

## **ABSTRACT**

for a dissertation submitted for the degree of Doctor of Philosophy (PhD) in the field of 6D010700 – “Biotechnology”

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### **"Microencapsulation of Probiotics in Polysaccharide Matrices for the Development of Functional Food Products from Mare's Milk"**

#### **General Characteristics of the Dissertation Work.**

The work is dedicated to the development of functional food products from dry mare's milk, enriched with probiotic microcapsules made of bacterial cellulose and pullulan with *Lactobacillus rhamnosus* GG ATCC 53103.

#### **Relevance of the Research Topic.**

Functional foods are the trend of the future, as modern biotechnology enables the processing of food raw materials into healthy foods, which meet consumer demands. Functional foods include foods which, in addition to the basic function of supplying the human body with nutrients, contain additional physiologically significant biologically active compounds for humans. Probiotic microorganisms occupy a prominent place among the food ingredients of functional nutrition.

The viability of probiotics, i.e. the number of living and active cells in a certain amount at the time of consumption is a key characteristic of the quality of these products, as it determines their effectiveness. It is therefore important to ensure high survival rates of probiotic bacteria during production, storage, and consumption of the product. One way to solve the problem of improving the viability of probiotics is to microencapsulate them. Microencapsulation is the process of locking biologically active substances into a thin shell of film-forming material. This technology avoids stress and ensures a high level of survival of microorganisms in such a "container".

The use of bacterial cellulose (BC) looks promising as a wall matrix. Along with its high adsorption capacity, it has a complete safety and biocompatibility, which allows its use in the food industry. In order to increase the stability of the probiotic immobilized in the BC matrix, an additional polysaccharide, Pullulan (PUL), can be included in it, which will be a prebiotic. Prebiotic is an undigested polysaccharide in the stomach and small intestine, which serves as selective food for saccharolytic microorganisms-probiotics. The presence of the prebiotic in the matrix is expected to stimulate the proliferation of the probiotic cells and to have a protective effect on the probiotic strain of *Lactobacillus rhamnosus* GG during its technological process and gastrointestinal conditions. Encapsulated probiotics are used in various fermented dairy products, such as yogurt, cheese, cream, frozen milk desserts, for biomass production, as well as in dry preparations.

Production and consumption of sour milk products is steadily expanding. However, their range is mainly represented by fermented products produced from cow's milk. Practically no such food matrix as mare's milk, which has a lot of beneficial properties, is getting used. It has high nutritional and biological value, as well as maximum digestibility. It should be noted that the protein composition, similar to human milk, has hypoallergenic properties and contains a rich amino acid structure. Mare's milk has therapeutic and preventive properties. It appears that,

mare's milk, due to the uniqueness of chemical composition and properties is a more preferred raw material for the production of consumer products. The traditional and so far the only product produced from mare's milk in Kazakhstan's industry is kumis. However, it contains alcohol, which limits the range of its consumers. Despite the fact that mare's milk is essentially Kazakh national "brand", the production of sour milk products from it is paid little attention.

The use of mare's milk for the production of functional foods based on it is limited to poor knowledge of milk processing technology, chemical stabilization and milk's technological properties. In addition, this is due to the seasonality and difficult accessibility. In this regard, the use of dried cow's milk in the production of acidic functional foods is an urgent issue.

In the dairy market, the most dynamic in terms of growth of consumption is yogurt - acidic milk product with a high mass percentage of dry matter. Existing technologies for the production of yogurt from cow's milk involve the use of only fermented starter cultures. The introduction of specially selected strains of probiotic bacteria into the formulation of this yogurt will attribute it to functional products, as it will have not only high nutritional, but also therapeutic properties.

Cottage cheese paste is a well-balanced and easily digestible milk product, containing high digestibility protein, which makes it highly valuable for children, elderly people, and athletes. It is an indispensable product of the full and healthy diet of modern man.

In this regard, the improvement of the technology and composition of probiotic products, which should be aimed at providing favorable conditions for bacteria during their production and storage, as well as during the passage of the stomach barrier, seems quite relevant.

**The goal of the work:** Creation of technology for obtaining products from mare's milk enriched with microencapsulated probiotic bacteria.

**Research objectives:**

- Modification of the nutrient medium for the producer pullulan A. pullulans C8 Production and preliminary preparation of biopolymers for microcapsulating probiotics;
- Selection and obtaining the optimal composition of probiotic microcapsules;
- Determination of the viability of microencapsulated probiotics in model gastrointestinal tract and temperature stress conditions;
- Development of technology for the production of yogurt and cheese paste based on dried mare's milk enriched with probiotic microorganisms in microencapsulated form;
- Determination of nutritional value, organoleptic parameters and biological safety of fermented milk products enriched with microcapsules;
- Production testing, development of a package of documents and obtaining state registration for functional fermented milk products.

**Objects of the study:** microcapsules with probiotic *Lactobacillus rhamnosus* GG, yogurt and cheese paste "Argymaq" based on dried mare's milk with microencapsulated probiotics.

**Subject of the study:** Determination of the morphological and structural

characteristics of microcapsules; determination of nutritional value, physical and chemical indicators and biological safety of yogurt and cheese paste enriched with microcapsules.

**Research methods:** Production of exopolysaccharides through surface and submerged cultivation of producers. Microencapsulation by extrusion. Investigation of microcapsule structure using scanning and transmission electron microscopy. Microcapsule strength tested with an Instron tensile tester. Resistance of microcapsules to stress factors assessed using a model gastrointestinal tract and high temperature conditions. Physico-chemical and organoleptic methods of product evaluation; determination of nutritional and biological value was carried out according to GOST standards.

**Scientific Novelty of the Research Results:**

- Optimized an economically viable nutrient medium based on molasses for the pullulan producer.

- Developed probiotic microcapsules containing *Lactobacillus rhamnosus* GG based on alginate, bacterial cellulose, and pullulan.

- Established that microencapsulation provides protection for LGG cells against stress factors (bile, gastric juice, and high temperature).

- Identified the optimal fermentation regime for the milk base and a method for yogurt stabilization.

**Theoretical Significance of the Research:**

Paths for economically efficient production of pullulan have been delineated. The composition of microcapsules was theoretically and experimentally justified. Potential uses for dry mare's milk were presented. Technological foundations for increasing the range of products based on biologically valuable dairy raw material - mare's milk - have been developed.

**Practical Value of the Research:**

The optimized nutrient medium based on sugar production waste – molasses, the strain *Aureobasidium pullulans* C-8, and its cultivation conditions can be used for scaling up pullulan production.

Technologies have been developed to produce a new yogurt and cottage cheese paste of the 'Argymak' series based on mare's milk with a microcapsulated probiotic. Experimental batches of yogurt and cottage cheese paste have been tested and released at the Meridian Group LLP industrial enterprise.

**Key Points for Defense:**

- The modified nutrient medium with molasses for cultivating the producer ensures a high level of pullulan biosynthesis and is economically efficient;

- The inclusion of LGG bacterial cells in microcapsules with a shell of BC and pullulan increases their viability in model gastrointestinal conditions;;

- Technological methods for correcting the physico-chemical properties of mare's milk enabled the production of dairy products (yogurt and cottage cheese paste) with a high dry matter content;

- The developed technology parameters and formulations for new mare's milk products with microcapsulated probiotics allow us to expand the range of dairy products.

### **Main research results and conclusions:**

1. The Chapek-Dox medium for the yeast-like fungus *A. pullulans* C8, a producer of pullulan, has been modified. The nutrient medium contains 10% molasses as a source of carbon and nitrogen. The maximum values of the economic coefficient are 37.83% at the level of accumulation of pullulan – 37.42 g/l.

2. Probiotic microcapsules were obtained by extrusion: Alginate - 2%, alginate 2% - BC 0.5%, alginate 2% - pullulan 2%/BC 0.5%. The immobilization efficiency of *L. rhamnosus* GG cells in Alg POOL/BC microcapsules was 81.2-89.1%, the titer of viable cells was 10<sup>10</sup> CFU/g. Microcapsules are spheres of 2500-3400 microns in size, white in color, with a smooth surface.

3. The survival rate of the encapsulated LGG strain in the model conditions of the gastrointestinal tract increased by 40-50% compared with free cells. Microcapsulation ensured the resistance of the strain to thermal inactivation. The viability of free cells was 37.5%, while the survival rate of LGG cells in microcapsules was 83.4%.

4. To obtain lactic acid products, SCM was reduced and enriched with 7.5% SOM. 1% starter culture YO-MIX 601 200 DCU was used for yogurt fermentation. During the study, the optimal fermentation mode was chosen – 5 hours at a temperature of 38-39 ° C. A pullulan of 1% has been added as a stabilizer. To obtain the curd mass, the sourdough "Cottage cheese" ("Useful batch") was selected. Microcapsules were added to fermented milk products in an amount of 3%. The optimal shelf life of products with probiotic microcapsules is 28 days.

5. The energy value of yogurt and cottage cheese paste is 58.8 and 125.3 kcal, respectively. The total amount of fatty acids in yogurt is 61.356%, and in cottage cheese paste – 61.649%; MNFA – 24.799% and 24.492%; PUFA – 3.448% and 5.028%. The amount of interchangeable amino acids in the new products was 32.31% in yogurt, and 34.06% in cottage cheese paste. The pool of essential amino acids is 69.64% and 67.57%. No antibiotics, pathogenic microorganisms, toxic elements, pesticides and radionuclides were found in the samples of yogurt and cottage cheese paste with microcapsulated probiotics.

6. Packages of regulatory and technical documentation for the production of yogurt and cottage cheese paste "Argymak" from mare's milk with microcapsulated probiotics have been prepared. The technology has been tested at the production facility of Meridian Group LLP. The products have passed state registration in the Eurasian Economic Union, the Committee for Sanitary and Epidemiological Control of the Ministry of Health of the Republic of Kazakhstan.

**Connection with the Plan of Main Scientific Works.** The dissertation was carried out within the framework of the project: AP05134040 "Microencapsulation of Probiotics in Polysaccharide Matrices for the Development of Functional Food Products Based on Mare's Milk".

#### **Validation of the Work.**

The materials of the dissertation were presented and discussed at the following conferences:

–IV International Farabi Readings. International Scientific Conference of Students and Young Scientists "Farabi World" (April 10-11, 2017, Almaty);

–V International Farabi Readings. International Scientific Conference of Students and Young Scientists "Farabi World" (April 10-11, 2018, Almaty);

–XV International Scientific and Practical Environmental Conference "Biological Species in the Structural-Functional Hierarchy of the Biosphere" (October 8-12, 2018, Belgorod, Russia);

–IEEE 9th International Conference on Nanomaterials: Applications and Properties, NAP 2019, Odessa, Ukraine;

–International Scientific Conference of Students and Young Scientists "Farabi World". (April 6-8, 2023, Almaty);

–"Women in Tech" Forum (March 15-16, 2023, Almaty).

### **Publications.**

Based on the dissertation, 15 works have been published, including 3 articles in peer-reviewed foreign scientific journals indexed in Web of Science or Scopus databases with a non-zero impact factor; 1 article in an international peer-reviewed scientific journal included in the first quartile according to JCR in the Web of Science Core Collection and having a percentile CiteScore in Scopus database of at least 35; 1 articles in international peer-reviewed scientific journals included in the second quartile according to JCR in the Web of Science Core Collection; 3 articles in republic journals listed by the Committee for Control in Education and Science of the Republic of Kazakhstan; 8 abstracts in the proceedings of international conferences; 2 patents for utility models and 2 Certificates of Product Registration issued by the Eurasian Economic Union, Committee of Sanitary and Epidemiological Control of the Ministry of Health of the Republic of Kazakhstan.

### **Volume and Structure of the Dissertation.**

The dissertation is presented on 135 pages of computer text and includes the following sections: symbols and abbreviations, introduction, literature review, materials and research methods, results and their discussion, conclusion, and a list of 255 references. It contains 32 tables, 27 figures, and 2 appendices.