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BIOTECHNOLOGICAL APPROACHES TO THE CREATION OF CHOCOLATE WHICH BASED ON MARES MILK WITH DIETETIC, PROPHYLACTIC PROPERTIES

ABSTRACT

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Relevance of the Research Topic

Traditional milk chocolate is produced using cow's milk and cocoa products (*Theobroma cacao L.*). While it possesses high sensory qualities, its nutritional value is often diminished due to an excess of saturated fats, sugars, and various additives. In this regard, the development of chocolate products with an improved nutritional profile, including through the incorporation of non-traditional types of dairy raw materials and biologically active components, has become increasingly relevant.

Among the promising ingredients for the creation of specialized foods for dietary and preventive nutrition, mare's milk is considered a viable option. Owing to its specific chemical composition—characterized by a predominance of albumin proteins, low levels of fat and protein, and high concentrations of lysozyme and lactoferrin—mare's milk exhibits pronounced functional properties. Its fatty acid profile is distinguished by a high content of polyunsaturated fatty acids of the omega-3 and omega-6 categories, particularly linoleic, α-linolenic, docosahexaenoic, and eicosapentaenoic acids, which contribute to reducing the risk of cardiovascular diseases. Furthermore, mare's milk is notable for its balanced composition of vitamins and minerals, containing elevated levels of B vitamins, ascorbic acid, tocopherol, cholecalciferol, magnesium, potassium, calcium, phosphorus, and other essential elements.

Thus, the incorporation of mare's milk into chocolate products represents a scientifically and practically substantiated direction in the development of a new generation of specialized foods.

Objects of the Study. The objects of the study are the composition of chocolate products based on mare's milk enriched with dry fucoidan extract, probiotic microorganisms, and resveratrol; the technological parameters of their production; the physicochemical and functional–technological properties; as well as their effects on the biological value and preventive efficacy of the final products.

Research Aim. The aim of the research is to develop biotechnological approaches for the creation of novel types of specialized chocolate based on mare's milk, possessing dietary and preventive properties.

Research Objectives

1. To develop a technology for producing powdered mare's milk that maximizes preservation of its nutritional and biological value, as well as shelf-life stability.

2. To provide a scientific rationale for the selection of raw materials and biologically active ingredients to be used in the development of dietary chocolate varieties.

3. To design and optimize formulations for novel types of chocolate, including the incorporation of functional ingredients.

4. To investigate the chemical composition, physicochemical, organoleptic, and consumer properties of the developed chocolate products.

5. To conduct an experimental assessment of the medico-biological properties of the chocolate using a toxic lead acetate poisoning model.

6. To develop regulatory and technical documentation and organize the production of pilot-industrial batches of the chocolate products.

7. To evaluate the clinical efficacy of specialized chocolate based on mare's milk enriched with resveratrol in individuals with stable angina and hypertension.

Research Methods

The assessment of the physicochemical and organoleptic properties, as well as the safety indicators of mare's milk under different conditions of pasteurization, drying, and storage, was carried out using the following methods:

– Sampling was performed in accordance with GOST 26809 and GOST 13928;

– Determination of moisture content — according to GOST 29246-91;

– Determination of solubility index — according to GOST 30305.4-95;

– Determination of fat content — according to GOST 29247-91;

– Determination of protein content — according to GOST 23327-98 (Kjeldahl method);

– Determination of acidity — according to GOST 30305.3-95;

– Determination of carbohydrate content — in accordance with R 4.1.1672-2003, Part III, Section 4, Method R 4.1.1672-03, Guidelines for Monitoring the Quality and Safety of Food Additives;

– Determination of ash content — according to GOST 15113.8-77, applicable to food concentrates and establishing methods for ash determination;

– Determination of microbiological parameters — according to GOST 9225-84; GOST 31659-2012; GOST 31747-2012; GOST 30347-2016; GOST 30705-2000; GOST 32031-2012, as well as methodologies approved in accordance with established procedures.

The vitamin composition of mare's milk was investigated in accordance with the following regulatory documents:

– Determination of vitamin C — according to GOST 30627.2-98 (colorimetric method);

– Determination of vitamin E — according to GOST 30627.3-98;

– Determination of vitamin PP — according to GOST 30627.4-98;

– General analysis of vitamin composition — according to GOST 30627.1-98.

During protein analysis, fractions were studied using Laemmli electrophoresis, with ImageJ software employed for processing the electrophoresis data. The peroxide value of milk fat was determined according to standard methodology. As a control, spray-dried mare's milk powder was used.

The content of trace elements was analyzed in accordance with GOST 26928-86.

Scientific Novelty of the Research. The scientific novelty of the study lies in the development and substantiation of an integrated biotechnological approach to the creation of specialized chocolate products based on mare's milk, ensuring balance in terms of key macro- and microelements, enrichment with highly effective functional ingredients, and the preservation of their biological activity during processing. The criterion of scientific novelty is reflected in the composition of the developed specialized chocolate products.

As functional ingredients, the following were utilized:

– Fucoidan — a sulfated polysaccharide derived from brown algae, exhibiting pronounced anti-inflammatory and general tonic effects;

– Probiotic microorganisms, which contribute to the normalization of gut microbiota and enhancement of the body's barrier functions;

– Resveratrol, a polyphenolic compound with targeted neuroprotective and cardioprotective properties.

The incorporation of mare's milk as a base with these bioactive components into the chocolate matrix represents a scientifically significant direction that has not previously been practically introduced within the field of food biotechnology and functional confectionery production.

Scope of Application. Confectionery industry enterprises.

Practical Significance of the Research. Technological solutions for the production of specialized chocolate based on mare's milk have been developed, which may be implemented in confectionery industry enterprises. The range of chocolate products created and proven to possess preventive efficacy can be applied within dietary and preventive nutrition programs designed for different population groups.

The regulatory and technical documentation developed for the novel types of chocolate products provides a legal foundation for their industrial-scale production and entry into the consumer market.

The results of the study enable the expansion of the raw material base of the confectionery industry through the utilization of mare's milk—a promising but underutilized type of dairy raw material—thus contributing to the resolution of import substitution challenges.

The identified patterns of interaction between functional ingredients and the components of the chocolate matrix may also be applied in the development of other types of specialized foods.

The research materials are of practical interest to specialists in food biotechnology, nutritionists, dietitians, food production technologists, as well as researchers engaged in the development of functional and specialized products.

Key Provisions Submitted for Defense

1. A technology for producing powdered mare's milk has been developed that maximizes preservation of its nutritional and biological value as well as shelf-life stability.

2. The feasibility of using mare's milk as a primary component in the development of specialized functional chocolate products has been scientifically substantiated, supported by a comprehensive analysis of its chemical composition and functional-technological properties.

3. The concept and formulation compositions of chocolate products based on mare's milk with defined properties and a modified nutrient profile have been developed and experimentally validated. These ensure the preservation of traditional organoleptic characteristics while simultaneously enhancing the biological value of the product.

4. The patterns of influence of functional ingredients (fucoidan, probiotic cultures, and resveratrol) on the physicochemical, rheological, and organoleptic parameters of the chocolate mass have been identified.

5. *In vivo* studies confirmed the preventive efficacy of specialized chocolate based on mare's milk enriched with resveratrol. A reliable positive effect was established on the functional state of major physiological systems and on the antioxidant status of laboratory animals exposed to lead acetate intoxication.

6. The clinical efficacy of dietary chocolate varieties based on mare's milk enriched with resveratrol was evaluated in individuals with stable angina and hypertension.

7. Regulatory and technical documentation for the novel types of specialized chocolate products has been developed and approved.

Author's Personal Contribution. The author's personal contribution encompasses the development of the research concept and methodology, the execution of the full cycle of laboratory and experimental studies, the analysis and interpretation of the obtained data, as well as the preparation of publications. All results presented in the dissertation were obtained by the author independently or with the author's direct participation. The scientific principles and conclusions of the dissertation are based on the author's original research.

Approbation of the Research. The main provisions and results of the dissertation were presented at the XXI International Scientific Conference "Challenges and Problems of Modern Science" (January 30–31, 2025, London, United Kingdom).

Publications. Based on the materials of the dissertation, 10 publications have been issued, including two articles in peer-reviewed journals indexed in the Scopus database, three articles in domestic journals recommended by the Science and Higher Education Quality Assurance Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan under Lists 1 and 2, and 5 articles published in the proceedings of international conferences held abroad and in the Republic of Kazakhstan.

Structure and Scope of the Dissertation. The dissertation comprises 126 pages of text and consists of an introduction, a review of the literature, materials and methods, results and discussion, a conclusion, and a list of 244 references. The main text includes 29 tables and 18 figures, while supplementary materials are presented in two appendix.

Conclusions of the Dissertation. In accordance with the aims and objectives formulated in the dissertation, a comprehensive analysis of modern literature and patent data was carried out to support the development of specialized confectionery products, in particular chocolate with targeted medico-biological properties.

Based on the chemical composition of mare's milk, a technology was developed for producing chocolate enriched with fucoidan, resveratrol, and dry cultures of lactobacilli. Optimal technological regimes were selected, and the organoleptic characteristics of various chocolate types were refined. The developed chocolate contains no added sugar; enrichment with sugar-containing ingredients was eliminated, while natural biologically active components were incorporated with specific preventive functions—normalization of intestinal microbiocenosis, enhancement of the body's defense mechanisms, and reinforcement of antioxidant capacity.

The Following Conclusions Were Drawn from the Research Results:

1. A technology for producing powdered mare's milk was developed, ensuring maximum preservation of its nutritional and biological value as well as extended shelf life.

2. The raw materials and biologically active ingredients to be used in the production of dietary chocolate varieties were scientifically substantiated.

3. Three formulations of novel chocolate products incorporating functional ingredients (fucoidan, resveratrol, and dry cultures of lacto- and bifidobacteria) were developed.

4. The chemical composition, physicochemical, organoleptic, and consumer properties of the developed chocolate products were analyzed.

5. An experimental evaluation of the medico-biological properties of the chocolate was conducted using a toxic lead acetate poisoning model.

6. Regulatory and technical documentation was developed, and the production of pilot-industrial batches of chocolate products was organized.

7. The clinical efficacy of specialized chocolate based on mare's milk enriched with resveratrol was evaluated in individuals with stable angina and hypertension.