Production of soft cheese enriched with functional ingredients

L G Krekker

East Siberia State University of Technology and Management, 40V, Klyuchevskaya str., Ulan-Ude, 670013, Russia

E-mail: krekker@mail.ru

Abstract. The article presents the results of a study on the possibility of obtaining soft cheese prepared on the basis of acid-rennet coagulation using calcium and magnesium salts, as well as a combination of starter cultures with the addition of streptobacteria. The production of valuable indicators of culture, including proteolytic, lipolytic activity and aromatization has been studied. The optimal conditions for obtaining acid-rennet curd under conditions of combined use of calcium and magnesium salts have been selected, which allow reducing the transition of protein to whey and increasing the content of magnesium and protein substances in the matured soft cheese.

1. Introduction

Soft cheese in Russia is a category of relatively new products. According to experts, from 2014 to 2018, the volume of domestic sales of soft cheese in Russia increased by 35.9%; from 29.6 thousand tons to 40.2 thousand tons [1]. And before 2020, this indicator showed an enviable growth rate. According to the results of 2019 and according to MilkNews agency, the demand for cheese in 2019 increased by 9%, despite the increase in prices by 10% [2].

Soft cheese occupies a special category among a large assortment of cheese. Their technology has several advantages: low milk consumption compared to semi-hard cheese, the possibility of using by-products, short ripening time, less than 14 days. These and many other factors actualize work on the development of the assortment of soft cheese, including using functional ingredients.

The purpose of this article is to increase the yield of protein substances in the production of soft cheese, enrich the product with calcium and magnesium, and create a symbiotic starter culture based on streptobacteria to improve the organoleptic and production-valuable parameters of the curd.

2. Research materials and methods

The use of probiotic starter cultures in cheese culture increases the nutritional and biological value of soft cheese. It is known that some probiotic cultures, including lactobacilli, have a unique ability to synthesize biologically active substances. For example, Lactobacillus plantarum have a pronounced proteolytic activity and independent ability to synthesize the essential amino acid arginine in large quantities [3]. Arginine regulates the level of nitric oxide in human blood, thereby preventing the deposition of cholesterol on the walls of blood vessels [4].

Soft cheese is a high-protein product. Milk proteins are a source of a large amount of minerals, including calcium, phosphorus, sulfur, etc. But at the same time they contain a small amount of magnesium, which has a significant effect on the absorption of calcium. In this regard, the introduction of this macrocell into functional protein products is relevant. According to the Institute of Nutrition...
RAMS, the need for magnesium for adults is 400 mg per day. In Russia, about 70-80% of people suffer from a lack of magnesium daily. Milk and cheese contain relatively little magnesium - 14 and 23 mg%, respectively.

In the process of obtaining cheese, ionized calcium in solution can act as a protein destabilizer without any heating, even as a result of acid-rennet coagulation. The effect of rennet is directed only to the casein-calcium phosphate complex [5]. It mainly takes part in rennet coagulation. Upon the production of cheese, the addition of calcium chloride to pasteurized milk causes a reaction of calcium ions with the constituent parts of the milk, which leads to more complete coagulation of casein. But it is known that magnesium is able to form colloidal forms with whey proteins, contributing to their more complete deposition.

Hydrophilic colloidal systems coagulate when a large amount of electrolyte is added. Protein macromolecules are folded into compact globules with a negative charge and very strong hydration shells. They are very stable in milk and do not coagulate when the casein isoelectric point is reached, although when pH decreases, they form associates of several monomers [5]. Whey proteins can be isolated by decreasing their solubility by introducing magnesium salt into milk. This technique was used in this work to obtain enriched soft cheese.

The principal difference of soft cheese is also active lactic acid fermentation, which is ensured by the use of cultures of lactic acid bacteria, increased doses of bacterial starter cultures, a long process of milk curdling and aging of cheese mass.

Starter microflora changes all the components of milk, determines the direction and intensity of biochemical processes, the quantity and spectrum of the resulting chemical compounds, and, as a result, the organoleptic advantages of the fermented product, its nutritional and biological value.

Traditionally, in the production of soft cheese, mesophilic lactic streptococci L. lactis, L. cremoris, L. diacetylactis are used as starter cultures; leucostats: Leuconostoc cremoris, Leuconostoc lactis, less commonly, L. helveticum and L. plantarum.

The use of L. plantarum culture is currently of particular interest, because a number of researchers have found that it promotes the accumulation in the medium of the amino acid arginine in a much larger amount compared to other probiotic cultures [3]. Arginine regulates the content of nitric oxide in the blood, which is responsible for controlling blood flow, immune function, communication between nerve cells, liver function, blood cholesterol level and its coagulability [4].

In cheese production, L. plantarum mesophilic lactic acid bacilli are mainly used to increase storage stability, because this microorganism has a pronounced antagonistic activity to Escherichia coli and a number of other extraneous microorganisms [6].

Due to its physiological and biological properties, in particular because of the low rate of acid formation, L. plantarum, according to A.V. Gudkov [8] and other researchers, can only be an additional component of starter cultures for cheese, in which the main role is played by lactic streptococci. In addition, it is known that during the fermentation of protein products by lactobacilli, milk calcium phosphates and citrates pass into more soluble calcium lactates. This contributes to better absorption of calcium by the human body when consuming a dairy product. All this and much more determine the need for the use of lactobacilli in the production of a new type of soft cheese.

3. Results and discussion

In the experiment for the production of soft cheese, the optimal ratios of starter culture for cheese making were selected, which included L. lactis, L. cremoris, L. diacetylactis in a ratio of 1:1:1 and culture of L. plantarum strain 8P-A3.

The culture was activated on a hydrolyzate-milk environment, at 30°C for (16-18) hours (according to the instructions of SAI VNIMI) and its physiological and biochemical characteristics were studied. The results are presented in table 1.
Table 1. Characterization of pure cultures \textit{L.plantarum}.

| Species of bacterium | Acidity, °T | Potential of ripening, h | VFA, ml 0.1 H NaOH | Carbon dioxide, mm | Diacetyl l, acetoin | Level of weeping, ml | Proteolytic activity, mg/100 ml | Maintenan ce of revivable bacterial flora, CFU/cm³ |
|----------------------|-------------|--------------------------|---------------------|-------------------|-------------------|---------------------|-----------------------------|----------------------------------|
| Sreptobacterium      | 82±2        | 16                       | 0.7                 | -                 | -                 | 47                  | 11.2                        | 1·10⁸                             |

The results of the studies showed that the restored cultures have good biochemical activity. Acidity in the starter of streptobacteria after 16 hours of fermentation reaches 82°T. \textit{L.plantarum} cultures have a fairly high viscosity, mild aroma; the content of volatile fatty acids in the starter is 0.7 ml. The degree of syneresis is moderate; the content of viable microflora is high 1·10⁸ CFU/g.

The morphology of bacteria with simple staining with methylene-blue aniline dye is shown in figure 1.

According to morphology, the culture is small cocci located in a microscopic preparation in the form of short curved chains, in the field of view of at least 167 cells in hydrolyzed milk.

![Figure 1. Culture \textit{L.plantarum} on a hydrolyzate-milk environment.](image)

At the next stage, the optimal starter ratios for soft cheese and streptobacteria were selected. The results are presented in table 2. To select the optimal ratio of starter cultures, various options were made and the activity of acid formation, proteolytic and lipolytic activity, and the accumulation of aromatic substances were studied.

According to the data in Table 2, the ratio of cultures 1:1 allows obtaining a clot for 13 hours with an acidity of 88°T, the number of mesophilic lactobacteria and streptobacteria cells is almost equal to 108 CFU/g. With an increase in the number of mesophilic microorganisms in the starter culture and their ratio to \textit{L.plantarum} 2:1, the fermentation process decreases to 11 hours, but the acidity of the clot also increases significantly.

Table 2. The selection of the optimal ratio of crops of combined culture.

| Ratio – starter for soft cheese: \textit{L. plantarum} | Acidity, °T | Number of cells, CFU/cm³ | Activity, h |
|-------------------------------------------------------|-------------|--------------------------|-------------|
|                                                       | pH          | Mesophilic streptococci | \textit{L. plantarum} |
| 1                                                     | 1:1         | 88                       | 4.82        | 3·10⁸               | 4·10⁸               | 13                       |
| 2                                                     | 2:1         | 92                       | 4.77        | 8·10⁸               | 2·10⁸               | 11                       |
| 3                                                     | 1:2         | 82                       | 4.89        | 5·10⁸               | 1·10⁹               | 14                       |

In this variant, the level of streptobacteria is reduced to 2·10⁸ CFU/g, which is undesirable for the starter culture, which is intended to be used to obtain a functional product. The most optimal crop ratio according to the studied parameters is the third version of the starter culture, since the content of
lactobacilli in it reaches $1 \cdot 10^9$ CFU/g, the variant is characterized by moderate acidity - 82°T and pH values of the clot. Thus, the research results showed that the optimal ratio of cultures of mesophilic lactic streptococci and streptobacteria in the preparation of starter culture for soft cheeses is 1:2.

Proteolytic activity is one of the most important properties of microorganisms, characterizing the ability to break up milk proteins with the formation of simpler nitrogen compounds: peptides and amino acids, including arginine [8]. Lactobacillus L. plantarum uses sodium caseinate if the medium contains cysteine in addition to it. For this culture, the presence of cysteine is sufficient for it to prove to be an energetic proteolite [7]. Lactic acid bacteria are involved in the enzymatic transformation of fat, which has a certain effect on the consistency of cheese. Lactic acid bacteria affect the taste and aroma of cheese by breaking up the lipid components of the cheese mass. Therefore, the microflora of modern bacterial starter cultures is selected taking into account the lipolytic activity of the strains. G.A. Belova and colleagues [7] recommend determining the lipolytic activity of lactic acid bacteria by the diffusion method - by the diameter of the lipolysis zones of the indicator medium.

It should be noted that the partial hydrolysis of proteins and fat carried out by lactic acid bacteria during the ripening of the cheese mass, not only contributes to the formation of specific organoleptic properties of cheese, but also leads to an increase in the digestibility of cheese, giving it certain biological properties. With the active participation of lactic acid microflora, free amino acids are accumulated in cheese, including essential amino acids, as well as peptides and oligopeptides with high biological activity. In this case, the synthesis of antibacterial substances, antibiotics and some vitamins occurs. The results of the study of the proteolytic and lipolytic activity of a combination of cultures are presented in table 3.

**Table 3. Biochemical indicators of the combined culture.**

| Ratio of crops | Proteolytic activity, mg /100 g of a product 24 hours | 48 hours | Lipolytic activity, mm |
|---------------|---------------------------------------------------|---------|-----------------------|
| Mesophilic lactic streptococci | 4.30 | 5.38 | 7 |
| L. plantarum | 7.27 | 9.0 | 4 |
| Combined culture 1:2 | 6.50 | 7.46 | 6 |

The results of the studies showed that the highest proteolytic activity among the studied cultures was possessed by L. plantarum lactic acid bacilli, which accumulate in 48 hours from 7.27 to 9.0 mg of tyrosine per 100 g of starter culture. But at the same time, the lowest level of lipolytic activity is observed in this sample - the zone width is 4 mm. Enzymatic hydrolysis of milk fat, the main source of which is lactic acid microflora, is most active in the first sample of 7 mm, which is associated with the presence of a larger number of lipolytic enzymes.

The research results also showed that the combined starter culture has a greater proteolytic and lipolytic activity than individual cultures: 6.5 mg/100 g of tyrosine product and 6 mm zone of enlightenment of the indicator medium.

The research data showed that the ferment obtained is characterized by rather high rates of proteolytic and lipolytic activity, which confirms the possibility of its use in cheese production.

Metabolism products formed by lactic acid bacteria during the fermentation of lactose and citrates (lactic, acetic acid, acetaldehyde, diacetyl-acetoin, ethanol, carbon dioxide, etc.), not only play an important role in the formation of taste, aroma, texture and pattern of cheese, but also to a large extent determine the direction of physicochemical, biochemical and microbiological processes [4]. In particular, a decrease in the active acidity of the cheese mass as a result of the accumulation of lactic acid causes the deactivation of alkaline proteases, which carry out proteolysis non-specific for cheese, and contributes to the transition of some protein compounds from the sol to gel state, which changes the structure of the cheese mass.
In the process of life, starter microorganisms accumulate aromatic substances in the environment (volatile acids, acetaldehyde, diacetyl-acetoin, etc.), which are involved in the formation of a typical taste and aroma of the finished product. Lactic acid and VFA give products a pronounced sour-milk taste, diacetyl, acetaldehyde give products a specific sour-milk flavor; CO\textsubscript{2} gives products a pleasant, refreshing taste. Various flavors of cheese are felt mainly due to the presence of acetaldehyde, diacetyl, as well as volatile fatty acids. The research results are presented in table 4.

### Table 4. Aroma and gas-forming ability of the combined culture.

| Type of culture                  | Availability of diacetyl-acetoin | Content of VFA, mg/100g | Availability of gas, mm |
|---------------------------------|----------------------------------|-------------------------|-------------------------|
| Mesophilic lactic streptococci  | +                                | 2.8                     | -                       |
| L. plantarum                   | +                                | 3.0                     | -                       |
| Combined culture 1:2            | +                                | 3.6                     | -                       |

Data from experimental studies have shown that diacetyl and acetoin are present in all samples and there is no carbon dioxide. The content of VFA is the highest in the combined starter culture, which is explained by the synergistic effect when cultures are combined with symbiotic relationships. The lowest VFA content is in the starter culture prepared on mesophilic lactic streptococci without the addition of streptobacteria.

The composition of bacterial starter cultures is an important factor determining the density and other structural and mechanical properties of protein clots. The introduction into the composition of bacterial starter cultures of energetic acid-forming agents contributes to the production of a denser clot with intensive separation of serum, and low-energy clot with a tenderer clot.

The results of studies conducted earlier [9] showed that when magnesium and calcium are used together with acid-rennet coagulation, a denser clot (Bogach density is 0.086 kg/cm\textsuperscript{3}) is formed compared to the traditional method (0.057 kg/cm\textsuperscript{3}). Coagulation time is reduced to 17 min, compared with traditional - 22 min. The use of calcium and magnesium salts together allows slightly reducing the amount of serum released and increasing the yield of protein mass. The protein yield is 20.18 g / 100 ml of milk. It is possible that the use of magnesium salts makes it possible to enlarge the colloidal structures of milk, which increases the size and mass of casein micelles and promotes the formation of colloidal complexes with whey proteins, which generally leads to a reduction in the processing time of cheese mass and an improvement in the use of milk solids [9].

In the experiment presented in this article, milk corresponding to cheese suitability was used, pasteurized at a temperature of (72±2) °C, for 20-30 seconds, cooled to the coagulation temperature of soft cheese (32±2) °C, a symbiotic culture was introduced into it with the addition of streptobacteria in an amount of 1%, the milk was aged until the acidity of 22-25°T for about an hour, after such maturation, the mixture does not require additional pasteurization. Then, acid-rennet coagulation of three samples was carried out: 1) control, with the addition of rennet 0.0015 g/l; 2) with the addition of rennet and CaCl\textsubscript{2} 0.6 g/l; 3) with the addition of rennet, calcium chloride and magnesium sulfate in an amount of 0.2 g/l [9]. During the development of soft cheese, the separation of whey from a clot is ensured by the maturity of milk, and its coagulation time, depending on acidity, is 50-90 minutes. In this regard, the samples were cultured at a temperature of 30°C. Characterization of clots and the process of syneresis are presented in table 5.

### Table 5. The effect of the type of acid-rennet coagulation on the process of obtaining and clot processing.

| Specified indicators | Type of acid-rennet coagulation |
|----------------------|---------------------------------|
|                      | 1 | 2 | 3 |
Titrated acidity is highest in the first sample: at the end of clot treatment, up to 52°T. In the second version, it is 38°T at the end of the treatment, and in the third version it is 44°T. The density of the clot increased when calcium and magnesium were used together from 0.081 to 0.092 g/cm³ and the synergistic ability of the clot decreased. The research results also showed that the use of magnesium together with calcium in the process of acid-rennet coagulation allows reducing the proportion of protein in serum by 15%.

Thus, the research results showed that the clot obtained using the combined starter culture differs in properties from control samples prepared on the basis of traditional coagulation. In the process of obtaining a prototype of finished cheese, quality indicators are within normal limits. Further, cheese was subjected to self-pressing and ripening for 3 days at a temperature of 10-12°C.

Further, the obtained cheese samples were investigated using Fourier-transform infrared spectroscopy. The absorption or transmission spectrum is unique for each substance, and the intensity of the bands in the spectrum is a direct indication of the amount of component in the material. The studies have shown that each protein compound is represented in the form of various bonds with the ability to absorb infrared radiation. The spectra obtained indicate that the use of magnesium as an additional coagulation factor gives greater efficiency. The spectra of this sample have pronounced peaks, suggesting the content of a higher concentration of peptide bonds, including and protein substances, compared with the sample obtained by the method of rennet coagulation without the use of magnesium. Raster microscopy of the surface of cheese was also carried out. Particle analysis provides information on the surface topography, as well as qualitatively and quantitatively characterizes the chemical composition of the product.

| №  | C    | O    | Na   | P    | S    | Cl   | K    | Ca   | Mg   |
|----|------|------|------|------|------|------|------|------|------|
| 1  | 76.8± | 18±  | 0.56± | 0.81± | 0.35± | 1.44± | 0.24± | 1.41± | 0.32± |
|    | 0.5   | 0.44 | 0.02  | 0.01  | 0.002 | 0.12  | 0.01  | 0.04  | 0.002 |
| 2  | 74.78± | 20.3± | 0.51± | 0.82± | 0.25± | 1.45± | 0.23± | 1.40± | 0.19± |
|    | 1.7   | 0.5  | 0.02  | 0.04  | 0.01  | 0.23  | 0.001 | 0.14  | 0.01  |

1 - acid-rennet coagulation using salts of Mg and Ca; 2 - acid rennet coagulation using salts of Ca.

The sample is placed in an apparatus where, under low vacuum, it is irradiated with electrons - X-ray microanalysis. An analysis of this radiation allows determining the elemental composition. The
results were mathematically processed. Table 6 presents the average values of the chemical compositions of soft cheese with a confidence error.

Experimental data indicate that magnesium promotes the binding of whey proteins, which leads to an increase in sulfur content in the first sample, since whey proteins contain sulfur-containing amino acids in larger quantities, including the first sample contains 0.32 mg/100 g of magnesium, which is the ratio with calcium in the product of 0.3:1. This is the closest value for the full absorption of calcium. Ideally, it is 0.5:1. When using only calcium for acid-rennet coagulation, these values are 0.1:1 [7].

4. Conclusion
Thus, the experimental data showed that the use of streptobacteria increases the proteolytic activity of the starter culture, the content of aromatic substances in it and the general level of probiotic microflora. The use of magnesium in combination with calcium in acid-rennet coagulation makes it possible to increase the total content of protein compounds, reduce the intensity of their transition to serum and increase the amount of magnesium in the finished product. The matured soft cheese has functional properties and is a source of a large number of protein substances. It is enriched with whey proteins, calcium and magnesium, as well as probiotic lactobacilli.

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