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ORIGINAL RESEARCH PAPER

QUALITY, SAFETY AND SHELF LIFE OF "RED COTTAGE CHEESE" PRODUCT

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Abstract: New recipes and production technologies for "Red Cottage Cheese" product was developed. The optimal technological conditions for the production of the given cottage cheese product were established. The product had a sour-milk, caramel taste and the smell of baked milk. The shelf life of "Red Cottage Cheese" product was determined by analyzing the dynamics of changes in organoleptic, physical, chemical, and microbiological quality indicators during its storage. The shelf life of "Kyzyl Eremsek" cottage cheese product with stevioside at a temperature of (4 ± 2 °C) was determined to be 15 days.

Keywords: *cottage cheese, natural cow's milk, organoleptic characteristics, physical and chemical characteristics, stevioside, technology*

INTRODUCTION

The most suitable basis for functional protein foods are dairy products, in particular low-fat cottage cheese and cottage cheese products. The protein contained in cottage cheese possesses a complete amino acid composition and is easily digested.
Production volumes and consumption of low-fat dairy products tends to increase both abroad and in our country. Leading foreign companies produce cheeses, cheese-like and gelled products from skim milk, buttermilk and whey, using various food flavorings. These products possess satisfactory organoleptic characteristics and a good combination of biologically valuable milk components: proteins, carbohydrates, essential amino acids, macro- and microelements. Biologically active substances in the composition make these products a good supplement in a balanced diet for people of different age groups.
The interest in cottage cheese as a national product has grown significantly in recent years, which has led to an expansion of variety and an increase in the volume of its production. Previously, consumers preferred cottage cheese products with a high fat content (15 - 26 %). Now, semi-fat and fat-free cottage cheese products are in great demand, since they are a healthier alternative due to a higher content of essential amino acids of great bioavailability [1 – 5].
The developed production technology of «Red Cottage Cheese» product with stevioside has made for creating a greater variety of cottage cheese products. This technology allows to increase protein content, reduce calorie content and increase the shelf life of the cottage cheese product.
The production technology of "Red Cottage Cheese" is rooted in the deep past of the Turkic people. "Red Cottage Cheese", with its creamy caramel taste, has long been considered a delicacy. The Turkic people cooked it by a long-term heat treatment of a mixture of whole milk and «Qatiq» in order to obtain a curd clot, and then added sugar and butter [6, 7].
The aim of this research is to develop new recipes and production technologies for «Red Cottage Cheese», as well as establishing the shelf life of the product «Red Cottage Cheese» by analyzing the dynamics of changes in organoleptic, physical, chemical, and microbiological quality indicators during storage.

MATERIAL AND METHODS

The following served as the object of the research:
– natural cow's milk – the raw material – supplied by dairy farms of Kartalinsky, Bredinsky, Troitsky, Kizilsky districts of Chelyabinsk region;
– skim milk separated from natural cow's milk, as per the State Standard GOST R 52054-2003 [8], first grade or above, 19 °T acidity or lower;
– low-fat cottage cheese as per the State Standard GOST R 52096-2003 [9];
– model samples of cottage cheese products with components in different proportions;
– prepared samples of the developed "Red Cottage Cheese" product (with stevioside, with sucrose).
Other materials for analysis such as sodium hydroxide, sulfuric acid, isoamyl alcohol, phenolphthalein indicator, the BRT Inhibitor Test kit (manufactured by Chr. Hansen)

were purchased from Closed Joint-Stock Company "SoyuzChimProm" (Chelyabinsk, Russia).

Physical and Chemical Characteristics of Raw Milk for Production of «Red Cottage Cheese» Test Samples

Dry Solids

The principle of the method for determining dry solids in milk is based on drying a sample of the test product at a constant temperature. (GOST 3626-73. Milk and milk products. Methods for determination of moisture and dry substance) [10].

Nonfat Milk Solids (MSNF)

The method is based on the determination of the mass fraction of solids in the sample (%) and the mass fraction of fat in the sample (%) and the subsequent determination of the difference between these two values. (GOST R 54761-2011. Milk and milk products. Methods for determination of dry skim dairy residue mass fraction) [11].

Fat Content

The mass fraction of fat was determined by isolating fat from the product under the action of concentrated sulfuric acid and isoamyl alcohol, followed by centrifugation and measuring the volume of the released fat in the graduated part of the butyrometer (GOST 5867-90. Milk and dairy products. Method for determination of fat) [12].

Protein Content

The mass fraction of protein was determined by measuring the mass fraction of total nitrogen using the Kjeldahl method subsequently determining the mass fraction of protein. (GOST 25179-2014. Milk and milk products. Method for determination of protein) [13].

Lactose Content

The method to determine lactose is based on the hydrolysis of lactose contained in an aqueous extract of a milk or milk product sample freed from fat and protein, in the presence of β-galactosidase, to glucose and galactose, oxidation of the galactose present in the sample (free galactose plus one formed during the hydrolysis of lactose), under the action of β-nicotinamide adenine dinucleotide in the presence of enzyme β-galactose dehydrogenase and photometric measurement of the mass fraction of the resulting β-nicotinamide adenine dinucleotide (reduced form). (GOST 34304-2017. Milk and milk products. Method for determination of lactose and galactose content) [14].

Milk Density

The areometric method was used to determine milk density. This method uses a hydrometer and involves determining the volume of the displaced liquid and the mass of the hydrometer floating in it. (GOST R 54758-2011. Milk and milk products. Methods for determination of density) [15].

Titratable Acidity

Titratable acidity was determined by neutralizing acids contained in the product with sodium hydroxide solution in the presence of a phenolphthalein indicator. (GOST 3624-92. Milk and milk products. Titrimetric methods of acidity determination) [16].

Raw Milk Safety Indicators

Determination of Residual Activity of Antibiotics in Milk

The content of antibiotics in raw milk was determined according to GOST 51600 [17]. The method was based on a change in the color of the agar medium populated with spores of *Bacillus stearothermophilus* various calidolactis. The color changed from blue to yellow in the absence of antibiotics or other inhibitory substances in the tested milk, and remained blue in the presence of antibiotics. We used the BRT Inhibitor Test kit, which included hermetically sealed tubes with agar and nutrient medium containing spores of *Bacillus stearothermophilus* various calidolactis, Brilliant Black indicator, a control solution of milk with 0.004 $\mu g \cdot g^{-1}$ of penicillin G, and a control solution of milk without any antibiotics. During the analysis, 0.1 cm^3 of the control milk solution without antibiotics was added to one of the test tubes using a disposable pipette, 0.1 cm^3 of the control milk solution with penicillin G was added to other test tubes, and 0.1 cm^3 of the analyzed milk was added to the remaining test tubes in two replications. The tubes were corked and placed in a block of temperature-controlled wells at a temperature of 65.0 ± 0.5 °C and kept for 135 ± 15 minutes until the contents at the bottom of the tubes with a control milk solution with no antibiotics turned completely yellow.

Determining Shelf Life of Cottage Cheese Samples

We studied organoleptic, microbiological, physical, and chemical characteristics of cottage cheese products during storage [18].

Microbiological Indicators of Finished Products

The number of mesophilic aerobic and facultative anaerobic microorganisms, the total bacterial contamination of milk was determined according to GOST 9225 [19].
Bacteria of *Escherichia coli* group (coliforms) were determined according to GOST R 53430 [20]; *Staphylococcus aureus* – according to GOST R 52815 [21]; pathogenic microorganisms, including salmonella, – according to GOST R 52814 [22]; yeast and mold – according to GOST 10444.12 [23].

Organoleptic Characteristics of Finished Products

The tasting of samples of the cottage cheese products under study was carried out after obtaining positive results of laboratory tests of physical, chemical, and microbiological indicators. Coded samples of the tested product of days 1, 8, 12, 15, 20, 26 of storage alongside with similar freshly produced precooled products were presented for tasting simultaneously. We evaluated the appearance, texture, color, taste, smell using a 5 point scale: 5 – very good quality, 4 – good quality, 3 – not a good quality, 2 – poor quality, 1– very poor quality.
To ensure statistical validity of the results, there were 7 independent participants not familiar with the sample codes [24, 25].

Statistical Analysis

All analyses were carried out in triplicate unless otherwise stated and the average values were calculated. The results were expressed as mean value ± standard deviation. Significant differences between mean values at significance level $p < 0.05$ were established using the One-way analysis of variance and Student`s test. Microsoft Excel version 2010 was used as the statistical analysis software.

RESULTS AND DISCUSSION

At the first stage, we studied raw natural cow's milk from dairy suppliers of Kartalinsky, Bredinsky, Troitsky, and Kizilsky districts of Chelyabinsk region. The aim was to identify physical and chemical characteristics of the product at the acceptance stage (Table 1).

Table 1. *Physical and Chemical Characteristics of Raw Milk*

Indicator	Raw Milk from Districts of Chelyabinsk Region			
	Kartalinsky	Bredinsky	Troitsky	Kizilsky
Dry solids [%]	12.50±0.03	12.55±0.02	12.42±0.01	12.29±0.02
Mass fraction of nonfat milk solids [%]	8.64±0.02	8.67±0.01	8.62±0.02	8.56±0.02
Mass fraction of fat [%]	3.83±0.01	3.88±0.01	3.81±0.01	3.73±0.01
Mass fraction of protein [%]	3.18±0.01	3.22±0.01	3.12±0.01	3.09±0.01
Lactose [%]	4.66±0.02	4.68±0.02	4.69±0.01	4.67±0.01
Density [°A]	27.2±0.01	28.4±0.13	29.2±0.15	28.1±0.12
Acidity [°T]	16.8±0.11	16.4±0,12	16.5±0.09	17.2±0.11

Note: * denotes statistically significant difference at $p < 0.05$ level

From the point of view of nutritional value, raw milk from Bredinsky district was considered the best. It had the biggest amount of dry solids – 12.55 %, and fat – 3.88 %. The biological value of milk was determined by MSNF and total protein content. Milk from Kartalinsky and Bredinsky districts had higher levels of MSNF and total protein than milk from Troitsky and Kizilsky districts – 8.64 % and 8.67 %, respectively.

To determine the residual activity of antibiotics in raw milk, No. 1 BRT Inhibitor Test kit was used. This is a ready-to-use highly sensitive qualitative test that within 2 h 15 min determines antibiotics of beta-lactam, tetracycline, sulphonamide, macrolide, aminoglycoside and other antibiotic groups, including chloramphenicol and streptomycin. This method does not involve their differentiation or quantitative measurement.

Figure 1 shows the tubes of the BRT Inhibitor Test kit with samples containing raw milk supplied by different dairy farms, taken out of thermostatically controlled wells.

It can be seen from the figure that all raw milk samples under analysis (from 1 to 4) after thermostating at a temperature of 65.0 ± 0.5 ºC for 2 h 15 min turned yellow, indicating the absence of antibiotics.

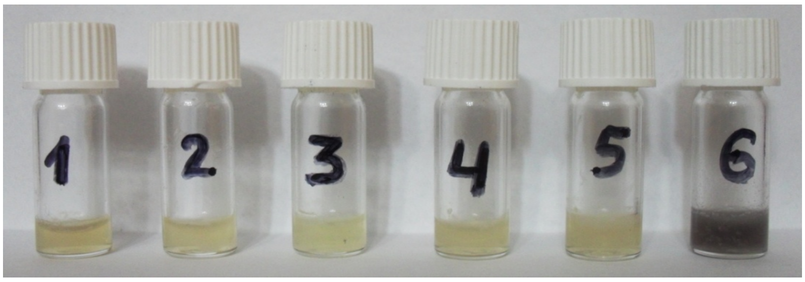

Figure 1. *Raw Milk Samples Tested for Presence of Antibiotics: 1 – Troitsky District, 2 – Kartalinsky District, 3 – Bredinsky District, 4 – Kizilsky District, 5 – Control Sample without Antibiotics, 6 – Control Sample with Penicillin G*

The findings on the presence of antibiotics in raw milk supplied by dairy farms of Kartalinsky, Bredinsky, Troitsky, and Kizilsky districts are presented in Table 2.

Table 2. *Safety Indicators of Raw Milk Samples Tested for Presence of Antibiotics*

Indicator	**Permissible levels, mg / kg (l), not more; in accordance with Federal Law dd.12.06.2008 No.88-FZ amended on 07/22/2010**	**Tested Raw Milk Samples from Districts of Chelyabinsk Region**			
		Kartalinsky	**Bredinsky**	**Troitsky**	**Kizilsky**
Antibiotics:					
Chloramphenicol	Less than 0.01	Not detected	Not detected	Not detected	Not detected
Tetracyclines	Less than 0.01 units/g	Not detected	Not detected	Not detected	Not detected
Streptomycin	Less than 0.5 units/g	Not detected	Not detected	Not detected	Not detected
Penicillin	0.01units/g	Not detected	Not detected	Not detected	Not detected
Inhibitors	Not allowed	Not detected	Not detected	Not detected	Not detected

According to the findings, natural raw milk supplied from Bredinsky district was of higher quality. Judging by the physical and chemical indicators, as well as the safety indicators, it can be classified as premium grade milk as specified in the Technical Regulation of the Customs Union “On the Safety of Milk and Dairy Products" (TR CU 033/2013) [26] and can be used as a raw material for manufacturing test samples of cottage cheese products.

Production Technology of "Red Cottage Cheese"

Based on the studies, we developed the recipe and production technology for "Red Cottage Cheese" product. We also determined the optimal technological conditions for the production of the cottage cheese product. A block diagram illustrating the production process of cottage cheese products is given in Figure 2.

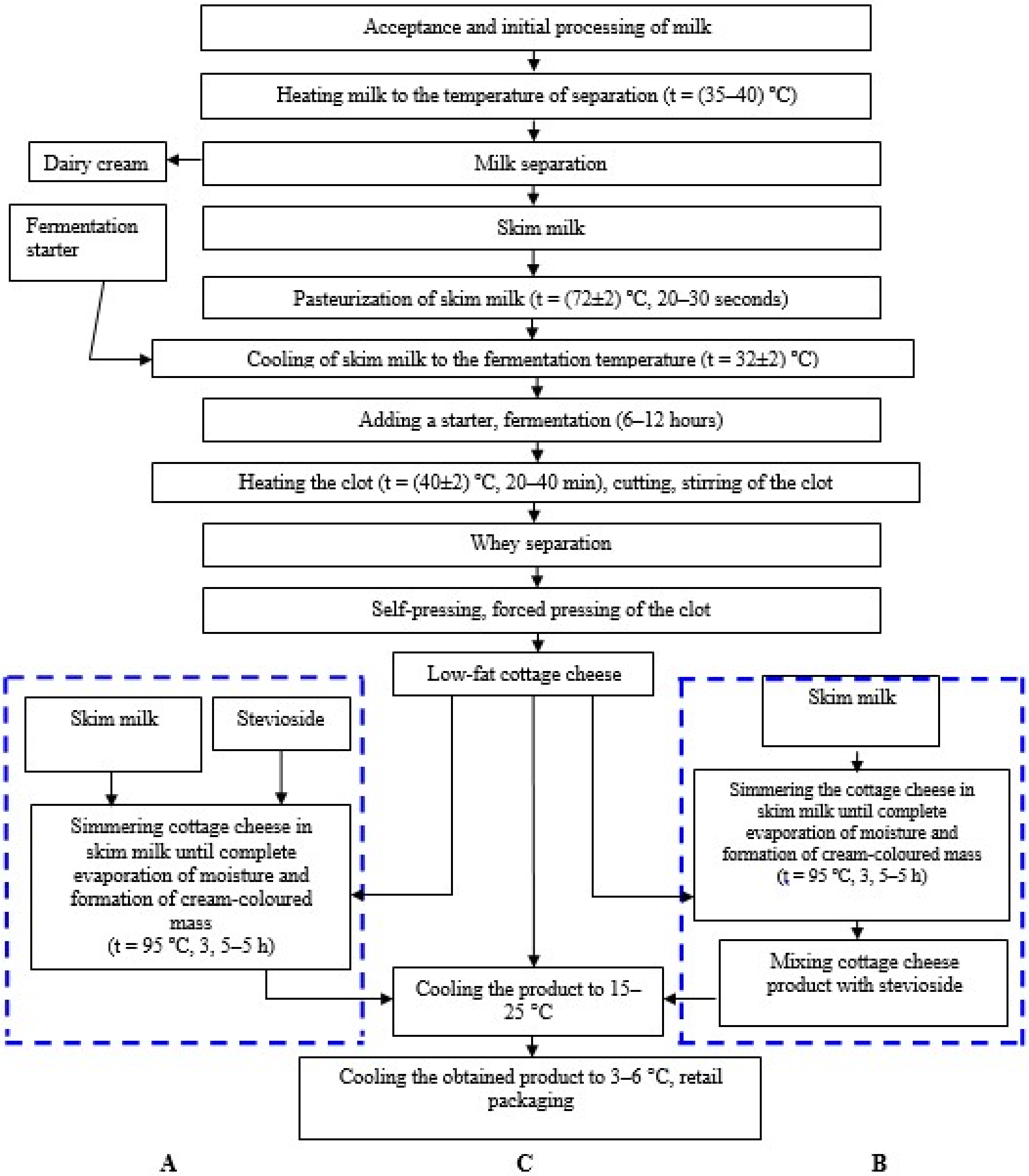

Figure 2. *Flowchart Illustrating Production of Cottage Cheese Products technology **A** – stevioside is introduced during the process of simmering of the mixture of milk and cottage cheese, technology; **B** – stevioside diluted in pasteurized whey is introduced into the finished cottage cheese product, technology; **C** – traditional technology for the production of low fat cottage cheese*

The pasteurization modes were chosen based on the modes generally accepted in the production of low-fat cottage cheese. We considered the following modes: 72 ± 2, 78 ± 2, 84 ± 2 °C, 20-30 seconds holding time.

Among the above specified pasteurization modes, we chose the one of 72 ± 2 °C with holding time of 20-30 seconds.

Direct starter cultures of two types were selected for the production of the cottage cheese product: LAT CW LT Lactina (*Lac. lactis, Lac. cremoris, Str. termophilus*) and F-DVS CC-06 (*Lactococcus lactis* subsp. cremoris and *Lactococcus lactis* subsp. Lactis). The temperatures of 28 ± 2, 32 ± 2, 36 ± 2 °C were chosen.

The analysis of the results revealed the following:

– F-DVS CC-06 was more active in comparison with LAT CW LT Lactina, it accelerated the increase in acidity in the process of fermentation by 0.64 %, but organoleptic characteristics of the curd clot deteriorated;

– increasing the fermentation temperature from 28 ± 2 to 32 ± 2 °C led to an acceleration of the increase in acidity by 3.74 %, an increase in synergistic properties of clots by 1.2 times, a decrease in the mass fraction of moisture in the curd clot by 7.6 %.

– increasing the fermentation temperature higher than 32 ± 2 °C led to the deterioration of organoleptic characteristics of the curd clot, while the temperature lower than 32 ± 2 °C slowed down the fermentation process by 1.5 hours.

Thus, the recommended starter culture is LAT CW LT Lactina, fermentation temperature 32 ± 2 °C.

We studied the effect of the clot processing parameters on the quality of the cottage cheese product. To state the end of the milk fermentation process and the value of active acidity when cutting the clot, test samples were fermented to the following active acidity values: 4.86 ± 0.2; 4.80 ± 0.2; 4.74 ± 0.2; 4.70 ± 0.2; 4.64 ± 0.2 *p*H units. The optimum active acidity value of the clot during cutting was established to be 4.70–4.74 *p*H units.

We also studied the modes of the simmering of the obtained low-fat cottage cheese in skim milk. Simmering gives the product a cream color, caramel taste and the smell of baked milk, which is characteristic of "Red Cottage Cheese". The simmering was carried out at 90, 95, 99 °C. Experimentally, we established the optimum temperature of the simmering of low fat cottage cheese in skim milk to be 95 °C, and the optimum duration 3.5–5 hours. An increase in the duration of the simmering led to burning of the product, the finished product had a stiff, granular consistency. Reducing the duration of the heat treatment resulted in the product having a high moisture content and uneven color.

To establish the shelf life of the cottage cheese product, we tested quality indicators during storage. 4 test samples of "Red Cottage Cheese" were studied: 1 – with sucrose; 2 – with sucrose and preservative (potassium sorbate); 3 – with stevioside (technology A); 4 – with stevioside (technology B).

Technology A – stevioside was added while boiling the cottage cheese and milk mixture. Technology B – stevioside diluted in pasteurized whey was introduced into the finished cottage cheese product [27-29].

When determining the shelf life of new types of cottage cheese products, we were guided by the requirements of MG 4.2.1847-04 "Sanitary and epidemiological assessment of the justification of shelf life and food storage conditions" [30].

Hygienic requirements for shelf life and food storage conditions (Sanitary Regulations and Norms 2.3.2.13.24-03) [31] classify cottage cheese and cottage cheese products as perishable products.

The estimated shelf life of cottage cheese products is 20 days. However, the research was carried out during 26 days, taking into account 1.3 allowed coefficient for perishable products.
The cottage cheese products were stored at the temperature of 4 ± 2 °C and relative humidity of 80 – 85 %. 200 g samples of cottage cheese products were packed in polystyrene containers with caps.
We studied organoleptic, physical, chemical, and microbiological parameters during storage.
The tasting of the samples under study was carried out after obtaining positive results of laboratory tests of physical, chemical, and microbiological indicators. The coded samples of the tested product of days 1, 8, 12, 15, 20, 26 of storage alongside with similar freshly produced precooled products were simultaneously presented for tasting. We evaluated the appearance, texture, color, taste, smell using a 5 point scale: 5 points – very good quality, 4 – good quality, 3 – not a good quality, 2 points – poor quality, 1 point – very poor quality.
To ensure statistical validity of the results, there were 7 independent tasting participants who were not aware of the sample codes.
The summary of the organoleptic evaluation of test samples of cottage cheese products during storage is presented in Figure 3.

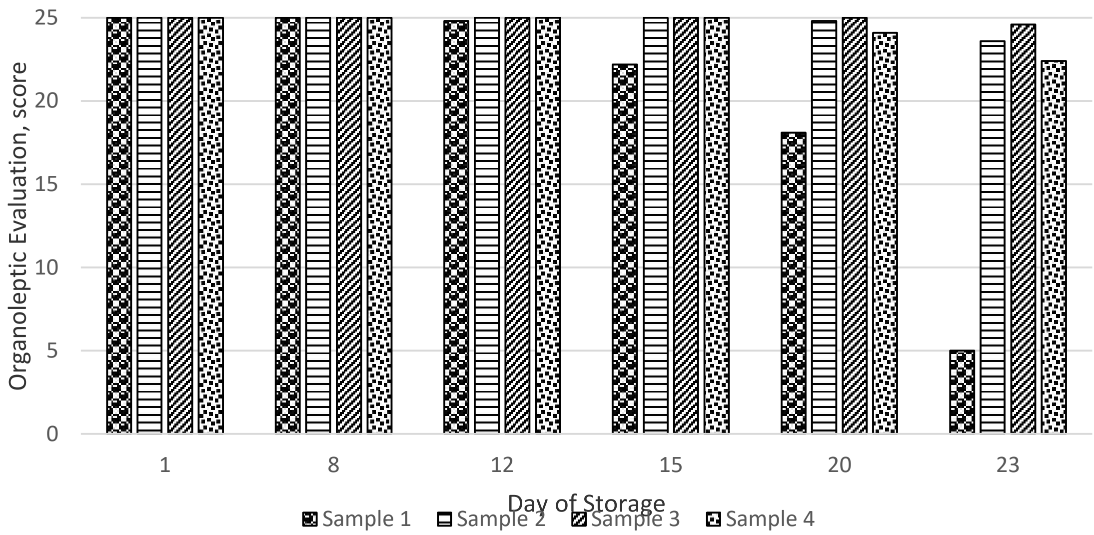

Figure 3. *The Summary of Organoleptic Evaluation of Test Samples of Cottage Cheese Products during Storage*

From day 1 to day 8, all test samples received a maximum of 25 points. On day 12 of the storage, sample 1 had 0.2 points decrease. On the 15th day of storage, sample 1 had a stale smell and taste, as a result, it scored 22.2 points, while samples 2, 3, 4 scored 25 points each.
On day 20, there was a decrease in organoleptic indices in sample 1: the consistency became loose, there was a release of serum, taste and smell became sourish. This sample scored a total of 18.1 points. In samples 2 and 3, as compared to sample 4, the

taste was more pronounced and balanced, as a result, samples 2 and 3 scored 24.8 and 25 points on day 20, respectively, and sample 4 scored 24.1 points.
Samples 3 and 4 underwent the least changes in organoleptic indices on the 23rd day of the storage, which indicates that stevioside, as a part of the product, may possess preservative properties. However, sample 3 scored more points than sample 4, which suggests that stevioside must be added during the simmering of the cottage cheese and milk mixture.
Profilograms of organoleptic characteristics of cottage cheese products on the 23rd day of storage are presented in Figure 4.

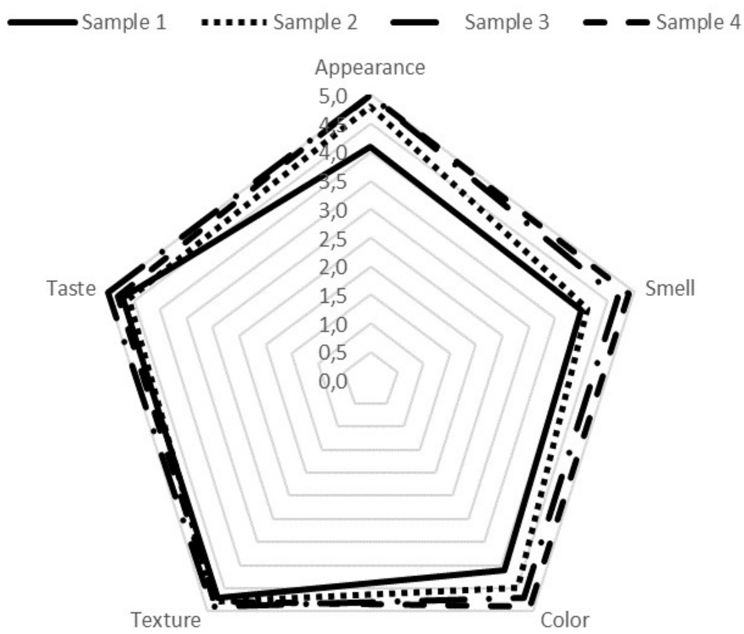

Figure 4. *Organoleptic Characteristics of Cottage Cheese Products on 23rd Day of Storage*

On the 26th day of storage, there was no organoleptic evaluation of the test samples due to unsatisfactory microbiological indicators (the amount of yeast and mold).
During the entire storage period, sample 1 had the greatest decrease in organoleptic indicators. Adding a preservative to the sample as a part of the product (sample 2) made it possible to reduce the intensity of changes in organoleptic indicators throughout the entire storage period. However, the use of stevioside in the recipe (sample 3) was more effective.
As for physical and chemical parameters, we determined titrable acidity. Figure 5 presents the change in the acidity of cottage cheese products during storage.

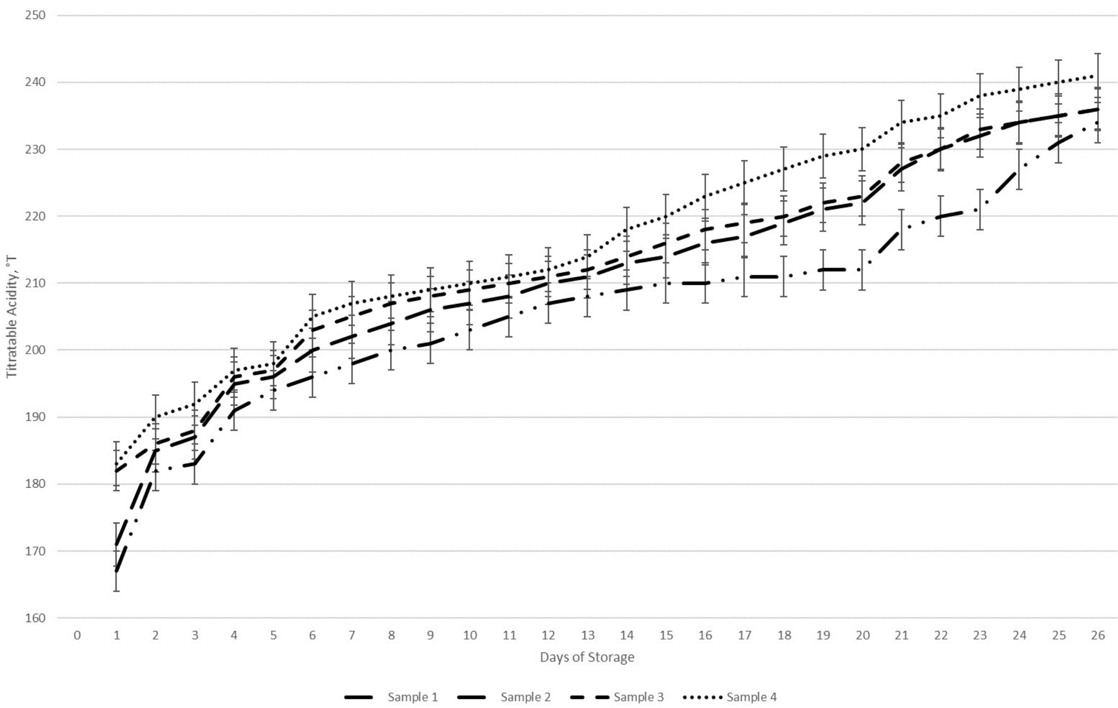

Figure 5. *Change in Acidity of Cottage Cheese Products during Storage*

During storage, titratable acidity in sample 3 grew more slowly compared to the other samples. The acidity increased fastest in sample 1. The use of a preservative in sample 2 also helped to slow down the increase in acidity.

It was found that in the sample made with technology B, titratable acidity increased faster than in the sample made with technology A. Therefore, technology A is recommended for production of a cottage cheese product.

In accordance with Federal Law No. 88-FZ “Technical Regulations for Milk and Milk Products” amended on July 22, 2010, in heat-treated cottage cheese products, including those with components, the total amount of yeast and mold is not to exceed 50 $CFU \cdot cm^{-3}$, coliforms are not allowed in 0.1 g of the product, pathogenic microorganisms, including salmonella - in 25 g, *S. aureus* staphylococci - in 1.0 g (when cottage cheese products go to market).

The presence and development of the above mentioned groups of microorganisms in cottage cheese products leads to their deterioration and adversely affects the general level of product safety.

Figure 6 shows the change in the amount of yeast and mold in the samples under study during storage.

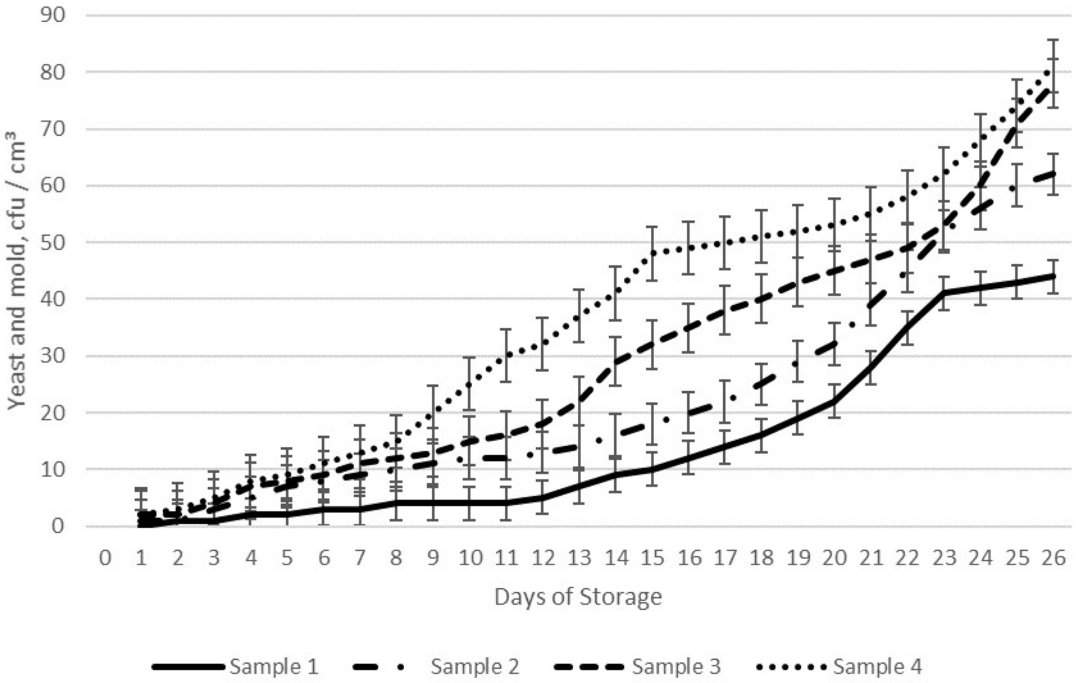

Figure 6. *Change in Amount of Yeast and Mold during Storage*

The minimal growth of yeast and mold during storage is observed in sample 3. The number of yeast and mold reaches a critical level of 50 (in accordance with the Technical Regulations for milk and dairy products) [26] only on the 26th day of storage. Throughout the entire storage period, in 0.1 g of the test samples no presence of Escherichia coli bacteria was detected, as well as no pathogens, including salmonella in 25 g and staphylococci (*S. aureus*) in 1 g.
As a result of the studies, it was found that sample 3 – "Red Cottage Cheese" with stevioside – had a longer shelf life compared to the other samples, which is 15 days (taking into account the allowed coefficient of past shelf life storage) at a temperature of 4 ± 2 ºC. The addition of stevioside, a natural sweetener, as a part of the cottage cheese product allowed us to reduce the intensity of changes in organoleptic, physical, chemical, and microbiological parameters throughout the entire shelf life.

CONCLUSIONS

New recipes and production technologies for "Red Cottage Cheese" product were developed. The optimal technological conditions for the production of the cottage cheese product were established. The physical and chemical parameters of raw milk for the manufacture of test samples of "Red Cottage Cheese" product were determined. By physical and chemical indicators, as well as safety indicators, natural raw milk supplied from Bredinsky district can be classified as premium grade milk as specified in the Technical Regulation of the Customs Union “On the Safety of Milk and Dairy Products" (TR CU 033/2013) and can be used as a raw material for manufacturing test samples of cottage cheese products.

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The analysis of the results revealed the following:
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Thus, the recommended starter culture is LAT CW LT Lactina, fermentation temperature 32 ± 2 °C.
Throughout the entire storage period, in 0.1 g of the test samples no presence of *Escherichia coli bacteria* was detected, as well as any pathogens, including salmonella in 25 g and staphylococci (*S. aureus*) in 1 g.
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