Bulletin of the *Transilvania* University of Braşov
Series II: Forestry • Wood Industry • Agricultural Food Engineering • Vol. 11 (60) No.1 - 2018

QUALITY AND NUTRITIONAL VALUE OF PASTEURED MILK ENRICHED BY SELENIUM AND VITAMINS

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Abstract: *The aim of our research was to study the complex effect of selenium-containing supplements and vitamin premixture on the quality and nutritional value of enriched pasteurized milk. We established for the first time that the combined introduction of selexen and a vitamin premixture 963/7 into the composition of pasteurized milk in the concentrations under study makes it possible to preserve the flavor and aromatic properties of the product when stored in a cooled state, and to reduce the rate of growth of titrated acidity and the number of mesophilic microflora, psychotropic micrococci, vegetative forms of spore-forming aerobic bacilli with slight increase in the number of lactobacilli and thermophilic streptococcus. Using a dietary ration of 200 ml of enriched milk will meet the need for an adult in the following micronutrients (%): selenium – 46, vitamins B_5 – 42, B_6 – 34, PP – 28, C – 23–22, B_9 – 21.*

Key words: *milk, selenium, vitamins, nutritional value.*

1. Introduction

Milk is an indispensable product of mass and daily consumption. It is well absorbed (95-98%) even with the smallest secretory work of the digestive glands of the body and stimulates the assimilation of nutrients from other food systems. Milk is a good foundation for creating functional products for specific needs of a consumer audience through changes to its composition [7].

The deficient trace element selenium is effective in any conditions accompanied by an increased load of free radicals and reactive oxygen species on the human body. Its immunostimulating properties have been established and a positive influence on the human reproductive function has been proven. Consumption of the necessary amount of selenium increases life expectancy [3], [9], [10]. For this reason, selenium-enriched milk produced in Sweden has been developed

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containing 32 mg/l of selenium (shelf life of 14 days), and the one produced in the UK contains 100 mg/l of selenium (shelf life 10 days) [1]. Employees of the North Caucasus Federal University conducted complex studies on the development of drinking milk and milk drinks enriched with nivalent selenium (stabilized by bovine serum albumin) [4], [7]. Despite the well-known engineering solutions for the enrichment of drinking milk with selenium, the widespread deficiency in human nutrition of essential micronutrients, including this microelement and vitamins, and related health problems dictate the need to improve the composition of functional products, to study their quality and effectiveness. The aim of our research was to study the complex effect of selenium-containing supplements and vitamin premixture on the quality and nutritional value of enriched pasteurized milk.

2. Materials and Methods

The material for the study were samples of drinking milk with a fat content of 2.5% which underwent dual pasteurization at a temperature of 76 ± 2 ° C with an exposure of 15-20 seconds. As the *control* we used normal pasteurized milk with a shelf life of seven days; and for the *experiment* – pasteurized milk with selexen and a vitamin premixture 963/7 containing vitamins B_9, B_6, PP, B_5 and C.

Selexen (produced by Medbiopharm, Obninsk, Kaluga region) is a synthetic heterocyclic organic selenium compound (containing not less than 95% selenopyran), is a stable beige-colored crystalline powder with a weak specific smell, soluble in milk cream. The product has 23-24% selenium content.

Premixture 963/7 (DSM Nutritional Products Europe Ltd (Switzerland)) is a light-beige micronized powder easily soluble in skim milk.

Enriching additives were introduced in the normalization stage of the milk mixture: 0.67 g selexen and 150 g vitamin premixture 963/7 per 1000 l of the final product. The milk samples were poured into 200 ± 9 ml Pure-Pack containers and stored at an air temperature of 4 ± 2 °C with relative air humidity no more than 75%.

Appearance, consistency, and color of model milk samples were determined visually, and taste and odor were determined during testing.

The protein content was determined through the nitrogen content using the Kjeldahl method. The conventional method for determining purity groups for the milk was used. Fat content was determined with concentrated sulfuric acid and isoamyl alcohol, followed by centrifugation and measurement of the volume of fat released in the graduated section of the byrometer. Milk density was determined by the hydrometric method. Temperature was determined with a glass liquid thermometer.

The mass fraction of the skimmed milk powder was determined by drying a sample of the product at a temperature of 102 ± 2 ° C to constant weight, followed by subtracting the fat content.

Phosphatase was determined by reaction with sodium phenolphthalein phosphate.

Acid was calculated by titrating a suspension of the solution of sodium hydroxide dissolved in 0.1 N solution of sodium hydrate with the addition of 5 drops of 1% phenolphthalein solution until

the solution turned pink and remained pink for 1 minute.

The selenium content was determined by the fluorimetric method. Vitamin content was determined by high-performance liquid chromatography.

The daily intake of selenium and vitamins for an adult was taken from the current standards [12].

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, followed by identification of all the visible colonies that grew, using biochemical and serological tests.

The presence of bacteria of the E. coli group was determined by sowing the product into a selectively diagnostic nutrient medium, incubating the crops at a temperature of 37 ± 1 °C for 24 ± 3 hours, then counting typical and atypical colonies and determining the possibility of bacteria from these colonies to ferment lactose to form gas.

The presence of *Listeria monocytogenes* was determined by sowing the product into a liquid selective culture medium (with pre-enrichment), incubating the crops at a temperature of 30 ± 1 °C for 24 ± 2 hours, reintroducing it to agarized selective diagnostic medium, incubating the crops at a temperature of 37 ± 1 °C for 48 hours, and identifying all grown visible colonies by studying their biological properties.

The presence of *S. aureus* was determined by sowing the product on the surface of a dense nutrient medium, incubating the crops at a temperature of 37 ± 1 °C for 24-48 hours, and counting typical colonies with subsequent confirmation of their belonging to *S. aureus* according to their plasma-coagulating ability.

The group composition of the microflora was determined by the unified cup method on a nutrient medium (meat-peptone agar), changing the conditions of cultivation. For the detection of mesophilic microorganisms, the crops were incubated at 30 ± 1 °C for 72 hours, for psychotropic - at 7 ± 1 °C for 10 days, and for thermophilic - at 44 ± 1 °C for 72 hours.

Isolated lactic acid microorganism species were determined conventionally using the Berji bacteria determinant and the morphological, tinctorial, cultural, and biochemical properties were studied.

All measurements were repeated three times. Statistical analysis was performed using Microsoft Excel XP and Statistica 8.0. Statistical error did not exceed 5 % (with a 95 % confidence level).

3. Results and Discussion

3.1. Pasteurized Milk Sample Quality

In Russia and abroad, there is a constant increase in demand for pasteurized milk with improved storage time. The persistence of pasteurized milk is characterized by a time during which microbiological, biochemical, and organoleptic indices are maintained at the level established by regulatory documents. At the first stage of the research, the organoleptic quality indicators of samples of pasteurized milk were evaluated. The results of the studies are presented in Table 1.

The results of organoleptic studies of freshly-developed samples of pasteurized milk show that both the control and test samples for organoleptic indicators met the requirements of normative

documentation and the level of "excellent" quality. In appearance and consistency, the milk was an opaque, homogeneous, non-sticky liquid with no flakes or sediment, white in color, with characteristic, clean, pleasant, slightly sweetish taste and odor, with no off-taste, odors and odors in cow's milk.

The first signs of reduced quality of the *control*, namely the absence of a characteristic sweetish smell, appeared on the fourth day of storage. However, by the end of the experiment (on the 10th day of storage), the taste and smell had become "empty" and "stale", and the consistency changed – it became heterogeneous with small flakes of protein, which corresponded to the "unsatisfactory" quality level.

On the eight day of storage, the taste and aromatic characteristics of the *experiment* samples changed somewhat, but this did not significantly affect the consumer properties even after the expiration of the shelf life and allowed samples enriched with selenium and a complex of vitamins to correspond to an "excellent" quality level.

Organoleptic characteristics of pasteurized milk samples Table 1

Parameter	**Research results**	
	Control	*Experiment*
Freshly prepared samples		
Appearance and consistency	opaque, homogeneous, non-sticky liquid without flakes and sediment	
Color	white, homogeneous throughout	
Taste and smell	pure, slightly sweet, without extraneous, not characteristic of cow's milk, flavors and odors	
On the 10th day of storage		
Appearance and consistency	opaque, non- homogeneous, non-sticky liquid with small flakes of protein	opaque, homogeneous, non-sticky liquid with no flakes or sediment
Color	white, homogeneous throughout	
Taste and smell	empty, stale	clean, without impurities uncharacteristic of cow's milk, no aftertastes or smells

Additives used in the production of food products as technological factors or enriching components can influence the physicochemical parameters of the finished product. The influence of enriching additives on the physicochemical parameters of freshly processed pasteurized milk was studied. 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 of pasteurized milk did not have a negative effect on the formation of its quality, since the quantitative characteristics of the investigated indicators of freshly developed prototypes 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 a complex of enriching additives on the increase in titrated acidity of pasteurized milk samples during storage.

With an initial titratable acidity of 16.7 ° T, an increase within one degree occurs in the *control* samples on the fourth day of storage, while in samples with added selexan and vitamin preparation - on the eighth.

Table 2

Physicochemical parameters of samples of pasteurized milk

Parameter	Norm	Research results	
		Control	*Experiment*
Fat mass fraction, %	no less than 2.5	2.52 ± 0.02	
Density, kg/m^3	no less than 1028	1028.2 ± 0.1	
Protein mass fraction, %	no less than 2.8	2.82 ± 0.02	
Acidity, °T	no more than 21	16.7 ± 0.2	
Phosphatase	Absent	Absent	
Clean group	no less than I	I	
Mass fraction of skimmed milk powder, %	no less than 8.2	8.5 ± 0.2	
Product temperature at release from manufacture, °C	4 ± 2	4 ± 2	

By the end of the experiment (on the 10th day of storage), the acidity in the control increased by 5.1 ° T and exceeded the norm, while it increased by 2.6 ° T in the *experiment* and continued to meet the established requirements. The reduced rate of acidity growth can be explained by the fact that the lactic acid microflora is able to accumulate selenium, which slows the growth of the titrated acidity of the product.

In the study of the microbiological quality indicators of pasteurized milk samples, *E. coli* bacteria, salmonella, *S. aureus*, and *Listeria monocytogenes* were absent in a certain volume of the control and experimental samples throughout the entire experiment.

According to the results of bacterial contamination studies of pasteurized milk, it was determined that the amount of mesophilic aerobic and facultative anaerobic microorganisms (AMAFAM) both in the control and in the experiment was within the norm (not more than 1.0 × 10^5) even after the end of their shelf life (10 days). However, in experimental trials, the growth of AMAFAM was less intensive: the amount of mesophilic microflora on the 10th day of storage increased 42 times, while in unrefined milk it increased 56-fold. Singular use of Selexen did not significantly affect the development of the mesophilic microflora of pasteurized milk during storage.

Given that the total number of bacteria in pasteurized milk is an indirect indicator of its storage stability, and the organoleptic characteristics of the product change when 5 to 10 million cells per 1 cm3 of pasteurized milk are contaminated, the appearance in the control of "empty" and "stale" taste and smell, as well as heterogeneous

consistency on the 10th day of storage, may be due to the development of psychrotrophic bacteria which enter the pasteurized milk during its secondary seeding and multiply at the same storage temperature, 2 ... 4 ° C [8]. Up to 75% of raw milk psychrotrophs belong to the genus *Pseudomonas.* However, some representatives of mold fungi, yeast, enterococci, micrococci, and CB [6] show psychrotrophic properties. To study the group composition of the "wild" microflora and assess the safety of milk enriched with selenium and a complex of vitamins, the original indicators - the number and composition of psychotropic aerobic and facultative anaerobic microorganisms (AMAFAM) and heat-resistant aerobic and facultative anaerobic microorganisms AMAFAM) (Table 3) - were also studied.

Table 3

Comparative composition of the dominant microflora pasteurized milk samples

Indicator name	**Research results, CFU/cm^3**			
	Freshly prepared samples		*On the 10th day of storage*	
	Control	*Experiment*	*Control*	*Experiment*
AMAFAM:	7.3×10^2	7.2×10^2	4.1×10^4	3.0×10^4
yeast	not detected		not detected	
molds	not detected		not detected	
spore forming aerobes	< 10		0.8×10^2	0.7×10^2
lactobacilli	not detected		not detected	
cocci	6.3×10^2	6.3×10^2	3.2×10^4	2.2×10^4
CB	not detected		not detected	
AMAFAM:	3.0×10^2	3.0×10^2	6.1×10^2	6.2×10^2
yeast	not detected		not detected	not detected
molds	not detected		not detected	not detected
spore forming aerobes	< 10		2.0×10^1	1.8×10^1
lactobacilli	< 10		5.5×10^1	6.0×10^1
cocci	2.6×10^2	2.6×10^2	4.5×10^2	4.9×10^2
CB	not detected		not detected	
AMAFAM:	not detected		not detected	
yeast	not detected		not detected	
molds	not detected		not detected	
spore forming aerobes	not detected		not detected	
lactobacilli	< 10		1.6×10^4	not detected
cocci	not detected		not detected	

When assessing the safety and quality of milk, it is necessary to know the overall level of bacterial contamination and the nature of the bacterial landscape. In all pasteurized milk samples, mesophilic microorganisms prevailed throughout the entire experiment period (up to 70%), namely coccal forms (micrococci, *S. saprophyticus, S. capitis*), and in terms of species diversity - heat-resistant (more than 60%), which were represented not only by cocci (*Str. termophilus, Ent. fecium, Ent. durans*), but also by asporogenous chopsticks (*Lbm.*

bulgaricum, Lbm. acidophilum) and vegetative forms of spore-forming bacteria (*B. pumilus, B. subtilis*).

The quantitative composition of the microbocenosis of freshly developed *control* and prototype pasteurized milk did not differ, but on the 10th day of storage the difference in the number of individual microorganisms was significant.

The number of Mesophilic cocci (due to a decrease in the number of micrococci) in the *experiment* was 31% lower than in *control*, and *B. megaterium bacilli* - 12.5% lower; from the heat-resistant microflora, the number of vegetative cells of spore-forming aerobes was 10% lower in the experiment, and no psychotropic micrococci were found in the enriched milk samples. This is apparently due to the ability of ascorbic acid, as an antioxidant, to intercept a greater number of free radicals with the action of selexen and to create less favorable conditions for the growth of aerobic microorganisms, which are the isolated micrococci, *B. pumilus,* and *B. subtilis* [13]. The total number of lactic acid microorganisms increased in the *experiment* by 18%, which is explained by well-known data on the beneficial effects of vitamins (pyridoxine, pantothenic, nicotinic, and folic acids) present in the premixture on their life activity [11]. However, in general, this circumstance did not worsen the quality of enriched milk samples and also stabilized the number of mesophilic staphylococci due to their antagonistic activity in intermicrobial interactions [2].

Thus, for the first time, it was established that the complex use of selexen and vitamin premixture 963/7 in the technology of enriching pasteurized milk helps to reduce the number of micrococci, vegetative forms of spore-forming aerobic bacilli (*B. pumilus, B. subtilis, B. megaterium*) with a slight increase in the number of lactobacilli (*Lbm. bulgaricum, Lbm. acidophilum*), and thermophilic streptococcus.

It was found that the appearance of specific defects in the control samples on the 10th day of storage is due to the action of exoenzymes - proteases and lipases produced by psychotropic micrococci [5].

Since the technique of sowing with meat-peptone agar reveals only aerobic and facultative-anaerobic microorganisms, excluding the possibility of growth of obligate anaerobes, which are bacteria of the genus *Clostridium,* their number was not taken into account.

3.2. Nutritional Value of Pasteurized Milk Samples

At this stage of the study, the possibility of replenishing the deficiency of individual micronutrients in the diet through the use of an average daily intake (200 ml) of enriched milk was studied. The results of the study are presented in Table 4.

Table 4

Maintaining physiological need (PN) in micronutrients using a daily portion pasteurized milk samples

Nutrient	**PN, mg/day**	**Research results, mg/200 ml**					
		Freshly prepared samples				*On the 10th day of storage*	
		Control		*Experiment*		*Experiment*	
		content	% of PN	content	% of PN	content	% of PN
Selenium	0.07	0.0056 ± 0.0003	8.0	0.0322 ± 0.0002	45.7	0.0322 ± 0.0002	45.7
Vitamin B_5	5.0	0.64 ± 0.02	12.8	2.14 ± 0.03	42.8	2.12 ± 0.02	42.4
Vitamin B_6	2.0	0.134 ± 0.001	6.7	0.68 ± 0.02	34.0	0.68 ± 0.02	34.0
Vitamin PP	20.0	0.68 ± 0.02	4.8	5.62 ± 0.02	28.1	5.62 ± 0.02	28.1
Vitamin C	90.0	2.56 ± 0.03	2.8	21.0 ± 0.2	23.3	20.4 ± 0.05	22.7
Vitamin B_9	0.4	0.0146 ± 0.0002	3.6	0.084 ± 0.001	21.0	0.084 ± 0.001	21.0

Calculations show that the use of a daily portion of a freshly prepared, basic milk composition provides a low level of micronutrient intake (% of PN): selenium - 8, vitamins B5 - 13, B_6 - 7, PP - 5, B_9 - 3, C - 3. Therefore, attention is drawn to the low micronutrient value of pasteurized milk, produced by traditional technologies, due to the low content of vitamins in the feedstock and the impact of technological factors on them. The use of the contents of 1 unit (average daily portion) of enriched milk, depending on the shelf life, will satisfy the nutritional needs of an adult for the following micronutrients (% of PN): selenium - 46, vitamin B_5 – 42, B_6 – 34, PP – 28, C – 23–22, and B_9 – 21.

4. Conclusions

The complex introduction of selexen and vitamin premixture 963/7 into the composition of pasteurized milk in the concentrations studied makes it possible to preserve the flavor and aromatic properties of the product when stored in a cooled state, reduce the rate of growth of titratable acidity, and the number of mesophilic microflora, psychotropic micrococci, and vegetative forms of spore-forming aerobic bacilli (*B. pumilus, B. subtilis, B. megaterium*) with a slight increase in the number of lactobacilli (*Lbm. Bulgaricum, Lbm. Acidophilum*) and thermophilic streptococcus. Based on the obtained results, the shelf life of the enriched pasteurized milk can be extended to 10 days.

Using a portion of 200ml of enriched milk in the daily ration, depending on the shelf life, will meet an adults needs for the following micronutrients (% of PN): selenium - 46, vitamins B_5 – 42, B_6 – 34, PP – 28, C – 23–22, and B_9 – 21.

Acknowledgement

The work was supported by Act 211 of the Government of the Russian Federation, contract № 02.A03.21.0011.

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