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SWEET WHEY AS A RAW MATERIAL FOR DIETARY SUPPLEMENTS OBTAINING WITH IMMUNOMODULATORY EFFECT

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Abstract. This article presents the results of the study of literary sources to prove the viability of the idea of using sweet whey to deep its fractionation, and to obtain biologically active proteins with immunomodulatory effect. We demonstrated methods for fractionation of milk whey (membrane and chromatographic), as well as the technological scheme of concentration of sweet whey. We introduced the composition of sweet whey and protein content of immunomodulatory action. Modern methods of processing whey, which include, basically, only the process of dehydration and concentration of whey and its use in the complete component composition, which limits its use for food purposes are shown. The necessity of processing of secondary resources in a catastrophic ecological situation on the planet and full use of the composite processing of raw materials for food purposes, and also shows properties of proteins immunomodulating actions which are part of the whey are grounded.

Keywords: immunomodulators, whey protein, sweet whey, lactoferrin, lactoperoxidase, chromatography.

ПІДСИРНА СИРОВАТКА ЯК СИРОВИНА ДЛЯ ОТРИМАННЯ ДІЄТИЧНИХ ДОБАВОК ІМУНОМОДУЛЮВАЛЬНОЇ ДІЇ

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Анотація. У статті представлено результати дослідження літературних джерел з метою доведення спроможності ідеї використання у ресторанному господарстві молочної сироватки для глибокого її фракціонування, і отримання біологічно активних білків імуномодулювальної дії. Висвітлено способи фракціонування молочної сироватки (мембранні та хроматографічні) та наведено технологічну схему концентрації молочної сироватки. Представлено склад молочної сироватки та вміст білків імуномоделювальної дії. Наведено сучасні методи переробки молочної сироватки, які передбачають, в основному, тільки процеси зневоднення та концентрування сироватки і використання її в повному компонентному складі, що обмежує її застосування в харчових цілях. Обґрунтовано необхідність переробки вторинних ресурсів у зв'язку з катастрофічною екологічною ситуацією на планеті та повного використання складових переробки сировини на харчові цілі, а також показані властивості білків імуномоделюючої дії, які входять до складу молочної сироватки.

Ключові слова: імуномодулятори, сироваткові білки, молочна сироватка, лактоферин, лактопероксидаза, хроматографія.

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Introduction

Modern economic conditions contribute to the emerging role of technology oriented to the use or processing of secondary raw materials of different origin. This approach is driven by the need to solve environmental problems and improve the economic performance of primary production by recycling and producing additional competitive products. One of the basic salvage of food production is the sweet whey, which is formed during the processing of milk into protein-fat products (curd cheese, cheese, casein). Every year, more than 130 million tons of sweet whey is produced in the world. Ecological and economic calculations show that 1 ton of sweet whey can cause environmental damage the same as 100 m^3 of utility sewage. In Ukraine, less than 50 % of sweet whey is being processed, so the problem of its rational use is particularly acute. Use of vital sweet whey in kind is limited by the terms of its storage. The easiest way of sweet whey processing is drying. A more promising approach is the fractionation of sweet whey to obtain whey cream, whey protein concentrate, milk sugar. However, such technologies are usually aimed at the partial processing of secondary raw milk. In recent years, the greatest interest is caused by the deep processing of sweet whey, which provides for the extracting of its individual components and obtaining their derivatives. Such products may become widely used in the food, medical and pharmaceutical industry.

The most valuable components of sweet whey are immunoglobulins, lactoferrin and lactoperoxidase, although being presented in small quantities, but having protective, antimicrobial, antioxidant, immunomodulatory and regulatory functions. These compounds can be used as a basis for obtaining therapeutic and prophy-

lactic products. In sweet whey, β-lacto globulin and α-lacto albumin containing optimally balanced set of amino acids are present in the largest quantities. Due to the high biological value, these proteins can be used to produce baby foods and diet food.

Thus, it is expedient to develop technologies for integrated processing of sweet whey, which aims at the extraction of valuable protein components and obtaining related products with important biological functions.

Problem statement

The problem lies in the inefficient and incomplete use of food, biological and psychological resources (components) of sweet whey in food technologies due to its unsatisfactory organoleptic properties. The solution to this problem is connected with the great energy consumption of the proposed technologies and their complicated instrumentation, which is not inherent to the enterprises of the restaurant business.

To solve this problem, it is necessary to consider the chemical composition of sweet whey and to analyze the possibility of its fractionation into its components in order to extract therefrom minor bioactive substances of protein origin – lactoferrin and lactoperoxidase with specific physiological activity, and their subsequent use in food technologies. The embodiment of the proposed technology can be achieved through the organization of restaurant business complex enterprises with procuring storage.

Literary review

Sweet whey is obtained during the process of hard cheese, curd cheese and casein production. Depending on the type of targeted product, there are curd whey, cheese whey and casein whey. About 50% of milk dry substances (DS) are transformed into sweet whey. Composition and properties of sweet whey depend on the type of target product and its technology characteristics (Table 1). The most valuable component of whey are proteins. The content of protein compounds in sweet whey ranges from 0.5 to 1.0 %, depending on the method of coagulation of milk proteins during target product production. Sweet whey is a source of complete proteins, which do not contain limited amino acids. The content of amino acids-antioxidants (methionine, cystine) in the whey proteins is almost 1.5 times higher than in skim milk. Whey protein is a valuable source of arginine, histidine, methionine, tryptophan and leucine; they are used by the human body for the structural exchange processes, mainly for liver proteins regeneration, as well as formation of hemoglobin and blood plasma [1].

After separation of fat and casein, from 0.5 to 0.8 % protein (from 15 to 22 % of all milk proteins) remain in milk. The main whey proteins are β-lacto globulin, α-lacto albumin, blood serum albumin, immunoglobulins and components of proteose-peptone fraction. There are trace amounts of proteins which, along with immunoglobulins, substantially define the immune status of the animal organism (lactoferrin and lactoperoxidase).

Table 1– Composition of sweet whey

Indicator	Sweet whey		
	Cheese whey	Curd whey	Casein whey
DS content, % including	4.5 – 7,2	4.2 – 7.4	4,5 – 7,5
lactose	3,9 – 4,9	3,2 – 5,1	3,5 – 5,2
nitrogenous substances	0,5 – 1,1	0,5 – 1,4	0,5 – 1,5
mineral substances	0,3 – 0,8	0,5 – 0,8	0,3 – 0,9
milk fat	0,2 – 0,5	0,05 – 0,4	0,02 – 0,1
titratable acidity, T^0	15 – 25	50 – 85	50 – 20

The following methods of whey processing are known currently: complete utilization of dry substances and separate use of the components [2]. Sweet whey has long been used in its natural form as an ingredient of bakery products, dietic nutrition drinks for elderly people, desserts and ice cream, as well as for the preparation of nutrient media, animal feed and fertilizers, detergents, cosmetics and health baths. However, such use of whey is not practiced because of rapid deterioration, large volumes of production, the imbalance in the basic composition of nutrients and low organoleptic indicators [3].

The most promising area of complete utilization of whey dry substances is based on dehydration by vacuum evaporation and drying. It enables to obtain condensed and dry whey concentrates, dry demineralized whey, dry delactosed whey, dry whey with filler ingredients, blocked whey (intermediate moisture foods), granular whey [4]. Separate use of whey components allows to obtain a mixture of individual components: cream cheese, casein-albumin mixture, whey protein concentrates, lactose, protein concentrates with polysaccharides (pectin, chitosan), salts mineralizates [5-7]. One of the traditional areas of whey processing abroad is the production of milk sugar [2].

Another area of separate use of whey components is a deep processing: isomerization of lactose into lactulose, obtaining galactose, lactose urea synthesis, improving the production of alcohol and organic acids, extraction of mineral substances and whey proteins, enzymatic treatment of whey and its components.

In recent years, it is the deep processing of sweet whey that is of greatest interest, the purpose of which is to expand the use of the obtained products in the food, medical, pharmaceutical industries, in the production of baby foods and diet food, in therapeutic practice [8].

One of the important components of whey protein, which can be obtained by the methods of fractionation, is lactoferrin. Lactoferrin is a multifunctional protein of transferrin family, being in charge of iron transfer to

the cells and controlling the level of free iron in the blood and in external secretions. Lactoferrin (LF) molecule is comprised of a single polypeptide chain containing 692 aminoacid residues folded into two homologous globular lobes (N-(amino) and C-(carboxy) particles), with terminal regions connected by a short α-helix. Each domain has one iron binding site and one site of glycolysis. The degree of glycosylation can vary, so the molecular weight of the protein on different data ranges from 76 to 80 kDa. Each molecule of lactoferrin binds two Fe^{+} ions over bicarbonate ions, forming a reddish complex:

$$2\ Fe^{3+} + 2HCO_3^- + LF(H_3)^2 \rightarrow LFFe_2(HCO_3)_2 + 6H^+$$

Under certain conditions, lactoferrin is capable of binding also Cu^{2+}, Zn^{2+}, Cr^{3+}, Co^{3+}, Mn^{2+}, Cd^{2+}, Ni^{2+}. Thus, lactoferrin molecule exists in two forms. Holo-lactoferrin – a closed, stable form, relatively rigid and resistant to proteinase action, formed with metal binding. Apo-lactoferrin – an open form, flexible and more sensitive to proteinase, in the absence of metal. In both forms, most of the surface of the lactoferrin remains the same, but the fact of binding iron ions to this protein alters its isoelectric point of pH 8.0 to pH 8.5 by the simultaneous addition of negatively charged bicarbonate ions [9]. It is known that lactoferrin affinity to iron compared with transfer – 100 °C for 5 minutes, which can be used in pasteurization. Lactoferrin forms highly stereo-specific dimers at neutral pH in solutions [10].

Lactoferrin is present in milk, saliva, tears, pancreatic juice, bronchial, gastro-intestinal and genital secretions, in blood serum and leucocytes. However, its largest amount was found in colostrum (6.7 – 7.0 mg/cm^3), in human milk (2.6 mg/cm^3), in mature milk (up to 1.0 mg/cm^3). Lactoferrin content in colostrum of cows is also high (5 mg/cm^3), there is about 0.2 mg/sm^3 of this protein in usual cow milk and 15 – 50 mg/cm^3 in sweet whey [11]. It is known that less than 10 % of lactoferrin in milk is saturated with iron, that is most part of it is located in Apo-form. Lactoferrin is synthesized by glandular epithelial cells and neutrophils [12].

Lactoferrin possesses a unique set of biological properties of different nature. Since lactoferrin is a transferrin protein, it not only regulates the concentration of iron ions in the blood and secretions, but also has a strong antimicrobial and antiviral effect. Lactoferrin is involved in protective reactions of the human body and regulates the functions of immune cells. It was found that lactoferrin hydrolyzes RNA.

The most studied is the mechanism of antibacterial activity of human and bovine lactoferrin *in vivo* and *in vitro* on a broad spectrum of Gram-positive and Gram-negative bacteria. Lactoferrin binds iron and other metal ions of variable valency from surface structures of microorganisms' membranes of and deprives them of vital elements, included in respiratory chain cytochromes, catalases, peroxidases and superoxide dismutases, moreover, reduces their resistance to the toxic effect of chemical reactive oxygen derivatives. Lactoferrin binds to lipopolysaccharide of bacterial walls which affects the membrane permeability and results in the cell lysis. Its binding to the bacteria wall is associated with lactoferricin – peptide, which is located at the N-lobe of lactoferrin molecule and is produced by *in vitro* cleavage of protein with protease. Lactoferrin acts on a wide range of human and animal viruses. Currently, there has been studied protein activity against herpes simplex virus 1 and 2, cytomegalovirus, HIV, hepatitis C virus. The main mechanism of lactoferrin antiviral activity is its compound with glycosaminoglycans and lipoproteins of eukaryotic cells' membranes, which prevent the penetration of virus particles. Thus, lactoferricin peptide providing basic lactoferrin antimicrobial properties, practically does not show antiviral activity. In most cases, the Apo-form shows much greater antiviral effect than Holo-form [13].

Lactoferrin and lactoferricin show antifungal activity against human pathogenic fungal diseases. Lactoferrin inhibits the growth of Candida, Aspergillus fumigatus, Trichophyton mentagrophytes. The fungicidal properties are due to the direct interaction of the protein with a pathogen, but not to the ability to bind free iron. For example, the action of lactoferrin on Candid albicans *in vitro* leads to changes in membrane potential and oxidation of cytoplasm of Candida cells, which means the direct or indirect interaction of lactoferrin with the plasma membrane [14]. Lactoferrin also has an antioxidant effect. This is primarily due to the fact that it binds the iron, which is a strong oxidant. Lactoferrin prevents lipid peroxidation of the cell membrane. Thus, it prevents the cell from the distortions of membrane and vital processes, as the cell receives nutrients and communicates with other cells through membrane. Lactoferrin also plays an important role in the activation of the immune response. Thus, lactoferrin stimulates germ-eating cells (macrophages) and regulates the activity of natural killer cells which destroy harmful bacteria and tumor cells [15].

Unique antibacterial, antiviral, antifungal, immunomodulatory, antioxidant, detoxifying and anticarcinogenic properties of this natural iron-binding glycoprotein make promising its use as an active basis of pharmaceutical broad-spectrum drugs, food additives, therapeutic and prophylactic products.

Research, that is widely held in the different countries of the world, have shown significant therapeutic potential of bovine lactoferrin: maintenance of iron homeostasis, treatment of iron-deficiency anaemia and infections, preventing development and metastasis of tumors, preventing neonatal sepsis, an antioxidant effect. Lactoferrin helps to reduce the RNA titer of hepatitis C virus in the blood of the patient, and can be used in the treatment of chronic hepatitis C [16].

Concentrate of whey proteins, obtained by the method of ultrafiltration

Sweet whey, as well as milk, presents more than 250 substances and contains about 100,000 molecular structures that are in a soluble (nanoscale) and colloid-dispersed (clusters) states, as well as in the form of a suspension (casein dust) and emulsion (milk fat).

Nutritional value of sweet whey is characterized by a complete set of food products: high purity (harmlessness), sufficient food energy value, good digestibility, the optimal ratio of nutrients, biological and physiological full value. In terms of its organoleptic qualities, cheese whey can be categorized as satisfactory (specific flavour).

In terms of its food energy value, sweet whey corresponds to the whole milk (WM).

Preparation of individual proteins from sweet cheese whey and whey protein concentrates (WPC) is based on the difference in molecular weight, isoelectric point and solubility, for which methods of membrane fractionation and chromatography are used.

To obtain whey protein concentrates, membrane methods of raw materials processing are used, which provide the native state of protein concentrates in the liquid or dry form that preserves their biological activity. In accordance with [17] we propose such a technological process of obtaining WPC-UF (concentrate of whey proteins, obtained by the method of ultrafiltration) with varying concentrations of proteins (fig. 1).

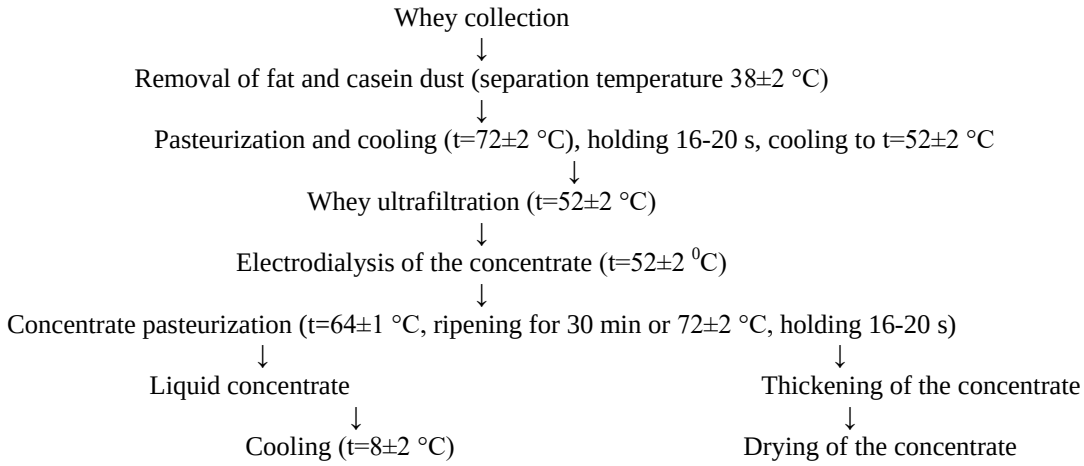

Fig. 1. Structure scheme of technology of obtaining CWP-UF

After membrane processing of the whey, scientists propose to use the technology of obtaining immunomodulatory proteins through chromatographic techniques.

During fractionation of proteins by ion-exchange chromatography, great attention is paid to the choice of ion exchanger (matrix origin and ion exchanger capacity) and buffer solution in which the proteins sorption is carried out (pH volume and ionic strength, buffer origin and buffer capacity). In the chromatography of proteins, synthetic ion-exchange resins based on polystyrene are practically not applied. This is due, firstly, to a high content of crosslinking, which render such materials substantially impermeable to proteins, and, secondly, to the sorption of proteins, sometimes irreversible, on the hydrophobic surface of polystyrene. Most commonly used are ion exchangers based on cellulose, dextran and agarose. These are the basis for ion exchangers with different functional groups, such as carboxymethyl (CM-), diethylaminoethyl (DEAE-), sulfoethyl (SE-), sulfopropyl (SP-). The most widely used ion exchangers for anionic proteins sorption are (DEAE) – cellulose and its analogues. The best for cationic proteins distribution are ion exchangers with carboxymethyl functional groups as: Sepabeads FP-CM13, CM-Sephadex C-50, CM-Sepharose-FF, Hiprep 10/16 CM FF, CM-Toyopearlpak 650 [18].

In the studies [19-20], there was proposed to split off lactoferrin and lactoperoxidase from cheese whey, previously filtered through a membrane with 1.4 mkm pore size to remove microorganisms, fat residues and casein. Further, the whey was passed through the ion exchanger SP Sepharose Big Beads with 100 – 300 mkm pore size, balanced by 0.025 M phosphate buffer pH 6.5 at a rate of 60 – 90 volumes per hour. Elution of lactoferrin and lactoperoxidase was carried out by NaCl solution in a phosphate buffer at a salt concentration of 0.2 – 0.5 M and 0.6 – 1.0 M respectively. Lactoferrin and lactoperoxidase fractions, desalted by gel filtration, were freeze-dehydrated.

From literary sources, it is known that lactoferrin and lactoperoxidase are cationic proteins, unlike immunoglobulins. Isoelectric points of lactoferrin and lactoperoxidase correspond to pH 8.0 – 8.8 and 8.6 – 9.6 respectively. Literary analysis shows that the most appropriate way for splitting off cationic and anionic protein is ion-exchange chromatography. To split off cationic proteins, it is preferable to apply ion exchangers with carboxymethyl groups [21]. This study proposes to conduct the process of lactoferrin and lactop-

eroxidase sorption in dynamic conditions; dilution of purified concentrate with phosphate buffer in 10 times to lactoferrin content of 0.02 g/dm^3 and lactoperoxidase content of 188 u/dm^3 that allows to achieve more than 90 % protein sorption. For cationic proteins desorption, 0.05 – 1.0 M sodium chloride solutions are most commonly used. Desorption process was conducted with the previous washing of chromatographic column with phosphate buffer. As a result of the received data, there was defined that lactoperoxidase elutes better with 0.2 M sodium chloride solution, and lactoferrin – with 0.5 M sodium chloride solution.

For cleaning obtained concentrates from salt, diafiltration method is used as the quickest and simple in instrumentation. For this purpose, it is enough to hold a 6-fold diafiltration. As a result of this process, lactoferrin specimen with concentration of 3.7 g/dm^3 can be obtained.

Received purified concentrates of lactoferrin (3.7 g/dm^3) and lactoperoxidase (34000 u/dm^3) can be directed to drying without additional processing. Output of lactoferrin and lactoperoxidase, relative to the original content in sweet whey, is 93 % and 90 % respectively [21].

Conclusion

Therefore, the information studies can lead us to the conclusion that sweet whey is an excellent source of biologically active substances of protein origin with specific properties.

There are plenty of technologies for the production of substances from the whey in the native form, but there are no technologies of whey complex processing using all its components in the culinary production.

Application of different methods of sweet whey fractionation and the inclusion of its components in culinary products in the restaurant business enterprises will only be possible if the restaurant complexes, comprising procuring storage where you can provide and implement the technologies of obtaining biologically active substances of protein nature.

Currently in Ukraine, we can increasingly observe the design of restaurant and hotel complexes, which provide their own culinary and beverages production, such as beer production to feed it to the pub.

It should also be noted the economic feasibility of using sweet whey for the production of biologically active substances, because this raw material is cheap and is produced in Ukraine in large enough quantities in the cheese-making enterprises. Thus, the use of such raw material will contribute not only to obtain additional revenue from the sale of new products, biologically valuable and important for health, but also to solve such important problems of all food enterprises as the complex processing of raw materials together with directly related problem of environment protection.

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ПОДСЫРНАЯ СЫВОРОТКА КАК СЫРЬЕ ДЛЯ ПОЛУЧЕНИЯ ДИЕТИЧЕСКИХ ДОБАВОК ИММУНОМОДУЛИРУЮЩЕГО ДЕЙСТВИЯ

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Аннотация. В статье представлены результаты исследования литературных источников с целью доказать состоятельность идеи использования молочной сыворотки для глубокого ее фракционирования, и получения биологически активных белков иммуномодуляторного действия. Показано способы фракционирования молочной сыворотки (мембранные и хроматографические), а также технологическую схему концентрирования молочной сыворотки. Приведен состав молочной сыворотки и содержание в ней белков иммуномодуляторного действия. Представлены современные методы переработки молочной сыворотки, которые, в основном, предусматривают только процессы обезвоживания и концентрирования сыворотки и исспользование в полном компонентном составе, что ограничивает ее применение в пищевых целях. Обоснована необходимость переработки вторичных ресурсов в связи с катастрафической экологической ситуацией на планетне и полного исспользования составных переработки сырья на пищевые цели, а также показаны свойства белков иммуномодулирующего действия, которые входять в состав молочной сыворотки.

Ключевые слова: иммуномодуляторы, сывороточные белки, молочная сыворотка, лактоферрин, лактопероксидаза, хроматография.

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