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II.3. BIOCHEMICAL APPROACHES FOR GOAT MILK YOGURT PRODUCTION

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Abstract

Goat milk production is a dynamic and growing industry that is fundamental to the wellbeing of hundreds of millions of people worldwide and is an important part of the economy in many countries. In this research goat milk samples, obtained from the individual farms were investigated to chemical composition and physico-chemical properties. 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 milk. The scheme for preparing starter cultures and recommendations regarding the use of consortia of symbiotic cultures for the production of goat milk yogurt have been developed. Starter cultures were obtained for the manufacture of goat milk yogurt, with biotechnological properties characteristic for fermented dairy products.

Key words*: goat milk, native lactic acid bacteria, yogurt production.*

INTRODUCTION

Goat milk production is a dynamic and growing industry that is fundamental to the wellbeing of hundreds of millions of people worldwide and is an important part of the economy in many countries (Li X.Y. et al., 2020; Wang Y. et al., 2019). Goat milk has a promising source of protein, vitamins, minerals and fatty acids (Chen X. et al., 2020; Asresie A. et al., 2014; Beshkova D.M. et al, 2011). Goat milk is known for its better digestibility, lower allergenic potential due to the low content of lactose, as well as the presence of health-promoting compounds (Verruck S. et al., 2019; Hassan F.A.M. et al., 2014; Paz N.F. et al., 2014). From goat milk usually is obtained butter, yogurt, sour milk etc. (Picon A. et al., 2019; Shori A.B. et al., 2015). Fermented dairy products have delicious sensory properties, fine consistency and pleasant specific taste (Muelas R. et al., 2018). Especially fermented goat milk products have significant commercial potential, large destination and multiple health benefits for population (Yurchenko S. et al., 2018; Innocente N. et al., 2016). Considering the importance of fermented dairy products from goat milk, which are in demand on internal and external markets, elaboration of the technological process for manufacturing of the products is necessary (Saha B.N.P. et al., 2016; Serhan M. et al., 2016).

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. Goats farming have an important food potential, which must be exploited at industrial level.

At the international level, the proposed topic carries researches on the preparation and optimization of goat milk yogurt technology and manufacture (Caleja C. et al., 2016; Garcia V. et al., 2014). 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 properties and the harmlessness as a raw material (Zhao X. et al., 2020; Sun Y. et al., 2020; Sousa Y.R.F. et al., 2019; Zhu Z. et al., 2019).

Currently, the theoretical and applied research is insufficient for an objective assessment of the

effect of the constituent components of goat milk on the quality of products, which hinders the use of goat milk when creating new generation food products of high biological and nutritional value, including the functional area (Deshwal G.K. et al., 2020; Feng C. et al., 2019; Verruck S. et al., 2019; Yangilar F., 2013).
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.

MATERIALS AND METHODS

Fat content was performed in accordance with ISO 2446: 2008. Concentrated sulfuric acid was used as the main reagent, which converts insoluble calcium salts of milk into soluble sulfuric acid casein compound. The latter dramatically reduces the amount of adsorption of fat globules and thereby contributes to their merger. The butyrometer was mounted on a stand and 10 ml of H_2SO_4 was poured. Slightly tilting the device, acid was carefully poured over its wall to 11 ml of a well-mixed product (20°C). To avoid mixing the product with H_2SO_4, 1 ml of isoamyl alcohol was added. Butyrometer was taken by the neck and, holding the cork, shaken several times until a homogeneous mass without flakes was obtained in the tube. After that, the butyrometer was placed (holding the stopper down) for 5 minutes in a water bath at 65-70°C. Then, the fat released on the butyrometer was counted on a scale.
Protein content was performed in accordance with ISO 8968-1: 2014. The method included the quantitative determination of nitrogen in the samples under study. The nitrogen contained in the test samples was heated with concentrated sulfuric acid in the presence of catalysts and transferred to ammonium sulphate, the sample itself was completely destroyed. Ammonia was squeezed out of ammonium sulphate with concentrated base, which was distilled into a receiver with a certain volume of titrated acid. Measuring the amount of acid remaining in the receiver after the end of the distillation, the amount of ammonia in the sample was calculated and, therefore, the amount of fixed nitrogen in the sample under study. Calculations are made according to the formula:

$$WN = \frac{1{,}4007(Vs - Vb)Mr,}{m} \quad [\%]$$

where:
WN - the mass fraction of nitrogen in the sample [%];
Vs - the volume of hydrochloric acid (accurate to 0.05 ml) [ml];
Vb - the volume of hydrochloric acid (with an accuracy of 0.05 ml) [ml];
Mr - the molecular weight of hydrochloric acid;
m - the mass of the test portion (with an accuracy of 0.1 mg) [mg];
1.4007 - coefficient of calculation for the expression of the nitrogen content in the sample [%].

Lactose content of the tested samples was determined in accordance with the standard method ISO 22662: 2007, which provides high performance liquid chromatography (HPLC) with refractometric detection. 10 ml of a clear sample obtained after centrifugation was measured. This sample was filtered through a 0.45 μm membrane. Through a cartridge for filtering C18 was loaded with 10 ml of ethanol and 10 ml of distilled water. Then the sample was passed through a C18 cartridge. After each filtration, the cartridge was washed with 10 ml of ethanol, and then with 10 ml of distilled water. Then 1 ml of a standard solution of lactose, previously prepared at a concentration of 2.4%, was measured and injected into the injection device. When the device lever was moved 90°, the quaternary pump sucked 10 μl of the solution and switched it into the acetonitrile phase. Further, the separation of water and lactose occurred depending on the retention time. The refractometric detector determined the value of the peak areas of water and lactose, then the information was transferred to the software database. The analysis time was 12 minutes. Therefore, 1 ml of the sample was taken for analysis (clear phase after centrifuging the milk) and subjected to qualitative and quantitative analysis in the same way as the standard solution.
Free fatty acid content was determined on a Hewlett-Packard chromatograph (model 5890, Palo Alto, CA, USA), with a flame ionization detector (FID) and connected to a ChemStation computer (Hewlett-Packard, Palo Alto, CA,

USA). This method allows you to set the mass fraction of fatty acids to their total content in triglycerides. The separation of fatty acids was carried out depending on the chain length and the degree of their unsaturation, by analogy with their closest standards. The mass fraction of each acid was calculated on the basis of the obtained chromatogram over the areas of the peaks using a standard graph.

Physico-chemical properties of the goat milk was recorded using the EKOMILK Total Bulteh 2000 automatic milk analyzer. All the determinations were performed in duplicate.

Statistical analysis of the results was carried out by least square 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 ± 1ºC. Experimental results are represented according to standard rules.

RESULTS AND DISCUSSIONS

Analysis of the chemical composition and physico-chemical properties of goat milk

In this study, a comparative analysis of the fat, protein, fat and ash content of the goat milk was made. Table 1 presents experimental data of goat milk evaluation.

Table 1. Chemical composition and physico-chemical properties 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 was determined the mass fraction of proteins (%), which constituted 4.28 ± 0.03. The fatty acid composition of goat milk was studied using gas-liquid chromatography. Experimental data showed the content of 23 fatty acids, represented by saturated, monounsaturated and polyunsaturated fatty acids. It is known that the chemical composition of milk differs depending on season, feeding, age, lactation period. The high content of skimmed dry matter in goat's milk indicates that it is better for technological processing.

Development of starter culture strains for goat milk yogurt production

Goat milk products with valuable nutritional properties for humans can be positioned as a healthy diet product. 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 sensory 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 our previous studies isolation and selection of valuable lactic acid bacteria strains for the goat milk fermentation has been performed (Popovici C. et al., 2019). Starter cultures have been prepared according to the proposed technology (Figure 1). In the next step, the research was intent on the association between *Streptococcus thermophilus* strains within the species. In order to create combinations between strains of the same species, the strains selected by *Streptococcus 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.

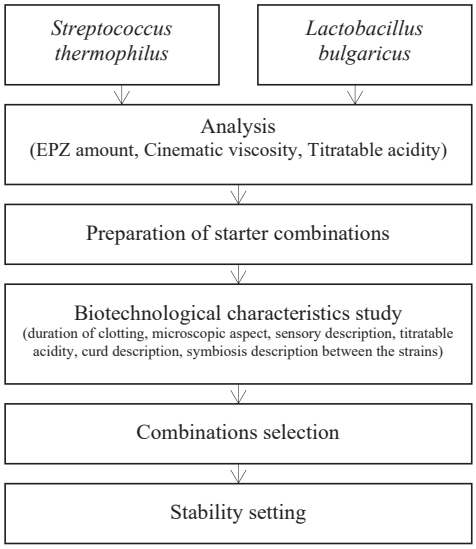

Figure 1. Steps of starter culture preparation for goat milk yogurt production

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 *Streptococcus thermophilus* and *Lactobacillus 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, 3 combinations of of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* species were created, 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 2.

Table 2. The associations description of native strains for goat milk yoghurt

No	Characteristics	Association I	Association II	Association III
1	Duration of clotting, hours	3.5±0.5	3.5±0.5	4.0±0.5
2	Titratable acidity, °T	112±2	118±1	98±2
3	Viscosity, cSt	43.97±1.3	70.25±1.72	47.49±1.3
4	Amount of EPZ, mg/100 ml	58.43±1.9	106.51±1.0	0
5	Appearance of the clot	O, V, F, D, fz	O, V, F, D, fz	O, D, nV, 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 *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in starter cultures for the production of goat milk yogurt, it is obvious that the starter culture consisting of 2 *Streptococcus thermophilus* strains CNMN-LB-50 + *Lactobacillus bulgaricus* CNMN-LB-42 has a higher viscosity and faster clotting time than the starter culture consisting of 3 *Streptococcus thermophilus* strains CNMN-LB-50 + *Streptococcus thermophilus* CNMN-79 + *Lactobacillus bulgaricus* CNMN-LB-42.

In all varieties, the elimination of the whey was not detected, the titratable acidity was within the 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 *Streptococcus thermophilus* and *Lactobacillus bulgaricus* cultures. The results are shown in Figure 2.

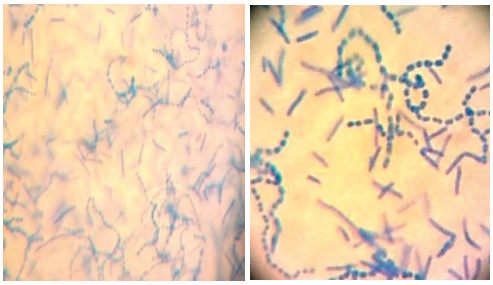

Figure 2. Microscopic aspect of the formed associations

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 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

In the presented research, the chemical composition and physico-chemical properties of goat 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 milk, corresponding to the requirements for lactic bacteria intended for the yogurt production. The scheme for preparing starter cultures and recommendations regarding the use of consortia of symbiotic cultures for the goat milk yoghurt production have been developed. Starter cultures have been obtained for the goat milk yogurt production with biotechnological properties characteristic for fermented dairy products.

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