



Physico-Chemical and Antioxidant Properties of Skimmed Varenets (Slavic Baked Milk Yogurt) Mixed with Enzyme-Modified Potato Starches

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Abstract

The present studies have examined the effect of potato starches treated with amylase *B.licheformis* (Bl) (laboratory sample) or *Amylosubtilin*® (AM) (Berdsk Factory of Biological Preparations (now: Sibbiofarm), Russia) in different concentrations on the quality of Slavic skim milk drink *Varenets* made from baked milk. The baked milk is milk heated to 98 °C for 3 hours, it has a sweet aroma and taste and creamy hue. The presence of enzyme-modified potato starches (Bl or AM) has been found to promote the activation of lactic acid fermentation, the accumulation of exopolysaccharides, and such stabilized products have a higher viscosity, a lower percentage of syneresis compared to the control sample. The antioxidant capacity of the Varenets samples after fermentation was evaluated by two assays: analysis of radical capture activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH •); and the ability to restore Fe + 3 (Iron Reduction Antioxidant Ability Assay, FRAP). All Varenets samples showed different values for DPPH, FRAP assays depending on the starches used. The Introduction of pre-fermented starches into defatted jam promotes formation of enhanced antioxidant properties of milk product. The use of starches modified with enzymes improves sensory characteristics, in particular, as a fat imitator, forms full taste of the drink.



Article History

Received: 22 July 2020

Accepted: 23 December 2020

Keywords

Antioxidant Properties;
Enzyme-Modified
Potato Starches;
Organoleptic;
Rheological;
Varenets.

Introduction

Varenets is a traditional dairy product of Slavic (Russian) cuisine, in particular the Urals and Siberia. Very similar drinks can be found in other countries. For example, in Ukraine - ryazhenka, in Georgia

such a product is called mazoni, in Egypt - leben, and in Bulgaria and among the Turkic peoples - kатык. For its preparation, traditional starters for yogurt with the bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp bulgaricus*. are used.

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Doi: 10.12944/CRNFSJ.9.1.09

A feature of *Varenets* is the use of melted cow's milk for its preparation. Melted milk is milk heated to 98°C for 3 hours, as a result, the fermented milk product obtained from such milk has a creamy tint and a specific pleasant smell of heating. Traditionally, the fat content of *Varenets* ranges from 2.5 to 4%, such a drink has a thick consistency, a full saturated taste. Due to changes in the lifestyle and diet of a large part of the population, the problem of the growth of non-communicable diseases, in particular obesity, diabetes, cardiovascular disease and cancer, is acute. In 2014, the results of a systematic analysis of the dynamics of overweight and obesity in 1980-2013 of adults and children were published, which showed that 39% of adults over 18 years of age were overweight, 13% were obese. The prevalence of obesity among children and adolescents is increasing in both developed and developing countries.¹

This research reported estimates for 188 countries, 21 regions, and development status (developed or developing). One of the main problems associated with excessive consumption, including fats, is the development of obesity and, as a result, an increased risk of type 2 diabetes, coronary heart disease, hypertension, gallbladder pathologies, some types of cancer, dyslipidemia and other insulin resistance.²

This situation contributes to the development of low-calorie, low-fat or low-fat food. Such foods should

meet the dietary needs of obese people, people at risk of cardiovascular disease, diabetics and people following a diet for weight loss, and encourage them to buy such products.^{3, 4} Here are forecasts that the need for low-fat and low-fat food will steadily steadily.⁵

In this regard, it is relevant to obtain the *Varenets* fermented milk product popular in Russia, the fat content of which is reduced to a minimum value of less than 0.5%. However, the rheological and physical characteristics of defatted dairy products are reduced because the reduction in the proportion of fat changes its mechanical and sensory characteristics.⁶ Thermostatic fat-free yogurt has a low hardness and a high degree of syneresis or serum separation on the surface.⁷

One approach that can be implemented in the technology of making *Varenets* is the use of stabilizers and thickeners of a polysaccharide nature. Carboxymethyl cellulose, gelatin, various starches can be used as stabilizer.^{8, 9} Starch is widely used in the production of yoghurts and other dairy products, their presence improves the texture, gives the defatted product a better and more complete flavor of.^{10, 11} potato starch is also used for this purpose.^{12, 13} The purpose of this work was to identify the influence of enzymatically modified potato starches (EMS) on the physicochemical, rheological, sensory and antioxidant properties of fat-free *Varenets*.

Table 1: Code of EMS and Varenets samples13

Varenets samples with Starches	Amylase activity (U/g starch)	Amylosubtilin (g/100 ml reaction mixture)	Amylase <i>B. licheniformis</i> (ml/100 ml reaction mixture)
Control (without starch)	-	-	-
Native (potato starch)	-	-	-
AM-0.05	0.415	0.01	-
AM-0.1	0.83	0.0201	-
AM-0.5	4.15	0.1005	-
AM-1	8.3	0.201	-
BI-0.05	0.415	-	0.05
BI-0.1	0.83	-	0.1
BI-0.5	4.15	-	0.5
BI-1	8.3	-	1

Material and methods

Materials

The cow's skimmed milk (fat 0.05%, Valio, Russia) was used for *Varenets*. Native and enzyme-modified potato starches (modification by *Amylosubtilin*® (AM) and Amylase of *Bacillus licheniformis* (B)) have been described by us earlier^{13,14}. Enzyme preparation of Amylase of *Bacillus licheniformis* have been described earlier¹⁴. *Amylosubtilin*® (Berdsk Factory of Biological Preparations (now: Sibbiofarm), Russia) is obtained by drying the fermentation broth upon the in-depth cultivation of *Bacillus subtilis*. These starches (Tabl.1) have been investigated by us earlier.¹⁴

Production of *Varenets*

The starches were added to the milk at a concentration of 1% and heated, followed by heating at 98 C for 3 hours to obtain melted milk, and the mixture of melted milk and starch were mixed for the even distribution of starch. Similarly, a control sample of milk without starch was heated too. Starter culture of lactic acid bacteria (*Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophiles*) was fermented at 37 C for 16 hours. The sourdough is sated (5% vol/vol.) into the control milk or milk-starch mixture after cooling at 40 C. Incubation at 40 C for 6-7 hours before reaching pH 4.5 and stabilizing at 6 degrees. After stabilization, each *Varenets* sample was evaluated for physicochemical, textural, sensory, antioxidant properties.

Physicochemical Analyses

The amount of protein, lactose (carbohydrates), content of solid substances and density were measured using InfraLUM® FT-12 (Russian Federation) as described earlier.¹³ Proteins, salts in whey were tested on the milk analyzer Clover-2M (Russia). The whey was obtained by centrifugation of samples (at 3000 g 15 min). A pH meter (HI 2216, Hanna Instruments, Germany) was used to measure the pH. The titrative acidity was evaluated by titration of the suspension (10 g yoghurt in 20 ml distilled water). The samples were boiled to remove carbon dioxide and cooled. The sample was titrated with 0.1 M sodium hydroxide (NaOH) to pink in the presence of 1% phenolphthalein as an indicator. Methods for testing the water - saving capacity (WHC)¹³ and syneresis were described by us earlier. The apparent viscosity of the *Varenets* (300 ml, at 8 C) was measured with a Brookfield concentric cylinder

viscometer (Model RV-DVIII, Inc., China) equipped with rotor No. 3 at 60 rpm. The measurements were carried out in triplicate for each treatment and the results were recorded in mPa • s.

The colour parameters (L*, a*, and b*) of the yogurt samples were measured using a colorimeter Chroma Meter (China).¹⁰

Determination of the Total Amount of Polysaccharides in *Varenets*

The isolation of exopolysaccharides and polysaccharides (total TAP polysaccharides) and their determination were carried out as described by Feldman *et al.* (2014)¹⁵ with modifications. 10-15 g of the sample was placed in a laboratory flask and boiled in a water bath at 100°C for 30 minutes. The samples were cooled and centrifuged at 4000 rpm for 30 minutes and 0.7 ml of 85% trichloroacetic acid was added to 4 ml of the supernatants. These mixtures were cooled to 4°C and centrifuged again at 8000 rpm for 10 minutes. The deposition of TAP (1 ml) from the supernatant was carried out using cold ethanol (-20°C, 3 ml). The Samples were kept in a refrigerator for 48 h and then centrifuged (4°C, 8000 g, for 10 min), the precipitate was re-dissolved in distilled water (volume is equal to the sample volumes), and the TAP was determined as described by Feldman *et al.* (2014).¹⁵

Sensory Evaluation

Varenets samples were evaluated by panelist as described by Nikitina *et al.* 2019.¹³ Sensory assessment was conducted by ten trained participants (5 men, 5 women, age 18-45 years, healthy subjects tolerant to lactose). Hedonistic scale from 1 (very dislike) to 10 (very pleasant) was used for taste, from 1 (poor) to 3 (excellent) for odour, from 1 (poor) to 10 (excellent) for texture and consistency and 1 for evaluation of yogurt appearance. Defects were also requested if found. Each participant received 30 ml of yoghurt (at 12°C) in a 100-ml glass bottle with a twisting lid.

Evaluation of Antioxidant Capacity

1 DPPH (2,2-di-phenyl-1-picryl hydrazyl) Radical Scavenging Assay

Antioxidant scavenging capacity was analyzed according to the procedure described by Brand-Williams *et al.*, 1995¹⁶, with some modifications. Samples of *Varenets* and their whey's were

previously diluted 10 times, after which 1 ml of diluted samples were mixed with 1 ml of a freshly prepared solution DPPH (0.12 mM) in ethanol. Reaction mixtures and control (2 ml; DPPH in ethanol) were incubated at room temperature in the dark for 30 minutes. The mixtures were centrifuged for 2 minutes, 10,000 rpm. The absorbance of the supernatant was measured at λ_{max} 517 nm using a spectrophotometer and DPPH16 radical capture activity was calculated¹⁶.

Ferric Reducing Antioxidant Power Assay (Frap)

The reduction power analysis was carried out according to the procedure described by Lertittikul *et al.*, 2007,¹⁷ with modifications. 1 ml of the test product (whey) was mixed with 1 ml of 0.2 M potassium-sodium phosphate buffer (pH 6.5) and 1 ml of 1% potassium ferricyanide. The reaction mixture was incubated for 20 minutes at 50°C, cooled, and then 1 ml of 10% trichloroacetic acid was added. The mixture was centrifuged at 2000 g for 10 minutes at room temperature. 2 ml of distilled water and 400 μ l of 0.1% FeCl₃ were added to the supernatant (2 ml). The absorption of the reaction mixture was measured at 700 nm. The reducing force was expressed as absorption at 700 nm relative to the control.

Statistical Analysis

All experimental data were obtained from at least three parallel experiments, the results of analytical determinations for each sample in three or five parallel experiments. A simple classification variance analysis was applied, and whenever it was adequate, the Tukey’s test was used to determine differences between samples. Reliability was established at $p \leq 0.05$. Data analysis was performed using GraphPad software.

Results and Discussion

Physicochemical Composition Varenets Sample

The pH and titratable acidity of the samples of *Varenets* are shown in figure 1. The pH of the control and native samples was at the level of 4.35–4.4, whereas samples with BI starches had lower pH (4.28–4.32). The lowest pH was in AM samples. Titratable acidity reflects dependence, the lower the pH, the higher the acidity. The exception is sample AM-1. The introduction of enzyme-modified starch obtained using *Amylosubtilin* stimulates acidity growth during lactose fermentation. Possibly the starch, that has undergone preliminary hydrolysis acts as an additional source of energy.

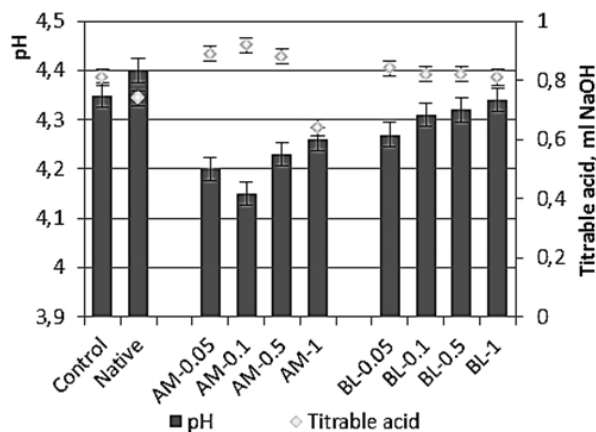


Fig. 1: pH measurements and titratable acidity of non-fat *Varenets* sample with added different starches: Control (without starch), Native (potato starch), AM-0.05, AM-0.1, AM-0.5, AM-1 (modification by *Amylosubtilin*®), BI-0.05, BI-0.1, BI-0.5, BI-1 (modification by Amylase of *Bacillus licheniformis*)

The chemical composition of *Varenets* is shown in Table 1. In general, there is no significant difference in the total amount of protein in the control *Varenets*

and EMS samples, however the amount of protein in the Native sample is 0.4% less than in the samples of others ($P > 0.05$). The amount of whey protein in the

AM and BI samples in total increased by about 0.2%, which may be due to the more active development of lactic acid bacteria and the manifestation of their proteolytic activity, which increases the amount of peptides as a product hydrolysis of casein. The amount of carbohydrates and the total dry matter content of the starch samples naturally increased as

a result of the addition of 2% starch carbohydrates. The increase in whey salts and beverage density was due to the addition of starch. Other studies have revealed similar trends, increased acidity and decreased pH in fermented milk beverages and yoghurts with the addition of modified corn and cassava starch.^{18,19,20}

Table 2: Physicochemical composition Varenets sample with added different starches: Control (without starch), Native (potato starch), AM-0.05, AM-0.1, AM-0.5, AM-1 (modification by *Amylosubtilin*®), BI-0.05, BI-0.1, BI-0.5, BI-1 (modification by Amylase of *Bacillus licheniformis*). The data presented is the average of three replicates with standard deviation.

Sample	Protein, %	Whey protein, %	Carbo-hydrates, %	Solids contents, %	Salts, %	Density, kg/m ³
Control*	3.46±0.23	1.96±0.02**	4.06±0.04**	8.21±0.3**	0.69±0.01**	1033±1**
Native	3.09±0.21	2.04±0.04	6.09±0.03	10.72±0.29	0.71±0.01	1036±0.5
AM-0.05	3.50±0.32	2.3±0.04	6.06±0.07	10.87±0.44	0.77±0.01	1036±1
AM-0.1	3.48±0.25	2.25±0.06	6.06±0.07	10.84±0.39	0.76±0.01	1037±1
AM-0.5	3.47±0.24	2.26±0.03	6.06±0.06	10.83±0.34	0.76±0.01	1036±1
AM-1	3.46±0.26	2.22±0.03	6.05±0.06	10.73±0.36	0.76±0.01	1036±1
BL-0.05	3.47±0.26	2.27±0.10	6.07±0.10	10.88±0.47	0.76±0.01	1036±1
BL-0.1	3.51±0.24	2.25±0.04	6.06±0.10	10.83±0.39	0.76±0.01	1036±1
BL-0.5	3.48±0.20	2.28±0.04	6.06±0.08	10.83±0.33	0.76±0.01	1036±1
BL-1	3.52±0.35	2.27±0.04	6.06±0.08	10.88±0.48	0.76±0.01	1036±1

*(not fat Varenets), ** - indicate significant differences among groups (P ≤ 0.05).

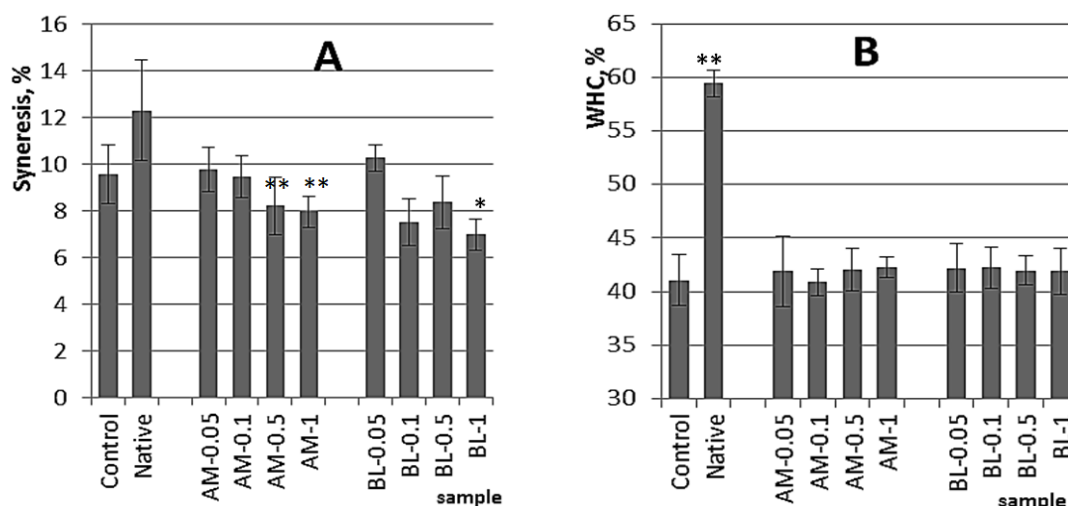


Fig. 2: Syneresis (A) and WHC (B) of non-fat Varenets samples added with different starches: Control (without starch), Native (potato starch), AM-0.05, AM-0.1, AM-0.5, AM-1 (modification by *Amylosubtilin*®), BI-0.05, BI-0.1, BI-0.5, BI-1 (modification by Amylase of *Bacillus licheniformis*). The data presented is the average of three replicates with standard deviation.

* - indicate significant differences control group and others (P ≤ 0.05), ** - indicate significant differences native group and others (P ≤ 0.05).

Syneresis and WHC

The increase of the dry residue due to the addition of starch should have a positive effect on the textural characteristics of *Varenets*. It was found that the introduction of enzyme-modified starches AM-0,5, AM-1, BI-0,1, BI-0,5, BI-1 positively affects the level of syneresis of samples (Figure 2A). The native starch sample had the highest syneresis. A long chain molecule of the native starch could inhibit the close association of casein, and a more open structure could be expected (Schmidt *et al.*, 2001). Such a positive effect of EMC can be due to their lower viscosity, increased water absorption capacity (WAS) and solubility at room temperature (WSI), as well as morphological rearrangements, loosening of starch granules¹⁴, respectively, an increase in the total contact area with dairy components. The behavior of syneresis observed in this study coincides with that of fermented milk beverages and starch-added yoghurts.²⁰

The WHC of the control sample and the EMS samples were at the same level, whereas native

starch increased this capacity in the milk clot by 18% (Figure 2B). This appears to be due to the high gelling capacity of native potato starch after heating and, accordingly, the greater ability to retain water in the matrix after heat treatment. Native starch has a high swelling capacity at high temperatures, which provides high WHC.

Apparent Viscosity

The viscosity of the starch-added samples was significantly higher than the control (Fig. 3). The viscosity of *Varenets* with EMC was higher than that of native *Varenets* starch, especially when using starches AM-0,5, AM-1, BI-1 in *Varenets* technology. The mechanism for increasing viscosity and, as a result, the textures of fermented milk beverages varies depending on the degree of hydrolysis of EMS. Some of the BL samples had low viscosity and syneresis, the starch samples had a higher water absorption capacity¹⁴ than native starch. This property provides a better ability to bind water in the syneresis test when the mechanical stress is low.

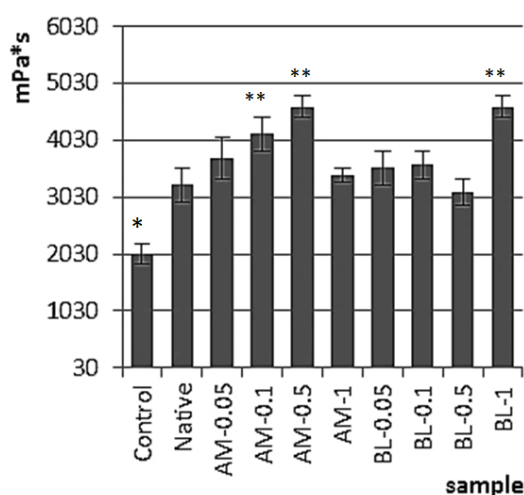


Fig. 3: Viscosity of non-fat *Varenets* sample with different potato starches: Control (without starch), Native (potato starch), AM-0.05, AM-0.1, AM-0.5, AM-1 (modification by *Amylosubtilin*®), BI-0.05, BI-0.1, BI-0.5, BI-1 (modification by *Amylase of Bacillus licheniformis*). The data presented is the average of three replicates with standard deviation.

* - indicate significant differences control group and others ($P \leq 0.05$). ** - indicate significant differences native group and others ($P \leq 0.05$).

Modified starches are generally neutral stabilizers that do not interact with proteins in pH-dependent processes, but improve the consistency and

texture of the beverage by increasing the viscosity of the aqueous phase of the system.^{21,22} Although the viscosity of the native starch gel is higher,¹⁴

the viscosity of the fermented product with native starch is lower than with EMS, this may be due to the looser and micelle-like structure of the EMS gel in milk and its ability to form more homogeneous matrices. An increase in the concentration of the enzyme used to produce starch makes it possible to obtain starch with a lower molecular weight. This structure of partially hydrolyzed starch seems to allow the starch to react more effectively with milk proteins, especially during a 3-hour heating process. *Amylosubtilin* and *B.licheniformis* amylase have been used for modification in the form of complex preparations having little activity, such as beta-amylase, cellulase, protease, etc.¹⁴ For this reason starches with different structure and characteristics have been obtained.

Total Amount of Polysaccharides in Varenets

Exopolysaccharides (EPS) are important metabolites of LAB. They can be emitted into the environment as EPS mucus or adhere to the bacterial surface, forming capsule EPS. EPS dairy products typically

act as natural and safe thickeners, emulsifiers or stabilizers, resulting in a significant improvement in texture.^{23,24} In the case of skimmed dairy products, natural EPS may not be sufficient to create the desired texture. The addition of starch to the Russian defatted *Varenets* resulted in an increase in extractable polysaccharides, the difference between the control and the native sample was 16 mg/g of product (Fig. 4). For EMS samples, the increase in TAP total concentration was 20-25 mg/g of the product. The largest amount of TAP was found in all BI and AM-1 samples. Apparently, EMS appears to act as an additional substrate or stimulating agent for the synthesis of polysaccharides by lactic acid bacteria.

Increased synthesis of EPS positively affects viscosity and syneresis, EMS further hydrophilizes casein aggregates. As a result the probability of phase division at which the size of the areas of the dispersive environment reducing sample durability decreased.

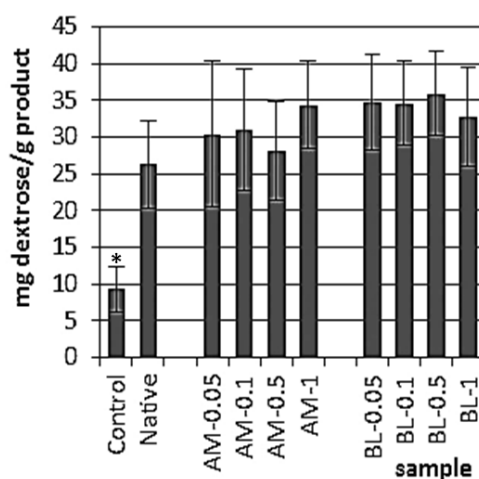


Fig. 4: TAP of non-fat Varenets sample with different starches: Control (without starch), Native (potato starch), AM-0.05, AM-0.1, AM-0.5, AM-1 (modification by *Amylosubtilin*®), BI-0.05, BI-0.1, BI-0.5, BI-1 (modification by Amylase of *Bacillus licheniformis*). The data presented is the average of three replicates with standard deviation.

* - indicate significant differences control group and others (P ≤ 0.05).

Effect of Ems on Sensory Characteristics and Color

Table 3 shows the results of the study of the sensory properties of *Varenets* (taste, appearance, texture, aroma, and in general). Most dairy consumers are

aware of the positive effects of low-fat yogurt, but the texture and taste are not always satisfactory¹⁸. In this regard, low-fat or fat-free dairy products are less popular among consumers due to the loss of rheological and sensory properties, as well as an

increase in syneresis²⁵. In the present study, EMS had a positive effect on the sensory evaluation of fat-free *Varenets* samples. The native starch sample was the least preferred in appearance; the remaining starch samples did not differ from the control. When the aroma was tested, the most intense pleasant smell of heating in combination with fermented milk was in samples AM-0.1 and BI-1, in the Native sample the aroma was unimpressed, without an intense taste of lactic acid. When evaluating the texture, it was found that the smoothest, homogeneous and viscous consistency is characteristic of samples AM-0,1, AM-0,5, as well as B1-0,5, B1-1.

The control sample was less viscous and viscous, with a less pronounced heating aroma. The average taste score of samples with EMS was higher than that of the control sample; *Varenets* AM-0,1, AM-0,5 and БЛ-0,5, БЛ-1 had high taste qualities. The native sample had a taste of starch and was poorly lubricated on the palate, since it had a ragged consistency. During heating milk, the melanoidin formation reaction occurs, as a result of which the milk acquires a cream tint and a specific taste. After fermentation with lactic acid bacteria, the taste intensity of the Maillard reaction products decreased due to acidic taste.

Table 3: Sensory scores and color measurements of non-fat *Varenets* sample added with different EMS: Control (without starch), Native (potato starch), AM-0.05, AM-0.1, AM-0.5, AM-1 (modification by *Amylosubtilin*®), BI-0.05, BI-0.1, BI-0.5, BI-1 (modification by Amylase of *Bacillus licheniformis*)

Sample	Appearance (0-1)	Odour (1-3)	Texture (1-10)	Taste (1-10)	Overall	Color		
						L ¹	a ¹	b ¹
Control	1±0	1.6±0.6	5.8±0.8	5.4±0.6	13.8±1.9	71.23±1.98	3.26±2.54	19.37±2.68
Native	0±0	1.0±0.7	4±0.7	4.6±0.6	9.6±1.9	52.77±2.11*	5.12±1.22	31.05±2.20*
AM-0.05	1±0	1.8±0.5	6.6±0.6	5.8±0.5	15.2±1.4	65.64±3.44	5.11±1.08	25.14±2.45
AM-0.1	1±0	2±0	8.2±0.5	7.8±0.5	19±0.9	59.94±2.41	5.09±1.52	24.73±2.14
AM-0.5	1±0	1.6±0.6	8±0.7	7.4±0.6	18±1.8	66.92±4.23	4.84±2.02	26.13±2.54
AM-1	1±0	1.8±0.5	7.2±0.5	6.8±0.5	16.8±1.3	66.38±4.21	4.65±1.10	25.81±3.02
BL-0.05	1±0	1.6±0.6	6.8±0.5	7±0	16.4±1	67.69±3.98	4.61±1.08	25.78±3.24
BL-0.1	1±0	1.6±0.6	7.8±0.5	7.2±0.8	17.6±1.8	64.30±1.98	4.87±0.88	14.84±1.24
BL-0.5	1±0	1.8±0.5	8.2±0.5	7.2±0.5	18.2±1.3	65.88±2.12	4.83±2.02	26.35±2.80
BL-1	1±0	2±0	8.6±0.6	8.2±0.5	19.8±1.0	68.62±2.14	4.56±1.82	25.44±2.78

¹L, darkness-lightness (0–100); a, greenness-redness (–60 – +60); b, blueness-yellowness (–60 – +60).
* - indicate significant differences control group and others (P ≤ 0.05).

Lactic fermentation did not affect color characteristics, but the presence of starch slightly changed the color characteristics of *Varenets* (Table 3). Compared to the control sample, *Varenets* with starch was darker, the native sample had the greatest darkening, enzymatically modified starches did not lead to such intense darkening. Studies of starch thermal stability have shown higher resistance to EMS than native starch (data not shown). This may cause more intense staining of native starch *Varenets* because a 3-hour heating period resulted in partial decomposition of native starch and release of glucose to form reactive milk proteins. In AM and

B1 samples, indices a and b are more shifted to the red and yellow sides, respectively, than in the control sample. The Maillard reaction when EMS is added to milk is less intense than when native starch is added. From the point of view of the technological process, in order to prevent a more intense color of the *Varenets*, it is possible to reduce the heating time, which will reduce economic costs.

Effect of EMS on Antioxidant Capacity

The radical capture activity of DPPH indicates the ability of antioxidants to donate hydrogen or electrons, resulting in more stable species²⁶ The B1

samples had the greatest potential for binding free radicals (Fig. 5A); in these samples, the serum also had high DPPH-activity. The Native sample showed the least activity. Whey of AM samples in 3 out of 4 cases and native whey showed comparable DPPH activity with control.

The ability to reduce Fe^{3+} in Fe^{2+} was higher than that of the fermented milk product with EMS (Fig. 5B), while the control and native showed weaker strength. The addition of starches to *Varenets* resulted in an increase in the regenerative capacity of the product whey. The greatest reducing potential was shown by BL *Varenets* starch whey.

The high antioxidant potential of lactic acid bacteria has been reported in many sources,^{27,28} the ability to bind peroxides and free radicals is found not only in the supernatant of the culture fluid, cell-free extracts, but also in fermented dairy products, individual protein components isolated from dairy products.²⁹⁻³¹ However, studies of the role of starch in the formation of the antioxidant potential of lactic acid products are not enough. Wu *et al.*³² described that raw potato starch hydrolysates have high antioxidant activity and can be used as a food antioxidant. In our case, hydrolysis products do not play a significant role in the formation of antioxidant potential, since they are present in starch in a residual amount.

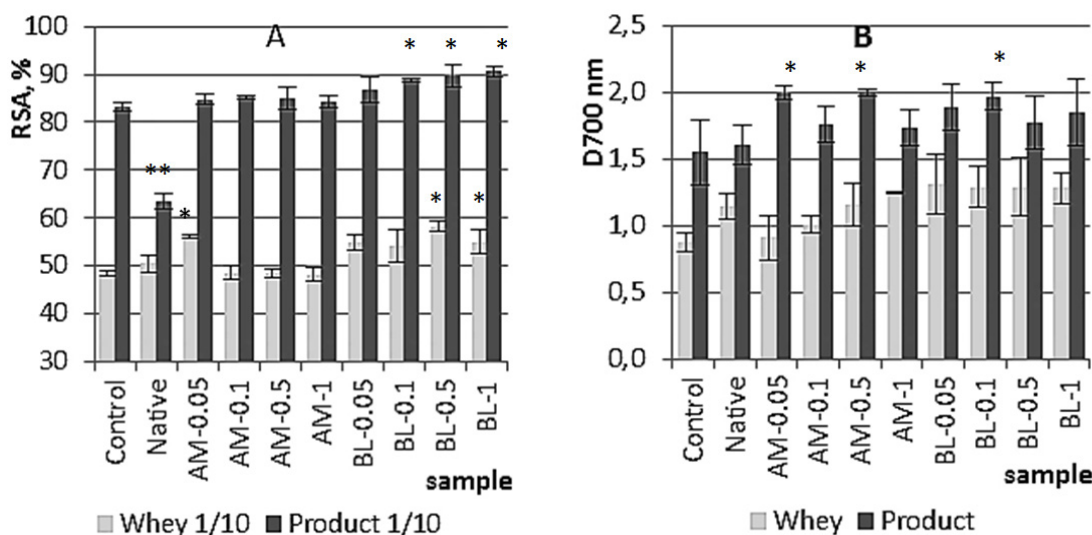


Fig. 5: DPPH scavenging activity (A) and FRAP-power (B) of whey and non-fat *Varenets* samples added with different EMS: Control (without starch), Native (potato starch), AM-0.05, AM-0.1, AM-0.5, AM-1 (modification by *Amylosubtilin*®), BI-0.05, BI-0.1, BI-0.5, BI-1 (modification by Amylase of *Bacillus licheniformis*). The data presented is the average of three replicates with standard deviation.

* - indicate significant differences control group and others ($P \leq 0.05$). ** - indicate significant differences native group and others ($P \leq 0.05$).

Starches do not have significant antioxidant potential, but partially fermented starch, as a representative of the low molecular weight polysaccharide in the composition of *Varenets*, stimulated the accumulation of lactic acid (Fig. 1), the accumulation of exopolysaccharides (Fig. 4). That is, such starch can be considered as an additional carbon source that contributes to the development of starters and the formation by lactic acid bacteria of a complex of substances with antioxidant properties.

Conclusion

The use of enzyme-modified potato starches to stabilize skimmed *Varenets* results in a higher protein level than native starch, while increasing whey protein levels. In particular, EMS, especially those treated with amylase *B. licheniformis* added to skimmed milk, do not interfere with the starting culture of *Lactobacillus delbrueckii* subsp. *bulgaricus* strain, and *Streptococcus thermophilus* fermenting lactose to lactic acid in the production of *Varenets*

stimulate the synthesis of exopolysaccharides. *Varenets* with EMC has a lower level of syneresis than the native and control sample, also due to enhanced EPS synthesis.

Under the influence of EMC, the viscosity of *Varenets* increases, the texture of the drink improves, and the ability of the milk gel to bind whey improves. EMS at a concentration of 1% can be added to the *Varenets* mixture to produce a more creamy taste of melted defatted yogurt products from baked milk. *Varenets* prepared from EMS had a higher level of antioxidants than that prepared from native starch. *Varenets* samples with BL starch showed the highest antioxidant capacity for DPPH and FRAP tests. Such starches contribute to the formation of a greater antioxidant potential in the final fermented milk

product, which increases the utility of the defatted stabilized *Varenets*. In addition, *in vivo* studies are needed to assess the possible health benefits of a *Varenets* dairy product with EMS, such as free radical damage protection.

Acknowledgment

We thank Russian Foundation for Basic Research for a financial and Kazan National Research Technological University for technical support.

Funding

The reported study was funded by RFBR, project number 20-016-00025.

Conflict of Interest

The authors declares no conflict of interest.

References

1. Ng M., Fleming T., Robinson M., *et al.* Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2014;30;384(9945):766-81. doi: 10.1016/S0140-6736(14)60460-8.
2. Ramachandran A. Diabetes and obesity—The Indian angle. *Indian Journal of Medical Research*. 2004;120:437–439.
3. Sandrou, D. K., Arvanitoyannis I. S. Low-fat/calorie foods: current state and perspectives. *Critical Reviews in Food Science and Nutrition*. 2003;40:427–447.
4. Paz N. F., Oliveira de E. G., Kairuz de M. S. N., Ramon A. N. Characterization of goat milk and potentially symbiotic non-fat yogurt. *Food Sci. Technol (Campinas)*. 2014;34(3):629-635. dx.doi.org/10.1590/1678-457x.6409.
5. Low-Fat Yogurt Market size. Analysis By Origin (Non-Organic, Organic), By Type (Flavored, Fruit, Plain), By Region (North America, Europe, Asia Pacific, Central & South America, MEA), And Segment Forecasts, 2018–2025. Trends, *Global Industry Report*. 2017. Report ID: GVR-1-68038-895-4.
6. Sandoval-Castilla O.; Lobato-Calleros C.; Aguirre-Mandujano E.; Vernon-Carter E.J. Microstructure and texture of yogurt as influenced by fat replacers. 2004;14:151–159.
7. Walstra P., Geurts T.J., Noomen A. Dairy Technology: principles of milk properties and processes. Marcel Dekker: New York, NY, 1999;533–535 pp.
8. Alakali J.S.; Okonkwo T.M.; lordye E.M. Effect of stabilizers on the physico-chemical and sensory attributes of thermized yoghurt. *Afr. J. Biotechnol*. 2008;7:158–163.
9. Pang Z., Deeth H., Yang H., Prakash S., Bansal N. Evaluation of tilapia skin gelatin as a mammalian gelatin replacer in acid milk gels and low-fat stirred yogurt. *J. Dairy Sci*. 2017;100:3436–3447. doi.org/10.3168/jds.2016-11881.
10. Kategunya R.; Sanguansri C. Physico-chemical properties of jackfruit seed starch (*Artocarpus heterophyllus*) and its application as a thickener and stabilizer in chilli sauce. *LWT-Food Sci. Technol*. 2011;44:1309–1313. doi.org/10.1016/j.lwt.2010.12.019.
11. Januario, J.G.B.; da Silva, I.C.F.; de Oliveira, A.S.; de Oliveira, J.F.; Dionísio, J.N.; Klososki S.J.; Pimentel T.C. Probiotic yoghurt flavored with organic beet with carrot, cassava, sweet potato or corn juice: Physicochemical and texture evaluation, probiotic viability and acceptance. *Int Food Res J*. 2017;24:359–366.
12. Altemimi A. B. Extraction and optimization

- of Potato Starch and Its Application as a Stabilizer in Yogurt Manufacturing. *Foods*. 2018, 7, 14; doi:10.3390/foods7020014
13. Nikitina E., Rifqi Ahmad Riyanto, Vafina A., Yurtaeva T., Tsyganov M., Ezhkova G. Effect of fermented modified potato starches to low-fat yogurt // *J Food Nutrition Research*. 2019, 7(7), 549-553. DOI: 10.12691/jfnr-7-7-10.
 14. Vafina, A., Proskurina, V., Vorobiev, V., Evtugin, V.G., Egkova, G. Nikitina, E. Physicochemical and morphological characterization of potato starch modified by bacterial amylases for food industry applications. *J Chemistry*. 2018;ID 1627540:9 pages. doi.org/10.1155/2018/1627540.
 15. Feldmane J., Ciprovica I., Semjonovs P., Linde R. The influence of fermentation temperature on the development of exopolysaccharidea in yoghurt production. "Food for Consumer Well-Being" FOODBALT. 2014. *Conference Proceedings. Jelgava*, LLU, 2014:226-270.
 16. Brand-Williams W., Cuvelier M. E., Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*. 1995;28(1):25-30. doi.org/10.1016/S0023-6438(95)80008-5/.
 17. Lertittikul W., Benjakul S., Tanaka M. Characteristics and antioxidative activity of Maillard reaction products from a porcine plasma protein-glucose model system as influenced by pH. *Food Chemistry*. 2007;100:669-677. doi.org/10.1016/j.foodchem.2005.09.085.
 18. Sahan N., Yasar K., Hayaloglu A. A. Physical chemical and flavour quality of non-fat yogurt as affected by a β -Glucan hydrocolloidal composite during storage. *J Food Hydrocolloids*. 2008;22(7):1291-1297. doi:10.1016/j.foodhyd.2007.06.010.
 19. Cui B., Lu Y., Tan C., Wang G., Li G.-H. Effect of cross-linked acetylated starch content on the structure and stability of set yoghurt. *Food Hydrocolloids*. 2014;35:576-582. dx.doi.org/10.1016/j.foodhyd.2013.07.018.
 20. Lobato-Calleros C., Ramírez-Santiago C., Vernon-Carter E. J., Alvarez-Ramirez J. Impact of native and chemically modified starches addition as fat replacers in the viscoelasticity of reduced-fat stirred yogurt. *Journal of Food Engineering*. 2014;131:110-115. http:// dx.doi.org/10.1016/j.jfoodeng.2014.01.019.
 21. Hansen P. M. T. Food hydrocolloids in the dairy industry. In Nishinari K., Doi E. (Eds.), *Food hydrocolloids*. US: Springer. 1993;211-224.
 22. Nguyen P. T. M., Kravchuk O., Bhandari B., Prakash S. Effect of different hydrocolloids on texture, rheology, tribology and sensory perception of texture and mouthfeel of low-fat pot-set yoghurt. *Food Hydrocolloids*. 2017;72:90-104. doi.org/10.1016/j.foodhyd.2017.05.035.
 23. Han X., Yang Z., Jing X. P., Yu P., Zhang Y. C., Yi H. X., Zhang L. W. Improvement of the texture of yogurt by use of exopolysaccharide producing lactic acid bacteria. *BioMed Research International*. 2016;ID7945675;6 pages. doi: 10.1155/2016/7945675.
 24. Zhang H., Ren W., Guo Q. B., Xiong Z. Q., Wang G. Q., Xia Y. J., Lai P., Yin B. X., Ai L. Z. Characterization of a yogurt-quality improving exopolysaccharide from *Streptococcus thermophilus* AR333. *Food Hydrocolloids*. 2018;81:220-228. doi.org/10.1016/j.foodhyd.2017.12.017.
 25. Nouri M., Ezzatpanah H., Abbasi S. Application of renneted skim milk as a fat mimetics in nonfat yoghurt. *Food and Nutrition Sciences*. 2011;2:541-548. doi:10.4236/fns.2011.26077.
 26. Prior R. L., Wu X., Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agricultural and Food Chemistry*. 2005;53(10):4290-4302. DOI: 10.1021/jf0502698.
 27. Ding W., Wang L., Zhang J., Ke W., Long R. Characterization of antioxidant properties of lactic acid bacteria isolated from spontaneously fermented yak milk in the Tibetan Plateau *J Functional Foods*. 2017;35:481-488. doi.org/10.1016/j.jff.2017.06.008.
 28. Del Carmen S., de LeBlanc A. de Moreno, Levit R., Azevedo V., Langella P., Bermúdez-Humarán L. G., LeBlanc J. G. Anti-cancer effect of lactic acid bacteria expressing antioxidant enzymes or IL-10 in a colorectal cancer mouse model // *International Immunopharmacology*. 2017;42:122-129. doi: 10.1016/j.intimp.2016.11.017.
 29. Ayyash M., Al-Nuaimi A. K., Al-Mahadin S., Liu S.-Q. In vitro investigation of anticancer

- and ACE-inhibiting activity, α -amylase and α -glucosidase inhibition, and antioxidant activity of camel milk fermented with camel milk probiotic: A comparative study with fermented bovine milk. *Food Chemistry*. 2018;239:588–597. DOI: 10.1016/j.foodchem.2017.06.149.
30. Moslehisad M., Ehsani M. R., Salami M., Mirdamadi S., Ezzatpanah H., Naslaji A. N., Moosavi-Movahedi A. A. The comparative assessment of ACE-inhibitory and antioxidant activities of peptide fractions obtained from fermented camel and bovine milk by *Lactobacillus rhamnosus* PTCC 1637. *International Dairy Journal*. 2013;29:82-87. doi.org/10.1016/j.idairyj.2012.10.015.
31. Abdel-Hamid M., Romeih E., Gamba R. R., Nagai E., Suzuki T., Koyanagi T., Enomoto T. The biological activity of fermented milk produced by *Lactobacillus casei* ATCC 393 during cold storage. *International Dairy Journal*. 2019;91:1-8. doi.org/10.1016/j.idairyj.2018.12.007.
32. Wu S., Lu M., Fang Y., Wu., Xu Y., Wang S. Degradation of potato starch and the antioxidant activity of the hydrolysates. *J Food Processing Preservation*. 2017; 41:e13068; 5 pages. doi.org/10.1111/jfpp.13068.