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Microbiological characteristics of hard cheese with flax seeds

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ABSTRACT

Highly nutritious dairy products such as hard cheeses are considered a good source of protein, fats, mineral substances, and vitamins and are consumed in significant quantities. At the same time, the disadvantages of cheeses include the presence of a large amount of saturated fatty acids in their composition, which are associated with the development of cardiovascular diseases. Therefore, modifying the composition of fatty acids in hard cheese by increasing the content of unsaturated fatty acids and reducing the amount of saturated fatty acids is extremely important for consumers' health. This research aimed to determine the dynamics of microbiological indicators in the ripening technology of hard rennet cheese with different contents of flax seeds as a source of omega-3 fatty acids. The technology of hard rennet cheese with 3-5% flax seed content was investigated. A 1.3 times higher content of lactic acid microflora was found in samples of hard cheese with 5% flax seeds during the first 10 days of ripening compared to the control sample of cheese. On the 60th day of ripening of cheese with a content of 5% flax seeds, the number of lactic acid bacteria was 9.4 ±0.3×10^9 CFU/g, and in the control cheese – 7.8 ±0.3×10^9 CFU/g. During the production and ripening of cheese with different content of flax seeds, no exceedance of normative values was found for the number of *Enterobacteriaceae* and *Staphylococcus aureus* bacteria. A method (washing the seeds in a sodium bicarbonate solution and drying at a temperature of 95 ±1 for 20 min) of processing flax seeds before adding them to the cheese mass was proposed, which reduced the number of mesophilic microorganisms by approximately 200 times to 3.9 ±0.1×10^1 CFU/ g, fungi by 160 times to 0.3 ±0.1×10^1 CFU/g and aerobic mesophilic bacilli by 78 times to 1.1 ±0.1×10^1 CFU/g. Therefore, the developed hard rennet cheese with flax seeds can be consumed as an additional source of omega-3 fatty acids and dietary fiber.

Keywords: rennet cheese, cheese technology, flax seeds, cheese ripening, lactic acid microflora

INTRODUCTION

Today, the industry is trying to produce food products intended not only to satisfy hunger and provide the necessary nutrients but also to prevent food-related diseases **[1]**, **[2]**. Milk and milk products are considered the primary sources of nutrients in the human ration, offering quality proteins, minerals, vitamins, and energy **[3]**. In addition, milk is an excellent matrix for the release of bioactive compounds **[4]**, and various dairy products are the basis for enrichment with other nutrients **[5]**, **[6]**. Currently, the development of new formulations of dairy products that consumers would like very much is one of the main forces in the dairy industry. Dairy products such as cheeses, ice creams, and yogurts are consumed worldwide, and improving the composition of these products with vitamins, antioxidants, fiber, and polyphenols can be achieved by integrating rich sources **[7]**, **[8]**, **[9]**, **[10]**. Additional components can be carrot paste **[11]**, **[12]**, **[13]**, broccoli **[14]**, grape extract and extracts **[15]**, **[16]**, **[17]**, sesame **[18]**, spinach powder **[19]**, tomato extracts **[20]**. In addition, consumers worldwide demand the development of cheeses with reduced content of synthetic additives such as flavors and colorants **[21]**, **[22]**.

Consequently, increasing knowledge of the relationship between nutrients and health has led to new product categories, such as functional foods and nutraceuticals. Among the various functional product groups, omega-3 fatty acids are popular **[23]**, **[24]**. Because lipids are considered one of the most essential nutrients for humans.

Among the fatty acids of food lipids, these are indispensable - α-linolenic (C18:3 omega-3), eicosapentaenoic (omega-3), docosahexaenoic (omega-3), and linoleic (C18: 2, omega-6), which are not synthesized by the human organism, so they must be got with food products **[25]**, **[26]**. Unsaturated fatty acids are used to replace saturated fatty acids in various products, as high consumption levels of the latter negatively influence people's health **[27]**.

It has been reported that modern dietary habits have significantly reduced the daily intake of omega-3 fatty acids to less than recommended. As a result, the need to fortify food with omega-3 fatty acids is increasing **[28]**. In most developed countries, health policy recommends reducing the intake of saturated fatty acids and increasing the intake of unsaturated fatty acids, especially α-linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3), and docosahexaenoic acid (22:6n -3), which are beneficial for health **[29]**, **[30]**. Research has shown that the daily intake of long-chain polyunsaturated omega-3 fatty acids in the UK, USA, Canada, and Australia is below the recommended norm (less than 100-200 mg/day). According to the International Society for the Study of Fatty Acids and Lipids, their content in the ration should be about 650 mg/day of polyunsaturated fatty acids and 2.2 g/day of alpha-linolenic acid **[31]**. Omega-3 polyunsaturated fatty acids provide significant nutritional and health benefits, such as by preventing coronary heart disease, hypertension, type 2 diabetes, rheumatoid arthritis, and obstructive pulmonary disease **[32]**, **[33]**, **[34]**, **[35]**, **[36]**.

Including unsaturated fatty acids in dairy products interests food industry enterprises. These products can greatly strengthen health and prevent diseases **[25]**.

The main food sources of eicosapentaenoic and docosahexaenoic acids are tuna and salmon **[37]**; for alpha-linolenic acid, there are plant sources such as dark green leafy vegetables and flaxseed oil **[38]**. Flaxseed oil contains 52% alpha-linolenic acid, is an excellent source of omega-3, and is a good carrier of vitamin D_3 **[39]**. The disadvantage of introducing animal fats (fish oil) into dairy products as a source of omega-3 fatty acids is its fishy taste. At the same time, using vegetable oil as a source of omega-3 can avoid this and get enriched products that meet consumers' requirements in terms of taste properties **[39]**, **[40]**. Thus, sources of enrichment in unsaturated fatty acids can be vegetable raw materials such as nuts, chia seeds, flax, canola, and soybeans and oils such as olive, canola, soybean, chia, linseed, palm, and corn **[41]**, **[42]**. Significant concentrations of omega-3 alpha-linolenic acid, up to 9.5%, were found in wheat bran oil **[43]** and in rye bran oil (7.6%) **[44]**. However, flax is considered one of the best and most affordable additives for making cheeses with an increased content of essential omega-3 fatty acids **[45]**. Flaxseeds are grown worldwide for fiber, oil, medicinal purposes, and as a food product **[46]**. Flaxseed's nutritional, functional, probiotic, and phytoactive properties are attracting the attention of health food consumers and producers **[38]**. Flaxseed is considered a potential functional food ingredient as it provides a variety of health benefits along with nutritional value **[46]**. Flaxseed consists of 37-41% fat, 28-29% total dietary fiber, 20% of protein, 6.5-7.7% of moisture, and 2.4-3.4% of ash **[46]**. Worldwide, flaxseed is recognized as the well-known richest plant source of omega-3 fatty acids, containing alpha-linolenic acid (18:3), which accounts for 39.00 to 60.42% of total fatty acids (including polyunsaturated fatty acids 73%, monounsaturated fatty acids 18% and saturated fatty acids 9%), followed by oleic (18:1n-9) 13.44-19.39%, linoleic (18:2n-6) 12.25-17.44%, palmitic acid (16:0) 4.90-8.00% and stearic acid (18:0) 2.24-4.59% **[47]**.

The development of a yogurt recipe that was enriched with omega-3 fatty acids by adding flax and blackcurrant oils was reported to provide 10% of the recommended value for α-linolenic acid **[48]**. Researchers **[49]** added 10% linseed oil to the curd paste as a source of α-linolenic acid. To increase polyunsaturated fatty acids in cheese and various dairy products, researchers **[50]**, **[51]**, **[52]** added feed rich in these acids, flaxseed, and rapeseed to the ration of cows.

Thus, the demand for omega-3-rich foods is increasing worldwide and is expected to grow. This forces the food industry to constantly work on developing dairy products with good taste properties and significant demand. Therefore, the enrichment of hard cheeses with various sources of essential acids is promising, since cheeses have a significant demand and are consumed by different population categories in many countries. Therefore, developing flaxseed cheese will expand the range of dairy products rich in omega-3 fatty acids.

The work aimed to determine the dynamics of microbiological indicators in the ripening technology of hard rennet cheese with different contents of flax seeds as a source of omega-3 fatty acids.

Scientific Hypothesis

To develop a technology for producing hard rennet cheese with flax seeds and to set whether the added content of flax affects the ripening process of the cheese.

MATERIAL AND METHODOLOGY

Production researches were conducted at the Chortkiv cheese factory (Chortkiv, Ukraine), and laboratory research (physicochemical and microbiological) at the Department of Food Biotechnology and Chemistry of the Ivan Pulij Ternopil National Technical University.

Samples

20 samples of hard rennet cheese with different flaxseed content were investigated (5 samples with 1.5% flax, 5 samples with 3% flax, 5 samples with 5%, and 5 were controls without flax).

Chemicals

To produce hard cheese, milk of 3.5% fat was used; mesophilic sourdough CHN-19 (Chr. Hansen, Denmark), consisting of *Lactococcus lactis subsp. cremoris*, *Lactococcus lactis subsp. lactis*, *Leuconostoc mesenteroides ssp. cremoris* and *Lactococcus lactis subsp. diacetylactis*; rennet enzyme (Natural Extra, Italy), which consists of 95% of chymosin and 5% of pepsin; calcium chloride (Novokhim, Ukraine) and "Debut" flax seeds.

Laboratory Methods

Microbiological testing was performed according to standard methods, which included preparing samples for the research, carrying out tenfold dilutions, and sowing them on selective and accumulating media. In particular, bacteria of the *Enterobacteriaceae* genus on the Endo medium (Pharmaktyv, Ukraine), lactic acid bacteria on the MRS Agar medium (HiMedia, India) according to the national standards of Ukraine DSTU 7357:2013 **[53]**, bacteria of the genus *Salmonella* and *Listeria* according to DSTU EN 12824:2004 **[54]** and DSTU ISO 11290-1:2003 **[55]** respectively. Staphylococci were isolated on BD Baird-Parker Agar medium (HiMedia, India). Bacteria of the genus Bacillus were determined by sowing cheese and its dilutions on meat peptone agar, followed by incubation at 30 0C for 72 hours. The samples were kept in a water bath at 85 °C for 15 minutes. Fungi on Saburo's medium (Pharmaktiv, Ukraine). The number of mesophilic bacteria on meat peptone agar medium with incubation of crops at a temperature of 30 ±1 °C for 72 hours.

Description of the Experiment

Sample preparation: The preparation of flax seeds: flax seeds (5-10 g) were washed in a 1% solution of baking soda (100 ml) at a temperature of 55 ±5 °C for 5 min, followed by washing in sterile tap water (100 ml), followed by drying in a drying cabinet in a Petri dish at a temperature of 95 ±1 °C for 20 min and stored in a sterile package.

Briefly, the cheese production technology with linseed was as follows. Pasteurized cow's milk was heated to a temperature of 34-35 °C, and dry leaven CHN-19 was applied to its surface, left for 3-4 min, and stirred for uniform distribution of the leaven and left alone for 30-35 min. Calcium chloride and rennet enzyme were added, previously dissolved in 50 ml of water. The mixture was evenly mixed and left for 40-45 minutes to form a clot. After that, the curd was checked for readiness (whey separation test), and the cheese clot was cut into cubes 1-1.5 cm in size. The cut cubes were mixed in a circular motion for 5 minutes and left to settle to the bottom. Then, 10% of the serum was removed, and the same volume of water was added at a temperature of 65 ±1 °C; the mass was kneaded for 10 minutes and left alone again for the grains to settle to the bottom. Then, a third of the whey was drained, and the same amount of water was added at a temperature of 42-43 °C; the cheese mass was stirred for 20 min, and the cheese grain was separated by draining the whey. We added sterilized flax seeds according to the method we developed and mixed the curd grain for uniform distribution of flax. The cheese grains were collected in molds and given 5-10 minutes for the whey to drain, then put under a press for 12 hours. After pressing, the cheese head was placed in a brine bath for 12 to 18 hours, depending on the duration of exposure. After half of the elapsed time, the cheese head was turned over. Then the heads of cheese were laid out on drainage mats to remove residual moisture and form a dry crust for 3-4 days, turning the head of cheese 2 times a day. After forming a dry crust, the head of cheese was placed for ripening at a temperature of 8-12 °C for 60 days.

The technology of preparation of the control sample of cheese was similar to that of cheese with flax seeds. Only flax was not added to the cheese grain.

Number of samples analyzed: we analyzed 20 samples.

Number of repeated analyses: All measurements of instrument readings were performed three times.

Number of experiment replication: The number of repetitions of each experiment to determine one value was three times.

Design of the experiment: In the first stage, the microbiota of flaxseed was studied, and a disinfection method was developed. In the second stage, the technology of hard cheese with flax seeds was developed. On the third, the dynamics of microbiological changes in hard cheese with flax seeds during its ripening were studied.

Statistical Analysis

Statistical processing of the results was carried out using methods of variation statistics using Statistica 9.0 (StatSoft Inc., USA). Non-parametric methods of research were used (Wilcoxon-Mann-Whitney test). The arithmetic mean (x) and the mean (SE) standard error were determined. The difference between the comparable values was considered significant for $p < 0.05$.

RESULTS AND DISCUSSION

In the first stage of the work, the microbiota of flax seeds was researched before washing and after our proposed processing in a 1% solution of baking soda with subsequent drying at a temperature of 95 ±1 °C for 20 minutes. The results are given in Table 1 and Figure 1.

Table 1 Characteristics of flaxseed microbiota during preparation for use in the production technology of hard cheese with an increased content of omega-3 fatty acids, x ±SE; n =5.

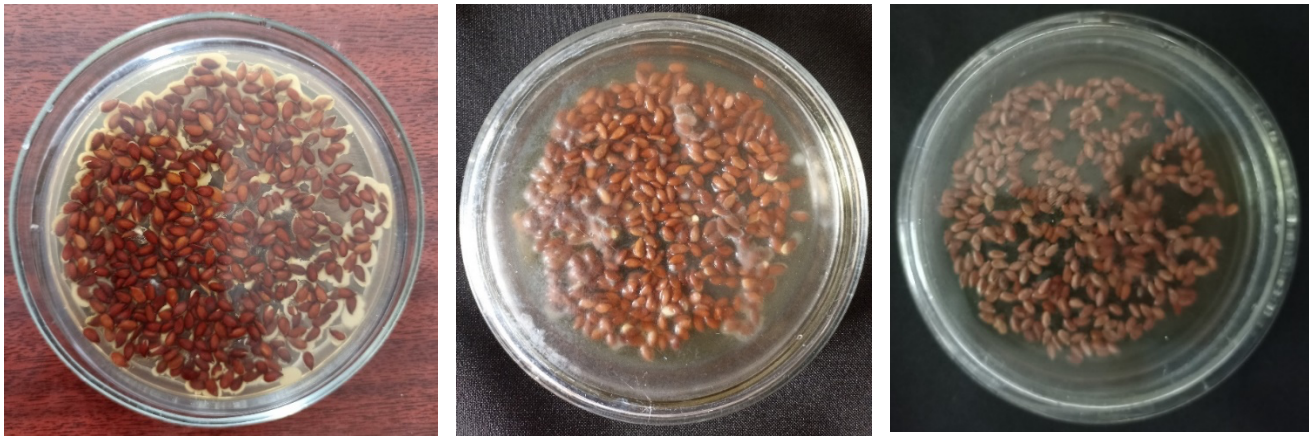
Indicator	Technological operations of flax seed preparation		
	before processing	after soaking in a 1% soda solution	after drying in a drying cabinet
Number of mesophilic bacteria, CFU/g	$7.8 \pm 0.3 \times 10^4$	$5.9 \pm 0.2 \times 10^{3*}$	$3.9 \pm 0.1 \times 10^{1**}$
Number of *Bacillus* spp., CFU/g	$8.6 \pm 0.3 \times 10^2$	$8.5 \pm 0.3 \times 10^{1*}$	$1.1 \pm 0.1 \times 10^{1**}$
Number of fungi, CFU/g	$4.8 \pm 0.1 \times 10^2$	$5.1 \pm 0.2 \times 10^{1*}$	$0.3 \pm 0.1 \times 10^{1**}$

Note: * - $p < 0.05$, ** $p < 0.001$ – compared to the amount before processing.

It was found that the surface of ordinary linseed is quite significantly contaminated with bacterial and fungal microflora. Usually, such seeds can only be used with disinfecting processing in dairy product production technology. In particular, insemination with mesophilic bacteria before processing was $7.8 \pm 0.3 \times 10^4$ CFU/g, which is a significant amount. Reducing microbial insemination by soaking in a 1% sodium bicarbonate solution for 10 min followed by washing in running drinking water provided an average of 13.2 times ($p < 0.05$) decrease in mesophilic microbiota on the seed surface. The next processing, which included drying the flax seed in an oven at a temperature of 95 ±1 °C for 20 min, ensured its almost complete disinfection since a small amount of mesophilic microorganisms – $3.9 \pm 0.1 \times 10^1$ CFU/g – was isolated from the surface. That is, the number of mesophilic microorganisms was 150 times ($p < 0.001$) less than after washing.

The processing proposed by us also had a significant influence on the reduction of spore-forming microflora since their content after soaking in sodium bicarbonate solution was 10.1 times lower ($p < 0.05$) compared to the amount on the surface of flax seeds before processing – $8.6 \pm 0.3 \times 10^2$ CFU/g. Excessive content of spore-forming microflora in the raw material can affect the cheese ripening process because this microbiota may contain anaerobic representatives that cause defects (swelling of the cheese head) in the later stages of its ripening. Temperature processing of seeds in a drying cabinet did not have such a significant influence on reducing the number of spore-forming microbiota as on the mesophilic one. Since the content of Bacillus spp. after such processing was decreased by 7.3 times ($p < 0.05$), and their number was $1.1 \pm 0.1 \times 10^1$ CFU/g. This content of *Bacillus* spp. is non-essential and cannot influence cheese production technology when adding flax seeds, as they belong to aerobic microflora.

The influence of the proposed processing on the fungal microflora was also investigated because these microorganisms are ubiquitous and can show their activity in a wide range of temperatures and pH of the environment. Fungal microbiota was also well washed off during soaking in the soda solution, as their number was decreased on average by 9.4 times ($p < 0.05$) to $5.1 \pm 0.2 \times 10^1$ CFU/g. This content of fungal microorganisms on the surface of flax seeds is still quite significant, such seeds cannot be introduced into the production technology of rennet cheeses, where the temperature and humidity conditions will be favorable for their growth. Using the drying mode in the cabinet under the selected mode made it possible to reduce the contamination of fungal microbiota on the surface of flax to $0.3 \pm 0.1 \times 10^1$ CFU/g, i.e., 17 times ($p < 0.001$). The results of microbiological research are shown in Table 1 and are partially illustrated in Figure 1. It can be seen that before processing, flax seeds are covered with continuous colonies of microorganisms that permeate the thickness and surface of the nutrient medium (Figure 1a). After soaking in a sodium bicarbonate solution and washing in water, the number of microorganisms on the surface decreased significantly, as indicated by the growth of colonies (Figure 1c). Sowing flax seeds after the drying process did not reveal the growth of microflora on the surface of the nutrient medium (Figure 1c).



a b c

Figure 1 Growth of flaxseed microbiota in Petri dishes after the proposed processing. Note: a) before processing; b) after washing; c) after drying at 95 ±1 °C for 15 min.

In general, our proposed processing of flax seeds to reduce the number of microorganisms on its surface is quite effective. It allows us to introduce raw materials into the recipe for producing hard rennet cheese enriched with omega-3 fatty acids.

Adding flax seeds to the rennet cheese production technology can affect microbiota development and maturation. Therefore, research was conducted to substantiate the parameters of the microbiological process for the production technology of hard rennet cheese with different amounts of flax seeds. Mathematical modelling of the cheese recipe according to the content of omega-3 fatty acids showed that the optimal amount of flax seeds as a source of essential acids would be from 1.5 to 5%. In the given range of flaxseed concentrations, three samples of rennet cheese with a flaxseed content of 1.5%, 3.0, and 5.0% were produced (Figure 2).

Figure 2 Hard cheese with 3% flax seeds after ripening.

Research of changes in lactic microbiota in cheese samples with different flaxseed content (Figure 3) revealed a more intense microbiological process with the participation of lactic microflora in cheese samples with flaxseed during the first 10 days of ripening, compared to the control sample of hard rennet cheese.

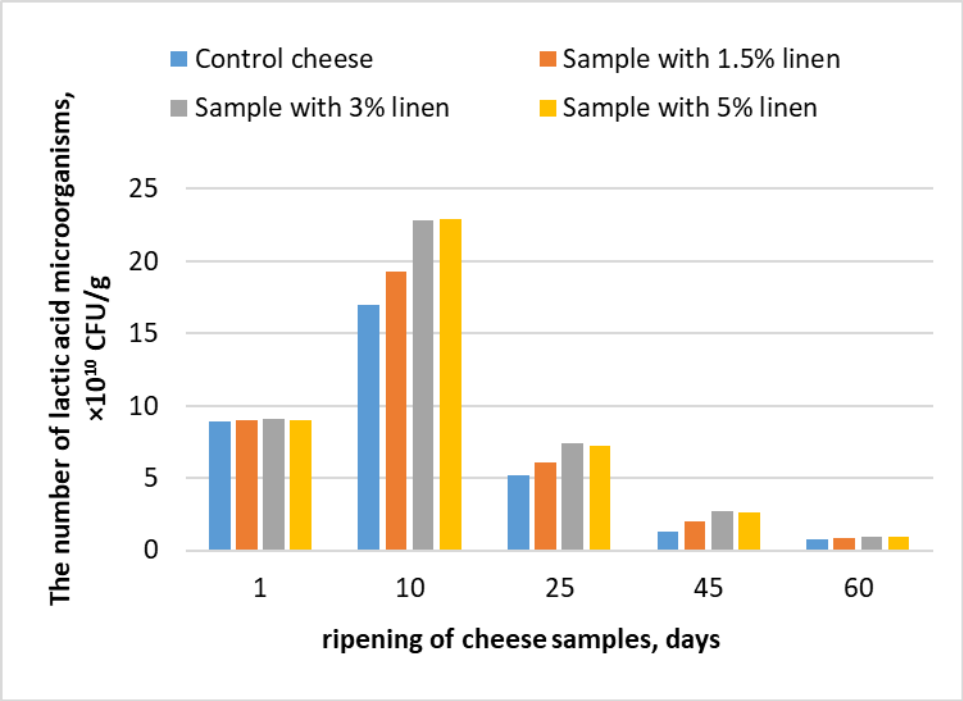


Figure 3 Development of lactic acid microorganisms in cheese samples with flax seeds.

In particular, with an almost identical amount of lactic microflora on the first day – 9.0 ±0.1×10^{10} CFU/g, after ten days of maturation, their number in the sample of rennet cheese with the highest content of flax seeds (5%) increased by 2.5 times (p <0.05), which is on average 1.2 times more lactic acid bacteria than in the cheese sample with the lowest flaxseed content (1.5%). In the control cheese sample, the content of lactic acid bacteria was 1.3 times (p <0.05) less than in the cheese sample with 5% linseed oil. The ripening of cheese from 10 to 25 days revealed a rapid decrease in lactic acid microflora, both in experimental samples of rennet cheese with flax and in the control sample. At the same time, a statistically significant difference between the dynamics of the reduction of lactic acid bacteria in the experimental cheese samples and the control was not set since the content of lactobacilli in the tested cheese samples during this period was decreased by an average of 3.2 times (p <0.05). This indicates that during this period, the lactic acid microflora has insufficient carbohydrates for nutrition and intensive development, so it gradually dies.

From the 25th to the 45th day of ripening of the experimental cheese samples, the process of dying of lactic acid bacteria continued. At the same time, in experimental samples of cheese with flax content, the number of lactobacilli during this period was decreased by 3.0 and 2.7 times (p <0.05), against 4.0 times (p <0.05) in the control sample of rennet cheese.

Research of the microbiota of experimental samples of hard rennet cheese with flax on the 60th day from the beginning of ripening revealed a decrease in the rate of death of lactic acid microbiota compared with the previous periods of the research. That is, on the 60th day of ripening of experimental samples of hard rennet cheese with flax, the number of lactobacilli in the cheese was 0.85-0.94×10^{10} CFU/g, which depended on the concentration of added flaxseed in the control cheese their number was 0.78 ±0. 03×10^{10} CFU/g.

Technically harmful fungal microflora, which accidentally enters the product during production in the process of vital activity, produces a much more diverse number of enzymes than lactic acid. Therefore, due to enzymatic processes, a complex of chemical substances is formed, which negatively influences the quality of rennet cheese. We determined the dynamics of fungal microbiota development in hard rennet cheese with different amounts of flax seeds during its two-month ripening process (Figure 4).

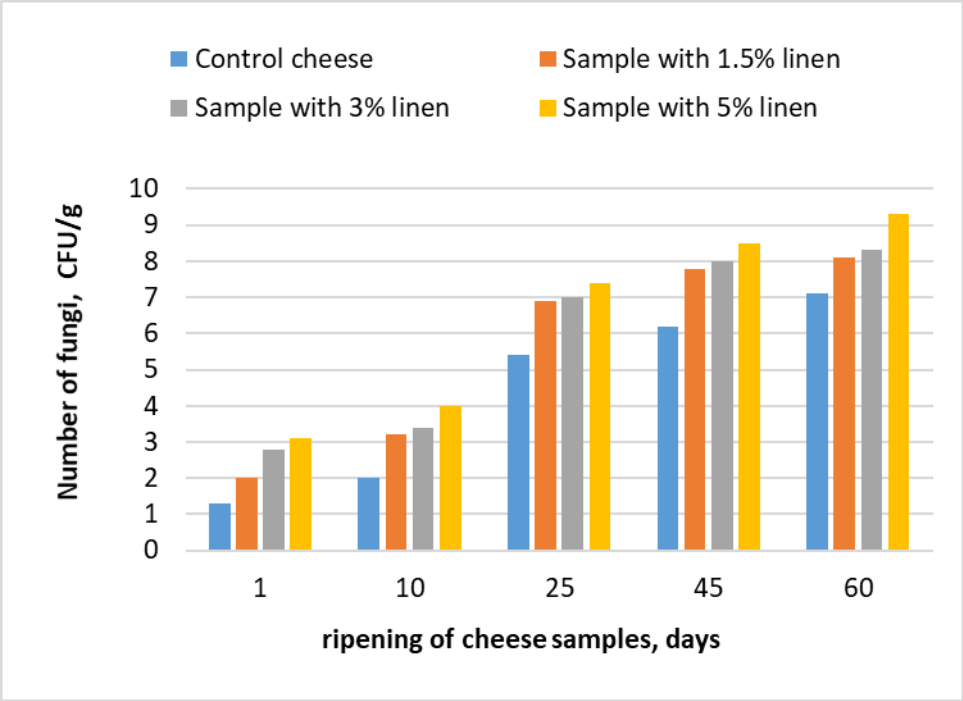


Figure 4 Development of fungal microbiota in samples of cheese with flax seeds.

After the first day of the technological process, the amount of fungal microflora in experimental cheese samples with flax seeds was 2 to 3.1 CFU/g. At the same time, the highest contamination was noted in the sample with the highest content of flax, and in the control sample of cheese, the number of fungi was, on average, 1 cell per g. During the 60-day ripening process of the experimental cheese samples, the number of fungal cells gradually increased, and in the finished product, it was within the range of 8-10 cells per 1 g. This content of fungal microflora in rennet cheese poses no threat to the deterioration of its microbiological and organoleptic evaluation, as in fresh cheese, as well as during storage. As such, several fungi cells cannot show significant enzymatic processes.

Because flax seeds are contaminated with spore-forming bacilli that belong to the epiphytic microbiota, and the frugal method of reducing the microflora from the seeds proposed by us does not entirely inactivate them, we determined the possibility of their development in cheese during the ripening process (Figure 5).

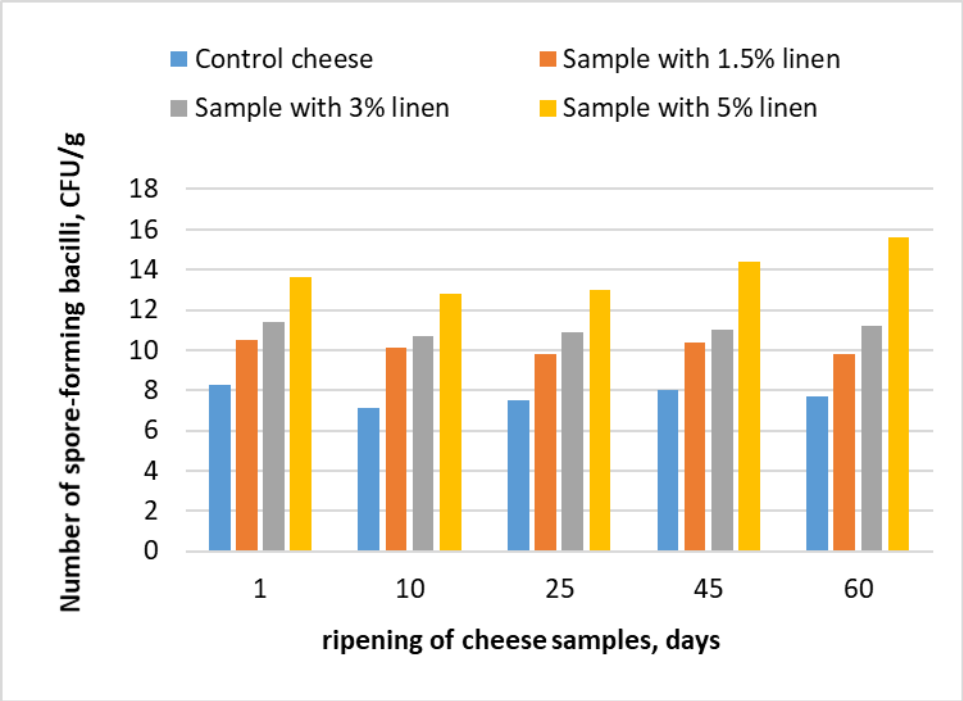


Figure 5 Development of spore-forming bacilli in samples of cheese with flax seeds.

We observe the dependence between the content of spore-forming bacilli on the first day of cheese production and the amount of flaxseed added to the cheese mass. In particular, with an increase in the concentration of linseed in cheese samples, the content of Bacillus bacteria increased. On the first day of the research, the number of spore-forming bacteria in the sample with 5% flaxseed content was 1.6 times greater (p <0.05) than in the control sample of cheese and 1.3 times (p <0.05) greater compared with the sample with 1.5% flaxseed content. It becomes evident that these bacilli are sources of flax seeds, which maintain their vitality during the first day of the production process. During this, there are favorable conditions for their development since, at this stage, the technological process takes place with good aeration of the environment, which is essential for the vital activity of aerobic bacilli. However, even this amount of Bacillus bacteria at this stage of cheese production is insignificant and does not influence the beneficial microbiota of sourdough and biochemical changes in the product.

During the quantitative evaluation of bacilli on the 10^{th}-60^{th} day of ripening of samples of hard rennet cheese with different flaxseed content, their development was stopped, as the content was almost at the same level as the value of the first day, within 10-15 CFU/g.

The influence of hard rennet cheese production technology with added flax seeds on the quantitative changes of sanitary-indicative and pathogenic microorganisms was determined (Table 2).

Table 2 Research of changes in sanitary-indicative microorganisms and pathogenic ones according to the production technology of hard rennet cheese with flax seeds, x ±SE, n = 5.

Indicators	The term maturation, day	Norms (DSTU 6003: 2008)	Control cheese	Experimental samples of hard rennet cheese with flax seeds		
				1.5 %	3.0 %	5.0 %
Enterobacteriaceae	1	in 0.01 g of the product is not allowed	>1	>1	>1	>1
	10		>1	>1	>1	>1
	25		>1	>1	>1	>1
	45		>1	>1	>1	>1
	60		>1	>1	>1	>1
Staphylococcus aureus	1 10 25 45 60	no more than 500 CFU/g	Not found	Not found	Not found	Not found
L. monocytogenes, *Salmonella* spp.	1 10 25 45 60	in 25 g of the product is not allowed	Not found	Not found	Not found	Not found

It was found during hard rennet cheese production and ripening technology with flax seeds. These microorganisms indicate compliance with the entire complex of sanitary requirements - the *Enterobacteriaceae* genus was not detected in 1 g of the product.

In general, from the results of a series of experiments on the influence of the added different amounts of flax seeds on the microbiological processes of ripening hard rennet cheese, we found that the main representatives of the autochthonous microbiota – lactic acid microflora develop somewhat more intensively in samples with 5% flax, compared to 1.5% content and in the control sample. However, the general dynamics of the growth of lactic acid microorganisms in the experimental samples corresponds to the dynamics in the control. At the same time, developing technically harmful microorganisms (spore-forming and fungal microbiota) in cheese samples with flax and control does not lead to the deterioration of their microbiological indicators. This makes it possible to use flax seeds in the production technology of hard rennet cheeses at 5%.

Highly nutritious dairy products such as hard cheeses are considered a good source of protein, fats, minerals, and vitamins **[56]**, **[57]** and are consumed in significant quantities in the traditional European diet **[50]**. At the same time, the disadvantages of cheese include the presence in their composition of a large amount of saturated fatty acids **[58]**, the consumption of which causes the development of cardiovascular diseases **[30]**, **[32]**, **[33]**, **[34]**, **[35]**, **[36]**. Therefore, modifying the composition of fatty acids in hard cheese by increasing the content of unsaturated fatty acids and reducing the amount of saturated fatty acids is extremely important for consumers' health. Enriching cheese with sources rich in unsaturated fatty acids increases the quality of the product by improving its fatty acid profile **[38]**, **[39]**. Alternative sources of unsaturated fatty acids include flaxseed and oil,

echium, walnut, algal oil, etc. **[41]**, **[42]**, **[44]**. This research shows that the problem of consumption of foods rich in polyunsaturated fatty acids can be partially solved by developing a hard rennet cheese with flax seeds.

It has been set that flax seeds are contaminated with saprophytic microflora of plants and the environment. Therefore, it can only produce hard cheese with prior disinfection processing. In particular, insemination with mesophilic bacteria before processing was 7.8 $\pm 0.3 \times 10^4$ CFU/g, which is a significant amount. The normative indicator of the content of mesophilic microflora for non-traditional plant raw materials (which includes flax seeds) should not exceed the amount of 5 $\times 10^4$ CFU/g **[59]**. The method proposed by us (washing the seeds in a solution of sodium bicarbonate and drying at a temperature of 95 ±1 for 20 min) of processing flax seeds before adding them to the cheese mass reduced the number of mesophilic microorganisms to 3.9 $\pm 0.1 \times 10^1$ CFU/g. This microbial number of mesophilic bacteria is not significant for breaking the production technology of rennet cheese **[60]**. Also, with this processing, the amount of fungal microbiota on the surface of the seeds was decreased approximately 160 times to 0.3 $\pm 0.1 \times 10^1$ CFU/g. Such content of fungal microflora on the surface of flax seeds is relatively safe for its introduction as an additive rich in omega-3 fatty acids in hard rennet cheese production technology.

Of interest was research on the influence of different amounts of added flaxseed on the microbiological processes in cheese during its ripening. It revealed a 1.3 times higher content of lactic acid microflora in samples of hard cheese with 5% flax seeds during the first 10 days of ripening compared to a control sample of cheese. Probably, the more intensive development of lactic acid bacteria in samples of cheese containing flax seeds is associated with additional enrichment of the cheese with minerals and B vitamins, which flax is rich in **[28]**, **[46]**, **[47]**, and which are necessary for the development of lactic acid microbiota **[11]**, **[12]**, **[13]**, **[61]**. In addition, the growth of a more significant number of lactic acid microflora in the experimental samples will contribute to faster ripening of the rennet cheese.

Compared to control cheese, an average of 1.4 times slower dying off of lactic acid microbiota was also revealed in the period from 25 to 45 days of ripening of experimental cheese samples. In our opinion, this decrease in the intensity of the death of lactic microflora in rennet cheese with flax during ripening is related to its enrichment with biologically active substances of the seeds, which serve as an additional source of nutrition for lactic acid bacteria.

So, it follows from the research that the ripening process of hard rennet cheese with the addition of 1.5 to 5% of flax seeds to the cheese grain had a beneficial influence on the development of lactic acid microflora, as their number was greater than in the control sample of cheese, and the dying process was slower. This indicates that adding 1.5 to 5% of flax seeds in the production technology of hard rennet cheese does not disrupt the general dynamics of the development of lactic acid microflora inherent to this type of cheese.

Adding various phytosupplements to the technology of hard rennet cheese can contribute to additional contamination with fungal microbiota since the spores of these microorganisms are usually present on raw plant materials **[62]**, **[63]**. In our research, when adding flax seeds to the hard cheese production technology, the fungal microbiota increases to 10 cells per g in experimental samples during 60 days of ripening, against 7 CFU/g in control. At the same time, the largest amount was in the sample of cheese, which contained 5% of flax seeds. This content of fungal microbiota did not exceed the permissible content of 50 CFU/g for most dairy products provided by the national standard **[60]**. Therefore, we believe that the use of flax seeds according to our proposed method of disinfection, which involves washing in a soda solution, drying, processing at pasteurization temperatures, and adding to the production technology of hard rennet cheeses, does not lead to a statistically probable growth of fungal microflora in the test samples during the entire ripening process.

At the beginning of cheese ripening (the first day), a 1.6 times higher content of spore-forming aerobic bacilli was found in the sample with the highest flax seeds compared to the content in the control cheese. At the same time, during 60 days of ripening of the cheese, no process of their reproduction was detected since their content practically corresponded to the initial amount of 10-15 CFU/g in the experimental samples and about 8 in the control. This indicates that anaerobic conditions are created in the middle of the cheese head during the technological process, unfavorable for developing this type of bacteria. Such data are consistent with research **[2]** that aerobic spore bacilli cannot reproduce in hard cheese technology. At the same time, their number should be at most 10 cells in raw materials **[59]**. Therefore, a small (up to 10 CFU/g of product) amount of aerobic bacilli introduced with flax seeds does not harm the production technology of hard rennet cheese with flax content.

In standard 6003:2008, hard rennet cheese is evaluated according to sanitary indicator microorganisms – *Enterobacteriaceae* and pathogens: *Staphylococcus aureus*, *Listeria monocytogenes,* and *Salmonella*. Bacteria of the *Enterobacteriaceae* genus should not be detected in 0.01 g of cheese at the time of sale, and the species Staphylococcus aureus in 1 g of rennet cheese should not exceed 500 CFU/g. At the same time, the pathogenic bacteria *Listeria monocytogenes* and *Salmonella* spp., traditionally used in dairy products, should not be in 25 g. Evaluation of cheese samples according to microbiological indicators of safety, respectively **[60]** during the entire

ripening process with different content of flax seeds did not reveal an excess in the number of bacteria of the genus *Enterobacteriaceae*, *Staphylococcus aureus*, and such pathogenic bacteria as *L. monocytogenes* and *Salmonella* spp. were not detected in 25 g of the product. This indicates that all cheese samples have a significant reserve of storage stability. It can also be stated that according to indicators of microbiological safety, all test samples were not inferior to the control cheese, and no pathogenic microorganisms were isolated from them.

In general, more and more data have recently appeared, and research was conducted on the enrichment of various types of cheese [48], [49] with vegetable oils to improve their lipid composition **[64]**, **[65]**. The addition of oils, particularly linseed, is a perspective in developing recipes for new types of products. In contrast, in hard cheese technology, adding vegetable oils will change the product category from cheese to cheesy, which consumers do not like very much. Therefore, in our research, flax seeds were added as a source of omega-3 fatty acids during the production of hard rennet cheese, increasing the product's biological value and enriching it with dietary fibers. The data received is consistent with investigations **[66]**, which studied the addition of whole flax seeds and their flour to the technological parameters of soft cheese. It was found that enriching the cheese with flax seeds is more expedient since the proportion of polyunsaturated fatty acids decreases during grinding. At the same time, adding flax seeds and flax flour slightly enhanced the development of the cheese's bifidobacteria and lactic acid microflora. Enrichment of such dairy products as yogurt and curd paste with linseed oil did not influence microbiological processes during production and storage **[48]**, **[49]**. At the same time, researchers **[67]** showed that adding linseed mucilage to cream cheese increased protein, ash, and total solids content, while moisture content and pH values decreased. In addition, it was found that the combination of flaxseed mucilage and probiotic bacteria enhanced the antibacterial action against pathogenic bacteria such as *Pseudomonas aeruginosa* and *Yersinia enterocolitica* **[67]**. In general, we can conclude that flax seeds, flax oil, or flax products positively influenced the microbiota of dairy products. Although we support the opinion of scientists **[50]**, **[51]**, **[52]**, **[68]** that a promising direction for the production of dairy products rich in polyunsaturated fatty acids is the correction of the ration of animals to get milk of improved lipid profile. At the same time, this direction is just emerging. It needs scientific justification and the possibility of industrial production of a sufficient amount of such milk.

Therefore, the disinfection of ice seeds according to our proposed method significantly reduces its contamination with epiphytic microorganisms, which allows it to be added to the technological process of production of hard rennet cheese in an amount of up to 5%. With such a quantity of flax seeds in the hard cheese recipe, no significant changes in the microbiological process occur during two months of ripening.

CONCLUSION

The proposed method (washing the seeds in a solution of sodium bicarbonate and drying at a temperature of 95 ±1 for 20 min) of processing flax seeds before adding them to the cheese mass reduced the number of mesophilic microorganisms to 3.9 $\pm 0.1 \times 10^1$ CFU/g, fungi in 160 times up to 0.3 $\pm 0.1 \times 10^1$ CFU/g and aerobic mesophilic bacilli 78 times up to 1.1 $\pm 0.1 \times 10^1$ CFU/g. The technology of hard rennet cheese with a flax seed content of 3-5% has been developed. A 1.3 times higher content of lactic acid microflora was found in samples of hard cheese with 5% flax seeds during the first 10 days of ripening compared to the control sample of cheese. On the 60th day of ripening of cheese with a content of 5% flax seeds, the number of lactic acid bacteria was 9.4 $\pm 0.3 \times 10^9$ CFU/g, and in the control cheese – 7.8 $\pm 0.3 \times 10^9$ CFU/g. During the technology of production and ripening of cheese with different content of flax seeds, no excess of the normative values was found for the number of bacteria of the genus *Enterobacteriaceae*, *Staphylococcus aureus*, and such pathogenic bacteria as *L. monocytogenes* and *Salmonella* spp. were not detected in 25 g of the product. Therefore, the developed hard rennet cheese with flax seeds can be consumed as an additional omega-3 fatty acids and dietary fiber source.

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