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7TH EUROPEAN MEETING HIGH PRESSURE TECHNOLOGY

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Supercritical method of obtaining lactoferrin from mare's and camel's milk

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1. Introduction

Lactoferrin (LF) is a highly valuable multifunctional protein from the transferrin family found in milk. The need for LF is manifested in newborns from the first hours of life. To meet the needs of the newborn in this protein, the mammary glands of the mother for several weeks secrete a rich substance of this protein, which is called colostrum. LF is one of the components of the immune system of the body, takes part in the system of nonspecific humoral immunity, regulates the functions of immunocompetent cells. To maintain the immune system at a good level, a person feels the need for this protein throughout his life. In addition to milk, LF is found in various secretory fluids: in saliva, tears and secretions of the nasal glands.

LF is used as a biologically active additive in food, and is part of the drugs used in the treatment and prevention of a number of diseases. The ability of LF to easily form complex compounds with iron ions Fe^{2+} and other transition metals, usually with a high positive charge (Si^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Co^{3+}, Cr^{3+}, Mn^{3+}, Al^{3+}, Ga^{3+}), underlie the biological function of the protein. LF has antifungal, antiviral, antibacterial, anti-inflammatory, anti-cancer, anti-parasitic, antioxidant and immunological effects. In addition, LF has a positive effect on the nervous system and bone tissue, is a major component of many drugs.

Taking into account the great importance of LF in human life, currently more than 20 plants have been launched for its production in the world. LF is recognized in the European Union as a safe protein for its use as dietary supplements in food products by the decision of the European Commission on food additives №258/97 of 22.11.2012 (published in the Official Journal of the European Union of 27.11.2012). Sources of raw materials for these plants are cow's milk and its whey. It should be noted that cow LF is 30% different from human and often causes an allergic reaction. An attempt was made to obtain human LF from the serum of genetically modified animals, for example, cows and goats, but for its industrial production it did not come due to the lack in most countries of the legal framework for the use of raw materials of transgenic animals. Another problem was that during industrial processing of whole milk, LF was obtained in non-activated form ("peptide drop") due to pasteurization of milk at a temperature of 62.5°C for 30 minutes or UHT treatment 134°C for a few seconds. It is known that heat treatment significantly affects the quality of products, lowering the content of vitamins, impairs the nutritional properties, appearance, color and taste.

2. Results and discussion

In this regard, especially promising for the industrial production of physiologically active form of LF can be supercritical fluid technology (SFT), where carbon dioxide is used as an extractant. Supercritical carbon dioxide ($scCO_2$) has several advantages compared with traditional solvents: it has low critical parameters (t=31,06°C; p=72,9 atm.), higher than liquids, penetrability, non-toxic, sterile and bacteriostatic, non-explosive, safe for the environment, does not produce wastewater and waste solvents. With low critical parameters, $scCO_2$ is an ideal solvent for working with labile biological objects. Extremely attractive is the use of $scCO_2$ for cold pasteurization or sterilization of liquid and solid objects, which is able to completely or partially replace the widely used in the industry heat treatment. Analysis of the literature data shows that in the treatment of $scCO_2$, the main parameters determining the survival of microorganisms are pressure, temperature, and processing time[1]. Has been proposed as another mechanism of inactivation of the microflora in the environment of $scCO_2$, which was to acidification of the environment, as well as lowering the pH inside the pathogenic cells due to the formation of carbonic acid.

Taking into account the above advantages of SFT, we have developed an innovative technology for obtaining highly purified biologically active LF from dry mare and camel milk. At the initial stage of processing of mare's or camel's milk, their degreasing was carried out by the method of traditional separation. Then the liquid milk was dried using freeze-drying. Considering that proteins in the environment of $scCO_2$ are insoluble, extraction of dry milk $scCO_2$ was carried out. At the same time, residual fats, as well as other bioorganic hydrophobic products, which were carried away with the flow of $scCO_2$, were extracted from the milk powder sample. Thus, the separation of the protein fraction of milk powder and liquid

containing hydrophobic products soluble in $scCO_2$ was carried out. Mare or camel milk powder treated with $scCO_2$ was then dissolved in distilled water with pH 3.7-4.7. At these pH values, the solution precipitated casein proteins, while LF in an acidic environment remained in a soluble state, which was then separated by filtration or centrifugation. LF, which is in a solution at pH 3.7-4.7, was sorbed on a strongly acidic gel cation exchanger KU-2 or macrocellular KU-23 in the H^+ form. Desorption of concomitant proteins and LF with cationite was carried out by sodium chloride changing the strength of the solution 0.1; 0.5; 1.0 M. Protein precipitation after desorption with different concentrations of sodium chloride was separated by filtration or centrifugation. The LF fraction was obtained after desorption from the cationite with a 1 M solution of sodium chloride. To obtain high purity LF, the protein was dissolved in 0.015 M phosphate buffer solution with pH 7.4 and eluted through a preparative column filled with Sephadex G-100 or G-150. The solution containing LF fraction was dried using freeze-drying. The above method was able to obtain biologically active LF purity of 95-98%, which was investigated for the presence of pathogenic microflora.
The basic physical and chemical characteristics of mare's milk LF obtained by the above-described technology were determined. The purity of the preparation and the molecular weight of LF from mare's milk were determined by electrophoretic method. As an eluent, 0.015 M phosphate buffer solution with pH 7.8 was used. As markers used proteins with known molecular mass: bovine albumin (MM 30 000); alcoholdehydrogenase from the liver of a horse (MM 84 000); lactoferrin from human milk (MM 80 000), cytochrome C (MM 35 000). The electrophoresis process was carried out at a potential of 200 V. As a result of the experiments, the molecular mass of LF from mare's milk was established, which was equal to 78 000 daltons. The titration method was used to determine the isoelectric point at which the precipitation of LF from mare's milk was observed – it turned out to be pI=7.8.

3. Conclusions

In carrying out the work, the following results were obtained: an innovative, environmentally safe technology was developed to produce mare and camel milk powder, as well as high-value LF protein from milk powder using the supercritical fluid technology method. The possibility of using supercritical carbon dioxide for cold sterilization of dry milk, ensuring the maintaining of the physiological activity of LF, is shown. The basic physicochemical properties of LF obtained using the new technology have been studied.

References

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