μm column. The mobile phase consisted of aqueous 0.1% formic acid (A) and 0.1% formic acid in acetonitrile (B). The flow rate was 0.4 mL min^{-1} and gradient elution was performed according to the following program: 0 min, 0% B; 11 min, 0% B; 32 min, 40% B; 38 min, 90% B; 41 min, 90% B; 42 min, 0% B. The injection volume was 10 μL. High resolution mass spectra (HRMS) were taken on an Agilent 6230 TOF LC/MS (Agilent Technologies, Germany). The mass spectrometry operating conditions were as follows: positive ionisation mode, gas temperature 320 °C, nitrogen flow rate 12 L min^{-1}, nebulizer pressure 40 psi, capillary voltage 3,500 V and applied fragmentor 130 V. The full scan mass range was set to 100–1,000 m z^{-1}. Internal reference masses 121.0509 m z^{-1} and 922.0098 m z^{-1} (G1969-85001 ES-TOF Reference Mass Solution Kit, Agilent Technologies & Supelco) were used. Spectrum extraction and peak detection were performed with MassHunter 7.00 Software (Agilent).

Results were expressed as μg per 1 g of plant dry weight (μg g^{-1} dw).

Data analysis

The results were expressed as mean ± standard deviation (SD). Statistical analyses were done using one-way ANOVA followed by the Tukey's test. Statistical significance was defined as $P < 0.05$. Correlation between ARA and TPC was calculated as Pearson's correlation coefficient.

RESULTS AND DISCUSSION

Production of callus cultures

Biotechnological production of phytochemicals from plants requires availability of stable and uniform *in vitro* cultures, which are also optimised for production of specific compounds. *In vitro* techniques for micropropagation of *V. corymbosum* are well established, e.g., (Ostrolucka et al., 2004), however, detailed protocols for stable callus production of blueberry leaves are limited, e.g., (Dembinska-Migas et al., 1998). Here, we describe protocol for production and maintenance of callus cultures from *V. corymbosum* varieties 'Bluecrop' and 'Duke'.

Callus induction was achieved from leaves of varieties 'Bluecrop' and 'Duke' on MS media containing 2,4-D, BAP, TDZ, KIN and NAA in $0.1–5.0$ mg L^{-1} concentrations. After 35 days of incubation varying degree of callus formation was observed on all tested media and plant growth regulator mixtures. Subsequently, calluses were transferred also to WP medium containing selected plant regulator mixtures and grown for 14 months. Best callus growth was observed on MS or WP media containing 0.2 mg L^{-1} 2,4-D (MS-D and WP-D), however good growth was observed also on MS media containing 1.5 mg L^{-1} 2,4-D, 2.0 mg L^{-1} BAP, 1.0 mg L^{-1} NAA and 0.1 mg L^{-1} TDZ (MS-NDBT), and 1.0 mg L^{-1} 2,4-D, 0.5 mg L^{-1} BAP, 2.0 mg L^{-1} NAA and 1.0 mg L^{-1} KIN (MS-NBKD) and 0.5 mg L^{-1} 2,4-D and 0.2 mg L^{-1} BAP (MS-DB). Mature calluses that were selected for extract preparation were pale brown to yellowish green, compact and quick to oxidize, if cut with sharp knife. For 'Bluecrop', comparable callus growth was achieved on four different variants of media (MS-D, MS-NDBT, MS-NKBT, WP-D) for 'Duke' – on two (MS-D, MS-DB).

Total phenolic content in blueberry leaves and calluses

Phenolic compounds are essential for plant growth, development and defence reactions, while their properties as natural antioxidants can have beneficial health effects in humans (Tanase et al., 2019). It is generally accepted that phenolic compounds are the substances directly responsible for the antioxidant capacity of blueberries and their positive health effects. Although most of the research concentrates on blueberry fruits, also the blueberry leaves have been shown to be a rich in polyphenols and to exhibit antioxidant capacity (Piljac-Žegarac et al., 2009). In this study total phenolic content (TPC) in the leaves of varieties 'Bluecrop', 'Chandler', 'Duke' and 'Patriot' were compared (Fig. 1, A). The lowest TPC level was found in leaves of 'Bluecrop' (83.46 ± 21.0 mg g^{-1} DW), and the highest in leaves of 'Duke' (142.10 ± 56.36 mg g^{-1} DW). Varieties 'Patriot' and 'Chandler' exhibited slightly lower TPC in leaves compared to 'Duke' (136.70 ± 47.82 mg g^{-1} DW and 119.10 ± 36.21 mg g^{-1} DW, respectively). Limited amount of information on the TPC in blueberry leaves is available, however, our results appeared to show comparable, if somewhat higher, concentrations than found in leaves of three blueberry varieties 'Bluecrop', 'Duke' and 'Nui' grown under two different management regimes in Serbia (Fotirić Akšić et al., 2019).
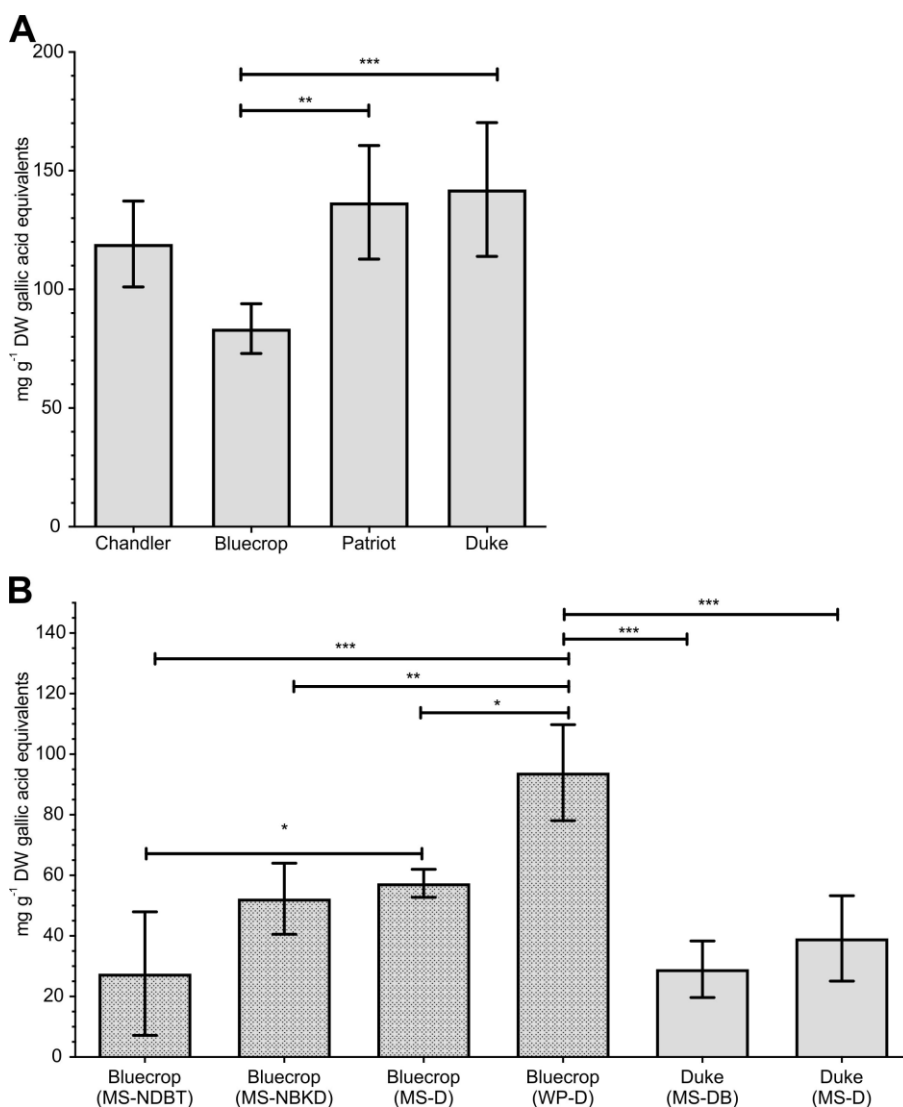
Figure 1. Total phenolic content in leaves of *V. corymbosum* varieties (A) and calluses grown on different cultivation media (B).

Results are expressed as mean $\pm$ SD mg g^{-1} DW, n = 3, * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$ (ANOVA). MS-NDBT – MS media with 1.5 mg L^{-1} 2,4-D, 2.0 mg L^{-1} BAP, 1.0 mg L^{-1} NAA and 0.1 mg L^{-1} TDZ; MS-NBKD – MS media with 1.0 mg L^{-1} 2,4-D, 0.5 mg L^{-1} BAP, 2.0 mg L^{-1} NAA and 1.0 mg L^{-1} KIN; MS-D – MS media with 0.2 mg L^{-1} 2,4-D; WP-D – WP media with 0.2 mg L^{-1} 2,4-D; MS-DB – MS media with 0.5 mg L^{-1} 2,4-D and 0.2 mg L^{-1} BAP.

In contrast to the TPC in leaves, the highest concentrations in callus cultures were achieved in calluses induced from 'Bluecrop' leaves, while calluses from 'Duke' exhibited lower TPC (Fig. 1, B). Modifications in culture media showed clear effect on the TPC. In the case of both varieties, addition of the 2,4-D alone to MS media increased production of phenolic compounds compared to combinations of 2,4-D with the other hormones. The highest TPC was observed in 'Bluecrop' callus cultures grown on WP

media supplemented with 2,4-D (93.89 ± 15.86 mg g^{-1} DW). There is a lack of information on specific effects of 2,4-D in plants from genus *Vaccinium*. However, effects of this plant growth regulator have been studied in other species. 2,4-D modulated gene expression in auxin, ethylene and abscisic acid pathways, as well as regulated a wide variety of other cellular functions in *Arabidopsis* based on an Affymetrix microarray study (Raghavan et al., 2005). Increase of antioxidant production and expression of antioxidative enzymes in response to 2,4-D has been shown in peas (Pazmiño et al., 2011). Regulatory effects on flavonoid biosynthesis has been shown in wheat (Pasquer et al., 2006). In callus cultures, 2,4-D showed concentration dependent effect on production of phenolic compounds. In *Moringa oleifera* callus cultures yields of phenolic compounds decreased with increasing 2,4-D concentrations, whereas combination of 2,4-D and kinetin showed positive correlation (Hamany Djande et al., 2019). Our data shows that 2,4-D alone at low concentration (0.2 mg L^{-1}) boosts production of phenolic compounds and increases antioxidative capacity, whereas when used at higher concentration (1.5 mg L^{-1}) in combination with other growth regulators, phenolic compound yields are lower. Observed effects might be due to induction of specific biosynthesis and defence pathways; however, additional gene expression and metabolic profiling analyses are needed to further substantiate this observation. Overall, our results emphasise both the importance of micronutrient and macronutrient content in the MS and WP media for induction of plant secondary metabolite production, and the choice of plant growth regulators and their concentrations.

Antiradical activity in blueberry leaves and calluses

Table 1. Antiradical activity of *V. corymbosum* leaf and callus extracts. Activity is expressed as mean±SD of IC50 μg ml^{-1} extract and μM Trolox g^{-1} DW, n = 3

Sample	IC50 (μg mL^{-1}) ± SD	TEAC (μM Trolox g^{-1} DW) ± SD
Leaves		
'Chandler'	218.40 ± 7.56	705.16 ± 112.5 *
'Bluecrop'	$439.04 \pm 18,13$	455.73 ± 86.08
'Patriot'	228.63 ± 56.49	1066.01 ± 180.46 *+
'Duke'	141.66 ± 43.64	1302.90 ± 284.81 *+
Callus cultures		
'Bluecrop' (MS-NDBT)	856.44 ± 175.30	83.59 ± 61.75
'Bluecrop' (MS-NBKD)	454.27 ± 34.88	340.48 ± 95.52
'Bluecrop' (MS-D)	456.97 ± 121.77	296.52 ± 223.75
'Bluecrop' (WP-D)	178.82 ± 69.41	750.86 ± 196.30 ¶
'Duke' (MS-DB)	1176.44 ± 674.47	123.14 ± 37.76
'Duke' (MD-D)	1028.22 ± 215.48	164.68 ± 4.49

* Statistically significantly higher TEAC compared to 'Bluecrop' ($p < 0.01$); + Statistically significantly higher TEAC compared to 'Chandler' ($p < 0.01$); ¶ Statistically significantly higher TEAC compared to 'Bluecrop' (MS-NDBT) ($p < 0.001$); 'Bluecrop' (MS-NBKD) ($p < 0.05$); 'Bluecrop' (MS-D) ($p < 0.01$); 'Duke' (MS-DB) ($p < 0.001$); 'Duke' (MD-D) ($p < 0.001$).

Antiradical activity of leaf and callus extracts was expressed as μM Trolox equivalent antioxidant activity (TEAC) and IC50 of each extract were calculated. Results show that antiradical activity strongly correlated with TPC (Pearson's correlation

coefficient $r = 0.966$, $p < 0.0001$). Leaves of 'Bluecrop' showed the lowest activity among leaf samples, while leaves of 'Duke' exhibited the highest antiradical activity (Table 1). In calluses low antiradical activity was observed for both variants of calluses produced from 'Duke'. In the case of 'Bluecrop', the highest activity was observed in calluses grown on WP-D media (750.86 ± 196.30 µM Trolox g^{-1} DW), while the lowest in MS-NDBT calluses (83.59 ± 61.75 µM Trolox g^{-1} DW) (Table 1). IC50 values are shown in Table 1. In general, correlation between TPC and antiradical activity was observed, similarly to the study with *V. corymbosum* and *V. myrtillus* in Italy (Giovanelli & Buratti, 2009).

Content of organic and phenolic acids in blueberry leaves and calluses

Organic acids in berries play an important role in determining the organoleptic properties of fruits; however, these phytochemicals are also useful as antioxidants, preservatives, acidulants, and drug absorption modifiers in pharmaceutical and other industries (Mikulic-Petkovsek et al., 2012). Organic acids in leaves of blueberry varieties 'Bluecrop', 'Chandler', 'Duke' and 'Patriot', and calluses obtained from varieties 'Bluecrop' and 'Duke' were determined with two HPLC-MS methods described in the Methods section.

Differences in organic and phenolic acid content between leaves and callus cultures were observed (Table 2). Tartaric acid was detected only in leaf extracts and in 'Bluecrop' calluses grown on MS-NBKD media. Oxalic acid was found only in calluses. Concentrations of maleic acid, shikimic, chlorogenic and caffeic acids were higher in leaves. Both calluses and leaves contained high concentrations of citric acid, however in 'Duke' calluses concentration was lower compared to leaves. In 'Bluecrop' calluses citric acid concentration varied depending on the media used for cultivation with the lowest yield in MS-NDBT calluses ($6,488 \pm 317$ µg g^{-1} DW) and the highest in MD-D calluses ($13,696 \pm 155$ µg g^{-1} DW). Calluses contained more malic acid than leaves, except for 'Bluecrop' grown on WP-D media. Higher yields of succinic acid were also achieved in calluses. Chlorogenic acid was the dominant phenolic acid in leaves, while its concentration in calluses was significantly lower and varied depending on the media composition. Cultivation on MS-D media allowed to reach higher chlorogenic acid content in 'Bluecrop' calluses, but this effect was not observed in 'Duke' calluses. The highest production of chlorogenic acid was observed in 'Bluecrop' calluses grown on MS-NBKD media. Caffeic acid concentrations widely varied amongst leaves of different varieties, but all were significantly higher than in calluses. There are few studies that have reported the effects of growth regulators on production of caffeic acid derivatives in *in vitro* cultures. In *M. oleifera* callus cultures, 2,4-D upregulated production of caffeic acid derivatives, with some derivatives being present only in calluses grown on 2,4-D (Hamany Djande et al., 2019). In our study no significant differences in caffeic acid production were detected between *V. corymbosum* calluses grown in presence of different growth regulators and their combinations. Additional phenolic compound analyses and quantification are needed to elucidate effects of media and growth regulators on specific secondary metabolites.

Table 2. Content of organic and phenolic acids in blueberry leaves and calluses. Data expressed as mean ±SD (n = 3), LOD – limit of detection, LOQ – limit of quantification. Values with the same letter within a column are not significantly different at $P < 0.0$

	Malic acid	Chlorogenic acid	Quinic acid	Citric acid	Oxalic acid	Succinic acid	Maleic acid	Tartaric acid	Shikimic acid	Caffeic acid	o-coumaric acid	p-coumaric acid	m-coumaric acid
'Chandler'	12,233 ± 71[a]	39,282 ± 223[a]	31,976 ± 363[a]	7,578 ± 69[a]	< LOD	1,121 ± 49[a]	1,080 ± 85[a]	600 ± 38[a]	318 ± 8[a]	325 ± 30[a]	24.4 ± 7.8[a]	16.4 ± 5.6[a]	17.3 ± 2.8[a]
'Duke'	9,873 ± 62[b]	39,261 ± 526[a]	28,645 ± 237[b]	9,425 ± 67[b]	< LOD	694 ± 28[b]	608 ± 38[b]	180 ± 21[b]	288 ± 6[b]	32 ± 9[bc]	1.3 ± 0.4[b]	0.4 ± 0.2[b]	0.4 ± 0.2[b]
'Patriot'	11,281 ± 198[c]	49,264 ± 834[b]	34,253 ± 572[c]	5,474 ± 144[c]	< LOD	915 ± 21[c]	737 ± 55[c]	349 ± 19[c]	417 ± 4[c]	61 ± 2[c]	5.3 ± 2.4[b]	5.0 ± 0.8[b]	3.7 ± 1.5[c]
'Bluecrop'	11,521 ± 225[ac]	31,303 ± 372[c]	32,290 ± 490[a]	7,720 ± 119[a]	< LOD	646 ± 23[b]	407 ± 31[d]	176 ± 32[b]	242 ± 3[d]	147.3 ± 1.4[d]	1.1 ± 0.3[b]	4.7 ± 0.3[b]	0.36 ± 0.12[b]
Duke (MS-DB)	22,313 ± 662[d]	17.3 ± 0.6[d]	99 ± 25[d]	5,902 ± 107[c]	6016 ± 181[a]	855 ± 91[bc]	39 ± 3[e]	< LOD	< LOQ	9.2 ± 1.3[b]	0.9 ± 0.3[b]	0.6 ± 0.1[b]	0.72 ± 0.11[b]
Duke (MS-D)	19,385 ± 47[e]	25 ± 3[d]	485 ± 15[d]	5,129 ± 82[c]	5287 ± 67[b]	848 ± 21[bc]	59 ± 5[e]	< LOD	4.2 ± 1.0[e]	11 ± 3[b]	0.80 ± 0.07[b]	0.3 ± 0.1[b]	0.7 ± 0.2[b]
Bluecrop (MS-NDBT)	13,554 ± 342[f]	40 ± 3[d]	< LOD	6,488 ± 317[d]	5467 ± 151[b]	2,225 ± 6[d]	74.9 ± 1.2[e]	< LOD	16.9 ± 0.6[f]	14 ± 2[b]	1.1 ± 0.2[b]	3.1 ± 0.6[b]	1.0 ± 0.2[bc]
Bluecrop (MS-NBKD)	24,503 ± 459[g]	467 ± 33[d]	396 ± 95[d]	7,970 ± 99[a]	4070 ± 191[c]	1,425 ± 34[e]	368 ± 30[d]	62.0 ± 0.3[d]	17.3 ± 0.8[f]	17 ± 3[b]	1.68 ± 0.10[b]	2.9 ± 0.4[b]	0.39 ± 0.13[b]
Bluecrop (MS-D)	23,836 ± 281[g]	168 ± 60[d]	922 ± 45[d]	13,696 ± 155[e]	4299 ± 314[c]	1,258 ± 166[a]	338 ± 8[d]	< LOD	25.8 ± 0.8[f]	8 ± 3[b]	2.5 ± 0.3[b]	1.5 ± 0.5[b]	0.07 ± 0.03[b]
Bluecrop (WP-D)	7,972 ± 37[h]	86 ± 12[d]	900 ± 8[d]	8,702 ± 196[f]	1752 ± 73[d]	764 ± 28[b]	203 ± 22[f]	< LOD	7.89 ± 0.10[ef]	7.2 ± 0.2[b]	1.43 ± 0.05[b]	3.2 ± 0.2[b]	0.20 ± 0.07[b]

Biosynthesis and accumulation of organic acids in various *Vaccinium* species have been described before (Laaksonen et al., 2010; Phillips et al., 2010; Forney et al., 2012), but there have not been any reports about modulation of organic acid composition in calluses. Mikulic-Petkovsek et al. (2012) reported that tartaric acid was not detected, and malic acid was found at low levels in *V. corymbosum* berries. Another study reported tartaric acid in *V. corymbosum* 'Bluecrop' and 'Patriot' berries (Çelik et al., 2012). In this study we showed very high level of malic acid in both leaves and calluses and comparatively high levels of tartaric acid in leaves. In general, our results clearly indicated that production of organic acids in *V. corymbosum* calluses can be boosted by modifications in cultivation media. In this study, increase in citric, succinic, malic and oxalic acids was achieved. In addition, even though the highest total phenolic content and antiradical activity was reached in 'Bluecrop' calluses grown on WP-D media, low concentrations of chlorogenic and caffeic acids were found in these samples. Since these compounds are generally implicated in the high antiradical activity of plant extracts, this finding indicates that other phenolic compounds might be responsible for the observed high antioxidative capacity in our study.

CONCLUSIONS

Successful establishment of callus cultures from *V. corymbosum* serve as a precondition for further metabolic engineering to boost production of valuable biologically active compounds. Modifications of micronutrient, macronutrient and plant growth factor composition and concentrations significantly affect production of organic and phenolic acids in callus cultures.

Total phenolic content clearly correlates with antiradical activity. Further in-depth chemical composition analyses are necessary to identify the phenolic compounds that are responsible for this activity.

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The influence of the flour amylolytic enzymes activity, dosage of ingredients and bread making method on the sugar content and the bread quality

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Abstract. The aim of this study was to study the effect of the sugar dosage, improver dosage, type of bread making methods and the amylolytic activity of five different types of wheat flours on the sugar content and the bread quality. The sugar content in the bread crumb was determined using the Bertrand's method and was counted for sucrose. When the dough was prepared using accelerated technology, the improver affected the sugar content in the bread due to the starch enzymatic hydrolysis. The effect of improver dosages and sugar dosages on the sugar content in the bread was established. When using the improver, the sugar content exceeded the permitted amount in 1.25 times. No correlation was found between sugar dosage in recipe and bread quality when accelerated bread making way was used because of short fermentation time. The influence of wheat flour amylolytic activity (falling number) on the sugar content in bread was established, including when sugar was absent in the formulation. When sugar presented at bread formulation, the flour amylolytic activity did not significantly affect the bread quality, except the acidity. The bread making way had a greater influence on bread quality than falling number of flour. When sugar absent at bread recipe, the higher was the flour amylolytic activity, the higher was the sugar content in bread made by traditional way due to the starch deterioration. Obtained data have shown that when a baking method is selected, the flour amylolytic activity must be taken into account.

Key words: bread, sugar content, sucrose, bread making technology.

INTRODUCTION

Sugars currently used by the bread making industry are sucrose, glucose, glucose syrup, fructose and lactose. Sucrose is most commonly used in many types of bread as an ingredient in the formulation. In bread, sugars may act as sweeteners, texture modifiers, bulking agents, flavor enhancers, flavour precursors, colour precursors and appearance modifiers (Puchkova et al., 2005; Cauvain & Young, 2007; Trinh et al., 2016).

In the Russian Federation, Ukraine and Belarus, the sugar content in the bakery products is regulated by normative documentation. Practice has shown that even if the sugar dosage was in accordance with the recipe, bread sugar dosage could not conform to the standard. It is one of serious problems to the bread making industry. Regardless of the type of sugar used in bread making process, the sugar content is determined in terms of sucrose. Methods for determining the mass fraction of sugar is based on the Bertrand reaction end the results obtained during the reaction are recounted for sucrose. That is why not only added sugar, but also flour's own sugars can contribute to the result. The flour contains reducing sugar (glucose, fructose and maltose disaccharide) and sugars that become reducing after hydrolysis of starch, sucrose, arabinose (Puchkova et al., 2005; Struyf et al., 2017).

Flour amylases make a big contribution to sugar content in the bread. The majority of fermentable and reducing sugars in dough are generated by α- and β-amylases degradation of damaged starch (Van der Maarel et al., 2002; Cauvain & Young, 2007; Codina & Leahu, 2009; Struyf, N. et al., 2016). α-amylases and β-amylases, both endogenously present in wheat, have been described extensively in literature (Cauvain & Young, 2007; Struyf, N. et al., 2016). α-amylases are endo-amylases that hydrolyze the α-(1, 4)-linkages inside the starch chain more or less randomly, thereby generating oligosaccharides and a-limit dextrins. The β-amylases are exoenzymes that cleave the penultimate α-(1, 4)-inkage from the nonreducing end of the polymeric chains and release the disaccharide maltose. β-amylase, acting alone, can degrade amylose completely to maltose. α-amylase degrades starch to smaller dextrins, thereby generating more non-reducing ends susceptible to β-amylase attack (Van der Maarel et al., 2002; Struyf, N. et al., 2016). Maltose has a reducing form, as one of the two units may have an open-chain form with an aldehyde group. Maltose is involved in the Bertrand`s reaction, so it is included in the total amount of sugar in bread.

The α-amylase activity in flour is often estimated by the Falling Number (FN) test. The FN represents an important quality characteristic of grain products. The FN of flour is related to the amount and activity of cereal enzyme α-amylase, which is present in the wheat after harvesting. Flours with a low FN result insufficient bread quality (low specific volume, sticky crumb). Every & Ross (1996) have shown that FN less than 250 s leads to sticky crumbs. Puckova et al. (2005) have meant about FN less than 200 s. In accordance with the Russian standard for flour with an ash content 0.55–0.75%, the FN should be no less than 200 s (State Standard of the Russian Federation, 2017).

Flours with a high FN (more than 350 s) have a reduced capacity to form fermentable sugars (Codina & Leahu, 2009; Struyf et al., 2016). To avoid quality deterioration, wheat with a high FN is sourced by the miller and α-amylases are added (Goesaert et al., 2009; Popper et al., 2006).

α-amylase is consistently used by the baking industry to improve dough properties and bread quality. It can be used as pure enzyme and as part of an improving additive. The beneficial effect of improvers with α-amylase on bread texture and elasticity is well known (Patel et al., 2012; Barrera et al., 2016). A fungal α-amylase from or malt are frequently used for this purpose (Goesaert et al., 2005; Cauvain & Young, 2007; Codina & Leahu, 2009). When the amylase is used, it leads to the maltose formation and to sugar content increasing in bread.

That is why the aim of present work was to study the effect of the sugar dosage, improver dosage, type of bread making methods and the amylolytic activity of five different types of wheat flours on the sugar content and the bread quality.

MATERIALS AND METHODS

Characteristic of ingredients

Five wheat flour samples obtained from different milling companies located in Russia were used for this study. Flour had different amylolytic activity (FN). The higher was the FN value, the lower was the amylolytic activity of flour.

The ash content and the FN were determined according to ICC approved method no: 104/1 and 107/1 respectively (ICC−standard methods: 104/1 (1990), 107/1 (1995). The wet gluten quantity and gluten quality were determined according to Russian Standard (State Standard of the Russian Federation, 2013), because this method commonly used all over the Russian Bread Industry.

The amount of gluten was determined in the following way. Crude gluten was washed from dough mixed with flour and water, which has been aged in water for hydration and the formation of intra- and intermolecular bonds in substances that form gluten (mainly proteins – gliadin and glutenin), followed by washing by a working body of a mechanized device (MOK–1M, Russia) using water to remove watersoluble substances, starch and brans from the dough. The resulting gluten was weighed and the percentage of crude gluten was calculated relative to the mass of the analyzed flour sample.

The gluten quality was determined as the compression strain index of raw gluten under the influence of a load (120 g) for a 30 s. It reflects the degree of gluten deformation. A special device IDK (Russian) was used for this. The results of the gluten elastic properties measuring were expressed in arbitrary units of the device IDK. According State Standard (State Standard of the Russian Federation GOST 26574, 2017) the gluten quality is divided into the following groups: unsatisfactory strong (less than 32 units of the device), satisfactory strong (33–52 units of the device), good (53–77 units of the device), satisfactory weak (78–102 units of the device), unsatisfactory weak (more 103 units).

The basic quality parameters characterized the wheat flour are presented in Table 1.

Table 1. Quality parameters of wheat flour

Indicators	Wheat flour				
	F1	F2	F3	F4	F5
Falling number, s	352 ± 13^a	318 ± 8^b	304 ± 7^c	282 ± 10^d	216 ± 11^f
Ash content, %	0.53 ± 0.03^a	0.71 ± 0.03^b	0.55 ± 0.03^a	0.69 ± 0.03^b	0.74 ± 0.04^b
Wet gluten, %	29.2 ± 1.9^a	29.9 ± 2.0^a	27.8 ± 1.6^b	30.3 ± 1.6^c	25.0 ± 2.5^d
Gluten deforma tion index, units of device IDK	65 ± 3^a	65 ± 3^a	65 ± 3^a	65 ± 3^a	60 ± 3^b

a–c = Means ± SD within the same row with different lowercase superscript letters are significantly different ($p \leq 0.05$).

Potable grade water was used in the study, as well as edible sodium chloride (Russia), dried or pressed baker's yeast (Lesaffre, Russia), complex baking improver Magimix 'Soft sandwich bread' (Lesaffre, France), enzyme 'Fungamil Super AX' with fungal α-amylase activity of 60 FAU/h (Russia), sunflower oil and sugar (sucrose). Mass fraction of sucrose in sugar 99.80%.

Bread preparation

The investigation was carried out in two steps. At the first step the impact of sugar dosage, improver dosage, type of bread making methods on the sugar content and bread quality was investigated. At the second step the impact of the amylolytic activity of five different types of wheat flours on the sugar content and bread quality was studied.

I. **Bread making process when investigated impact of the impact of sugar dosage, improver dosage, type of bread making methods on the sugar content and bread quality**

When investigated the impact of the technology, sugar dosage and improver dosage on sugar content and bread quality, the dough was prepared by the next ways.

Only one sample of flour was used in this study (F1 with falling number 352 s).

The dough was prepared using three techniques widely used at Russian bread industry:

(I) one-step method using dried baker's yeast. All ingredients were mixed and the dough was then fermented for 3 h;

(II) two-step method using a prefermented dough. The prefermeted dough was prepared on the first step using a bakery yeast, 50% of wheat flour and yeast. The dough was prepared on second step using prefermented dough and other ingredients (Table 2);

(III) accelerated way of bread making with liquid sourdough (10% of dough mass) and improver Magimix 'Soft sandwiche bread'. Dough was prepared using a two-step method with sourdough and bakery yeast. The first step was the preparation of starter. The sourdough was prepared at first step using commercial starter (1% of total flour mass), 5% of the total amount of flour (6 g) and 17% of the total amount of water (9 g). These ingredients were carefully mixed, then fermented for 24 h at 37 °C in a thermostat (TC/80, Russia). After that, part of the sourdough was used to knead the dough.

Commercial starter for sourdough (State research institute of baking industry, Russia) contained a mixture of *Lactobacillus casei*, *Lactobacillus fermentum*, *Lactobacillus plantarum* and *Lactobacillus brevis*.

It should be noted that the rest of the sourdough is mixed with flour and water and fermented. Part of fermented sourdough is usually used for bread making and small part is used for new sourdough. Sourdough allows getting the dough and bread in a quick way. Although the sourdough ferments for a long time, the method of dough preparing is very fast. And since sourdough is always continuously conducted at bakeries enterprises it can be used for bread making at any time. So the dough is prepared very quickly. Therefore, this method is called accelerated.

Dough was prepared by adding flour, water, improver Magimix 'Soft sandwiche bread', yeast and other ingredients (Table 2).

Improver Magimix 'Soft sandwiche bread' was used only in bread making process III, because of the deterioration in the dough quality in accelerated method. Magimix 'Soft sandwiche bread' is a bread improver used for preparing quality, standardized

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bread. The composition includes a complex of enzymes, emulsifier, ascorbic acid, L-cysteine.

Table 2. Formulations of the dough

Ingredients and parameters	I	II 1st step	2nd step	III 1st step	2nd step
Ingredient amounts					
Wheat flour, g	100	50	50	6	94
Dried yeast, g	0.7	0.3	0.2	-	1.0
Salt, g	1.5	-	1.5	-	1.5
Sugar, g	4.0	-	4.0	-	4.0*)
Sunflower oil, g	3.0	-	3.0	-	3.0
Starter sourdough, g				0.06	
Sourdough, g	-	-	-	-	15
Prefermented dough, g	-	-	78.7	-	-
Magimix 'Soft sandwiche bread', g	-	-	-	-	0.7**)
Water, g	54.4	28.9	25.3	9	45.4
Process parameters					
Moisture content, %	41.5	45	41.5	-	41.5
Fermentation time, h	3	3	1	24	0.17
Proofing time (fermentation before baking), min	71	-	82	-	64

*) In this study, when the impact of sugar dosage was investigated, the sugar content in the composition was decreased by 0, 10, 20, 50%. I.e. the sugar content in the formulation was 4 g, 3.6 g, 3.2 g, 2 g, while the dosage of the other ingredients was not changed.

**) the dosage of improver Magimix 'Soft sandwiche bread' was decreased on 15, 30, 50 and 100% in this study. I.e. the improver content in the formulation was 0.60 g, 0.21 g, 0.35 g, 0 g while the dosage of the other ingredients was not changed.

Fermentation time was different for each technique. Dough with sourdough was fermented only for 10 min because sourdough usage allowed obtaining high dough acidity. If the fermentation time was increased, the bread could have too high acidity, exceeding the requirements of Russian standards.

After fermentation all dough samples were shaped into 450 g round-shaped loaves, placed at aluminium pans, and leavened at 30 °C until the volume was twice the initial volume. The leavened dough was cooked in an oven SvebaDahlen (Sweden) at 200 °C for 23 min.

II. Bread making process when investigated impact of the flour amylolytic activity and type of bread making methods on the sugar content and bread quality

When investigated the influence of the amylolytic activity of five different types of wheat flours and tipy of technology on sugar content and bread quality, the dough was prepare by three ways:

(IV) one-phase method using pressed baker's yeast. All ingredients were mixed and the dough was fermented for 1 h;

(V) two-phase method using a prefermented dough. The prefermeted dough was prepared using bakery yeast, 50% of wheat flour and yeast. The dough was prepared on second step using prefermented dough and other ingredients (Table 3);

(VI) one-phase method using pressed baker's yeast and improver – fungal α-amylase 'Fungamil Super AX'. Fungal α-amylase was used only in this method to avoid quality deterioration due to accelerated technology.

Table 3. Formulations of the dough

Ingredients and parameters	IV	V 1st step	2nd step	VI
Ingredient amounts				
Wheat flour, g	100	50	50	100
Pressed yeast, g	1.5	0.5	1.0	1.5
Salt, g	1.5	-	1.5	1.5
Sugar, g	4.0	-	4.0	4.0
Sunflower oil, g	3.0	-	3.0	3.0
Prefermented dough, g	-	-	79	-
Enzyme 'Fungamil', g	-	-	-	0.002
Water, g	53.0	28.5	25.0	25.0
Process parameters				
Moisture content, %	41.5	45	41.5	41.5
Fermentation time, h	3	3	1	1
Proofing time (fermentation before baking), min	71	-	82	82

Assessment of baked bread

The quality of bread was evaluated by following parameters.

Mass proportion of moisture of the dough was determined by drying at a temperature of 130 °C during 40 minutes in drier (SHS–1M, Russia). The mass fraction of moisture was calculated as the ratio between the mass of evaporated water and the initial mass and was multiplied by 100%.

Acidity was determined by titration, using 0.1 N solution of NaOH (State Standard of the Russian Federation, 1996). For determination of titratable acidity, a 10 g sample was mixed with 100 mL of distilled water and the suspension was titrated to a final pH of 8.5 with 0.1N NaOH (PH-meter-millivoltmeter, Russia). One degree of acidity equal to the 1 ml of 0.1 N NaOH consumed. Acidity analysis were done in duplicate.

Porosity – was determined as the ratio between pore volume and 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 between product volume and mass of whole bread (cm g^{-1}) (Puchkova, 2004).

Compressibility was determined on the automatic penetrometer Labor (Hungary) (Puchkova, 2004). To determine compressibility, 40 mm thick slice was cut from the middle of the bread loaf (cut planes must be strictly parallel). The plastic penetrating body of the device (diameter 25 mm, weighing 300 g) was raised up to the upper position. Units of device were measured. The bread slice was put on the surface of the lifting table. The lifting table of the penetrometer was raised until the penetrating body was in contact with the bread. For a 5 s, the body penetrated bread. Units of device were measured again. The compressibility of bread was calculated as a difference between the initial and final units of the device.

The diameter D and the height H of the round pan bread was measured in millimeters. For hearth (pan) bread, the minimum and maximum diameters was measured. The shape stability indicator was counted as the ratio between the height and the diameter – H : D (Puchkova, 2004).

The sugar content deterioration

The sugar content in the bread crumb was determined in accordance with the Russian standard using the Bertrand's method (State Standard of the Russian Federation, 1968).

Bertrand's method of analysis remains a widely used industrial method to estimate the total sugar and reducing sugar content of different food products and bread. In this work, the assay itself involves collecting the precipitate of cuprous oxide formed by reduction of the copper-alkaline liquor in the presence of reducing sugars and assayed by the manganimetric method.

To determine the sugar 300 g of bread without crust was used.

The bread was thoroughly crushed. 25 g of crushed bread was introduced into a volumetric flask with a capacity of 200 cm^3. Water was added into the flask over 2/3 of the volume and flask was left to stand for 5 minutes with frequent shaking. After that, 10 cm^3 of a 15% solution of $ZnSO_4$ and 10 cm^3 of a 4% solution of NaOH were added into the flask, mixed well. Then water was addes until the mark of 200 cm^3, flask was shaked again and left for 15 minutes. Then liquid was filtered through filter paper into a dry flask. For hydrolysis of sucrose, 50 cm^3 of the filtrate was taken into a volumetric flask with a capacity of 100 cm^3 and 5 cm^3 of 20% hydrochloric acid was added. The flask was kept at 70 °C for 8 minutes. Then it was quickly cooled to room temperature (20 ± 1) °C. Solution was neutralized with a 10% sodium hydroxide solution. 20 cm^3 of the solution, 20 cm^3 of a 4% solution of $CuSO_4$ and 20 cm^3 of an alkaline solution of potassium-sodium tartrate were introduced into a conical flask. The flask was heated to a boil. They boiled for exactly 3 minutes from the moment of bubble formation. The liquid was filtered through an asbestos filter. The precipitate in the flask and on the filter was washed several times with hot water.

The Cu_2O precipitate was dissolved in 20 cm^3 of an ammonium-iron sulfate (III) solution. The solution was filtered by suction and the precipitate was washed. The resulting green colored solution in a suction flask was titrated with Potassium permanganate until a faint pink color. Bertrand tables in Russian Standard (State Standard of the Russian Federation, 1968) give a direct correspondence between the volume of potassium permanganate (0.1 N) used and the sucrose content of the sample.

Statistical analysis of the data

All of the experiments were carried out a total of five times. Statistical analysis was performed using Excell software. Comparison of the influence of factors was carried out by the method with significance tested at the 95% confidence level and differences among means were determined using the least significant difference and Duncan's test of two-factor analysis of variance with one repetition (ANOVA). The confidence intervals shown in the histograms and in the table reflect the accuracy of the used methods.

The accuracy of the experimental data was evaluated by using mathematical statistical methods in Microsoft Excel (2010 version) at a theoretical frequency of 0.95. Results were given as mean ± standard deviation.

RESULTS AND DISCUSSION

For research, bread 'Nareznoi' traditional and commonly produced in Russia, Ukraine and Belarus was used. The mass fraction of sugar in the dry matter of the wheat long-loaf bread 'Nareznoi' from flour of the first or higher grade should not exceed 4.2 ± 1% according Russian Standard (State Standard of the Russian Federation GOST 27844–88, 1988). The effects of the technology, sugar dosage and improver dosage on sugar content and bread quality were investigated. The sugar content depended on the method of the dough preparation (technology).

The sugar quantity didn't exceed the high normative level in breads prepared without improver by one-stage technology and two-stage technology with prefermented dough (Formulation at Table 2). When using sourdough and improver (III way according Table 2), the sugar content was the highest and exceeded the standard highest level by 1.17–1.25 times (Table 4). This may be due to the fact that the dough with the sourdough was fermented for only 10 minutes. Sugar was not fermented by yeast during such a short time (Verheyen, 2016). And the improver affected the sugar content in the bread due to the starch enzymatic hydrolysis (Van der Maarel et al., 2002). In terms of porosity, specific volume, shape stability and compressibility of the crumb, this sample was slightly inferior to bread prepared using a baking improver.

The bread making procedure using sourdough and short fermentation is commonly use in Russian industry, therefore the bread with excess sugar content may be found on the shelves. That is why the effects of dosage of sugar and improver on sugar content and bread quality when using this dough preparing method were investigated.

When the sugar dosage was reduced on 10 and 20%, the sugar content in bread steel exceeded the standard norm in 1.25 times (Table 4). Only when the sugar dosage was reduced by 50%, the amount of sugar in the bread met the standard requirements and was 4.1% of dry weight.

It should be noted that the sugar dosage reduction did not significantly affect the porosity, specific volume and compressibility of the crumb during 5 days of storage. This may also be due to the short dough fermentation time: sugar did not participate in fermentation, therefore, did not contribute to the formation of carbon dioxide, which determines the porosity and specific volume (Verheyen et al., 2015; Trinh et al., 2016; Verheyen, 2016). So, when sugar was reduced or eliminated it did not affect the fermentation and carbon dioxide formation.

When reducing the improver dosage by 15, 30 and 50%, the sugar content practically unchanged and exceeded standard norm in 1.25 times. The sugar content met the requirements only when improver was completely excluded. Porosity, specific volume, shape stability and compressibility of bread crumb were worse when not using the improver. This is understandable with such a short dough fermentation time (Verheyen, 2016; Van der Maelen et al., 2017).

Table 4. Impact of type of bread making methods on physico-chemical indicators of the bread

Indicators	I	II	III							
			dosage of sugar, % of the amount according to the recipe				dosage of improver, % of the amount according to the recipe			
			100	90	80	50	85	70	50	0
Mass proportion of moisture, %	39.7 ± 0.3^a	39.4 ± 0.3^a	39.0 ± 0.2^a	39.4 ± 0.3^a	39.5 ± 0.3^a	39.4 ± 0.3^a	38.2 ± 0.3^b	38.2 ± 0.3^b	38.4 ± 0.3^b	39.6 ± 0.3^a
Mass proportion of sugar, %	3.3 ± 0.1^a	4.2 ± 0.2^b	6.5 ± 0.3^c	6.5 ± 0.2^c	6.3 ± 0.2^c	4.1 ± 0.2^b	6.2 ± 0.2^c	6.2 ± 0.3^c	6.1 ± 0.3^c	4.9 ± 0.2^d
Acidity, degrees N	1.0 ± 0.1^a	1.0 ± 0.1^a	1.4 ± 0.1^b	1.6 ± 0.2^c	1.4 ± 0.1^b	1.5 ± 0.1^c	1.4 ± 0.1^b	1.4 ± 0.1^b	1.4 ± 0.1^b	1.6 ± 0.1^c
Porosity, %	83 ± 2^a	82 ± 2^a	85 ± 2^b	83 ± 1^a	85 ± 2^b	84 ± 2^b	85 ± 3^b	85 ± 2^b	83 ± 2^a	80 ± 2^c
Compressibility, units of the device										
20h after baking	64 ± 3^a	52 ± 2^b	69 ± 4^c	68 ± 3^c	71 ± 4^d	80 ± 4^e	98 ± 5^f	84 ± 3^g	66 ± 3^h	29 ± 2^i
48 h after baking	48 ± 3^a	37 ± 2^b	71 ± 3^c	59 ± 4^d	49 ± 2^a	58 ± 3^d	74 ± 5^e	78 ± 4^f	52 ± 3^g	20 ± 2^h
120 h after baking	31 ± 2^a	27 ± 2^b	41 ± 3^c	42 ± 3^c	45 ± 3^d	48 ± 3^e	34 ± 3^f	32 ± 4^a	27 ± 2^b	15 ± 2^g
Specific volume, cm^3 g^{-1}	3.8 ± 0.2^a	3.7 ± 0.2^a	4.1 ± 0.3^b	4.0 ± 0.3^b	4.0 ± 0.4^b	4.1 ± 0.3^b	4.3 ± 0.3^c	4.5 ± 0.2^d	4.0 ± 0.3^b	3.1 ± 0.3^e
Shape stability indicator, H : D	0.46 ± 0.05^a	0.44 ± 0.04^a	0.45 ± 0.05^a	0.45 ± 0.05^a	0.45 ± 0.06^a	0.48 ± 0.04^b	0.50 ± 0.05^b	0.49 ± 0.05^b	0.43 ± 0.02^a	0.40 ± 0.05^b

a–k = Means ± SD within the same row with different lowercase superscript letters are significantly different ($P \leq 0.05$).

Compressibility decrease rand crumbs become more firm, hard and crumbly during the bread storage, due to the changes in starch fractions (Gray & Bemiller, 2003). These changes are mainly ascribed to gradual amylopectin retrogradation, which occurs during the five storage days. Generally, the best crumbs compressibility (which characterizes the freshness of the product) had bread made with sourdough and improver (III). The crumb compressibility of bread made using the one-stage technology (I) was better than that of bread with prefermented dough. This can be explained by a more uniform thin-walled structure of porosity resulting from longer dough fermentation. Bread prepared in one stage had a large specific volume and better porosity. This means that a more uniform thin-walled porosity of the crumb contributes to a better preservation of the freshness of the bread.

It should be noted that the bread prepared with long fermentation had a distinct pleasant aroma in comparison with the loaves prepared with sourdough and improver, despite the fact that the sourdough contributes to the formation of taste and smell (Jensen et al., 2011; Onishi et al., 2011; Plessas et al., 2011; Demin et al., 2013, Savkina et al., 2019). The fermentation was too short, and the sourdough dosage was too small for enough aromatic substances accumulating in the dough (Birch et al., 2013; Verheyen, 2016).

Table 5. Impact of the flour amylolytic activity (FN) and type of bread making methods on physico-chemical indicators of the bread

Wheat flour (FN, s)	Way of bread making*	Acidity, degrees N	Porosity, %	Specific volume, $cm^3\ g^{-1}$	Compres-sibility, units of the device	Shape stability indicator, H : D
F1 (352 s)	IV	1.0 ± 0.2^a	83 ± 2^a	3.8 ± 0.3^a	47 ± 3^a	0.46 ± 0.05^a
	V	1.0 ± 0.2^a	81 ± 2^b	3.7 ± 0.2^a	45 ± 2^b	0.42 ± 0.04^b
	VI	0.8 ± 0.1^b	83 ± 2^a	3.5 ± 0.2^b	34 ± 3^c	0.41 ± 0.03^b
		x	x	x	x	x
F3 (318 s)	IV	1.6 ± 0.2^c	85 ± 2^c	4.0 ± 0.3^c	52 ± 2^d	0.43 ± 0.03^b
	V	1.4 ± 0.2^d	80 ± 3^b	3.7 ± 0.2^a	42 ± 1^e	0.39 ± 0.05^c
	VI	1.3 ± 0.1^d	81 ± 2^b	3.4 ± 0.2^d	40 ± 2^e	0.38 ± 0.04^c
		y	x	x	x	x
F2 (304 s)	IV	1.2 ± 0.1^e	85 ± 2^c	3.9 ± 0.2^c	52 ± 3^d	0.40 ± 0.02^d
	V	1.4 ± 0.2^d	80 ± 2^b	3.7 ± 0.1^a	42 ± 2^e	0.34 ± 0.04^e
	VI	1.0 ± 0.1^a	81 ± 2^b	3.8 ± 0.2^a	42 ± 3^e	0.38 ± 0.05^c
		z	x	x	x	x
F4 (282 s)	IV	1.7 ± 0.2^f	83 ± 3^a	3.8 ± 0.1^a	48 ± 2^f	0.42 ± 0.05^b
	V	1.6 ± 0.2^g	81 ± 2^b	3.5 ± 0.2^b	35 ± 2^c	0.41 ± 0.04^b
	VI	1.5 ± 0.2^d	81 ± 2^b	3.2 ± 0.2^d	30 ± 2^g	0.43 ± 0.05^b
		xy	x	x	x	x
F5 (216 s)	IV	1.8 ± 0.1^f	82 ± 2^b	3.8 ± 0.1^a	52 ± 3^d	0.42 ± 0.04^b
	V	1.7 ± 0.2^f	81 ± 2^b	3.7 ± 0.2^a	42 ± 2^e	0.42 ± 0.03^b
	VI	1.6 ± 0.2^g	82 ± 3^d	3.8 ± 0.2^a	40 ± 3^e	0.48 ± 0.04^a
		xy	x	x	x	x

*according Table 3: a–g = Means ± SD within the same column with different lowercase superscript letters denote significantly different among dough types ($P \leq 0.05$) while letters 'x-z' denote significantly different values among type of flours (Tukey's test, $p < 0.05$).

The influence of the flour amylolytic activity on sugar content and physico-chemical quality indicators in wheat bread was studied. In this study, the dough was prepared in a according to the formulation in Table 3. Flour samples with different amylolytic activity (FN) were used.

Flour amylolytic activity did not significantly affect the bread quality, except the acidity. The bread making way had a greater influence (Table 5). The data obtained confirm that there is no significant correlation between falling number and any of the quality traits, except acidity (Newberry et al., 2018).

The higher was the flour amylolytic activity, the higher was the bread acidity. This may be due to the fact that as a result of amylolysis, own sugar (maltose) was formed from flour starch. Maltose participated in lactic acid and alcohol fermentation in the dough and acidity was accumulated (Table 5). Porosity was higher when one-stage bread making way (IV) was used. This may be due to the dough fermentation propagation. In a two-stage process, the prefermented dough fermented without sugar. When the dough is kneaded using prefermented dough and sugar, it was fermented for only 1 hour. And with method IV, the dough with was fermented sugar for 3 hours. Sugar was fermented by yeast and was involved in the formation of gas to loosen the dough.

It was found that the higher was the flour amylolytic activity, the higher was the sugar content in bread (Fig. 1). Sugar exceeded the norm in any bread making way when flour had high amylolytic activity (FN 216 s).
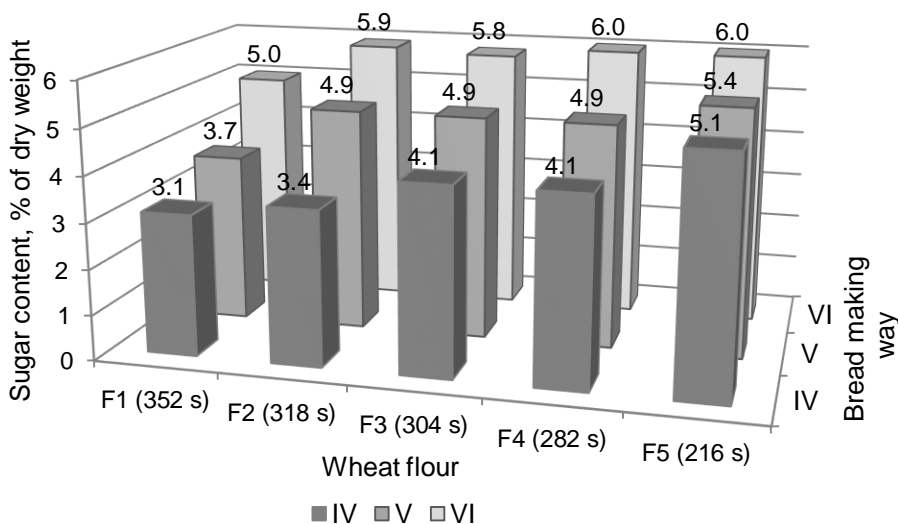
Figure 1. Sugar content in bread from different types of flour.

When bread was made using fungal amylase and flour possessing FN equal or lower then 318 s, the sugar content didn't depend on the flour amylolytic activity. Significant influence on the sugar content had only flour possessed FN more than 318 s.

When the amylolytic enzyme (VI) was used, the sugar content in all samples, regardless of the flour amylolytic activity, corresponded to the upper limit of the norm or exceeded it.

Sugar content was at the lower level of standard norm when bread was made by traditional way (IV) without fungal amylase and using flour possessing low amylolytic activity (falling number 352 s). It can be assumed that if the flour FN will be greater (amylolytic activity will be worse), then the sugar content will be below normal. A decrease in sugar content will lead to a decrease in the nutritional value of bread.

Flour possessing a FN greater than 216 s may be recommended for bread made with sugar in the recipe. If the flour has a higher amylolytic activity (the FN is equal to or less than 216 s), then it is not recommended to use an improver with amylolytic activity.

The influence of amylolytic activity of wheat flour on the sugar content and bread quality was established in bread prepared without sugar in the formulation (Table 6, Fig. 2). When sugar (sucrose) was excluded from the (Fig. 2), sugar was still found in bread. It was because the flour enzymes (for way IV, V) and fungal amylase (for VI way) affected the sugar generation in the dough from the starch (Van der Maarel et al., 2002; Cauvain & Young, 2007; Codina & Leahu, 2009; Struyf, N. et al., 2016).

Table 6. Impact of the flour amylolytic activity (FN) and type of bread making methods on physico-chemical indicators of the bread made without sucrose

Wheat flour (Falling number, s)	Way of bread making*	Acidity, degrees N	Porosity, %	Specific volume, $cm^3 \ g^{-1}$	Compressi- bility, units of the device	Shape stability indicator, H : D
F1 (352 s)	IV	1.0 ± 0.2^a	77 ± 3^a	3.2 ± 0.3^a	36 ± 2^a	0.32 ± 0.05^a
	V	0.8 ± 0.1^b	82 ± 3^b	3.4 ± 0.3^b	32 ± 2^b	0.38 ± 0.05^b
	VI	0.8 ± 0.1^b	79 ± 2^c	3.5 ± 0.2^b	34 ± 3^a	0.40 ± 0.04^c
		x	x	x	x	x
F3 (318 s)	IV	1.4 ± 0.3^c	84 ± 2^d	4.0 ± 0.3^c	49 ± 4^c	0.42 ± 0.03^d
	V	1.4 ± 0.2^c	80 ± 2^a	3.4 ± 0.2^b	39 ± 4^d	0.40 ± 0.04^c
	VI	1.4 ± 0.2^c	80 ± 2^a	3.0 ± 0.3^d	31 ± 3^b	0.44 ± 0.03^e
		y	y	y	y	y
F2 (304 s)	IV	1.2 ± 0.1^d	83 ± 3^d	3.1 ± 0.2^a	42 ± 4^d	0.35 ± 0.04^f
	V	1.0 ± 0.2^a	81 ± 1^a	3.1 ± 0.1^d	36 ± 2^a	0.47 ± 0.05^g
	VI	1.0 ± 0.1^a	82 ± 2^b	3.5 ± 0.2^d	33 ± 3^a	0.35 ± 0.03^f
		z	y	x	z	z
F4 (282 s)	IV	1.5 ± 0.2^c	82 ± 3^b	4.0 ± 0.3^c	48 ± 3^c	0.42 ± 0.04^d
	V	1.5 ± 0.2^c	80 ± 2^a	3.2 ± 0.3^d	37 ± 4^d	0.43 ± 0.05^c
	VI	1.6 ± 0.3^e	79 ± 2^c	2.9 ± 0.2^d	30 ± 3^b	0.42 ± 0.03^d
		xy	y	y	y	xy
F5 (216 s)	IV	1.8 ± 0.2^f	83 ± 2^d	4.1 ± 0.3^c	48 ± 4^c	0.43 ± 0.04^e
	V	1.7 ± 0.3^f	81 ± 2^a	3.3 ± 0.3^b	39 ± 3^d	0.42 ± 0.03^d
	VI	2.0 ± 0.2^g	80 ± 2^a	3.3 ± 0.2^b	31 ± 2^b	0.48 ± 0.02^g
		yx	y	z	y	yx

*according Table 3: a–g = Means ± SD within the same column with different lowercase superscript letters denote significantly different among dough types ($P \leq 0.05$) while letters 'x-z' denote significantly different values among type of flours (Tukey's test, $p < 0.05$).

Deterioration in the specific volume, shape stability and compressibility of the crumb for bread made using flour F1 and F3 was noted. These flour samples had a high falling number and low ash content. Therefore, we can conclude that when sugar was

excluded from the recipe, there was not enough flour own sugar (maltose) for fermentation. And since yeast needs minerals for life and fermentation (Kurtzman, 2011), the low ash content, together with the low maltose content in the flour, led to a deterioration in fermentation and a decrease in specific volume and compressibility.
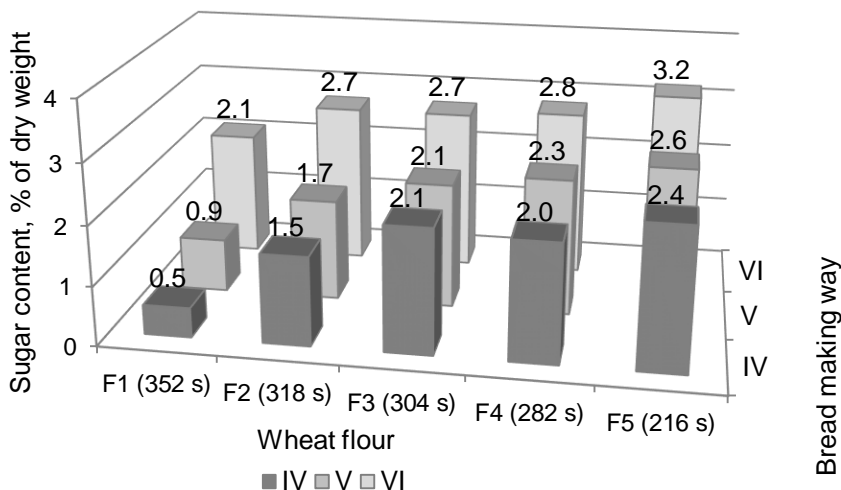

Figure 2. Sugar content in bread prepared without sugar in the formulation from different types of flour.

But it should be noted, that al parameters met the requirements of the standard for this bread (State Standard of the Russian Federation GOST 27844–88, 1988).

When flour with a falling number of 216 s (F5) and fungal amylase was used, the sugar content was at the lower limit of the standard norm for this type of bread (Fig. 2). Any wheat flour possessing low FN or high α-amylase levels is automatically considered a poor bread wheat (Newberry et al., 2018). But obtained data allows to assume that when the flour possessing FN below 216 s will be used with fungal amylase in the dough, the bread 'Nareznoi' can be prepared without sugar in the recipe. And in this case, the bread quality will not deteriorate, and the sugar content will meet the requirements of the standard.

CONCLUSIONS

The sugar content in the bread met the requirements of regulatory documents when dough was prepared in the traditional way. The sugar content exceeded the permitted amount in 1.25 times when bread was made by accelerated way using improver. This may be due to the starch amylolytic hydrolysis.

It was shown that sucrose dosage in formulation had not a significant effect on the porosity, specific volume and compressibility of the crumb during 5 days of bread storage when bread was made by accelerated method. The sugar content in the bread met the requirements only when the improver was completely excluded, but at the same time, the bread physico-chemical indicators (porosity, specific volume and crumb compressibility) were worse.

The bread making way had a greater influence on bread physico-chemical indicators (except the acidity) than FN of flour.

When sugar was excluded from formulation, sugar was still found in bread, because the flour enzymes activity and fungal amylase affected the sugar generation in the dough from the starch.

The deterioration in the specific volume, shape stability and compressibility of the crumb was observed when flour possessed a high FN and low ash content. It may be due to the deterioration of fermentation because of lower feed content for yeast.

In order to meet normative documentation requirements in sugar content, it is necessary to take into account the flour amylolytic activity (falling number) and bread making method.

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Quality and safety problems of sports nutrition products

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Abstract. The purpose of this work was to study the quality and safety of some sports nutrition products. The objects of study were sports nutrition products: protein bars of 'ProteinBar' (Russia) and 'Bombbar' (Russia); capsule forms of dietary supplements ω–3, ω–6, ω–9 firms 'Sportline' (Russia), 'Multipower' (Germany) and 'Maxler' (USA). According to the research results, the normalized safety indicators of the fat component of the studied products for sports nutrition (acid number and peroxide) are within acceptable values. The standardized safety indicators of the fat component do not fully reflect the safety requirements for the fat component of sports nutrition products, since there are no standards for the most important indicators of fat safety – the content of secondary oxidation products – copolymers insoluble in petroleum ether and epoxides. The results obtained in the course of the work showed that in almost all of the studied samples are content of epoxides (7.5–47.6 g^{-1}) and secondary oxidation products – 1% or more.

Key words: carcinogenic effect, epoxides, fat component, oxidation products, safety, sports nutrition, quality.

INTRODUCTION

Throughout the world, the development of evidence–based food technology and its production is given particular importance. A lot of research is being done in this perspective (Bazarnova et al., 2019; Laukaleja & Kruma, 2019). Separately, the production of the sports nutrition product line is singled out. Sports nutrition is a constantly developing area in which hundreds of scientific papers on various topics related to the studies of effectiveness of minor components of food are published annually (Kerksick et al., 2018; McGinnis et al., 2019); studying the influence of the main nutrients on optimizing the athlete's functional status, increasing its performance, accelerating post–load recovery, increasing the 'buffer' capacity of the body when performing extreme loads (Stendig-Lindberg et al., 1987; Nieman et al., 2002; Bilsborough & Crowe, 2003; Tambovtseva & Soshnikov, 2018; Tiller et al., 2019). A lot of papers are dedicated to the development of methods and rules for rehydrating the body in the training process and recovery phase (Cox & Clarke, 2014; De Oliveira et al., 2014; Leckey et al., 2017; O'Malley et al., 2017). There is a wide range of studies of polyunsaturated fatty acids

(Sinitskaya et al., 2018), their particular importance in sports nutrition has been stated in connection with their participation in the synthesis of eicosanoids, which are the precursors of prostaglandins and leukotrienes which inhibit the development of atherosclerosis, regulate inflammatory processes in the body, reduce cholesterol, have cardioprotective and antiarrhythmic and oncoprotective properties (Benito et al., 2006; Apte et al., 2013). However, there are practically no papers dedicated to the study of their safety. Nevertheless, they can be a potential source of danger to the body, due to the fact that during the oxidation process, oxidation products, such as glycidol esters (Irwin et al., 1996; Appe et al., 2013; Aasa et al., 2019), epoxides (Li et al., 2015; Wang et al., 2017), secondary oxidation products insoluble in petroleum ether are formed, they are teratogenic, mutagenic and carcinogenic effects (Rogozin et al., 2018). Some scientific papers (Maughan et al., 2018; Baltazar-Martins et al., 2019) have shown that athletes do not have a clear idea of the effects and risks of food, do not know the platforms for testing their safety and quality, and rely on trainers, family and teammates when choosing products, rather than objective scientific data. In this regard, the safety aspect of sports nutrition products remains open. Thus, the study of the quality and safety of products for sports nutrition throughout the "product lifecycle" is of high importance.

The aim of this paper was to study the most significant safety indicators of polyunsaturated fatty acids and the fat component of some sports nutrition products.

MATERIALS AND METHODS

The objects of study were sports nutrition products.

Protein bars of the firms 'ProteinBar' (Russia) and 'Bombbar' (Russia). Technology which involves the preparation of used dry raw materials, its dosing, mixing with sugar invert syrup, fat component, vitamin premix, rolling, calibration, molding, molding and packaging. Capsule forms of dietary supplements ω–3, ω–6, ω–9 by 'Sportline' (Russia), 'Multipower' (Germany) and 'Maxler' (USA). There are a variety of technologies for the production of encapsulated materials: spray drying to obtain capsules, spray freezing, inclusion in matrix, co–extrusion, encapsulation in a gel, encapsulation in a fluidized bed. Vegetable oils for encapsulations are pre–cleaned. The manufacturer as follows indicates the storage conditions of the test products: 'Keep away from direct sunlight, in a dry, cool place, inaccessible to children'. The sell–by–date of the control sample is 12 months. Studies of the fat component were carried out on the 3rd month of product storage. The research took place at a given shelf life for the purpose of modeling assessment of real safety when consuming sports nutrition products. Subsequently, research results in the dynamics of storage of this product line will be reflected.

Research methods

Generally accepted regulated methods that normalize the safety and quality of fats were used. Fat was extracted from the finished product by the extraction–weight method according to GOST R 54053-2010 'Methods for determining the mass fraction of fat'. The following indicators were studied:

– concentration of epoxides by reaction with concentrated phosphoric acid (Stopskij et al., 1986);

− acid number was determined by standardized methods to GOST R 52110-2003. Vegetable oils. Methods for determination of acid value;

− the peroxide number of the fat component of the product was determined by the iodometric method;

− content of oxidation products (copolymers) insoluble in petroleum ether (CIPE) according to the method described in the manual of the All–Russian Research Institute of Fats with the following additions.

A mixture of oxidation products insoluble in petroleum ether was dissolved in hot ethyl alcohol, concentrated to a small volume in a water bath, transferred quantitatively to a 50 cm^3 volumetric flask, made up to the mark with ethyl alcohol and mixed well. The resulting solution containing CIPE from 5 g of the fat fraction was divided into 2 equal parts. One part was dried to constant weight, and then calcined to determine the ash, as described in the main method. According to the data obtained, the content of CIPE in oxidized fat was calculated in % of the fat mass, given that the treated solution contains CIPE of 2.5 g of fat. In the second part of the solution, the fatty acid content was determined by titration with a 0.1 mol dm^{-3} alkali solution. According to the obtained results, the content of CIPE in oxidized fat in mmol kg^{-1} of fat was calculated (Rzhehina & Sergeeva, 1967).

Statistical processing was carried out in accordance with GOST R (national standard) R ISO 5725-2-2002. The arithmetic average of the results of two parallel determinations made under the conditions of repeatability (convergence) is taken as the measurement result. The mathematical processing of the results included the determination of the arithmetic mean X, the standard deviation of the individual result (standard deviation) S and the standard deviation of the arithmetic mean (standard error) SX. The measurement results xi, the absolute deviation of which from the arithmetic mean x exceeded $3S$, were discarded as unreliable. Accuracy of the measurements (absolute error Δx) was determined with reliability $\alpha = 0.95$. The correlation dependence was calculated using the Microsoft Excel program.

RESULTS AND DISCUSSION

Results

Table 1 presents the nutritional data of the test samples indicated on the package. The content of the fat component in capsules is 25% of the daily intake for 'Sportline', 20.8% of the daily intake for 'Maxler' and 21.8% of the daily intake for 'Multipower'. The remaining PUFAs come with the main diet of athletes. The content of protein components in the bars is 32.1% of the daily intake for 'ProteinBar', 27.7% of the daily intake for 'Bombbar'.

The qualitative composition of sports nutrition products indicated on the label is shown in Table 2. Based on the labeling data, the analyzed 'ProteinBar' bars contain flavors, soya lecithin, soy protein isolate, glycerin. These components can have a negative effect on the human body, causing allergic reactions, exacerbation of the course of chronic diseases of the gastrointestinal tract. In addition, soy proteins are characterized by an unbalanced amino acid composition, they are imperfect.

In 'Bombbar' bars, the content of whey protein isolate and milk protein concentrate shows that the product contains complete protein and the product containing it does not cause any concern, except for milk protein intolerance.

The origin of fatty acids in capsules, such as eicosapentaenoic acid, docosahexanoic acid, alpha–linolenic acid, oleic acid, is not indicated.

Table 1. Nutritional value of control samples of bars and capsules

Product	Proteins, g	% of the daily intake	Fats, g	% of the daily intake	Carbohydrates, g	Energy value, kcal
'ProteinBar' (per 100 g)	35.0	32.1	20.0		39.0	476.0
'Bombbar' (per 100 g)	30.2	27.7	13.1		8.0	262.0
'Multipower' Capsules (in 3 capsules / daily rate)	0		3.1	21.8	2.0	15.0
'Sportline' (in 3 capsules / daily rate)	0		3.6	25.0	3.0	39.0
'Maxler' (in 3 capsules / daily rate)	0		3.0	20.8	0.6	10.0

Table 2. The qualitative composition of sports nutrition products indicated on the label

Product name				
Protein bars		Encapsulated Fats		
'ProteinBar'	'Bombbar'	'Sportline'	'Maxler'	'Multipower'
isolate, whole–grain cereals that do not need cooking, cocoa butter, fructose, caramel starch syrup, banana, emulsifier, chocolate icing, cocoa powder, soy lecithin, cream flavor.	protein complex (whey protein isolate, milk protein concentrate), sweetener maltitol, inulin, almond flour, cocoa butter substitute, milk fat, water–retaining agent, glycerin, milk powder, dried pear, yogurt powder, cinnamon, pear flavoring, soya lecithin, lemon acid, contains sugars of natural origin.	ω–3 – 340 mg, ω–6 – 42 mg, ω–9 – 271 mg, fish oil, unrefined linseed oil, unrefined olive oil, eicosapentaenoic acid, docosahexanoic acid, alpha–linolenic acid, oleic acid.	ω–3 – 540 mg, ω–6 – 150 mg, ω–9 – 210 mg, organic linseed oil, alpha–linolenic acid, oleic acid, linolenic acid, gelatin, glycerin, locust bean gum, purified water.	ω–3 – 340 mg, ω–6 – 42 mg, ω–9 – 271 mg, fish oil, unrefined linseed oil, unrefined olive oil, eicosapentaenoic acid, docosahexanoic acid, alpha–linolenic acid, oleic acid.

The results of the study of the fat component of protein bars for sports nutrition and ω–3, ω–6, ω–9 capsules are shown in Table 3.

In the study of the fat component, the content of free fatty acids was determined. This indicator in the Russian Federation is controlled by technical regulations in fat products. The content of free fatty acids value, mmol kg^{-1} of oil: the permissible level is 10.7–71.3 (according to the regulatory documentation of the Russian Federation).

Table 3. The results of the study of the fat component of protein bars for sports nutrition and ω–3, ω 6, ω–9 capsules

Sample name	Acid number, mEq0 kg^{-1}	Peroxide value, Mmol 0.5O kg^{-1}	Mass fraction of oxidation products insoluble in petroleum ether,%	The content of epoxides, mmol kg^{-1}
'ProteinBar'	33.9	0.9	0.4	47.6
'Bombbar'	30.03	2.0	0.3	13.6
Capsules ω–3, ω–6, ω–9 'Sportline'	7.1	4.1	1.1	8.5
Capsules ω–3, ω–6, ω–9 'Maxler'	30.3	4.0	1.0	9.6
'Multipower' Capsules	33.9	4.8	0.7	9.8

The peroxide value in the fat component in the product indicates the presence of peroxides, the primary oxidation products. Peroxides are toxic to the body and therefore are standardized by TR TS 021/2011 and TR TS 024/2011 on safety. Peroxides, when ingested in large quantities, cause necrosis of the cells of the gastrointestinal tract, the development of cancer cells. The peroxide value, mEq0 kg^{-1}: permissible level – no more than 10.0.

The content of copolymers insoluble in petroleum ether (CIPE, %): acceptable level is not more than 1.0 (Onishchenko, 2001). This indicator does not exceed the norm, but these values correspond to frying fats subjected to repeated thermal effects; for native fats, this indicator is not standardized.

The content of epoxides, mmol kg^{-1}: the permissible level is not more than 60–65 mmol kg^{-1} (Rogozin et al., 2018). The amount of epoxides of the Bombbar bar is 13.6 mmol kg^{-1}, which corresponds to the acceptable level of food safety. The amount of epoxides of the 'ProteinBar' bar is 47.6 mmol kg^{-1}, which is approximately twice the permissible level.

Specified parameters for the fat component of ω–3, ω–6, ω–9 capsules for sports nutrition have indicators that do not exceed the regulated norm.

The mass fraction of oxidation products in 'Maxler' products reached the maximum permissible value, while 'Sportline' products exceeded the indicators by 0.1%. In 'Multipower' capsules, all the studied parameters, excluding mass fraction of oxidation products, are normal. Thus, despite the encapsulated form, the fat component is susceptible to oxidation. Given the fact that athletes of many sports (Ronald, 2013; Kashapov & Kashapov, 2019) consume sports nutrition products daily, there is a need for further refinement studies of the safety of the lipid fraction.

Discussion

In scientific periodicals, there are no works describing the study of the safety of the fat component in the composition of products for sports nutrition, but there is a significant number of works concerning the safety of fats in general.

Hydroperoxides and free radicals are highly reactive compounds and undergo various complex transformations with the formation of other free radicals (reflects the value of the acid number) and secondary oxidation products – including peroxide radicals, epoxy compounds, as well as polymerization and polycondensation products.

In the study of the fat component, we determined the content of free fatty acids, which, although they do not affect the safety of the product, indicate a certain degree of oxidation – if their content is too high, then saponification of fats occurs, respectively, a change in the organoleptic characteristics of the product takes place. This information correlates with the data obtained by scientists of various countries conducting studies of the fat component of meat, fish products (Martınez-Yusta et al., 2014), drinks (Ajmal et al., 2019); vegetable oils from various raw materials (Shahidi, 2005). These are not stable components, they react with each other or with other oxidation products, and their content varies. In food compositions, free radicals cause impulsive oxidation and produce undesirable biochemical compounds that are associated with the development of a number of diseases in biological systems (Bienkiewicz et al., 2019). Excessive intake and limited elimination of free radicals, as a rule, lead to oxidative stress, triggers a chain reaction that violates the integrity of cells, leads to their damage or death, initiates oncological processes (Chesnokova et al., 2006; Dong et al., 2019), the development of arthritis and neurodegenerative diseases (Spagnuolo et al., 2015).

The amount of 'ProteinBar' bar epoxides is 47.6 mmol kg^{-1}. The number of epoxides is not standardized in any of the normative and technical documents, but scientific studies have proved that these products are toxic.

Researchers from different countries have confirmed the toxic effect of epoxides on various body systems. For many of them, toxicological studies have shown cyto– and genotoxicity, carcinogenicity and mutagenicity, this may become a precursor of leukotoxins, which can cause leukocyte degeneration and necrosis, disrupt the endocrine system, block the estrous cycle in rats, and stimulate the proliferation of human breast cancer cells (Greene et al., 2000; Gulyaeva et al., 2000). Epoxidation serves as the activation of many known chemical carcinogens; chemical carcinogenesis is a multi–stage process, which includes initiation, promotion and progression (Makarenko et al., 2018). Epoxides trigger initiation – the first critical and irreversible step in carcinogenesis, requiring covalent binding of the carcinogen to DNA. They are inserted into the nucleotide and change the body's genome, that is, they are mutagenic products (Gulyaeva et al., 2000).

One of the secondary products formed during the oxidation of oils and fats is polyoxyacids. The quantitative content of these compounds is defined as the total content of oxidation products insoluble in petroleum ether. According to literature data, a close correlation between the content of fat breakdown products insoluble in petroleum ether and the effect of oxidized fats on the body is known (Simakova et al., 2015). Negative effects on blood and biochemical parameters of fat metabolism were recorded in experiments on animals (Simakova et al., 2014). Oxidation products insoluble in petroleum ether have a proven toxic effect and have a carcinogenic and carcinogenic effect (Goicoechea & Guillen, 2010). Our experimental data obtained in the study of some indicators of the safety of the fat component of the selected sports nutrition products are highly correlated with the experimental data on the study of fat safety described in the literature.

CONCLUSION

Global guidelines for food producers are the Codex Alimentarius Standards, which are developed on the basis of the latest scientific research with the assistance of independent international organizations and specialized consulting risk assessment

centers established by WHO and WTO. This code regulates the following indicators in edible oils: acid value (refined fats and oils 0.6 mg KOH g^{-1} which is equivalent 10.7 mmol kg^{-1}; natural fats and oils and cold–pressed fats and oils 4.0 mg KOH g^{-1} is equivalent 10.7 mmol kg^{-1}), peroxide values (natural oil and cold pressed fats and oils up to 15 milliequivalents of active oxygen kg^{-1} of oil; other fats and oils up to 10 milliequivalents of active oxygen kg^{-1} of oil); determination of volatile substances at 105 ºC (0.2% m m^{-1}), insoluble impurities (0.05% m m^{-1}),soap content (0.005% m m^{-1}), iron (refined fats and oils 2.5 mg kg^{-1}; natural fats and oils, and cold–pressed fats and oils 5.0 mg kg^{-1}) and copper (refined fats and oils 0.1 mg kg^{-1}; natural fats and oils, and cold–pressed fats and oils 0.4 mg kg^{-1}). The main documents of the Russian Federation that regulate the safety of sports products are the Technical Regulations of the Customs Union (TR/TS 021/2012 and 027/2011), which give standards for microbiological indicators, the content of toxic substances and radionuclides. The safety indicators of the fat component regulated by the technical regulations of the Customs Union do not fully reflect the safety requirements for the fat component of sports nutrition products, as there are no standards for the most important indicators of fat safety – the content of secondary oxidation products – CIPE and epoxides. In our opinion, the studies shave shown the need for further study of the safety indicators of the fat component in sports nutrition products during storage. The data obtained indicate the need for a critical assessment of production technology, products for sports nutrition, as well as the feasibility of amending the regulatory documentation in order to further control the safety of the fat component.

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Acceptance of low-sugar yoghurt among Latvian teenagers

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Abstract. Over a thousand year history, yoghurt has become one of a widely consumed product in the world. Its reputation as a healthy food has been undermined recently by concerns over the high sugar content. The majority of consumers expects and prefers yoghurts to be sweet. However, governments across Europe are calling for significant cuts in the amount of added sugar used in yoghurt production. The aim of the study was to evaluate the acceptance of low-sugar yoghurt produced by different commercial β-galactosidases by teenagers. Standardised milk with fat content 2.0% (SC Tukuma piens) was pasteurized at $95 \pm 1\,°C$ 5 min, cooled down till $43 \pm 1\,°C$ and fermented with β-galactosidase and starter YC-X11 (Chr. Hansen, Denmark) and fermented till pH 4.50 ± 0.20. Different commercial β-galactosidases: Nola™ Fit 5500, Ha-Lactase 5200 (Chr. Hansen, Denmark), GODO-YNL2 (Danisco, Denmark) and BrennZyme (Brenntag PolskaSp, Poland) were used. Fermented samples were gently mixed and cooled down till $6 \pm 1\,°C$ and 5% (w/w) of sugar was added to each sample. Sensory evaluation of the yoghurt's samples was performed by teenagers (14–18 years, $n = 50$) at Aizputes Secondary School (Latvia). Lactose and monosaccharides concentration prior to sugar addition was detected by HPLC (Shimadzu LC 20 Prominence, Japan).
The lactose hydrolysis into glucose and galactose by the use of β-galactosidase helps to increase sweetness through an occurrence of natural sugars in milk. During sensory evaluation, teenagers admitted the yoghurt with reduced sugar as sweet, significantly sweeter ($P < 0.05$) was yoghurt sample with Nola™ Fit 5500. The results demonstrated that it is possible to reduce sugar in yoghurt production and to gain consumer acceptance through the occurrence of glucose and galactose, but it is problematic to offer lactose-free or reduced lactose products to consumers without lactose intolerance.

Key words: acceptance, sweetness, yoghurt, β-galactosidase.

INTRODUCTION

Over a thousand-year history, yoghurt has become one of a widely consumed product in the world. Its reputation as a healthy food has been undermined recently by concerns over the high sugar content. Many studies updated the relationship between sugar consumption and health concerns: obesity (Li et al., 2015), dental caries, type 2 diabetes, and cardiovascular diseases (Lluch et al., 2017). In Europe (Ginder Coupez et al., 2017) and in Latvia the majority of consumers expects and prefers yoghurts to be sweet. Dairy products, comparing to other food products, are the leaders in high sugar concentration, still children and teenagers prefer sweet yoghurts and dairy desserts

(Azaïs-Braesco & Maillot, 2017). In Latvia sucrose concentration in yoghurts ranged from 6 to 25 g per 100 g. Dairy manufacturers often add sugar in higher doses to compensate for the taste of post acidification, finding the balance between sweet and sour. An average citizen, according to Latvia Statistics, consumes 80 grams of sugar per day. This is almost three times higher than 25 grams recommended by WHO (Guideline…, 2015). However, governments across Europe are calling for significant cuts in the amount of added sugar used in yoghurt production. The Dietary Guidelines in Latvia has recommended to reduce sucrose concentration in yoghurt till 5% for children at educational institutions (Cabinet Regulation 172..., 2012). Sugar reduction negatively influences yoghurt consumption (Lluch et al., 2017), and this goal can be achieved differently: replacing by steviol glycosides (Li et al., 2015), by adding natural sweetening flavours, the use of oligo-fructose and applying lactose hydrolysis (Rogenhofer & Hauß, 2019), as well as novel starters (Chr. Hansen, 2019). The application of commercial enzymes in yoghurt production allows create sweetness hydrolysing lactose and yielding sweeter monosaccharides (McCain et al., 2018). Relative sweetness of lactose is low (16), comparing to monosaccharides: glucose (70) (Tiefenbacher, 2017) and galactose (65) (Hobbs, 2009), therefore splitting of lactose to monosaccharides sums up higher product sweetness. The aim of the study was to evaluate the acceptance of low-sugar yoghurt produced by different commercial β–galactosidases by teenagers.

MATERIALS AND METHODS

Yoghurt preparation

Standardised milk with 2% of fat and 4.6% of lactose (SC Tukuma piens) was pasteurized at 95 ± 1 °C for 5 min, cooled down till 43 ± 1 °C and fermented adding β-galactosidase (see Table 1); after one hour of hydrolysis freeze-dried starter YC-X11 (*Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus fermentum*), was added 0.02% (w/w) according to the producer recommendations and fermented until pH 4.50 ± 0.20. The optimum pH range for all β-galactosidases is 5.4–8.0 (Table 1) and decreasing substrate pH cause enzyme inactivation (Zhou & Chen, 2001). Yoghurt samples were gently mixed and cooled down till 6 ± 1 °C and 5% (w/w) of sugar (Dansukker, Denmark) was added to each sample. A control sample was prepared according to the previously described technology without the usage of enzyme.

Different commercial food grade β-galactosidases: Nola™ Fit 5500, Ha-Lactase 5200, GODO-YNL2 and BrennZyme DairyLact were used (Table 1). Their characteristics are summarized in Table 1.

Carbohydrates detection

Lactose and monosaccharides concentration was detected by HPLC.

Sample preparation: samples were transferred into 2 mL test tube and deproteinized by adding 50 µL of hydrochloric acid (10% w/w) to 1 mL of sample. Afterwards, samples were centrifuged at 10,000 rpm for 5 min and the supernatant was used for analysis (Samanidou et al., 2017).

Approximately 1.5 mL of filtered whey sample was placed into sampler vials and sealed for HPLC analysis. HPLC (Prominence HPLC system, Shimadzu LC-20, Torrance, CA, USA) was used for sugar determination, refractive index detector

RID-10A; column SUPELCOSILLC-NH2 (250 mm × 4.6 mm) 5 µm column; 35 °C temperature; gradient mobile phase acetonitrile: deionized water (80:20); volume of the injected sample: 10 µL; flow rate: 1.0 mL min^{-1} (Zolnere et al., 2018).

Table 1. β-galactosidases characteristics

Parameter	Enzyme[1,2]			
	BrennZyme DairyLact	Nola™Fit 5500	Ha-Lactase 5200	GODO-YNL2
Specific activity[3]	5400 oNPGU g^{-1}	5500 BLU g^{-1}	5200 NLU g^{-1}	5000 NLU g^{-1}
Recommended dose	0.5–1.2 mL L^{-1}	0.1–3.3 g L^{-1}	0.5–1.0 mL L^{-1}	0.5–1.0 mL L^{-1}
Amount added in the study	0.5 ml L^{-1}			
Temperature range, °C	5–45	35–50	35–45	4–45
Optimal pH	6.0–7.0	5.4–7.0	6.5–8.0	7.5–8.0
Origin	*Saccharomyces marxianus var. lactis*	*Bacillus licheniformis*	*Kluyveromyces lactis*	*Kluyveromyces lactis*
Producer	BrenntagPolskaSp., Poland	Chr. Hansen, Denmark		Danisco, Denmark

[1]Zolnere & Ciprovica, 2017; [2]Zolnere et al., 2018; [3]Units of β-galactosidase activity are defined differently by each manufacturer; BLU – bifido lactase units; NLU – neutral lactase units; oNPGU – o-nitrophenyl-β-D-galactoside units.

Sweetness and reduction of carbohydrates calculation

Sweetness of yoghurt (1) before sucrose addition and carbohydrates reduction (2) were calculated according the following equitation's:

$$Sw = galactose \times 65 + glucose \times 72 + lactose \times 16 \qquad (1)$$

where Sw – sweetness of yoghurt; *galactose* – concentration of galactose (%); *glucose* – concentration of glucose (%); *lactose* – concentration of lactose (%).

$$R = 100 - \frac{(galactose_y + glucose_y + lactose_y) \times 100}{lactose_m} \qquad (2)$$

where R – reduction of carbohydrates (%); $galactose_y$ – concentration of galactose in yoghurt (%); $glucose_y$ – concentration of glucose in yoghurt (%); $lactose_y$ – concentration of lactose in yoghurt (%); $lactose_m$ – concentration of lactose in milk (%).

Questionnaire

Teenagers completed the questionnaire, which consisted of: demographic questions, information about lactose intolerance, yoghurt consumption frequency, teenagers' preference in yoghurt choice.

Sensory evaluation

Sensory evaluation was performed by 50 teenagers as potential low-sugar yoghurt consumers, at the age from 14 to 18, (60% – female, 40% – male), 13% of teenagers have lactose intolerance.

The sensory evaluation has been performed at Aizputes Secondary School (Latvia) in a class with individual tables, the temperature of yoghurt was 16 ± 1 °C.

Before sensory evaluation panellists were instructed about evaluation procedure. Panellists received approximately 30 mL of low-sugar yoghurt sample at 16 ± 1 °C temperature in cups with volume 50 mL, coded with three-digit random numbers. Warm tea was provided to panellists for cleansing their palates between samples.

Five-point JAR (just-about right) method was used to determine mean overall liking of yoghurt samples sweetness. In the five-point JAR scale: 1 – not sweet, 2 – somewhat too weak sweetness, 3 – just about right sweetness, 4 – too much sweet, 5 – somewhat too much sweet.

Seven-point hedonic scale (ISO 11136:2014) was used to determine yoghurt overall liking. In the seven-point scale – 1 – dislike very much; 4 – neithr neither like nor dislike, 7 – like very much.

The data collection and statistical interpretation of data were processed with FIZZ Aquistion Ver.2.51 software (Biosystemes, France).

Data processing

All results were reported as mean ± standard deviation. Statistical analyses were performed using analysis of variance (ANOVA) and mean concentrations of parameters were carried out by Duncan's multiple range test. Differences were considered statistically significant with a confidence interval of $P < 0.05$.

RESULTS AND DISCUSSION

Our previous research (Zolnere & Ciprovica, 2019) confirmed that various concentrations of monosaccharides were obtained after lactose hydrolysis with different β-galactosidases. The lactose hydrolysis data are shown in Table 2.

Contradictory results were obtained with galactose, a significantly higher ($P < 0.05$) concentration of galactose was established in sample with GODO-YNL2, and significantly lower in sample with HA-Lactase 5200.

Lactose splitting by β-galactosidase provides higher carbohydrates (mostly lactose and glucose) reduction rate during fermentation (see Table 2) compared to control. In all samples carbohydrates reduction during hydrolysis and fermentation was significantly higher ($P < 0.05$).

Table 2. Characteristics of fermentation products, their values

Parameters	Control	Nola™Fit 5500	Ha-Lactase 5200	GODO-YNL2	BrennZyme
glucose, g L^{-1}	2.59 ± 0.05^a	22.20 ± 0.12^c	21.00 ± 0.11^{bc}	18.45 ± 0.11^b	21.60 ± 0.08^{bc}
galactose, g L^{-1}	2.84 ± 0.05^a	13.90 ± 0.10^c	11.70 ± 0.05^b	14.00 ± 0.09^d	13.10 ± 0.10^c
lactose, g L^{-1}	33.01 ± 0.11^d	0.007 ± 0.001^a	0.09 ± 0.001^c	0.04 ± 0.001^b	0.03 ± 0.001^b
reduction of carbo-hydrates during fermentation, %	16.4^a	21.5^b	28.7^d	29.4^d	24.5^c
pH	4.7 ± 0.05^c	4.4 ± 0.05^a	4.5 ± 0.05^b	4.5 ± 0.05^b	4.5 ± 0.05^b
sweetness	0.18^a	0.68^c	0.66^b	0.67^{bc}	0.68^c

Means marked with a different letter are significantly different ($P < 0.05$).

Lactose hydrolysis into glucose and galactose helps to increase sweetness through an occurrence of natural sugars in milk (Li et al., 2015; Ohlsson et al., 2017; Zolnere & Ciprovica, 2017; Cheng et al., 2020). Sweetness of analysed samples could be compared to 2.5% of sucrose addition (Harju et al., 2012). However, the sweetness of the product depends on the hydrolysis degree, variety and concentration of occurred monosaccharides and oligosaccharides.

A significantly higher sweetness ($P < 0.05$) was established in all samples with β-galactosidase. A higher sweetness was established in samples with Nola^{TM} Fit 5500 and BrennZyme.

According to the findings of different studies (Rosolen et al., 2015), β-galactosidase application in yoghurt production decreases fermentation time, this fact was proved by pH set during the current study. In all samples with enzyme pH was lower, comparing to the control one (see Table 2). The availability of a higher proportion of easily fermented monosaccharides (mainly glucose) promotes faster growth rate of lactic acid bacteria (Schmidt et al., 2016).

The focus of the questionnaire is to analyse teenagers' preferences in yoghurt choice, yoghurt dietary pattern, sensory properties, etc.

Figure 1. Teenagers' preferences in yoghurt market.

According to the questionnaire results a significant number of teenagers (94%) eat yoghurt at least once per week (59%), 93% of respondents has preferences in sweet strawberry yoghurt consumption (see Fig. 1). Li and co-authors (2015) reported similar results, their study has shown a positive correlation between increased sugar concentration in flavoured milk and dairy product consumption among adults and children. The overall questionnaire results show that teenagers are highly aware of yoghurt consumption. Yoghurt producers should be oriented, particularly on teenagers, by improving product composition, as well as reducing added sugar and lower lactose concentration.

Sensory evaluation of yoghurt samples was done and the results are presented in Fig. 2.

During samples evaluation teenagers admitted low–sugar yoghurt as sweet, significantly sweeter ($P < 0.05$) was yoghurt sample with Nola™ Fit 5500 and BrennZyme (see Fig. 2, a.). The sensory evaluation showed the same tendency in sweetness of yoghurts as in analysed monosaccharides composition and concentration data.

The degree of sweetness correlates ($r = 0.84$) with overall liking degree of yoghurt. The current research results proved the statement that teenagers admitted yoghurt with higher sweetness (Nola™ Fit 5500) as the best one, followed by yoghurt with BrennZyme (see Fig. 2, b).
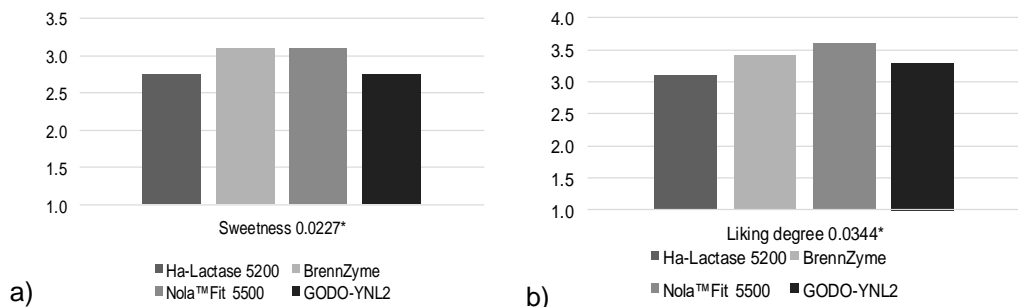
Figure 2. Yoghurt sensory evaluation results: a) Mean overall sweetness liking (JAR); b) Overall liking degree (Seven-point hedonic scale).

*Not significant at 5%.

Taking into account teenagers' preference, obtained results can be evaluated positively, still the largest part of teenagers (85%) has evaluated yoghurts with Nola™ Fit 5500 and BrennZyme as sweet enough. Sugar can be replaced only to a certain extent and the strategical objective should not be a reduction of consumer expectations in terms of sweetness intensity (Rogenhofer & Hauß, 2019).

Lactose hydrolysis could be a possible tool for sugar reduction in yoghurt production from a technological and nutritional point of view. Yoghurt with hydrolysed lactose is sweeter, faster fermented and more readily absorbed from intestine (Rosolen et al., 2015). However, the question: 'Could producers offer such product to lactose tolerant consumers?' is still debatable. The solutions could be the commercial starter, which allows to increase sweetness throught higher hydrolysis of lactose, yielding glucose comparing to classical yoghurt starters.

CONCLUSIONS

Sweetness of yoghurt can be regulated by commercial β-galactosidases. A significantly higher ($P < 0.05$) concentration of glucose was determined in yoghurt with Nola™ Fit 5500 and BrennZyme, which reflected in a higher (calculated and sensory evaluated) sweetness of the product. The results demonstrated that low-sugar yoghurt has gained consumer acceptance as sweet enough.

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