

ABSTRACT

of the dissertation for the degree of Doctor of Philosophy (PhD) in the specialty "6D070100 –Biotechnology"

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Biotechnological approaches to the creation of dairy products based on goat milk

General description of the work. The work is aimed at the study of antagonistic activity and technological characteristics of the new bacterial composition, as well as physicochemical parameters and biological value of the obtained dairy products.

Relevance of the research topic. One of the widely consumed food products in the world are dairy products, the biological properties of which are aimed at inhibiting pathogenic microorganisms, stimulating the growth of beneficial intestinal microflora and increasing immunostimulating properties. It should be noted that no matter how diverse the range of dairy products, including yoghurts, is, the fillers are mainly berries, fruits, cereals, and cow's milk is used as a raw material in 95% of cases.

Currently, goat milk products are gaining popularity in Europe and around the world, the consumption of which helps improve digestion and absorption of nutrients. Consumption of goat's milk significantly improves body weight and skeletal mineralization, and also increases the levels of vitamins, minerals and hemoglobin in the blood serum.

To develop new dairy products, one of the promising directions is the search for unique starter material and the development of technologies for synbiotic milk-based products, including optimal compatibility of consortia of probiotic microorganisms. Regular consumption of dairy products allows you not only to maintain good health, but also to slow down the aging process. Yoghurt is growing in popularity around the world, but the live bacteria in it only lasts one to two weeks.

Modification of dairy products by introducing plant components makes it possible to give traditional products new technological properties, save dairy raw materials and regulate the chemical composition in accordance with modern requirements of nutrition science. Berries are promising raw materials for the creation of dairy products. Hawthorn, rose hips, and rowan are among the most popular berries, which include a wide range of biologically active ingredients, such as essential oils, phenolic compounds, proteolytic enzymes, vitamins and microelements. Considering these qualities, the study of the joint use of berry syrups, dry extract of grape skins and starter probiotic culture in the production of new dairy products is of practical interest.

In this regard, it is relevant to develop dairy products from goat milk, enriched with plant fillers, with probiotic properties, preserving live bacteria until the end of the shelf life, which provides great benefits for maintaining health, intestinal microbiocenosis and will expand the range of domestically produced dairy products based on goat milk.

The purpose of research. Development of biotechnological approaches to the creation of dairy products with antioxidant properties on the basis of goat milk.

Research objectives:

1. investigation of physicochemical and microbiological parameters of goat milk;
2. isolation, genetic identification of lactic acid bacteria strains from goat milk from local producers using primers 8F and 806R;
3. selection and justification of the composition of lactic acid bacteria with high probiotic and fermenting properties;
4. justification of the dose of plant fillers and development of recipes for new dairy products;
5. assessment of the antioxidant properties of dairy products from goat milk.

Objects of the research. The following objects were used in the study: goat milk samples from the Almaty region; yoghurt starters YOMIX 495 LYO 100 DCU and YOMIX 883 LYO 50 DCU (Danisco, Germany); a bacterial composition containing *Streptococcus thermophilus* TA 45 (Danisco, Germany), *Lactobacillus bulgaricus* B6 (University of Food Technologies, Plovdiv, Bulgaria); biologically active food additives: syrup "KM - Rowan", syrup "KM - Rosehip" (PC "Firm "Kyzyl May", Almaty, Kazakhstan), syrup from hawthorn fruits (OJSC "Kemerovo Pharmaceutical Factory", Russia); food fortifier "Grape peel extract" (Healthlife Biotechnology Co., Ltd, China).

Male Wistar rats were used for the experiments.

Subject of the research. Evaluation of the antagonistic and technological characteristics of a bacterial composition for the production of domestically dairy products with probiotic properties from goat milk.

Research Methods: Methods for organoleptic evaluation of raw materials and finished products, areometric and pycnometric methods for determining density, titrimetric and potentiometric methods for determining acidity, method for measuring the mass fraction of total nitrogen and mass fraction of total protein according to Kjeldahl, acid method for determining fat according to Gerber, amperometric method for determining antioxidants, capillary method electrophoresis, microbiological analysis methods, diffusion method for determining the antagonistic activity of bacteria, Sanger 16S rRNA gene sequencing method, high performance liquid chromatography method, atomic absorption spectrometry for determining the toxic elements, immunological methods, Korolyuk method for determining catalase activity in erythrocytes.

For statistical processing of the obtained results, the programs "Statistica", «Microsoft Excel» were used.

Scientific novelty of the research. New strains of lactic acid bacteria have been isolated and identified. Utilizing thermophilic streptococcus, Bulgarian bacillus, and a strain isolated from goat milk, the bacterial composition was formulated, demonstrating suppression of the growth of pathogenic and conditionally pathogenic microorganisms. The ratio of *Lactobacillus bulgaricus* B6, *Streptococcus thermophilus* TA 45, and *Lactobacillus fermentum* 14 in a ratio of 1:5:1 has been justified. The introduced starter culture has shown a positive impact on the qualitative characteristics

and stability of the microflora during the storage of dairy products. A technology for producing dairy products from goat milk has been successfully developed.

The data, including nutritional and biological value, organoleptic, physico-chemical, microbiological, and antagonistic indicators of the finished products, were obtained.

The novelty of the proposed biotechnological solutions is substantiated by three patents of the Republic of Kazakhstan for utility models (№5613, №5614 and №8592).

Theoretical significance of research. Lactic acid bacteria with the antagonistic activity against bacterial test cultures were isolated. A bacterial starter composition with the most pronounced organoleptic characteristics and antagonistic properties has been created.

The use of the PCR method with random amplification of polymorphic DNA (RAPD) for the genetic identification of interspecific and intraspecific polymorphism and phylogenetic relationships in isolated strains is justified, which has significant advantages over other methods: ease of use for studying different species using the same primers; no need for preliminary cloning and sequencing of fragments to select primers; a universal set of input samples and rapid detection of variability in a large number of loci throughout the genome, as well as low cost and ease of use.

The results of the study can be used in the curriculum of universities in the preparation of bachelors, masters and PhD doctoral students in the specialty “Biotechnology”.

The practical value of research. A certificate of deposit of the probiotic culture *Lactobacillus fermentum* 14, isolated from goat milk, was received in the Republican Collection of Microorganisms (Astana), collection number of the strain is B-RCM 1020. Industrial testing of the developed dairy products was carried out on the basis of the Kazakh Academy of Nutrition. The novelty of the results obtained is confirmed by three utility model patents of the Republic of Kazakhstan No. 5613, No. 5614 and No. 8592.

The main provisions for the defense:

1. strains of lactic acid bacteria with the most pronounced acid-forming properties from goat milk were isolated and genetically identified;
2. the use of probiotic strain *Lactobacillus fermentum* 14 was substantiated by physicochemical parameters and antagonistic activity;
3. a bacterial composition with high organoleptic properties and antagonistic activity was prepared;
4. physico-chemical, microbiological indicators and biological value of finished dairy products were investigated;
5. the influence of dairy products on reduction of animals' toxic poisoning by cadmium chloride was evaluated.

The main research results and conclusions:

1. A comparative analysis of the protein composition of goat and cow milk showed the following ratio of casein proteins and whey proteins from the total amount of protein after 48 and 72 hours of storage: for goat milk - 77.9/24.4 and 76.2/25.1, for cow's milk - 76/25.5 and 75.5/26.2, and for both types of milk it is approximately 3:1,

which demonstrates the superiority of goat's milk over cow's milk in protein content and protein composition.

2. As a result of molecular genetic analysis of the types of nucleotide sequences, the values for the 16S rRNA gene were determined, and identification data for bacteria that showed the best acid-forming properties during milk fermentation were confirmed.

3. The bacterial compositions of *Lactobacillus bulgaricus* B6, *Streptococcus thermophilus* TA 45 and *Lactobacillus fermentum* 14 at ratios of 1:5:1 and 1:10:1 demonstrated antagonistic activity against *Micobacterium citreum* (32,51-35,0 mm), *Micobacterium rubrum* (31,43-32,3 mm), *Sarcina flava* U (25,71-26,9 mm), *Salmonella Dublin* (23.25-23.6 mm) and *Escherichia coli* test cultures.

However, based on the results of the technological and acid-forming properties of the studied bacterial starters, a combination of the above-mentioned microorganisms was selected in a ratio of 1:5:1.

The dosage of fermentation was selected in an amount of 3-5% by weight of the mixture, which contributes to good pH stabilization at the end of the ripening process and during storage of the dairy products. The dairy based on this starter had a delicate and uniform consistency.

4. The dose of vegetable fillers on the basis of organoleptic indicators and acid-forming properties of fermented milk was substantiated. It is revealed that the dosage of vegetable filler does not significantly affect the consistency of dairy products. The recipe is selected, the technological scheme of dairy products from goat milk is made.

5. The antioxidant activity of control and experimental samples of dairy products obtained on the basis of a bacterial composition was higher than the antioxidant activity of experimental samples of dairy products obtained on the basis of classical yogurt starter by 15.32% - 54.3% at the beginning of the shelf life and by 26,7 % - 55.8% at the end of the shelf life, that may be associated with the processes of ripening and coagulation by lactic acid bacteria, the accumulation of enzymes and vitamins, and the formation of water-soluble peptides.

5.1 Against the background of cadmium chloride poisoning of rats, dairy products enriched with vegetable fillers (syrops of hawthorn, rosehip, rowan and grape peel extract) from goat milk, had a beneficial effect on the general condition of animals. The experimental rats that received dairy products were more active, had good fur and normal respiration. The positive effect of fermented milk products was associated with normalization of intestinal microflora and improvement of intestinal peristalsis, as evidenced by the restoration of the defecation process, absence of liquid stools and increased appetite.

5.2 Total antioxidant activity in hepatocytes of animals consuming dairy products increased by 12%-40.2% in relation to group without dieting after toxic poisoning. The consumption of dairy products against the background of the rats' diet in the experimental groups contributed to the increase of the total antioxidant activity in blood serum by 17.2%–53.4%, as well as to the decrease of cholesterol level by 16.3%-23.7%.

5.3 The superoxide dismutase's activity of the experimental groups, compared to the 2nd group, increased by 31.9% - 77.3, in blood serum. A similar dependence was found on the side of catalase, which increased by 21 - 32.6 %.

Personal contribution of the author. The author contributed to the collection and analysis of data, to the determination of the goals and objectives of the work, to the planning and conduct of experiments, to the statistical processing and analysis of the data obtained, to the testing of research results, the preparation of publications, and the writing and design of research.

Coherence of the work with the program of the scientific research. The dissertation work was carried out within the framework of the funded project No. BR06249327 “Development and industrial production of baby and dietary food products based on goat milk”, carried out at the Kazakh Academy of Nutrition LLP in accordance with the budget program 267 “Increasing the accessibility of knowledge and scientific research” for 2018-2020 (Agreement No. 43/1 dated September 10. 2018).

Testing and implementation of research results. The research results and the main provisions of the dissertation work were presented and discussed at the following international scientific and practical conferences: “AGRITECH-2019: Agribusiness, Environmental Engineering and Biotechnologies” (2019, Krasnoyarsk, Russia); “Innovative development of the food, light industry and hospitality industry” (2023, Almaty, Republic of Kazakhstan); “Biotechnology: a look into the future” (2023, Stavropol, RF).

Publication of research results. There are 14 published works were published on the topic of the dissertation: 7 articles, two of which are included in the Scopus database (one of them with a percentile above 25), 4 articles were published in journals recommended by the Committee for Quality Assurance in the Field of Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan, three patents for utility models were received.

Structure of the dissertation. The dissertation work consists of references, designations and abbreviations, introduction, materials and research methods, results of research, conclusions. The results of the work are presented in 40 figures, 46 tables. The total volume of the work is 157 pages of computer text, including 29 pages of applications. The list of used literature contains 215 sources.