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Review Article

Influence of enriching additives on farmer's cheese formation and its functional properties

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Abstract

The aim of the research was to study the complex effect of selenium-containing supplements and a vitamin premixture on the quality, nutritional value of enriched farmer's cheese (*tvorog*). It was established for the first time that the combined introduction of selexen and the vitamin premixture ADE into the composition of farmer's cheese in the studied concentrations makes it possible to preserve the organoleptic properties of the product; to reduce the rate of titratable acidity growth; to increase microbiological safety by reducing the number of yeasts, molds, and enterococci with stabilization of the number of lactobacilli when stored in a cooled state. The shelf life of enriched farmer's cheese can be extended to 7 days. Consumption of a 100 g portion of the enriched product meets the percentage of daily adult needs in the following micronutrients: selenium - 47-46%, vitamin A – 43–42 %, vitamin E – 27–26 %, and vitamin D_3 – 16%.

Keywords: quality, selenium, farmer's cheese (tvorog), vitamins

1. Introduction

Farmer's cheese is a valuable dietary product. The proteins that make up the curd contain essential amino acids. Farmer's cheese promotes the formation of hemoglobin in the blood and the normalization of the nervous system, is recommended for the prevention of metabolic diseases, and strengthens bone and cartilaginous tissue (Kashina, 2013; Mentyukov, 2008).

Unfortunately, there is significant destruction of many biologically active substances during the high-temperature processing of raw milk, which indicates the need to enrich milk and dairy products with these essential components for the human body (Alzate, Perez-Conde, Gutierrez, & Camara, 2010; Petrova, Kharitonov, & Agarkov, 2002). Fermented milk drinks, pasteurized milk, kefir, puddings, farmer's cheese, and farmer's cheese cream enriched with vitamins (C, group B, carotene), iodine, calcium, phosphorus, magnesium, iron, and zinc are being produced. One well-known technology is that of farmer's cheese cream enriched with the mineral supplement "Neoselen" and a phytocomposition (peppermint, brown hips), the aggregate of which enriches the product with selenium and vitamins A, D, E, C, group B (Grechuk, 2000). Low-fat and low-fat farmer's cheese products have been developed - "Curative farmer's cheese yogurt" with condensed milk and vanilla, produced with the addition of vitamin-mineral premixture 12/03 and enriched with vitamins A, D, E, B_9, C and mineral components – calcium, phosphorus, selenium (sodium selenite) (Gralevskaya, 2006).

In spite of the well-known engineering solutions for the enrichment of dairy products with essential components, the widespread deficiency in human nutrition of irreplaceable micronutrients (Borodulina, Sannikova, & Malyamova, 2011), including the microelement selenium (Dolzhikova, 2016; Esmaeilis & Khosravi-Darani, 2014; Surai, 2006; Hu, Mcintosh,

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& Young, 2012; Shahidi & Chandrasekara, 2015; Williams & Harrison, 2010) and vitamins (Kodentsova, 2014; Kodentsova, Vrzhesinskaya, & Spirichev, 2010), and related health problems dictate the need for a better composition of functional products, and for study of their quality and effectiveness.

The bioavailability of selenium is from 50 to 80% and depends on the presence of other components in the diet. It improves under the influence of proteins and high doses of vitamins A and E, and decreases with deficiency of vitamins E, B_2, B_6, and methionine (Roman, Barbante, & Jitaru, 2014; Sun, Wu, & Luo, 2014; Tutelian, Knyazhev, & Khotimchenko, 2002). The purpose of these studies was to study the complex effect of selenium-containing supplements and vitamin premixture on the quality, nutritional value of enriched farmer's cheese.

2. Materials and Methods

2.1 Materials

The material for research was selenium-containing additive selexen (producer Medbiofarm, Obninsk, Kaluga region), which is a synthetic heterocyclic organic selenium compound (containing no less than 95% selenopyran). It is a stable, crystal-colored powder with a slight specific odor, soluble in dairy cream. The content of selenium in the preparation is 23-24 mg/100 mg. Selexen can be used as an antioxidant drug with a wide spectrum of action which simulates and stimulates the immune system, and is an adaptogenic, anti-stress, anticarcinogenic, antimutagenic and antiviral drug (Report on the study of the functional suitability of the domestic organic compound selenium-selexen, 2000);

Further, vitamin premixture ADE (manufactured by DSM Nutritional Products Europe, Ltd. (Switzerland)) was used, a clear, oily solution of light yellow color easily soluble in milk cream. Samples of farmer's cheese with 9.0% fat content made through acid-rennet production mode were made avialble. As the *control* we used basic farmer's cheese with a shelf life of five days; as *experiment* - with additional introduction of selexen and vitamin premixture ADE containing vitamins A, D_3, and E.

The additives were introduced in the normalization stage of the milk mixture: 1.30 g selexen and 130 g of the vitamin ADE premixtureture out of 1,000 kg of the finished product. The samples of 100 ± 4.5g each were packed in consumer containers (parchment and multilayer polymer film) and stored at an air temperature of 4 ± 2 °C and relative air humidity of not more than 75%. The study period was seven days.

2.2 Methods

A taste panel was formed to study the organoleptic properties of the experimental farmer's cheese samples. It consisted of seven tasters, who were active technologists at dairy enterprises. The meeting of the taste panel was closed-door.

The appearance, consistency, and color of the samples were determined visually, taste and smell - through sampling. A scale developed by Davydova (2012) was used during the farmer's cheese sampling. According to the scale, the maximum total score is 10 points, and it consists of the following: taste and smell - 5 points, consistency and appearance - 3 points, color - 1 point, external type of consumer packaging and labeling - 1 point. If the product gained the total score of 9-10 points, its quality was considered "excellent". A score of 7-9 points indicated "good" quality of the farmer's cheese, 6-7 points –"satisfactory" quality, 5-6 points –"unsatisfactory" quality.

Physicochemical and microbiological studies were carried out by classical methods.

The protein content was determined through the nitrogen content using the Kjeldahl method. The fat content was determined using concentrated sulfuric acid and isoamyl alcohol, followed by centrifugation, and the volume of fat released was measured in the graduated part of the butyrometer. Moisture content in samples was calculated using by drying the reground sample weight of the product over two hours at 100 ± 2°C until it reached a fixed mass, followed by cooling the sample and processing the results. The temperature was determined by a glass thermometer. Phosphatase was determined through reaction with sodium phenolphthalein phosphate. Acidity was calculated by titrating a suspension of a sample weight of the reground product in a 0.1 N solution of sodium hydroxide with the addition of 5 drops of 1 % phenolphthalein solution until it turned pink and remained pink for 1 minute (Merkulova, Merkulov, & Merkulov, 2010).

The selenium content was determined by the fluorimetric method. Vitamin content was determined by high-performance liquid chromatography (Skurikhin & Tutelian, 19 98). The daily intake of selenium and vitamins for an adult was taken from the current standards (Tutelian, 2009).

The presence of Salmonella bacteria was determined by sowing the product into a selective liquid, and then into a selective agarized nutrient medium, incubating the crops at a temperature of 37 ± 1 °C for 24 ± 3 hours, and identifying all of the visible colonies that grew using biochemical and serological tests. The presence of E. coli bacteria was determined by sowing the product in a selectively-diagnostic nutrient medium, incubating the crops at a temperature of 37 ± 1 °C for 24 ± 3 hours. The typical and atypical colonies were then counted and the ability of bacteria from these colonies to ferment lactose and form gas was determined. The presence of Listeria monocytogenes was determined by sowing the product into a liquid selective culture medium (with pre-enrichment) and incubating the crops at a temperature of 30 ± 1 °C for 24 ± 2 hours. It was then re-introduced to an agarized selective diagnostic medium, and the crops were incubated at a temperature of 37 ± 1 °C for 48 hours. All visible colonies were then identified by studying their biological properties. The presence of S. aureus was determined by sowing the product on the surface of a dense nutrient medium and incubating the crops at a temperature of 37 ± 1 °C for 24-48 hours. The typical colonies were counted and confirmed to belong to S. aureus according to plasma-coagulating ability. Yeast and mold count was determined by inoculating the product in Sabouraud dextrose agar, incubating the inoculated product at 24 ± 1 °C for 5 days, and counting all visible colonies (Stepanenko, 2003).

Isolated lactic acid microorganisms were determined conventionally using the Berji bacteria determinant (Holt, Krsh, & Snit, 1997).

2.3 Statistical analysis

Statistical analysis was performed using Microsoft Excel XP and Statistica 8.0. Statistical error did not exceed 5% (with a 95% confidence level). All statistical analyses were performed using SPSS statistics, version 17.0 (SPSS Inc., Chicago, USA).

3. Results and Discussion

3.1 Quality of farmer's cheese samples

The taste, smell and appearance of farmer's cheese plays a decisive role for the consumer, whereas its chemical composition and nutritional value are considered by most consumers to be a secondary concern (Kashina, 2013). The results of the farmer's cheese tasting are presented in Table 1.

When inspecting the appearance of consumer packaging and marking, no visible deformations and quality deviations were detected throughout the entire period of research. The polymer film was whole and clean with clear, bright markings. The results of the tasting studies showed that the introduction of enriching ingredients did not adversely affect the consumer characteristics of the samples. The fresh control and experimental samples of farmer's cheese were easily spread and the presence of perceptible particles of milk protein was noted. They had a pure fermented milk taste, with no foreign flavors or smells. The color was white and uniform throughout the mass. Based on the results of the tasting quality assessment, all the farmer's cheese samples scored a maximum of 10 points.

On the 5th day of storage (after the expiration date of the unenriched farmer's cheese) the characteristic taste and smell became "weakly expressed" in the control samples. In terms of consistency, the separation of whey was observed, which led to a decrease in the final score to 7.3 compared to 9.4 in the experiment.

On the 7th day of storage in the control samples of farmer's cheese, the samples had a stale taste and smell, the consistency changed to "slightly crumbly", and a grayish shade appeared in the color. As a result, the total score dropped to 5.5, and the quality level was noted as unsatisfactory. In the experimental farmer's cheese samples, the score for identical indicators decreased slightly, changed the overall score to 7.8 points, giving the enriched models a "good" quality level.

Thus, the introduction of a complex of enriching additives into the formulation of curds made it possible to stabilize the decrease in consumer characteristics (change in appearance, consistency, odor, and taste) during storage, and thereby improve the organoleptic properties of the experimental farmer's cheese samples.

Food additives used in the production of food products, not only as technological factors, but also as enriching components, can influence the technological parameters of production and the physicochemical indicators of the quality of finished products. Therefore, we studied the influence of enriching additives on the physicochemical parameters of freshly prepared farmer's cheese. The results of the studies are presented in Table 2.

According to the results of physical and chemical studies, it was found that the addition of enriching additives to the formulation did not adversely affect the formation of its quality, since the quantitative characteristics of the investigated indicators of the freshly-produced samples corresponded to the regulated requirements.

Since dairy products are subject to souring due to natural microbiological and chemical processes during storage, it was of interest to study the effect of enriching additives on the growth of titrated acidity in farmer's cheese samples during storage. It was found that in the control and experimental samples of farmer's cheese the titrated acidity corresponded to the regulated requirements (not more than 220 °T) throughout the entire experiment. Nevertheless, the acidity

Table 1. Scoring of organoleptic quality indicators of samples of farmer's cheese.

Storage period	Tasting results, scores				
	Appearance of packaging and labeling	Appearance and texture of farmer's cheese	color	taste and smell	total
			Control		
Freshly produced	1.0±0.0	3.0±0.0	1.0±0.0	5.0±0.0	10.0±0.0
1 day	1.0±0.0	3.0±0.0	1.0±0.0	5.0±0.0	10.0±0.0
3 days	1.0±0.0	2.7±0.2	1.0±0.0	4.4±0.3	9.1±0.2 [a]
5 days	1.0±0.0	2.2±0.3 [a]	1.0±0.0	3.1±0.2 [a]	7.3±0.2 [b]
7 days	1.0±0.0	1.5±0.3 [b]	0.8±0.2	2.2±0.2 [b]	5.5±0.2 [c]
			Experiment		
Freshly produced	1.0±0.0	3.0±0.0	1.0±0.0	5.0±0.0	10.0±0.0
1 day	1.0±0.0	3.0±0.0	1.0±0.0	5.0±0.0	10.0±0.0
3 days	1.0±0.0	3.0±0.0	1.0±0.0	5.0±0.0	10.0±0.0 [a]
5 days	1.0±0.0	2.9±0.1 [a]	1.0±0.0	4.5±0.2 [a]	9.4±0.1 [b]
7 days	1.0±0.0	2.5±0.2 [b]	1.0±0.0	3.3±0.3 [b]	7.8±0.2 [c]

Values (mean±SD, n=7) with different superscripts in the same row of control and experiment are significantly different ($P<0.05$).

Table 2. Physicochemical parameters of farmer's cheese samples.

Parameter	Norm	Research results	
		Control	Experiment
Fat mass fraction, %	no less than 9.0	9.1±0.1	9.1±0.1
Moisture content, %	no more than 73.0	72.0±0.2	72.1±0.2
Protein mass fraction, %	no less than 16.0	17.10±0.02	17.10±0.02
Acidity, °T	no more than 220	166.2±1.2	164.2±1.5
Phosphatase	Absent	Absent	
Product temperature when released from an enterprise, °C	4±2	4±2	

Values are expressed as mean±SD (n=5, P<0.05)

increased more slowly in the test samples than in the control samples. On the 7th day of storage, the acidity of the enriched farmer's cheese samples increased by 36.2 °T, in the unenriched - by 49.3 °T. The reduced rate of acidity increase can be explained by the fact that the lactic microflora is able to accumulate selenium, which slows the growth of the titrated acidity of the product (Sivakov, 1997).

Microbiological methods for researching products, determining the degree of their contamination with microbes, the composition of microflora, and changes to these indicators during the storage of products, make it possible to predict changes in quality, to predict possible storage times in specified conditions, and to sell products in a timely manner (Fedoreev & Popova, 2014; Merkulova, Merkulov, & Merkulov, 20 10; Stepanenko, 2003).

Bacteria of the E. coli group, salmonella and Staphylococcus aureus were absent in a certain mass of control and experimental samples of the product throughout the entire experiment.

The final group of microorganisms subject to control when assessing the safety of farmer's cheese-based products is yeasts and molds, the presence of which causes spoilage (deterioration) of farmer's cheese (Merkulova *et al*., 2010) and affects the overall safety level of the product. Therefore, we studied the microbiological stability of the experimental farmer's cheese samples during storage. Table 3 presents the results of the studies.

Table 3 shows that the samples lasted longer than the requirements for microbiological safety. In the control samples, the development of yeast was observed on the 5th day of storage and molds on the 7th day, whereas in the enriched products, no similar microflora was detected during the entire storage period of growth.

The joint application of selexen and vitamin premixture ADE into the experimental samples of farmer's cheese reduced the dynamics of undesirable microflora development, which contributed to the preservation of the microbiological stability of the enriched models throughout the seven days of storage.

The microflora of farmer's cheese consists of both microorganisms introduced with ferment and foreign microorganisms coming from pasteurized milk, equipment, packaging (Bannikova, Koroleva, & Semenikhin, 1987; Merkulova *et al*., 2010). From the microflora of pasteurized milk, the quality of farmer's cheese is mainly influenced by thermally-resistant lactobacillus, which develop the most common defect - an excessively sour taste (Merkulova *et al*., 2010; Ponomarev, Merzlikina, & Golubeva, 2014). The results of studies on the composition of the dominant lactic acid microflora of farmer's cheese enriched with selenium and a complex of vitamins (Experiment 1) are presented in Table 4.

It was established that selexen combined with the vitamin ADE premixture did not adversely affect the amount and composition of the lactic acid microflora of the experimental farmer's cheese samples. In the samples of the freshly prepared product, lactate streptococci: S. lactis, S. cremoris, and S. thermophilus predominated, accounting for slightly more than 60% of the total lactic acid microorganisms. Among lactobacillus, L. bulgaricum and L. acidophilus were found, accounting for 15-17%. Also in the control and experimental samples of farmer's cheese, Enterococci (E. facemium and E. durans) were also found in the control and experimental samples, accounting for less than 10% of the total number of microorganisms studied.

Lactic acid bacteria are beneficial to intestinal microflora, displacing pathogens (salmonella, escherichia, clostridium, and yeast). The effectiveness of their use in acute intestinal infections and inflammatory diseases has been proved.

Table 3. Indicators of microbiological safety of samples of farmer's cheese.

Storage period	Yeast, CFU/g (norm-not more than 100)		Mold, CFU/g (norm-not more than 50)	
	Research results			
	Control	Experiment	Control	Experiment
Freshly produced	not detected	not detected	not detected	not detected
1 day	not detected	not detected	not detected	not detected
3 days	not detected	not detected	not detected	not detected
5 days	less than 10	not detected	not detected	not detected
7 days	20	not detected	less than 10	not detected

Table 4. Comparative composition of the dominant lactic acid microflora of samples of farmer's cheese.

Types of microorganisms	Research results of cheese, CFU/g			
	Freshly produced		On the 7th day of storage	
	Control	Experiment	Control	Experiment
Lactococcus	1.2×10^{7}	1.3×10^{7}	$5.0\times10^{6\,a}$	$8.6\times10^{6\,a}$
Lactobacilli	3.0×10^{6}	3.5×10^{6}	$1.3\times10^{6\,b}$	$3.0\times10^{6\,b}$
Enterococcus	1.6×10^{6}	1.0×10^{6}	$8.0\times10^{5\,c}$	$3.9\times10^{5\,c}$

The studies were performed in seven replicates (n=7, P<0.05). Values with different superscripts in the same row significantly different (P<0.05).

Nitric oxide formed in the gastrointestinal tract by lactobacillus enzymes participates in the bacteriostatic function of the intestine and peristalsis, providing local immunity (Bannikova *et al*., 1987; Ermolenko, Isakov, & Zhdan-Pushkin, 2004; Sviridenko, 2009).

On the 7th day of storage, the amount of streptococci in the microbiocenosis of enriched samples decreased by 34%, but exceeded the content in the control by 42%. The number of lactobacillus decreased by 14%, but exceeded that of the control more than 2 times over. The number of enterococci decreased by 40%, and was 2 times lower than in the control. This is explained by the fact that anaerobic or microaerophilic conditions are more favorable for the growth of established lactic acid microorganisms, especially lactobacillus (Bannikova *et al*., 1987; Sviridenko, 2012), which were created by antioxidants present in the enrichment supplements.

The previously-established results on the lack of growth of yeasts and molds in enriched farmer's cheese are a result of the antagonistic activity of the lactic acid bacteria present in larger quantities than in unenriched samples (Bukharin, Ginzburg, & Romanova, 2005; Bukharin, Semenov, & Cherkasov, 2010; Ermolenko, 2009; Ermolenko, Isakov, & Zhdan-Pushkin, 2004).

The addition of selexan and the vitamin premixture ADE to farmer's cheese not only enriches the product with micronutrients, but also preserves its functional properties during storage at a higher level due to the stabilization of the number of lactic acid microorganisms.

3.2 Nutritional value and antioxidant properties of farmer's cheese samples

At this stage, we studied the possibility of replenishing individual dietary micronutrient deficiencies by using an average daily portion of 100 g of enriched farmer's cheese samples. The results of the study of the micronutrient composition of samples of farmer's cheese are presented in Table 5.

Calculations show that the consumption of 100 g of basic, freshly prepared farmer's cheese provides a low intake of selenium, vitamin A (5% each), and vitamin E (0.2% each). Therefore, not much attention is paid to the low micronutrient value of farmer's cheese produced according to traditional technologies due to the insignificant content of vitamins in the feedstock and the influence of technological factors on them.

Consumption of 1 unit of enriched farmer's cheese, depending on the shelf life, can meet the dietary needs of an adult in the following micronutrients (% of PN): selenium - 47-46%, vitamin A – 43–42%, vitamin E – 27–26%, vitamin D_3 – 16%.

4. Conclusions

It was established for the first time that the combined introduction of selexen and the vitamin premixture ADE in the composition of farmer's cheese in the studied concentrations makes it possible to preserve the organoleptic and physicochemical properties of the product when stored in a cooled state; increase its antioxidant activity; stabilize the numerical strength of lactic microflora due to the formation of anaerobic conditions. Given all of our results, the shelf life of enriched farmer's cheese can be extended to 7 days. Consumption of 100 g of the enriched product will meet the adult daily requirement in the following micronutrients (%): in selenium – 47–46%, in vitamins A – 43–42%, E – 27–26%, and D_3 – 16%.

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Table 5. Provision of physiological needs (PN) in micronutrients with the use of averaged daily portion of samples of farmer's cheese.

Micronutrient	PN, mg/day	Research results, mg/g					
		Freshly produced				On the 7th day of storage	
		Control		Experiment		Experiment	
		Content	% of PN	Content	% of PN	Content	% of PN
Selenium	0.07	0.0035±0.0003 [a]	5.0	0.0330±0.0002[a]	47.1	0.0325±0.0002	46.4
Vitamin A	0.9	0.043±0.003 [b]	4.8	0.390±0.020 [b]	43.3	0.380±0.020	42.2
Vitamin E	15.0	0.033±0.002 [c]	0.2	4.130±0.050 [c]	27.5	3.980±0.050	26.5
Vitamin D_3	0.01	Trace amount	–	0.00164±0.00002	16.4	0.00164±0.00002	16.4

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