ABSTRACT

of the dissertation of Ikombaev Talgat Dyusyumbekovich on the topic «Optimization of the production technology of national spreadable sor milk products»,
submitted for the degree of Doctor of Philosophy (PhD) in the educational program 8D05101 –«Biotechnology (by sectors and fields of application)»

General characteristics of the dissertation work.

The dissertation work is devoted to the development and scientific substantiation of a gentle technology for the production of national viscoplastic fermented milk products based on goat's milk using domestic low-acid-producing probiotic starter cultures, which are of great practical importance. The work takes into account the biochemical features of goat's milk and modern requirements for product quality and functionality. The developed technology preserves biologically valuable components, improves organoleptic properties and enhances the functional effect of the products.

The study used probiotic strains of *Lactobacillus paracasei, Lactococcus lactis* and *Bifidobacterium crudilactis.* isolated from traditional fermented milk products of the region, which ensures their adaptation to the local microflora and high efficiency in mild lactic acid fermentation. The use of these cultures makes it possible to obtain cottage cheese with reduced acidity, preserve vitamins and lipids of goat's milk, improve texture and taste, and also give the product pronounced probiotic properties.

Relevance of the research work. Current trends in nutrition, focused on health, functionality and food safety, actualize the need to develop new technologies for processing dairy raw materials with high biological value and probiotic effect. Particularly significant is the processing of goat's milk, which has unique properties such as low allergenicity, increased digestibility, and balanced amino acid and lipid composition. However, traditional technologies focused on processing cow's milk are ineffective when working with goat's milk, which requires adaptation and the introduction of gentle biotechnological solutions.

In the conditions of the Republic of Kazakhstan, where historically there have been traditions of consumption of fermented dairy products, as well as a growing interest in functional and dietary nutrition, the development of technologies that ensure the preservation of the natural properties of goat's milk and the formation of products with a targeted health-improving effect is especially relevant. The use of low-acid-producing probiotic cultures isolated from local traditional products and adapted to the microflora of the region opens up new opportunities for the creation of high-quality fermented dairy products. The use of the technology using low-acid-producing probiotic bacteria is due to the biochemical features of goat's milk, which contains less casein, especially as1-casein, which ensures its lower acid resistance and milder coagulation. The development of a gentle technology for the production of cottage cheese from goat's milk using domestic probiotic strains meets current requirements for the quality, safety and nutritional value of products, contributes to expanding the range of dairy products and increasing the competitiveness of domestic products. In addition, the implementation of this technology is of practical importance for stimulating the development of dairy goat breeding in Kazakhstan as a promising area of animal husbandry.

The purpose of the research: Optimize the technology of production of cottage cheese from goat's milk using low-acid-producing probiotic starter cultures.

The main objectives of the study to achieve this goal:

1. To study the physico-chemical and technological properties of goat's milk selected as a raw material for the production of a viscoplastic fermented milk product (cottage cheese).

2. To isolate and identify pure cultures of lactic acid bacteria (LAB) from traditional fermented milk products using modern microbiological and molecular genetic methods.

3. To study the biochemical, technological and probiotic properties of isolated lactic acid bacteria strains, including assessment of their antimicrobial activity, hydrophobicity of the cell surface, ability to autoaggregate and coagulate, biofilm formation, as well as tolerance to bile salts and antibiotic resistance.

4. To obtain experimental samples of cottage cheese from cow's and goat's milk using traditional technology, to analyze their organoleptic and physico-chemical characteristics.

5. To develop compositions of domestic low-acid-producing probiotic strains adapted to the characteristics of goat's milk.

6. Optimize the technological process of production of probiotic cottage cheese using low-acid-producing cultures and obtain probiotic cottage cheese from goat's milk using the developed starter cultures.

7. To study and evaluate the physico-chemical and microbiological parameters of the obtained probiotic cottage cheese based on goat's milk.

The objects of research: Goat and cow's milk, cottage cheese from goat and cow's milk, low-acid-producing probiotic strains of lactic acid bacteria (*Lacticaseibacillus paracasei, Bifidobacterium crudilactis, Lactococcus lactis*), viscoplastic fermented milk product - cottage cheese produced using a developed gentle technology based on goat's milk, as well as bacterial test cultures: *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa.*

Scientific novelty of the research results.

For the first time, five autochthonous strains of lactic acid bacteria with pronounced probiotic and technological properties were isolated and characterized from samples of goat's milk and cottage cheese taken in the East Kazakhstan region. Their molecular genetic identification was carried out by sequencing the 16S rRNA gene. The obtained nucleotide sequences are deposited in the international GenBank database of the National Center for Biotechnology Information (NCBI, USA) under the numbers: PX376708 (EF71346868), PX376985 (EF71346863), PX376986 (EF71346869), PX376954 (EF71346862), PX377005 — (EF71346864). Registration in GenBank confirms the originality of the strains and their potential as probiotics adapted to the microflora of the population of the Republic of Kazakhstan.

The possibility of using low-acid-producing probiotic starter cultures based on autochthonous strains, taking into account their key meatbolic characteristics, for mild lactic acid fermentation of goat's milk with the formation of a clot at an acidity of 51-79 °T, has been scientifically substantiated. Three probiotic consortia of *Lactobacillus paracasei, Lactococcus lactis* and *Bifidobacterium spp.* strains with low acid production and high biocompatibility, adapted to the microbiota of the population of the Republic of Kazakhstan, as well as providing stable fermentation, high viability of probiotic cells (up to 2.9×10^8 CFU/g) and improved organoleptic properties of the finished product, have been developed and tested.

The possibility of using low-acid-producing probiotic starter cultures based on autochthonous strains, taking into account their key metabolic characteristics, for mild lactic acid fermentation of goat's milk with the formation of a clot at an acidity of 51-79 °T, has been scientifically substantiated.

A comparative study of cottage cheese made from goat's milk and cow's milk produced using traditional technology has been conducted. Goat's cottage cheese with a comparable protein content (14.38–14.6%) is characterized by a higher caloric content (213.02–258.73 kcal/100 g) due to fat (up to 21.09%), an increased content of minerals: phosphorus — up to 0.214 mg/100 g, calcium — up to 0.169 mg/100 g, as well as water-soluble vitamins. The obtained indicators demonstrate the high biological value of goat's curd and substantiate its prospects for functional and dietary nutrition.

The technology of production of viscoplastic cottage cheese from goat's milk has been improved using a gentle regime and probiotic consortia of domestic low-acid-producing strains.

As a result, the acidity of the product was reduced (51-79°T versus 156°T in traditional cottage cheese), the fat content (up to 21.1%) and humidity (up to 66.2%) were increased. The developed cottage cheese has a soft consistency, a pleasant specific taste, high energy and biological value (213-259 kcal/100 g), probiotic activity (up to 2.9×10^8 CFU/g) and meets safety requirements.

The practical significance of the work.

As a result of the conducted experimental studies, a technology for the production of probiotic cottage cheese from goat's milk has been developed using domestic low-acid-producing strains of lactic acid bacteria isolated from traditional products of Kazakhstan. The strains used in the work were isolated from traditional fermented dairy products such as goat's milk, cottage cheese from goat's milk and cow's milk, selected from the East Kazakhstan region of the Republic of Kazakhstan. The probiotic strains registered in the GenBank database, identified by 16S rRNA sequencing, are valuable for subsequent use in the production of probiotic drugs and functional products.

During the study, an optimized starter culture composition was formed, including the best strains in terms of functional, technological and probiotic parameters: *Lacticaseibacillus paracasei* (KAN EI 4, Gch 5.2.1), *Bifidobacterium crudilactis* (7-2, 7-6C), *Lactococcus lactis subsp. lactis* (7-1M, 7-8M), obtained from traditional regional products. This composition was selected taking into account the biochemical characteristics of goat's milk - low acid resistance and a specific protein structure, which made it possible to adapt the fermentation process and create a gentle technology.

The developed technology of probiotic cottage cheese from goat's milk provides mild lactic acid fermentation, the formation of a viscoplastic texture, and an optimal level of acidity.

The finished product has a mild, balanced taste without pronounced sourness, high probiotic activity. The technology is especially relevant for farming and environmentally oriented production, where it is important to preserve the natural value of raw materials and apply gentle processing methods. The proposed technology can be successfully implemented both in processing plants and in small farms. It is characterized by reproducibility, technological reliability and corresponds to current trends in the field of functional and healthy nutrition.

The main provisions recommended for protection:

The technology of production of probiotic cottage cheese from goat's milk using domestic low-acid-producing strains of *Lactobacillus paracasei, Lactococcus lactis* and *Bifidobacterium crudilactis* has been scientifically substantiated.

The application of the developed technology using a consortium of *Lactobacillus paracasei Gch 5.2.1* and *Lactococcus lactis 7-8 M* ensures controlled mild fermentation (up to 79°T), contributing to the formation of a viscoplastic structure and stable organoleptic properties of cottage cheese.

Autochthonous strains of lactic acid bacteria used in the production of cottage cheese from goat's milk have high probiotic activity, confirmed by the results of in vitro studies (high enzymatic and antimicrobial activity, pronounced adhesive ability and resistance to stress factors).

Main results and conclusions of the study:

1. An analysis of the organoleptic and physico-chemical parameters of goat's milk from different regions of Kazakhstan has shown that milk from the village of Samar in the East Kazakhstan region, characterized by high fat content, balanced protein content, optimal consistency and pleasant taste, is the best quality for the production of viscous cottage cheese.

2. 23 isolates have been isolated from goat's milk and cottage cheese in the East Kazakhstan region, and 5 pure cultures of lactic acid bacteria strains with potential probiotic properties have been identified. The 16S sequencing method provides the highest accuracy than

the MALDI-TOF MS and API 50 CHL methods, and confirmed that the strains belong to the species *Lacticaseibacillus paracasei* and *Lactiplantibacillus plantarum*.

3. Autochthonous lactobacillus strains isolated from goat's milk demonstrated high enzymatic activity, technological stability and pronounced probiotic properties, including a decrease in pH to 3.8–4.6, an increase in abundance to 10^9 CFU/ml, antimicrobial activity against pathogenic microorganisms, high hydrophobicity and the ability to autoaggregate, coagulate and form biofilms. The results obtained confirm the expediency of their use in the production technology of probiotic cottage cheese.

4. Cottage cheese based on goat's and cow's milk has been developed using traditional technology. Goat's curd has several advantages over cow's curd. With a similar protein content (14.4–14.6%), it has a more delicate consistency and a characteristic taste. The energy value of goat cheese is 213-259 kcal/100 g. The product contains an increased amount of minerals — phosphorus (0.214 mg / 100 g), calcium (0.169 mg / 100 g), as well as water-soluble vitamins B and C (1.3 mg / 100 g), which increases its nutritional value and makes it promising for use in functional and preventive nutrition.

5. Three biocompatible starter consortia were formed using local low-acid-producing probiotic strains isolated from traditional fermented milk products, which ensured the production of a soft structure and high biological value of a dietary fermented milk product.

6. The technology of production of viscoplastic cottage cheese from goat's milk has been optimized using formed consortia of lactic acid and bifidobacteria, which made it possible to reduce the acidity of the product to 51-79°T (instead of 156°T for traditional cottage cheese), increase the fat content to 16.3–21.1% and humidity to 66.2%. The resulting probiotic cottage cheese, which has functional properties, was characterized by a soft consistency, pure fermented milk taste and an energy value of 213-259 kcal/100 g.

7. Microbiological studies of probiotic cottage cheese from goat's milk have shown the absence of coliform bacteria and total contamination in the range of $1.3–1.8\times10^4$ CFU/g. The content of lactic acid and bifidobacteria was at least 10^7 CFU/g, which meets the requirements of TR CU 033/2013 and confirms the safety and high viability of probiotics. The best characteristics were demonstrated by cottage cheese based on the consortium *L. paracasei Gch 5.2.1 + L. lactis 7-8 M:* high fat content, low acidity, up to 2.9×10^8 CFU/g of probiotics, pleasant taste and aroma, as well as increased calcium and phosphorus content.

The personal contribution of the dissertation to the development of the results of scientific work submitted for defense.

The dissertation independently formulated the main goals and objectives of the research, and developed a detailed experimental work plan. Personal sampling was carried out, as well as a full range of laboratory studies, including isolation, identification and comprehensive characterization of probiotic strains. The author carried out a comprehensive assessment of the technological and probiotic properties of the isolated microorganisms, developed functional consortia of starter cultures and an innovative gentle technology for the production of probiotic cottage cheese.

The author is a leading researcher at the IRN grant project AP26101141 «Development of starter cultures based on breeding of collection strains for the production of functional products enriched with postbiotics», implemented in 2025-2027 as part of the priority area «Sustainable development of the agro-industrial complex». This dissertation is a logical continuation and development of scientific research conducted within the framework of this project.

Approbation of the dissertation work.

The main provisions and results of the dissertation research were presented and discussed at the following scientific events: International Scientific and Practical Conference «Modern Challenges for Biotechnology, Veterinary Medicine and Medicine», Gvardeysky, 2020; International Youth Forum «Paradigms of Natural Science Education and Science for

Sustainable Development», Almaty, 2022; International Conference on Microbiology: Biology and Biotechnology of Microorganisms (ICMBB), 2021. In addition, the results of the study are reflected in scientific publications and recommended for implementation in the dairy industry and in farms engaged in the production of functional products from goat's milk.

In the second year of his doctoral studies, the author participated in the international seminar «Processing of agricultural and livestock products for the countries of the Belt and Road Initiative» (Harbin, China, June 6-26, 2018), where topical aspects of the dissertation research were presented and discussed.

The technology has been laboratory tested at the Institute of Animal Science and Veterinary Medicine of the Kazakh Agrotechnical Research University named after S. Seifullin and the positive results are confirmed by the test report. The developed technology can be recommended for use in the development and production of fermented dairy products based on goat's milk.

Scientific publications: Based on the materials of the dissertation, 14 scientific publications have been published, including: 2 articles in ranked international journals included in the Scopus database: Journal of Agriculture and Food Research (93 percentile, Q1); Studies in Systems, Decision and Control (60 percentile, Q3); 2 articles in journals recommended by the Committee for Quality Assurance in Science and Higher Education (CQASE); 4 articles in the materials of international scientific conferences and forums; 1 article in a scientific and technical journal. Additionally, the author is a co-author of 2 invention patents and 3 utility model patents.

The structure and scope of the thesis.

The dissertation consists of an introduction, four main chapters, a conclusion, a list of references, and appendices. The first chapter provides a literature review on the research topic. The second chapter describes the objects, materials, and research methods. The third chapter contains the results of the experimental work and their discussion. The final chapter summarizes the main findings and offers recommendations. The total volume of the dissertation is 150 pages, typed using a computer font. The work includes 38 tables and 33 figures. The reference list contains 204 sources.