ISOLATION AND CHARACTERIZATION OF LACTIC ACID BACTERIA STRAINS FROM GOAT MILK FOR POTENTIAL USE IN THE PRODUCTION OF YOUGURT

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Abstract: *In the presented research, the physico-chemical and microbiological characteristics of goat's milk were determined. The strains of valuable native lactic bacteria of the species Streptococcus thermophilus and Lactobacillus bulgaricus were selected with stable technological characteristics for the fermentation of goat's milk, corresponding to the requirements for lactic bacteria intended for the manufacture of yogurt. The scheme for preparing starter cultures and recommendations regarding the use of consortia of symbiotic cultures for the manufacture of goat milk yoghurt have been elaborated. Starter cultures were obtained for the manufacture of goat milk yogurt, with biotechnological properties characteristic for fermented dairy products.*

Keywords: *goat milk, lactic acid bacteria strains, yougurt.*

1. Introduction

Balanced nutrition is a priority concern, in order to ensure and maintain the state of health and good functioning of the human body. Recently (in last decades), nutrition, as a science, has progressed in understanding the physiological and genetic mechanisms by which nutrition and individual components of food influence health. At the same time, it is a paradox that nutrition is essential to maintaining life, it can also be a cause of many chronic diseases.

Goat's milk has a promising source of protein, vitamins, minerals, and fatty acids [1, 2]. Due to its low lactose content, goat's milk has better digestibility, reduced allergenicity, [3, 12, 24]. The advantages of goat's milk are expressed through an advanced dispersion of lipid globules which increases digestibility, has a richer content of Ca, Zn, Fe more easily assimilable and contains less cholesterol, has a higher antioxidant properties which is beneficial to the human body, the fractionated composition of proteins is more homogeneous. Goat milk contains more unproteinized nitrogen, the proteins are of better quality, with a higher thiamine content than any other food and practically does not cause allergic reactions and digestive disorders. It is known that αs1-casein - the main protein of cow's milk - is a potent allergen for humans. All these give goat milk products dietary and curative properties [4].

2. Materials and methods

2.1. Goat milk physico-chemical analysis

The main physico-chemical properties of goat's milk are: density, freezing point, pH, mass fraction of proteins, of skimmed dry matter, lactose and fat.

2.1.1. Density

Density is influenced by the dry substance content as well as by the ratio between the fat and non-fat matter. The milk density is between The title must be as short possible. If necessary, a subtitle is provided.

According to sanitary-epidemiological safety, the goats have a much lower disease risk, they do not suffer from brucellosis, tuberculosis and other diseases that affect cattle [5, 12]. From the goat's milk you get butter, yogurt, sour milk. A particular taste have fermented dairy products, that have a fine consistency and pleasant, specific taste. The goats farming has an important food potential, which must be exploited at industrial level.

On the national level, goat's milk is not used industrially, and there are no sour milk products from goat milk on the market place. In the Republic of Moldova, at the present time, no scientific results have been obtained regarding the use of goat milk.

At the international level, the proposed topic carries researches on the preparation and optimization of goat milk yogurt technology and manufacture [13-15]. In order to develop the industrial level of goat's farming, obtaining the high quality goat's milk products, is necessary to determine the milk quality clues and the harmlessness as a raw material. Therefore, the taken milk samples, obtained with technical-normative support from the individual household were investigated to physico-chemical and microbiological clues. The purpose of the research was the selection of lactic acid bacteria to determine the most promising combinations for use in the preparation of starter cultures for yoghurt manufacture.

The density increases simultaneous to the non-fat substance increase in the content. It decreases in proportion, opposite to the fat content increase. Knowing the density is important, both for detecting possible counterfeiting through milk diluting, as well as for determining the dry substance content.

2.1.2. Degreased dry substance

It is perforemed indirectly by calculation, respectively by reducing the fat content (F, %) from the total content of dry substance (D.S., %). Higher values may indicate: feeding with preserved fodder specific for the winter season; addition of sour cream in milk, addition of other substances (milk powder, starch, etc.) milk from animals at the end of the lactation period. Lower values may indicate: milk from animals at the beginning of the grazing period; excessive feeding with succulent fodder or green meal; counterfeiting by adding water.

2.1.3. Milk fat content

The fat content is determined according to MS ISO 11870 [25]. 10 ml of sulfuric acid is added in the butyrometer, as well as 5 ml of milk product and 6 ml of distilled water. Also 1 ml of isoamyl alcohol is added. The butyrometer is covered with a rubber stopper and then homogenized. After homogenization, the sample is centrifuged for 5 minutes at 1000-1200 rpm, then placed in the water bath at a temperature of 65º C. The fat content is read on the butyrometer stem and the indicated value is multiplied by 2,2.

2.1.4. Determination of milk protein substances

Protein substances in milk are an important source for both growing organism and the adult one, having a high biological and nutritional value due to its content in amino acids, both quantitatively and qualitatively, containing all the essential amino acids that the human body receives only with food. The goat milk proteins have a high level of digestion (95-97%). About 95% of the nitrogenous substances in milk are of protean (casein, lactalbumin, lactoglobulin.etc) and 5% of non-protean (amino acids, amides, etc.). The nitrogenous substances content gives information about nutritional and technological value of the milk as well as about its destination in processing [10].

2.1.5. Determination of lactose content in milk

Milk lactose is one of the components of the total dry extract representing about 35%. Lactose is an unstable compound undergoing fermentation under the influence of microorganisms contained in milk. In some cases, goat's milk contains a higher mass fraction of lactose, compared to the same indicator in cow's milk, but its molecule size in goat's milk is smaller and lactose contained in goat milk is more strongly attacked by the enzyme-lactase (β-galactosidase), produced by the starter culture. This could be an opportunity to use goat's milk for special adults and children diet [13, 14].

2.1.6. Freezing point or cryoscopic point of milk

The freezing point is defined as the temperature at which the milk freezes. For each percentage of added water the freezing point increases by 0.006 °C. The freezing point can be expressed in degrees (°H) or in degrees (°C). The value of the freezing point should be edited according to the acidity of the milk. The correction is progressive if the acidity of the milk is 7-8 °SH (Soxhlet-Henkel degrees) and regressive if the acidity of the milk is <8 °SH.

2.1.7. Ionic acidity (milk pH)

Due to the buffering capacity of proteins and mineral salts (citrates, phosphates), a sudden change in pH is observed, pH values may change from 4.5 to 6.5 [9]. The physico-chemical composition of goat's milk is conditioned by race, growth area and age, also by the nature of the feeding, the lactation phase, as well as the frequency and duration of the milking and the animal's health [15].

2.2. Microbiological analysis of goat milk

2.2.1. Number of coliform bacteria

The primary dilution is prepared from 1g of product through a series of decimal dilutions, so that it is possible to determine the estimated number of coliform bacteria or the quantity provided in the regulatory document for a particular product. Each dilution (in triplicate) is introduced into tubes with nutritional environment prepared according to GOST standard 30518 [19]. The tubes are thermostated at 37 ± 1 °C for 24 ± 2 hours. If gas bubble formation or turbidity is not observed, gas incubation is continued for a further 24 ± 2 hours. The tubes in which the formation of the gas is observed after 24 ± 2 hours or after 48 ± 2 hours are considered positive.

2.2.2. Number of coagulase-positive staphylococci (Staphylococcus aureus)

Contamination may occur during unhygienic milking, transmission of the pathogen agent through contaminated hands, and possible contamination of S. aureus (for example) in raw milk may occur from infection of the mammary glands.

2.2.3. Identification of pathogenic microorganisms, including Salmonella

Bacteria of the genus Salmonella can be present in the product in small quantities, with a large number of other bacteria in the family Enterobacteriaceae or other families. Therefore, prior enrichment of the culture, required for the detection of a small number of bacteria of the genus Salmonella, is mandatory. According to SM EN ISO 6579 Standard, 25 g of the product under analysis is heated to 37 ± 1 °C and suspended in buffered peptonate water, followed by incubation at 37 ± 1 °C for 18 ± 2 hours. The incubated cultures were seeded on the agar medium and thermostated at 37 ± 1 °C for 24 ± 3 hours [27]. Colonies suspected to be of the Salmonella genus are subsequently identified by specific biochemical and serological tests.

Determination of these bacteria in goat milk is mandatory. According to veterinary standards, Salmonella-like bacteria must be absent in 25 ml of milk [20]. The presence of this microbiological indicator in milk shows its epidemic risk.

2.2.4. Total number of aerobic mesophilic germs (NTG / ml or UFC / ml)

Assessing the quality of all microorganisms in goat milk is a very difficult problem. Therefore, as a rule, the quantitative assessment is used first, that is, the total number of germs (NTG) in 1 ml of milk, or from the contemporary method, the total number of mesophilic aerobic germs and facultatively anaerobic colony-forming units (NTUFC). In case the microbial load does not exceed 106 / ml NTG (Total Number of Germs) the decision is made that milk can be used in the manufacture of food.

2.3. Statistical analysis

The results variation analysis was carried out by the method with application of Microsoft Office Excel program. Differences were considered statistically significant if probability was greater than 95% (q <5%). All assays were performed at room temperature, 20 ± 1 °C. Experimental results are represented according to standard rules.

3. Results and discussions

3.1. Analysis of the physico-chemical properties of goat milk

The determination of the physico-chemical clues of the goat's milk was recorded using the EKOMILK Total Bulteh 2000 automatic milk analyzer. Physico-chemical characteristics of goat milk are shown in table 1.

Table 1. *Physico-chemical characteristics of goat milk*

No	Characteristics	Values
1	Fat mass fraction, %	3,58±0,19
2	Mass fraction of dry degreased substance (DDS), %	9,32±0,5
3	Protein mass fraction, %	4,1±0,11
4	Protein mass fraction, % (Kjeldahl method)	4,28±0,03
5	Lactose mass fraction,%	4,4±0,2
6	Cryoscopic temperature, ºC	- 0,530
7	Density, g/cm^3	1,031±0,0028
8	pH	6,5±0,70

In goat milk according to ISO 8968-1: 2014 (Milk and dairy products - Determination of nitrogen content - Part 1: Kjeldahl method and calculation of crude protein content) was determined the mass fraction of proteins,%, which constituted 4.28 ± 0.03.

It is known that the chemical composition of milk differs depending on season, feeding, age, lactation period. Also, at the beginning and at the end of the lactation, the fat content is higher, in the middle, when the productivity of the animals is maximum (the summer feed), the fat content decreases, the density of the milk grows at the beginning of the lactation period, the acidity (freshness) of the milk also grows during the summer period, which is related to the temperature of the environment [16-19].

The high content of skimmed dry matter in goat's milk indicates that it is better for technological processing. In some cases goat's milk contains the higher fraction of lactose, but its size in goat's milk is smaller and lactose of goat's milk is strongly attacked by the enzyme-lactase (β-galactosidase), produced by the starter microflora. This is a better opportunity to use goat's milk for special diets and for children nutrition [13, 14].

3.2. Microbiological analysis of goat milk

Milk samples collected under aseptic conditions were subjected to microbiological analysis. Microbiological control of raw materials is the most important step in the system of preventive measures to avert the infections through milk and dairy products consumption. Therefore it was necessary to determine the degree of contamination of goat milk by different groups of microorganisms, to establish the microbiological harmlessness.

Milk contamination can occur from various sources, and the microbial load can be quantitatively and qualitatively quite varied, depending on the conditions under which milk is produced, handled and processed. Microorganisms reach food from natural or external sources as a result of processing and handling, until consumption. Milk microbiota consists generally of non-pathogenic microorganisms as well as facultative pathogenic or pathogenic. The category of non-pathogenic microorganisms includes lactic-acid bacteria, widely distributed in nature, which play an important role in the fermentation of many foods and feeds. Contamination of milk with saprophytic germs increases the risk of including pathogenic germs. Many pathogenic bacteria do not multiply in milk (Mycobacterium tuberculosis, M. bovis, Brucella, Rickettsia), their danger is depending on the initial degree of contamination of the milk, its subsequent dilution, also treatments it is subjected to, time before consumption of milk and other factors. Other pathogenic germs (Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, etc.) can multiply in milk. The metabolic activity of most pathogenic germs is inhibited at low temperatures, this is why it is very important to immediately cool the milk after it is obtained, until it is heated and futher processed.

The microbiological characteristics of goat milk are shown in table 2.

Table 2. *Microbiological characteristics of goat milk*

No	Characteristics	Values
1.	Number of mesophilic aerobic and facultative anaerobic bacteria, UFC / $1cm^3$	$5.4 \pm 1.9 \times 10^3$
2.	Salmonella content, in 25g / product	not found
3.	Number of Staphylococcus aureus bacteria in 1.0g / product	not found
4.	Number of coliform bacteria, in 0.01g / product	not found

Analyzing the data presented in table 2 it was found that the number of aerobic mesophilic and facultative anaerobic bacteria in goat milk does not exceed 8.0×10^3, which represent an admissible content and the presence of pathogenic germs like Salmonella, Staphylococcus, coliform bacteria milk is not detected.

As a result of researches to determine the physico-chemical and microbiological indices, it can be seen that goat's milk is characterized by high technological properties and can be used to obtain yogurt.

3.3. Recognition and selection of valuable lactic bacteria strains for the goat's milk fermentation with high content of bioactive compounds

Particular attention has been paid in recent years to the selection of starter cultures of EPZ-producing lactic bacteria, which may not only be

a natural alternative source of food additives, which improve the rheological parameters of fermented dairy products, but also, important factors contributing to the adhesion of microorganisms and probiotics in the intestinal environment [21].

In the food industry EPZ plays an important role in the manufacture of dairy products such as yogurt, fermented cream, cheese. EPZs significantly improve the texture and stability of the final product, which increases the shelf life. With the help of EPZ-producing cultures, the synthesis (separation of whey) of yogurt and fermented cream is reduced [22]. EPZ is responsible for hydrophilic molecules that bind free water, which makes the final product less sensitive to synergy [23].

To prevent synergy, coagulated yogurt is prepared with higher concentrations of stabilizers (0.7%), as is the normal 0.3%. Yogurt drink is also mixed, being prepared from yogurt with a low dry matter content. The texture of the yogurt drink changes due to the mechanical action on the product, which requires the use of EPZ-producing starter cultures that ensure a stable structure.

The strongly filamentous stems generate the products with an unpleasant appearance when spinning from the spoon (that's why the spoon sensory analysis test was introduced); a similar sensation occurs by chewing when the clot is sticky and adherent to the palatin. Individual value has only the strains of the "thickening" group, the others will be used only in mixed cultures after preliminary tests [25].

EPZ starter cultures can be used for the production of yogurt drink as well as for the yogurt with low content of dry matter, lipids or even for the manufacture of skimmed yogurt. This type of crop forms have a more consistent (bonded) texture, higher viscosity, improves structure, prevents gel breakage and whey elimination.

The technology of acidic dairy products involves the fermentation of milk with pure cultures or bacterial consortia containing different strains of lactic bacteria. In this case, it is important that the strains used in the composition of the starter cultures are biocompatible and ensure the viable microbial cell number from 10^8 to 10^9 in 1 cm^3 (g). In the manufacture of yogurt, it is recommended to apply EPZ-producing strains in starter cultures, which provide organoleptic and rheological characteristics, that are necessary for the finished products without the use of food additives.

It is known that multicomponent starter cultures possess higher biochemical action with high resistance to different negative factors compared to the starter cultures prepared from monocultures and offer completely new probiotic properties to the products [11]. Therefore the native strains of S. thermophilus have been used in consortia for the manufacture of yogurt with high content of bioactive compounds.

When developing lactic acid bacteria consortia for starter cultures, it is important to consider the relationship between strains and possible changes in microflora during the subsequent cultivation of dairy products. By combining different species of lactic acid bacteria and regulating the fermentation temperature, it is possible to obtain a product with the desired taste and aroma, texture and dietary properties.

In this regard, at this stage, the purpose of the research was the selection of lactic acid bacteria to determine the most promising combinations for use in the preparation of starter cultures for yoghurt manufacture.

In order to obtain the starter culture from the Ramurala Collection, two strains of S. thermophilus, CNMN-LB-79 were obtained, which were selected from the goat's milk in the thesis of doctor by Bogdan Nina: “Harnessing the microbial strains isolated from goat's milk for industrial application” and a CNMN-LB-50 strain that was selected as an EPZ-producing strain in the thesis by Dr. Cartasev Anatolii: ”New native strains of Streptococcus thermophilus and their use in the manufacture of fermented dairy products”.

The cultures were restored from the lyophilized state and appreciated according to the main technological criteria: the restoration duration, the appearance of the clot, the microscopic aspect of the cells, the acidifying activity in whole milk with the inoculum of 5% culture. The selected strains correspond to the requirements of fermentative activity, acidification of the active milk, relative viscosity, high EPZ content. The test results are shown in table 3.

Table 3. *Technological properties of native lactic bacteria S. thermophilus strains*

No	Characteristics	*Streptococcus thermophilus Strains*	
		LB-50	**LB-79**
1.	The restoration duration, hours	6±0,5	6±0,5
2.	The microscopic aspect	Cocci, associated in diplococci and different lengths chains	
3.	The clot appearance	Homogeneous, viscous, filamentous	Homogeneous, viscous
4.	Consistency	Creamy, dense	dense
5.	Elimination of whey	no whey elimination	
6.	**Acidifying activity in whole milk, inoculum 5% culture**		
7.	Duration of clotting, hours	4,0±0,5	5,0±0,5
8.	The titratable acidity, ° T	77±1,5	71±0,3
9.	Cinematic viscosity, cSt	41,7±1,7	47,4±0,5
10.	EPZ, mg / 100 ml	44,5±1,2	0

According to results from table 3, the technological properties of the native strains of S. thermophilus lactic bacteria manifested in goat's milk, shows that the strains have the appearance of homogeneous, dense consistency, without eliminating the whey, and corresponds to the technological requirements for the lactic bacteria yoghurt manufacture. The microscopic aspect is shown in figures 1 and 2.

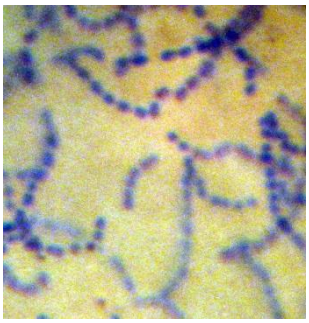

Fig. 1. Microscopy of S. thermophilus CNMN-LB-50 strain

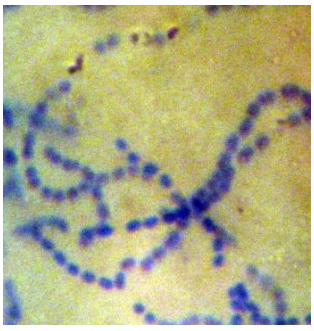

Fig. 2. Microscopy of S. thermophilus CNMN-LB-79 strain

According to the Technical Regulation the yogurt is a dairy product made by fermenting milk with starter cultures S. thermophilus and Lb. delbrueckii subsp. bulgaricus. Therefore, for the composition of the consortia of lactic bacteria in yoghurt manufacture (Food Technologies Directorate, IȘPHTA), native Lb. bulgaricus strains were selected and tested.

The Lb. bulgaricus bacteria is asporogenic, immobile, Gram positive, anaerobic or facultative anaerobic. They do not produce catalase, cytochrome oxidase - negative, do not reduce nitrogen, do not liquefy gelatin. They have a reduced proteolytic and lipolytic activity, fermenting lactose, maltose, sucrose (especially in the logarithmic development phase), glucose, fructose and galactose. It requires mineral substances and all the vitamins from B group to growth. It develops well in the environment with pH 5.5-5.8, but also at pH 5.0. They can develop within wide temperature limits (5-53 °C), but the optimal temperature is between 30 and 45 °C.

The relationship between the two species in starter cultures is symbiosis, which is important for the formation of lactic acid, the typical taste and aroma of the product. The cultures were also restored from the lyophilized state and appreciated according to the main technological criteria: the duration of restoration, the appearance of the clot, the microscopic aspect of the cells, the acidifying activity in whole milk with the inoculum of 5% culture. The results are presented in table 4.

Table 4. Technological characteristics of Lb. Bulgaricus strains

No	Characteristics	*Lactobacillus bulgaricus* CNMN - Strains				
		LB-40	**LB-41**	**LB-42**	**LB-43**	**LB-44**
1.	Restoration time, hours	18±0,5	18±0,5	18±0,5	18±0,5	36±1,0
2.	The appearance of the clot	Homogeneous, viscose				
3.	Consistency	dense			moderately dense	moderately
4.	Elimination of whey	without whey elimination				with whey elimination
5.	The microscopic aspect	Separated bacilli associated in chains of different lengths				
6.	**Acidifying activity in whole milk, inoculum 5% culture**					
7.	Duration of clotting, hours	4,5±0,5	3,5±0,5	4,5±0,5	4,5±0,5	7,0±0,5
8.	The titratable acidity, ° T	130±1,1	124±1,0	118±1,5	118±1,5	120±2,5
9.	Cinematic viscosity, cSt	17,41±0,5	15,41±0,8	26,84±1,0	18,55±0,5	11,13±0,1
10.	EPZ, mg / 100 ml	0	0	0	0	0

From the presented data we can conclude 4 Lb. bulgaricus cultures CNMN-LB-40, CNMN-LB-41, CNMN-LB-42, CNMN-LB-43 - were restored in the required time, according to the maximum requirements 20 hours. The restored cultures formed the shell in 3.5-4 ± 0.5 hours, with a homogeneous appearance, dense consistency, some slightly viscous, clean taste of fermented milk, with moderate titrable acidity between 89-94 °T. Stem Lb. bulgaricus CNMN-LB-44 was restored with a delay of 16 hours, forming a moderate coagulation, which does not meet the technological requirements and therefore cannot be applied in combinations for starter cultures intended for yoghurt manufacture. The product obtained by fermentation of the investigated strains had a low viscosity index between 11.13 - 28.84 cSt. Strains do not produce EPZ. For use in the composition of starter cultures intended for the manufacture of yogurt, the Lb. bulgaricus CNMN-LB-42 strain was selected. The microscopic aspect is shown figure 3.

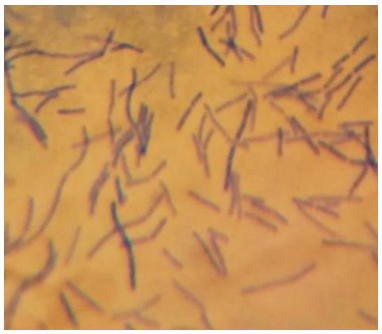

Fig. 3. Microscopy of the Lb. bulgaricus CNMN-LB-42 strain

3.4. Obtaining selected strains for starter culture used in manufacture of goat milk yogurt with high content of bioactive compounds

It is from practice known that the manufacture of the yogurt presents frequent complications for the producers, because the consistency of the products is slightly damaged at different mechanical actions in the technological process during transport and storage. Even more, the decrease in fat content influences the structural properties of the product, causing the elimination of whey and the formation of granules. As a rule, yogurt starter crops consist of S.thermophilus and Lb. bulgaricus having an average activity of acidifying the milk for 5-7 hours. Starter cultures were prepared according to the diagram shown in figure 4.

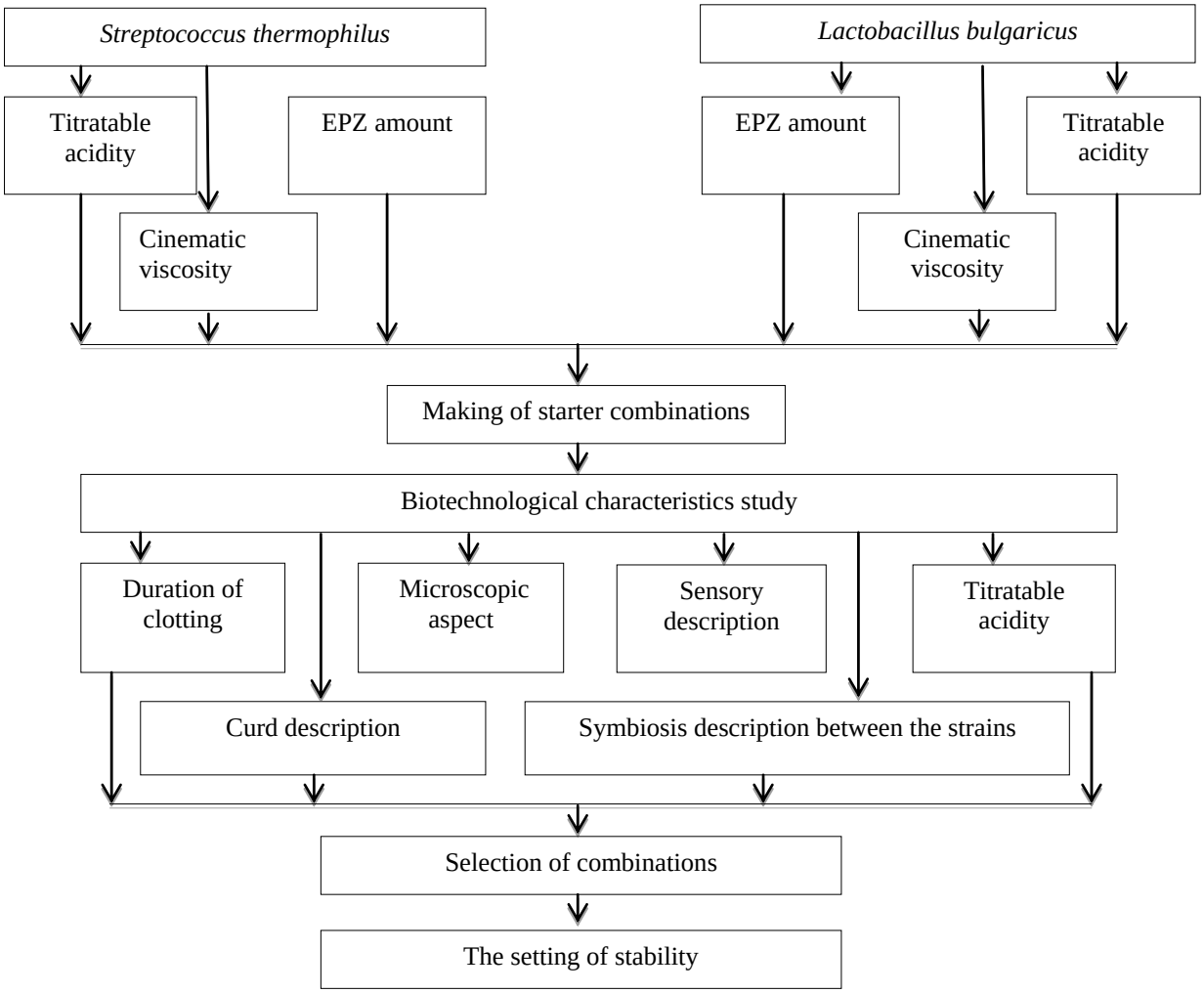

Fig. 4. Scheme of preparation of starter culture for the manufacture of goat milk yogurt

In the next step, the research was intent on the association between S. thermophilus strains within the species. In order to create combinations between strains of the same species, the strains selected by S. thermophilus were gradually associated in a 1:1 ratio, their compatibility at the level of acidifying and coagulating action being studied. The strains were inoculated into milk (20-30 ml). After incubation, until the clot was obtained, the obtained combinations were re-seeded twice in sterile skimmed milk. Were selected associations, which have demonstrated intensive acidogenesis action - within 5 hours, with the formation of a homogeneous, dense, creamy or viscous coagulation with moderate filant. An association of strains was developed within the species S. thermophilus and investigated according to biotechnological indices: EPZ culture - S. thermophilus CNMN-LB-50 + S. thermophilus CNMN-LB-79. The results of the investigations are shown in table 5.

Table 5. Characteristics of strain associations within the S. thermophilus species

Duration of clotting, hours	The titratable acidity, °T	Viscosity, cSt	Amount of EPZ, mg / 100 ml	The appearance of the clot
3,5±0,5	71±2	100,23±0,85	53,4±2,1	O, V, F, D, C, fz

Note: O - homogeneous, V - viscous, nV - non-viscous, F - filant, F + - very filant, D - dense, C - creamy, fz - without whey.

The obtained data show that the association of S. thermophilus strains CNMN-LB-50 + S. thermophilus CNMN-LB-79, consisting of a strain that is EPZ-producing and 1 non-EPZ-producing, exhibits high viscosity 100.23 cSt, fermenting milk in 4 hours, forming the dense shell, without eliminating the whey, with the titratable acidity 70 °T. As a result of examining

the association of S. thermophilus cultures it can be seen that the combination is successful and will be used in the manufacture of yogurt.

Fermented dairy products are very popular all over the world for their specific properties and beneficial effect on the human body. A crucial role in their manufacture is played by the biochemical processes caused by starter cultures. Therefore, the quality of dairy products depends on the quality of the starter cultures used in their production, which, in turn, is determined by the characteristics of the microorganisms within the starter culture.

The starter crops for the manufacture of yogurt should be made of S. thermophilus and Lb. bulgaricus species. Therefore, in the next stage, associations formed of lactobacilli and thermophilic streptococci were created and studied. Based on the associations formed within the species, combinations of strains for starter crops were created for the manufacture of yogurt:

1. S. thermophilus CNMN-LB-50 + S. thermophilus CNMN-79 + Lb.bulgaricus CNMN-LB-42 - EPZ starter culture;
2. S. thermophilus CNMN-LB-50 + Lb. bulgaricus CNMN-LB-42 - EPZ culture starter;
3. S. thermophilus CNMN-LB-79 ++ Lb. bulgaricus CNMN-LB-42 - starter culture without EPZ.

There were 3 associations of strains of S. thermophilus and Lactobacillus bulgaricus species, of which two EPZ starter cultures and one starter culture without EPZ as a control culture, which were investigated according to biotechnological clues. The results of the investigations are shown in table 6.

Table 6. The associations description of native strains for goat milk yoghurt

No	Duration of clotting, hours	The titratable acidity, ° T	Viscosity, cSt	Amount of EPZ, mg / 100 ml	The appearance of the clot
1	3,5±0,5	112±2	43,97±1,3	58,43±1,9	O, V, F, D, fz
2	3,5±0,5	118±1	70,25±1,72	47,49±1,3	O, V, F, D, fz
3	4,0±0,5	98±2	106,51±1,0	0	O, D, nF, fz

Note: O - homogeneous, V - viscous, nV - non - viscous, F - filamentous, D - dense, fz - without removing the whey.

Based on the data obtained from the formation of associations between the species S. thermophilus and Lb. bulgaricus in starter cultures for the production of goat milk yogurt, it is obvious that the starter culture consisting of 2 S. thermophilus strains CNMN-LB-50 + Lb. bulgaricus CNMN-LB-42 has a higher viscosity and faster clotting time than the starter culture consisting of 3 S. thermophilus strains CNMN-LB-50 + S. thermophilus CNMN-79 + Lb.bulgaricus CNMN-LB-42. In all the varieties, the elimination of the whey was not detected, the titratable acidity was within the permissible limits and contained 98-118 ° T. Therefore, the associations formed correspond to the requirements stipulated for the starter cultures intended for the manufacture of fermented dairy products. Also, the elaborated starter cultures were examined microscopically to determine the ratio between S. thermophilus and Lb cultures. bulgaricus. The results are shown in Figure 5.

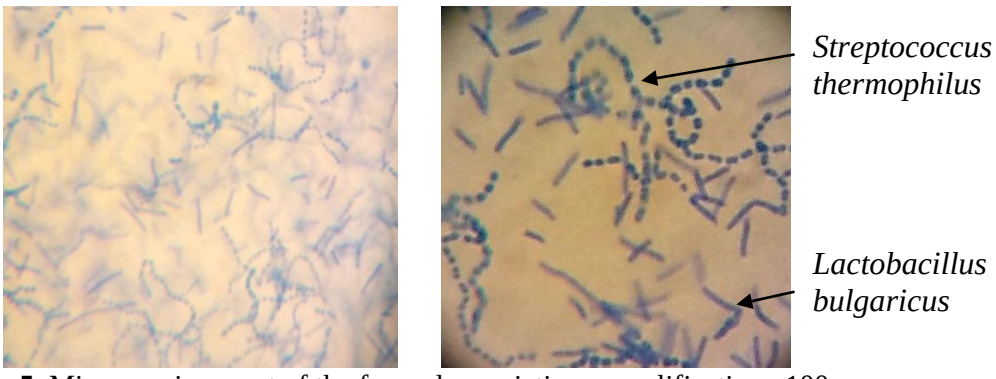

Fig. 5. Microscopic aspect of the formed associations, amplification x100

As a result of the research of the associations for the starter culture intended for the manufacture of yogurt, 2 starter cultures were selected:
1. YO1 - S. thermophilus CNMN-LB-50 + S. thermophilus CNMN-79 + Lb.bulgaricus CNMN-LB-42
2. YO2 - S. thermophilus CNMN-LB-50 and Lb. bulgaricus CNMN-LB-42;
3. YO3 - S. thermophilus CNMN-LB-79 and Lb. bulgaricus CNMN-LB-42.

The starter culture compounds were tested for the technological characteristics and symbiotic nature in the case of multicomponent cultures in order to select the ones with the highest prospects for use in the manufacture of fermented dairy products. The cultures were inoculated in goat milk within 5% amount.
At the initial stage, the acidogenesis activity of the native starter cultures was analyzed. The results obtained are shown in Figure 6.

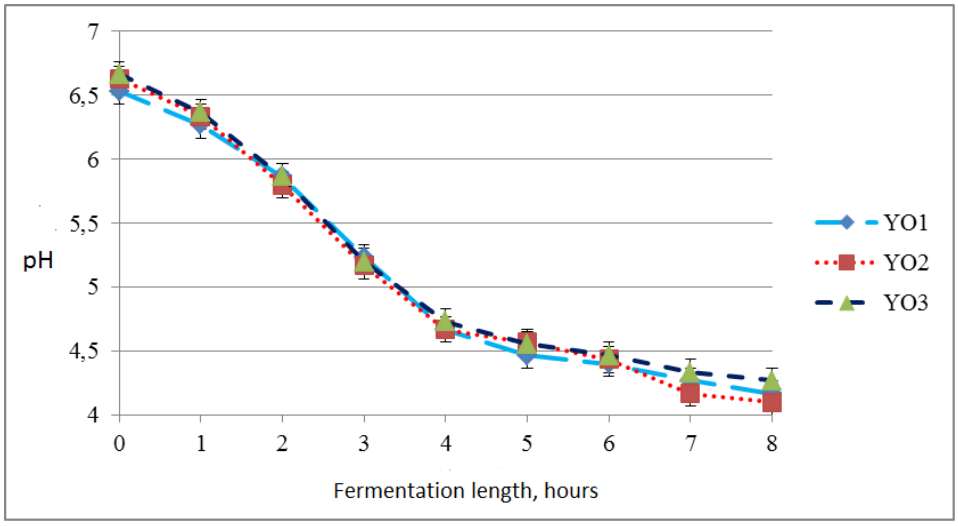

Fig. 6. Acidogenesis activity of elaborated native starter cultures

As a result of the studies on the acidification activity of the starter cultures for 8 hours of fermentation at temperatures of 40 ± 1 °C, it was found that the decrease of the acidity of the starter had a 5 hours of fermentation and values equal to 4.58 ± 0.02 - 4.31 ± 0.03 pH units. After 5 hours of fermentation, when the clot is already formed, the decrease of the activity of the starter culture continues in parallel with some small deviations.

After 8 hours of fermentation the active acidity has the following values: the pH of the cultures YO1 - 4.17 ± 0.05, YO2 - 4.15 ± 0.02, YO3 - 4.28 ± 0.01, which after 8 hours they have not changed fundamentally, from where we can conclude that starter cultures will not cause the deterioration of the finished product during storage.

Conclusions

The technology of dairy acid products involves the fermentation of milk with pure cultures or bacterial consortia containing different strains of lactic bacteria. In the manufacture of yogurt, it is recommended to apply EPZ-producing strains in starter cultures, which provide organoleptic and rheological characteristics, that are necessary for the finished products without the use of food additives. When developing lactic acid bacteria consortia for starter cultures, it is important to consider the relationship between strains and possible changes in microflora during the subsequent cultivation of dairy products. By combining different species of lactic acid bacteria and regulating the fermentation temperature, it is possible to obtain a product with the desired taste and aroma, texture and dietary properties. In the presented research, the physico-chemical and microbiological characteristics of goat's milk were determined. The strains of valuable native lactic bacteria of the species Streptococcus thermophilus and Lactobacillus bulgaricus were selected with stable technological characteristics for the fermentation of goat's milk, corresponding to the requirements for lactic bacteria intended for the manufacture of yogurt.

The scheme for preparing starter cultures and recommendations regarding the use of consortia of symbiotic cultures for the manufacture of goat milk yoghurt have been elaborated. Starter cultures were obtained for the manufacture of

goat milk yogurt, with biotechnological properties characteristic for fermented dairy products.

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