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DEVELOPMENT OF PROBIOTIC YOGURT FROM SMALL CATTLE MILK

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This paper considers the physicochemical parameters of sheep's and goat's milk as raw materials to produce live yogurt. Sheep's milk of 12 breeds (14 samples per breed) and goat's milk of 4 breeds (10 samples per breed) were examined. Milk was selected to produce yogurt from cow's, sheep's, goat's milk and combined mixtures of cow's and sheep's milk in different ratios. The milk was selected for the purpose of producing yogurt with long-term viability of dairy bacteria, thereby obtaining live yogurt without the addition of synthetic preservation agents. The viability of probiotics (Bifidobacterium and Lactobacterium) in the composition of yogurts from cow's, sheep's, goat's milk, and mixtures: cow's and sheep's milk in various ratios, cow's and goat's in similar ratios has been investigated. For consumer preference, organoleptic analyzes are very important, according to the results of which yogurt was selected in a ratio of 3:1 cow's milk and sheep's milk, respectively. An equally important indicator is the safety of the product, so microbiological and biochemical analyzes were carried out on day 1 and during storage (day 28 at 4 °C). Producing yogurt using sheep's milk is an interesting approach because of the improved nutritional value compared to cow's and goat's milk. Yogurt with the best indicators and a rational ratio of 2 types of milk was also identified. The physicochemical parameters of all types of yogurts were studied, the rational number of added starter cultures was selected. At the same time, yogurt from cow's milk received low marks on organoleptic analysis, the rest of the samples showed a good result. The best in terms of the organoleptic analysis are yogurts with the addition of sheep's milk. Yogurts have the ability to increase the body's resistance to harmful environmental factors, subject to the consumption of live yogurt

Keywords: viability of bacteria, sheep's milk, combined milk, and goat's milk, nutritional value, shelf life

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1. Introduction

Sheep's milk is a natural, very high-calorie and nutritious product.

In the production of sheep's milk, China ranks first in the world. In addition, it is also at the top of the list with the largest number of sheep in the world. China produces more than 1.5 million tons of sheep's milk annually. Most of the dairy sheep farming takes place in Qinghai and the Tibetan Plateau.

In 2020, according to FAO (Food and Agriculture Organization, FAO), milk production in the world increased by 1.4 % to 861 million tons, and according to the calculations of the International Franchise Consulting Network (IFCN), milk production in the world increased by 3 % in 2020, that is to 889 million tons. According to FAO, milk production in the world will grow primarily due to livestock, the average growth of the herd will be 1.1 % per year, milk yield will grow by an average of 0.7 % per year [1]. Traditionally, cow's milk is used in the production of yogurt but, in comparison with cow's milk, sheep's milk is characterized by a high content of fat, protein, and dry substances. Thus, it contains 18–20 % of dry substances, 7–10 % of fat. Therefore, this milk is better absorbed than cow's milk. The caloric content of sheep's milk is 109.7 kcal. Nutritional value of sheep's milk: proteins – 5.6 g, fats – 7.7 g, carbohydrates – 4.8 g. Among the fatty acids in sheep's milk, conjugated linoleic acid has a higher level. This may be an advantage as it may have beneficial effects in the body, such as preventing cardiovascular disease, anti-cancer and increasing muscle mass and lowering blood glucose levels due to hyperinsulinemia [2–4].

The development of functional milk-based products has become widespread, due to the ability to vary the ingredient composition and model the recipe. Modeling the formulation for amino acid and fatty acid composition makes it possible to obtain products that are balanced in composition [5–7].

Functional products, in particular the prebiotic category, play an important role in the modern food industry. Consequently, the study and production of new types of pre- and probiotic products is a hot topic.

2. Literature review and problem statement

The essence of the yogurt production technology is as follows. Special microbiological starters are added to pre-prepared, pasteurized milk [8]. Milk yogurt has a beneficial effect on the human body due to microorganisms and substances formed as a result of biochemical processes, as well as occurring when fermenting the milk. The biological properties of yogurt are expressed in inhibition of pathogenic microorganisms, stimulation of the growth of beneficial intestinal microflora and anticarcinogenic manifestation [9]. Despite this, no matter how diverse the range of yogurts is in its fillers, whether it is berries, fruits, cereals, cow's milk is used for production in 95 % of cases.

Milk mixtures that used different species to produce yogurt, such as goat and cow [10] were investigated; 5 variations were used but the authors did not investigate the viability of the bacteria in different proportions. The granularity and roughness of yogurt was studied by the authors of [11] who combined milk (cow's and goat's). However, the data are provided only by the characteristics, the disadvantage is the incomplete disclosure of the problem under study, in particular the lack of information on the rheology of yogurts. Work [12] studied the effect of the proportions of goat milk and sheep milk on the composition of fatty acids, as a result of which high-fat yogurt was obtained [13]. However, the study lacks information on the viability of dairy microorganisms and shelf life. In addition, yogurts made from sheep's milk have the desired organoleptic characteristics, including a pleasant creamy-sour taste, in demand by yogurt consumers. Previous studies have reported the beneficial effects of *Lactobacillus acidophilus* as a probiotic [14]; polymerized whey protein was also used. Polymerized milk whey protein interacts with casein micelles. The results showed that the addition of polymerized whey protein could be a new protein-based thickener to improve the consistency of yogurt from goat's milk and other products. The survival of *Lactobacillus acidophilus* bacteria under different variations of active acidity conditions was also studied. It was revealed that active acidity is not a critical factor for the survival of *Lactobacillus acidophilus* [15]. *Lactobacillus acidophilus* and *Bifidobacterium spp.* act on the gastrointestinal tract to enhance the therapeutic effect but the lack of viability and survival of these bacteria remain a problem in commercial foods [16].

The nutritional value of sheep's milk is 1.5 times higher than cow's milk, and the composition of this product is much richer in vitamins of groups A and B. Sheep milk is advised to use when the child is growing, as well as for pregnant women. This product is a wonderful antioxidant, thanks to it, cholesterol, amino acids, as well as vitamins A and D, are synthesized. In those who systematically drink sheep's milk, the brain works more actively, the cells of the body absorb more oxygen. If we compare the milk of a sheep and a cow, it is worth noting that cow's milk clearly loses in the amount of proteins, fats, and dry substances. Therefore, studying the physicochemical properties of sheep milk and processed products is of great scientific and practical importance as it can help solve the problem associated with protein deficiency and prevention of gastrointestinal diseases.

3. The aim and objectives of the study

The aim of this study is to develop a technology to produce probiotic yogurt with a long shelf life without loss of quality on a natural basis from sheep's milk and combined milk. This will make it possible to produce a useful product with a long shelf life.

To accomplish the aim, the following tasks have been set:

– to determine the physicochemical indicators of sheep's and goat's milk, taking into consideration the breed of livestock;

– to choose and substantiate the recipes and technological scheme to produce live yogurt;

– to analyze the microbiological indicators of the quality of yogurt.

4. The study materials and methods

4. 1. Making yogurt

We took milk of each type with a volume of 5 liters. Milk was obtained from goat's milk farms in the Almaty region, Karasai district, Farm "Gala milk"; sheep's milk was obtained from a peasant farm in the village of Mynbayevo. The milk was accepted according to the quality and quantity of production by the laboratory. The milk is cooled to a temperature of 4±2 °C, normalized in fat and dry matter content, and subjected to deodorization to remove foreign tastes and odors. In normalized milk, sodium caseinate was introduced, which contains up to 90 % protein and is characterized by emulsifying, water-binding properties. Next, the resulting mixture was sent to the homogenizer. Homogenization was carried out at a pressure of 20–25 MPa. Next, the mixture was pasteurized at a temperature of 85–92 °C with an aging of 2 to 10 seconds. The mixture was cooled to a fermentation temperature of 37±2 °C and sent to ripening tanks. Combinations of starter cultures of direct application (DVS) were introduced: thermophilic streptococcus, Bulgarian bacillus, and probiotics: acedophilic lacto- and bifido bacteria in a ratio of 1:1. The mixture was stirred for 10 minutes and left to ferment. The duration of fermentation was 4±2 hours before the formation of a sufficiently strong clot and an active acidity of 4.4±0.2. The fermented product is cooled to a temperature of 20±2 °C and sent for packaging. It was packed in plastic cups and sealed with the help of a sealer ADNK 39 (RF).

The prepackaged product was sent to prior refrigeration within 10±2 hours. When the product reaches the temperature of refrigeration storage, the technological process can be considered complete. Next, we carried out analyzes.

In each experiment (n=3), cow's milk (CM), goat's milk (GM), and sheep's milk (SM) were used to produce yogurts with a ratio of cow's (Y1), sheep's (Y2), goat's (Y3) or cow's and sheep's milk mixtures of 3:1 (Y4), 1:1 (Y5), and 1:3 (Y6).

4. 2. Physicochemical analysis

The quality of raw milk depends on the type and breed, the conditions of their maintenance, feeding, lactation stage, age, state of health, as well as the season of the year, which is due to the composition of the diet of sheep. Particular importance is attached to the content of biologically complete components of milk: protein, fat, and DSMR. The presence of dry matter (DM) in milk makes it possible to judge the nutritional value of raw milk. The presence of dry skimmed milk residue (DSMR) makes it possible to judge its biological usefulness since it contains all the necessary, indispensable substances for the human body.

Given this, the task was set to analyze the physicochemical and technological properties of sheep's milk depending on the breed of the animal, the season of the year, and the period of lactation.

Milk was taken from 10 ewes in the first month of lactation, in September. Milk collection was carried out in the morning milking. Milk indicators were studied using standard methods in the laboratory "Food Safety" at TOO "Kazakh Research Institute of Processing and Food Industry".

Total solids were determined by gravimetric method, Kjeldahl method was used to estimate protein content, Gerber's method was used to determine lipid content, and ash content was quantified using a muffle furnace at 550 °C (AOAC, 2012). Analyses were performed three times on the resulting products.

Thermal stability, the suitability of milk for high-temperature processing (sterilization, ultra-pasteurization), was established by an alcoholic sample based on the effects of different concentrations of ethyl alcohol on milk.

A digital pH meter was used to determine the pH values.

The number of somatic cells was determined on the viscometer "Expert Somatos-01".

Table 1

The physical-chemical parameters of sheep's milk from 12 breeds

Breed	Fat	DSMR	DM	Protein	Density	Lactose
Edilbayskaya	2.63±1.12	10.78±0.41	13.37±0.95	4.26±0.13	41.47±2.00	5.93±0.22
South Kazakhstan Merino	4.48±1.13	9.65±0.22	13.44±1.28	3.93±0.10	35.95±1.28	5.33±0.14
Ile de France	3.90±0.39	9.14±0.56	12.81±0.60	3.67±0.25	37.22±0.92	5.32±0.15
Kazakh fine-fleece	6.44±0.90	9.91±0.36	16.2±0.81	4.15±0.07	38.08±0.89	5.61±0.13
Meat merino	6.28±2.70	10.33±1.81	16.32±3.90	4.43±0.58	39.34±3.15	5.90±0.60
Akzhaik meat wool breed	8.20±2.10	9.71±0.40	17.68±2.06	4.14±0.19	33.70±2.70	5.34±0.27
Karakulskaya	8.90±3.70	10.08±0.87	18.97±4.00	4.44±0.34	35.63±3.68	5.54±0.52
Saryarka	12.16±3.17	10.41±1.00	22.43±4.03	4.54±0.56	33.73±3.40	5.73±0.60
Ordabasinskaya	9.50±4.10	10.42±0.73	19.80±4.30	4.52±0.36	35.54±4.38	5.73±0.40
Done Merino	6.10±0.38	10.03±0.79	16.00±0.98	4.27±0.35	38.70±3.43	5.62±0.45
Hissar	4.50±0.48	10.46±0.47	14.01±0.57	4.20±0.32	38.57±4.36	5.27±0.28
Hampshire	4.21±0.46	9.2±0.61	13.43±0.75	4.05±0.44	33.26±2.20	5.17±0.28

Table 2

The physical-chemical parameters of goat's milk

Breed	Fat	DSMR	DM	Protein	Density	Lactose
Zaaninskaya	3.50±0.66	6.80±0.61	9.80±1.10	2.77±0.16	22.99±2.85	3.95±0.36
Alpine	3.79±0.22	6.53±0.88	10.32±0.87	3.06±0.10	23.45±2.43	3.76±0.39
Nubian	3.18±0.37	6.75±0.52	9.23±0.56	3.04±0.19	23.48±2.12	3.71±0.47
BK	4.76±0.10	9.27±0.36	13.75±0.13	3.67±0.04	33.31±0.37	5.32±0.04

4. 3. Bacteriological analysis was performed according to [17]

Bacteriological analysis makes it possible to assess the viability of lactic acid bacteria. Prepared yogurts were stored for 28 days under refrigerated conditions, the slice was taken on days 1, 5, 13, 28. In each experiment, bacteriological evaluation of yogurts was carried out three times on the 1st and 28th day of storage (4 °C). It was evaluated using M17 agar and MRS agar. *L. Acidophilus* were evaluated using MRS agar with the addition of 0.15 % (wt%) bile salts followed by incubation at 37 °C for 72 h under aerobic conditions.

4. 4. Statistical estimation Data were analyzed using Ststistica12.0, MS Excel (USA)

Analysis of variance (ANOVA) was used to assess the effect of different ratios of milk types (cow's and sheep's milk, cow's and goat's milk) on the physicochemical and textural properties of yogurt, as well as on the acceptability and consumer properties.

5. Results of a study on the possibility of producing live yogurt from the milk of small cattle

5. 1. Determining the physicochemical parameters of milk of small cattle

The physicochemical parameters of the milk of small cattle were determined in order to identify the breed of sheep and goats with the best milk indicators to produce yogurt. Milk of sheep of 12 breeds and milk of goats of 4 breeds were selected. Each breed yielded 14 samples; Tables 2, 3 give the processed data, summarized for a median value and a standard deviation (n=14). Information on sheep's milk is summarized in Table 1.

Goat's milk was selected from 4 breeds; the data are given in Table 2; the results are given as mean value and standard deviation (n=10)

Correlation analysis showed that the most important indicator of the physicochemical indicators is DSMR. The amount of lactose and the density level are positively correlated with DSMR, 0.87 and 0.69, respectively. The connection is close, positive. Fat and solids show a positive close relationship r=0.92.

When choosing raw materials to produce live yogurt, we were guided by DSMR and the mass fraction of dry substances. Further, all studies were carried out within the selected breeds. According to the table, we stopped at the milk of sheep of the Sary Arka and Ordabasy breeds and the milk of goats of the BK and Alpine sheep breeds.

Heat resistance is the suitability of milk for high-temperature processing (sterilization, ultra-pasteurization). The technological properties of milk are given in Table 3.

Table 3

The technological properties of sheep and goat milk

Name	The state of the rennet clot	Heat resistance group	Coagulation time, min	Thermal stability of milk, min
Sheep	dense	II	12.3–30	42.3±1.2
Goat	dense	II	15–40	42.8±1.2

As can be seen from Table 3, the coagulability of goat's milk is quite well expressed and it is at the level of sheep's milk.

5. 2. The recipe and evaluation of live yogurt made from combined milk and sheep's milk

Sheep's milk has a specific smell and taste; with the help of deodorization and the selection of starter cultures, one can adjust these characteristics. Factors for variation chosen were a temperature range of 37–41 °C, as well as the varied dose of application of starter cultures and the ratio of milk types. Yogurt from the milk of various types of livestock is coded in the following order: yogurt from cow's milk (Y1), sheep's milk (Y2), goat's milk (Y3), followed by yogurts from combined milk: a mixture of cow's and sheep's milk in a ratio of 3:1 (Y4), in a ratio of 1:1 (Y5), and in a ratio of 1:3 (Y6). Further, yogurts were produced from the milk of selected breeds according to the selected recipe in line with the technological scheme (Fig. 1); we then studied the physicochemical parameters shown in Table 4. Yogurt is produced in two ways – reservoir and thermostat techniques.

In each experiment (n=3), cow's milk (CM), goat's milk (GM), and sheep's milk (SM) milk were used to produce yogurts with a ratio of cow's milk (Y1), sheep's milk (Y2), goat's milk (Y3), or cow's and sheep's milk mixtures of 3:1 (Y4), 1:1 (Y5), and 1:3 (Y6). The results are summarized in Table 4.

Table 4 demonstrates that yogurt with the addition of sheep's milk in terms of mass fraction of fat differs from all and shows the maximum values, yogurt from goat's milk takes average values. Despite the fat content of yogurt, one should pay attention to the protein content, the maximum values are also taken by yogurt from sheep's milk, almost twice as much as that of yogurt from cow's milk.

Information on organoleptic evaluation is shown in Fig. 2.

Fig. 2 demonstrates that the best ratings were received by yogurt Y4, which had a milky white color, a uniform consistency, and a pleasant taste. Y6 was worse in appearance, the consistency was heterogeneous. Compared to other yogurts, Y1 received the lowest ratings, all yogurts had a sweet taste except Y1 and Y3.

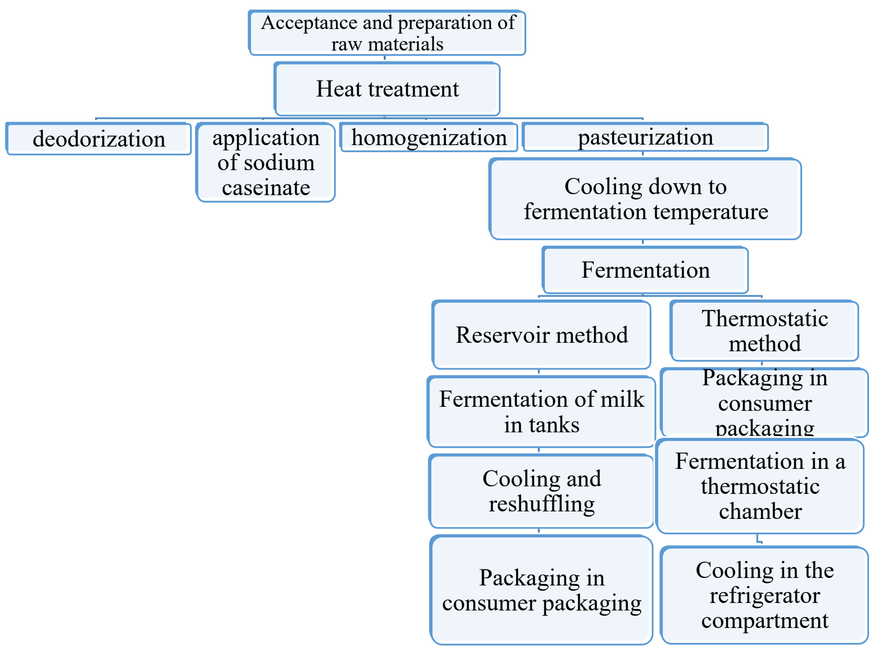

Fig. 1. Technology of yogurt production

Table 4

The physicochemical parameters of yogurt from the milk of sheep and goats of the selected breeds

Name of yogurt	Product code	Name of indicators, unit of measurements		
		Fat mass share, %	Protein mass share, %	Moisture mass share, %
Cow's milk yogurt (control)	Y1	3.50±0.00	3.39±0.00	87.25
Sheep milk yogurt	Y2	6.06±0.5	6.75±0.8	82.99±4.15
Goat milk yogurt	Y3	4.58±0.3	4.57±0.6	84.8±4.24
Combined milk yogurt (3C:1O)	Y4	3.78 ±0.1	4.01±0.0	86.6±0.00
Combined milk yogurt (1C:1O)	Y5	4.28±0.1	4.35±0.1	85.28±0.0
Combined milk yogurt (1C:3O)	Y6	5.06±0.1	4.56±0.1	83.52±0.12

Note: The data are given as mean value and standard deviation

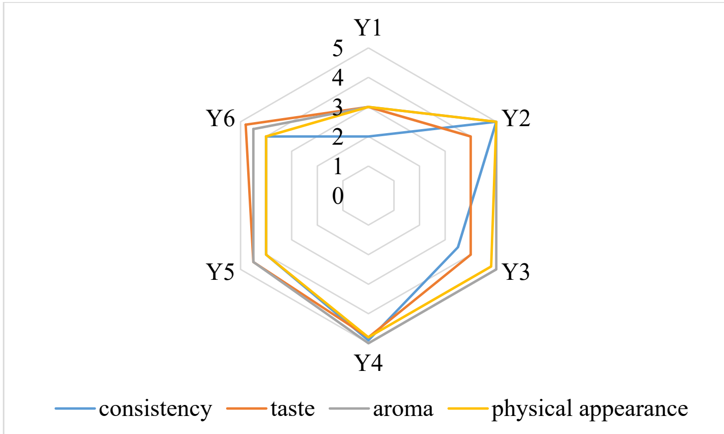

Fig. 2. Organoleptic evaluation of yogurts

5. 3. Microbiological analysis and biochemical analysis (indicators of bacterial viability on day 28)

The microbiological analysis of contamination with pathogenic microorganisms and viability of dairy bacteria is given in Table 5.

Table 5

The microbiological analysis of yogurts

Name of yogurt	Code	Name of indicators, unit of measurements					
		BGKP, 0,1 g/cm^3	The content of lactic acid bacteria, not less 1*10^6		Yeast, CFU/g/cm^3	Mold, CFU/g/cm^3
			day 1	day 28		
Cow's milk yogurt (control)	Y1	–	1*10^{10}	1*10^6	10	50
Sheep milk yogurt	Y2	–	1*10^{10}	1*10^9	<1.0*10^1	<5.0*10^1
Goat milk yogurt	Y3	–	1*10^{10}	1*10^8	<1.0*10^1	<5.0*10^1
Combined milk yogurt (3C:1O)	Y4	–	1*10^{10}	1*10^7	<1.0*10^1	<5.0*10^1
Combined milk yogurt (1C:1O)	Y5	–	1*10^{10}	1*10^8	<1.0*10^1	<5.0*10^1
Combined milk yogurt (1C:3O)	Y6	–	1*10^{10}	1*10^8	<1.0*10^1	<5.0*10^1

Table 5 demonstrates that the indicators of microbiological analysis are within the normal range and, on the 28th day of storage of yogurt samples in refrigerated conditions, the quality of the product is ensured. Table 5 also shows that lactic acid bacteria are viable on day 28, which indicates the usefulness of the product.

6. Discussion of results of studying the technology of live yogurt from the milk of small cattle

Based on Tables 2, 3, the choice was made according to the maximum amount of dry matter, the milk of sheep of the Sary Arka and Ordabasinskaya breeds was chosen, and the milk of goat breeds of sheep BK and Alpine. Further, yogurts were produced from sheep's milk of the Ordabasy breed and goat milk of the BK breed. Yogurt produced from cow's milk from the same farm was taken as control.

It should be noted that the coagulability property is best manifested in sheep's milk, which gives a dense clot that forms in a very short time of 12.3 minutes.

According to the thermal stability of milk, goat's milk turned out to be close to sheep's milk. In both species, approximately equal values for the thermal stability of milk were obtained, respectively, 42.3 and 42.8 minutes.

Table 4 demonstrates that the best result was achieved in samples Y2, Y3, Y6.

All samples showed a constant number of viable cells during 21 days of storage (Table 5).

The number of viable BB cells remained at 10^7 and 10^6 CFU/ml until storage day 28 at 4 °C. The difference between BB counts may be due to its sensitivity to oxygen. During the mixing of milk in the experiment, it was possible to add more oxygen.

L. acidophilus is more sensitive than bifidobacteria to media such as yogurt and acidified milk when considering pH ranges of 3.5 to 4.5.

In the technology of live yogurt there was the introduction of sodium caseinate, this gave an increase in the amount of proteins and improved the structure of yogurt.

A feature of our technology is the fermentation temperature of 37 °C rationally selected by regression analysis; it ranged from 36 to 42 °C. Also, yogurt differs in that without the addition of preservatives, sweeteners, thickeners, yogurts had a pleasant taste, homogeneous, moderately viscous consistency, and fermented milk aroma, which persisted for 21 days. A rational formulation and the ratio of types of milk in yogurt have been selected in order to preserve the longest viability of lactic acid bacteria. As can be seen from our results (Table 4), the fattest is yogurt from sheep's milk. The closest to the classic yogurt in terms of fat content is yogurt Y4 (the ratio of cow's and sheep's milk is 3:1). And in terms of protein content, after yogurt from whole sheep's milk, Y6 and Y3 are there with the ratio of cow's and sheep's milk 1:3 and from whole goat milk, respectively. Even though the mass fraction of protein Y4 is inferior to other types of yogurt, according to the organoleptic assessment, it received the highest score.

The conditions for the applicability of our results are the region of grazing and the lactation period – in the study, we selected sheep and goats from 90 days of lactation.

The disadvantages include the dependence of the production of live yogurt on the season, breed, and composition of the milk of small cattle. A development is the normalization of milk with cow's milk and the production of healthy yogurts with various additives (useful for health).

7. Conclusions

1. The results of our analyzes showed that the amount of dry matter in sheep's milk of all breeds is higher than in cow's and goat's milk, and ranged from 13.5 to 15.3. In goat's milk, the amount of dry matter ranges from 12.3 to 13.1, that is, it exceeds slightly that of cow's milk.

2. Physicochemical (pH, titrated acidity, total dry matter content, protein, lipids) and organoleptic (appearance, aroma, taste, and consistency) properties were evaluated. Overall, Y5 and Y4 showed elevated ($P<0.05$) approximate values of the composition with greater ($P<0.05$) hardness, apparent viscosity than Y1 and Y2; Y3 demonstrated intermediate values for texture parameters. It is established by organoleptic analysis that the greater the content of sheep's milk, the denser the structure of the gel (Y2) as a result of more interconnected protein clusters. While yogurt Y1 showed the lowest scores of organoleptic analyses ($P<0.05$) for overall impression, appearance; Y4 was the only yogurt to show a perfect consistency. Thus, a probiotic yogurt mixture containing a ratio of cow's and sheep's milk of 3:1 was selected and substantiated. Y4 yogurt is a viable technological alternative for the dairy industry. According to the proposed technological scheme to produce yogurt, the temperature of the starter is 37–38 °C.

3. Yogurts were compared on days 1, 5, 13, 28. The time of contact of the microorganism at low pH is a decisive factor for the viability of cells. In this context, milk and some of its derivatives may protect microorganisms by helping to maintain vitality during digestion when exposed to conditions with a low pH (below 2.0), which is due to this protection given the presence of fat globules and milk proteins, mainly casein. Thus, probiotic cultures may be more viable if they are bound to acidic bacteria and serve them as protective cultures. In addition, consumption associated with milk and certain derivatives, such as fermented milk, can help maintain or even increase the viability of probiotic cells during digestion.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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