ANNOTATION

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

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"The technology of producing biologically active peptides whey protein of mare's milk "

General characteristics of the work: Whey is a by-product of milk processing, and whey proteins serve as a source of both exchangeable and non-exchangeable amino acids, along with biologically active peptides. The biological properties of lactoferrin, a whey protein derived from mare's milk, encompass antioxidant, antimicrobial, chelating, and other qualities. These properties enhance its digestibility, attributed to the peptides formed through enzymatic hydrolysis in the gastrointestinal tract. The effectiveness of this process depends on the sequence of amino acids present in lactoferrin. The dissertation work is centered on the isolation of mare's milk whey protein lactoferrin and the determination of its biologically active peptides.

Relevance of the research topic:

Among all nutrients, protein plays the most important and unique role in the body's life. In terms of protein composition in domestic animals, mare's milk is the closest to mother's milk. The significant difference in composition from the milk of other animals is attributable to its high biological value and digestibility. Compared to the milk of other mammals, the proportion of whey protein in mare's milk is approximately 39%, surpassing that of cow's milk (18%). Consequently, it is classified as "albumin milk." The elevated whey protein content makes it particularly effective for nourishing children and the elderly. Whey protein serves as an additional source of arginine, histidine, tryptophan, leucine, as well as the essential amino acids phenylalanine and tyrosine in balanced proportions. Whey proteins can be classified as proteins utilized for the regeneration of liver proteins, the formation of hemoglobin, and blood plasma. The composition of mare's milk whey proteins includes β-lactoglobulin, α-lactalbumin, serum albumin, immunoglobulins, lactoferrin, and lysozyme. An important protein among these is lactoferrin, a globular protein present in milk, saliva, tears, and various other body fluids secreted by the body's epithelial mucosal cells. The amount of lactoferrin in mare's milk is several times higher (0.2-2 g/l) than in cow's milk (0.03-0.2 g/l) but is second only to mother's milk (1-7 g/l).

In recent years, heightened interest in lactoferrin is attributed to its multifunctional properties, including antibacterial, antioxidant, immunomodulatory, wound healing, antitumor, RNA, DNA, lactoperoxidase activity, and the ability to catalyze various biochemical reactions. These functions are geared towards maintaining the body's homeostasis. Furthermore, lactoferrin exhibits immunomodulatory effects by regulating free iron levels and binding to cellular receptors.

Proteins enter the body in their native form, initially appearing in the bloodstream as peptides or individual amino acids, whether essential or non-

essential. These are formed through the action of digestive enzymes from the gastrointestinal tract's microflora. The digestibility of the resulting peptides is closely tied to their amino acid composition. According to recent studies by Japanese scientists, peptides formed during proteolysis possess higher biological properties than native proteins. Consequently, the biological properties of lactoferrin may hinge on the constituent peptides it contains. Lactoferrin and its biologically active peptides contribute to improving immunity, maintaining normal blood pressure, and controlling inflammatory processes, among other benefits.

Exploring peptide complexes opens up avenues for creating new nutritional, medicinal, and prophylactic substances. In the food industry, peptides of varying lengths, such as di-, tri-, and polypeptides, can form an integral part of a strategy for rational human nutrition. Prolonged deficiencies in protein or specific amino acids in daily diets can lead to various diseases. Consequently, the study of the structural and functional properties of peptides necessitates the identification of biologically active peptides. Contemporary scientific research primarily focuses on preventing the development of various chronic diseases and utilizing peptide drugs to enhance the body's immunity. However, information regarding their preventive effects is often lacking. Therefore, there is significant interest in leveraging the biologically active properties of peptides to develop therapeutic, prophylactic substances, and food additives with diverse functional properties. These innovations are anticipated to play a crucial role in preventing and treating various diseases.

Peptides play a crucial role in protecting the body against the growth of pathogenic microflora. They stimulate the regeneration and renewal of the mucous membrane of the gastrointestinal tract and serve not only as a source of essential amino acids but also of minerals such as calcium, iron, zinc, etc. Peptides are highly sought after for their ability to bind mineral elements, enhancing their solubility and absorption through intestinal enterocytes. Additionally, peptides exhibit antioxidant and immunomodulatory properties, attributed to aromatic amino acids.

Addressing this urgent problem involves developing new and effective technologies for extracting peptides from whey protein, with a specific focus on identifying biologically active peptides. The results obtained contribute to enhancing our understanding of the fundamental relationship between nutrition and health. This is especially crucial during the therapeutic and prophylactic administration of peptides isolated from mare's milk whey protein.

Purpose of the study: Study of the biologically active properties of lactoferrin protein and its peptides from mare’s milk whey and development of isolation technology.

Research objectives:

1. Determination of physical and chemical parameters of mare’s milk and classification of whey proteins;
2. Isolation of lactoferrin protein from whey proteins and description of its amino acid sequence;
3. Study of the antioxidant, antimicrobial and metal-binding (chelating) properties of the lactoferrin protein;

4. Enzymatic hydrolysis of the lactoferrin protein and determination of the biological properties of peptides;

5. Development of a technological scheme for the production of lactoferrin and its peptides.

Objects of research: Mare's milk, lactoferrin, peptides.

Research methods: Biotechnological, biochemical, microbiological, molecular genetic, physicochemical and statistical methods were used in the work.

Scientific novelty of the research:

Whey, a by-product of dairy production, contains proteins and peptides with a high level of biological activity. Among these proteins, lactoferrin stands out for its antipathogenic and immunomodulatory properties. In a groundbreaking effort, lactoferrin was isolated from Kazakh mare's milk. The amino acid sequence of mare's milk lactoferrin was determined, and its biologically active properties (antioxidant, antimicrobial, chelating) were thoroughly studied. Active peptides derived from lactoferrin were extracted, and their antimicrobial and free iron ion binding effects were investigated. Through enzymatic treatment with trypsin, a total of 56 cationic, anionic, and neutral peptides were obtained, with 28 of them identified as peptides binding to iron ions.

As a result of the research work, a diagram of the technology for producing lactoferrin and its peptides is presented.

Theoretical significance of the work:

Whey protein and its biologically active peptides exhibit antioxidant activity, effectively cleansing the body of free radicals. Moreover, they play a crucial role in enhancing the absorption and bioavailability of mineral elements in the body. Peptides containing histidine, cysteine, serine, aspartic acid, and glutamic acid can bind divalent metal ions (such as calcium, iron, zinc, magnesium, etc.), forming soluble peptide-metal complexes. This process reduces the risk of mineral deficiency.

Additionally, peptides can serve as a natural source of ingredients for creating new functional foods that provide benefits to the body. Scientific research indicates that significant biologically active peptides can be derived from whey.

Practical significance of the work:

Taking into account the biological properties of mare's milk lactoferrin and its peptides determined in scientific work, the research results can be used to create physiologically effective drugs for medical, cosmetic and food purposes.

Based on the processing of mare's milk whey proteins, a technological scheme for the isolation of lactoferrin has been developed. Based on the results of the work, a patent of the Republic of Kazakhstan “Method for isolating and purifying lactoferrin from mare’s milk”, No. 6702 dated November 26, 2021, was received, as well as a copyright for milk drying. A certificate was received for entering data into the state register of rights to objects “Technology for the production of milk powder (technical instructions)”, No. 6859 dated 04/22/2021.

Based on the research results, methods for isolating pure proteins from whey and determining the purity of the isolated proteins have been improved. Protocols have been prepared for the determination of antimicrobial, antioxidant, and metal-

binding methods for lactoferrin and its peptides. Based on the rules and concepts of scientific research work, it is recommended to publish a methodological manual for students (undergraduates and undergraduates) in the disciplines “Food Biotechnology”, “Biochemistry”, “Microbiology”.

The results obtained have commercial potential.

Main provisions submitted for defense:

1. Physico-chemical parameters of mare's milk were determined and whey proteins were identified.
2. Lactoferrin protein was isolated from whey proteins and its amino acid sequence was characterized.
3. The antioxidant, antimicrobial and metal-binding (chelating) properties of the lactoferrin protein have been studied.
4. Enzymatic hydrolysis of the lactoferrin protein was carried out and the biological properties of the resulting peptides were determined.
5. A technological scheme for the production of lactoferrin protein and its peptides has been developed.

Author's personal contribution:

All experiments and results of the dissertation work were carried out with the personal participation of the dissertation candidate. The author of the work studied the literature data, determined the goals and objectives of the work, chose the object and concept of the study, and also conducted and planned experimental studies, statistical processing and analysis of the results, writing articles and patents.

Approbation of scientific work: The main provisions and results of the dissertation research were presented and discussed at the following international scientific conferences and symposia:

- International scientific conference “Farabi World” for students and young scientists (April 9-10, 2019, Almaty, Kazakhstan).
- The International Scientific conference of young scientists. “Fundamental research and innovations in molecular biology, biotechnology, and biochemistry” dedicated to the 80th anniversary of academician Murat Aitkhozhin (November 28-29, 2019, Almaty, Kazakhstan).
- 8th IDF International Symposium on sheep, goat, and other non-cow milk (November 4-6, 2020, Belgium).
- International scientific conference “Farabi World” for students and young scientists (April 6-8, 2021, Almaty, Kazakhstan).
- Polysac 2nd International Conference, Chelation of divalent metals and antioxidant activities of equine lactoferrine (December 11-13, 2021, Tunisia).
- The 3rd International Scientific and practical conference dedicated to the 85th anniversary of the Tashkent Pharmaceutical Institute “Modern pharmaceutics: actual problems and prospects” (November 2, 2022, Tashkent, Uzbekistan).

Publications. The results of the research work were published in 14 scientific papers, including in the journal LWT-Food Science and Technology (Q1), included in the Web of Science and Scopus database - 1 article, in scientific journals represented by the Committee for Quality Assurance in the Field of Science and Higher Education Education of the Ministry of Science and Higher Education of the

Republic of Kazakhstan - 3 articles, 8 theses were published in collections of international conferences and symposiums. In addition, 1 Patent and 1 Copyright Certificate have been prepared.

Structure of the dissertation. The dissertation is written on 114 pages of text and consists of symbols and abbreviations, normative references, introduction, literature review, materials and research methods, research results and their discussion, final sections, 189 references, 11 tables, 39 figures.