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Satbayev University

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ИЗВЕСТИЯ

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РЕСПУБЛИКИ КАЗАХСТАН
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HYDROLYTIC REGULATION OF COMPONENT PROPERTIES

Abstract. The article provides an assessment of the dairy farming need in the Russian Federation and the Republic of Belarus in calves feed. The main global trends aimed at providing young animals with high-quality food means are considered.

Various variants of directed hydrolysis of calf milk replacer (CMR) protein components intended for feeding young animals in the first months of life are analyzed.

The possibilities of reducing the soy proteins antigenic activity, which are widely used at present in the CMR formulations for feeding young farm animals, are discussed.

The results of experimental work and patents are presented, which describe the most widely used approaches to the production of enzymatic hydrolysates of proteins with desired properties, as well as the assessment of their biological activity and immunochemical properties. The issues of using various enzyme preparations of bacterial, fungal and animal origin for hydrolysis of colostrum proteins and plant sources of protein raw materials for the CMR production are considered.

Key words: CMR, soy proteins, hydrolysis methods, anti-nutritional substances, antigenicity, immunoglobulins, bacterial proteases.

Introduction. Optimization of the dairy herd development is largely determined by the cow's lactation duration, the availability of a forage base that provides balanced nutrition for animals of different age categories. In particular, in order to increase the commercial milk production, an annual replenishment of young animals in a dairy herd is required at the level of about 40 heads per 100 cows, and one calf needs to be fed balanced nutritional components equivalent to 300-350 liters of milk. Based on this and taking into account the total number of cows, the milk needs for feeding calves in the Russian Federation is 1.3 million tons, and in the Republic of Belarus - 0.4 million tons [1,2].

The determining factors for effective calves rearing are their health state, as well as the weight and age of the first calving, which largely depends on the colostrum and feeds quality in the first three months of life [3].

Soy proteins characteristics for calf milk replacer (CMR). In order to increase the milk marketability and the economy of agricultural production, calf milk replacers (CMR) are used for feeding young animals. Their release is carried out using a variety of technologies [4], which have their own advantages and disadvantages. The desire to improve the balance and nutritional value of CMR is the subject of ongoing research in this area, especially in the search for new sources of raw materials and biotechnological methods of their directed transformation. The development of research and theoretical generalizations in the field of new competitive feed types for young agricultural animals is relevant, including by obtaining products of higher quality, as well as the selection and implementation of optimal for this dehydration technology methods, resource and energy conservation [5,6].

In particular, to achieve the above goals and expand the range of functional properties, a promising direction is the use of specially treated soybean seeds. When creating and optimizing the composition of such specialized products, certain advantages can be achieved due to biotechnological transformation of components based on their fermentation [7]. This is due to the fact that this process, as is known [8,9], causes the proteins degradation with the formation of peptides with different molecular weights. This approach can be rather effective method for modifying the CMR protein components in the desired direction.

In addition, it should be borne in mind that a significant factor affecting the CMR protein components quality are their antigenic properties, the level of which can also be regulated by enzymatic treatment [10].

Soybean antigenic proteins include glycinin and β-conglycinin. When feeding young agricultural animals with diets containing soy protein, a small part of it is not digested and through the gaps between the terminal epithelial cells falls into the lymph and blood. These molecules, possessing significant antigenic activity, stimulate the immune system, leading to the occurrence of specific antigen-antibody reactions and delayed hypersensitivity mediated by T-lymphoid cells. Therefore, at present, the goal of research is to develop methods for reducing the soy proteins immunoreactivity. Several strategies have been developed to prevent food allergies, including feed handling and plant breeding. These researches indicate, inter alia, that certain types of immunomodulators, such as vitamin C and linoleic acid, can specifically block the IgE-mediated anaphylaxis, which may provide new insights into effective soy allergy prevention, and possibly other many food allergies [10].

In work [11], the antigenic soybean seeds activity subjected to heat treatment by boiling for 30 and 60 minutes was studied. It has been shown that heat treatment reduces the soy proteins ability to bind IgE, which contributes to an increase in the end products safety.

In work [12], the content of glycine protein in industrial soy products, which is an immunodominant soy allergen, was studied. Soy flour gave the highest protein antigenicity (32 mg/g of extracted protein), and soy protein isolate and concentrate, respectively, 29 mg/g of extracted protein and 24 mg/g of extracted protein. Among soybean consumer products, soy milk showed the greatest antigenicity, varying from 7 to 23 mg/g of extracted protein, followed by pace (8 mg/g of extracted protein), soy infant formula (3.4 mg/g of extracted protein) and soy "cheese products" (0.50 mg/g of extracted protein). In consumer products such as Korean miso, soy sauce, soy nuts, "soy meat" hamburgers, textured soy protein, antigenicity (detection limit = 0.45 ng) was not found [12].

It is significant that soy products subjected to severe heat treatment under high pressure (texturates) and fermented soy products (soy sauce) show a minimum level of antigenicity.

In connection with the above, these studies confirm that the use of protein hydrolysis technologies is of significant interest in the creation of new CMR types.

In general, the destruction of biopolymers by enzymes is one of the fundamental phenomena in nature, freeing structural elements for the new biopolymer molecules synthesis. Hydrolysates are widely used in food production, including as biologically active food additives, special food and feed products and ingredients. Hydrolysis technologies make it possible to more efficiently use of waste products that process agricultural raw materials.

In the patent literature for the soybean enzymatic hydrolysates production, various enzymes and technological methods are proposed that provide products with the specified properties.

There is a known method for intensifying mass transfer processes for obtaining a protein hydrolyzate from soy flour, which consists in preparing a suspension with a dry matter content of 6-12%, suspension hydrolysis with a mushroom protease to a hydrolysis degree of 20-40% and subsequent hydrolysis with papain to hydrolysis degree of 30- 45%. The total duration of the hydrolysis is 2.5-4 hours. The resulting hydrolyzate is separated and dried to a moisture content of 9.4%, the product contains 65-68% crude protein [13].

To obtain a protein hydrolyzate from soy flour, a method is proposed [14], processing flour from cereals, legumes and oilseeds, or a mixture thereof for enzymatic treatment when obtaining protein hydrolysates, including grinding the specified flour to a particle size of at least one dimension less than 50 microns, with provided that at least 50% of these particles are less than 25 microns in size.

The known method [15] of soy product obtaining, based on the hydrolysis of soybean raw materials suspension by pectinase and cellulase for 5 hours. The resulting product contains 22% of sugars, while their content in the starting material is 0,77%.

The patent [16] describes the preparation of a milk replacer from vegetable protein and carbohydrate sources using an enzyme having multiple carbohydrase activities.

To obtain protein-carbohydrate solutions, mixtures of enzyme preparations with both proteolytic and cellulase or amylase activities are also used. Thus, the patent [17] proposes a method for obtaining soluble (not forming colloidal masses) proteins fractions and carbohydrates by sequential hydrolysis of proteins and carbohydrates.

According to the method [18], the components are extracted from a pre-barothermally treated raw material with a developed surface area, which facilitates the enzyme's access to the attack sites. In the described process, the initial substrate concentration during enzymatic hydrolysis can reach 25-30%, which leads to the formation of more concentrated protein and carbohydrate nature products.

The characteristic methods described above, naturally, have their advantages and disadvantages. At the same time, the variety of approaches in order to ensure effective soybean components biotransformation by fermentation indicates that the achievement of truly optimal solutions in this area is still ahead and requires further research.

In the literature, the regularities and properties of soy protein hydrolysates are investigated, aimed in particular at reducing the soy proteins antigenic activity.

In a review article [8], the physicochemical, organoleptic, immunochemical (residual antigenicity) characteristics of proteins enzymatic hydrolysates from various sources are given. It is argued that the necessary decrease in the hydrolysate's antigenicity used in the hypoallergenic food products composition for medicinal purposes is achieved at values not higher than 10^{-5} relative to the original protein antigenicity, which requires a membrane ultrafiltration stage.

In the work [19], enzymatic hydrolysis of defatted soy flour was carried out with three different proteases (Flavourzyme 1000 L, Novozym FM 2.0 L, and Alcalase 2.4 L FG). The highest hydrolysis degree (DH 39.5) was observed with the Flavourzyme enzyme. Soy flour hydrolysates have been used to study the functional proteins properties (foaming and gel formation). Hydrolysis with Flavourzyme enzyme showed the best foaming and gelling properties of soy proteins. It was also found that the enzymes used degraded both conglycinin and soybean glycinin. During fermentation with Alcalase and Novozym proteases, the highest concentration of the following free amino acids was observed in the hydrolyzate - histidine (30%), leucine (24%) and tyrosine (19%), and during Flavourzyme hydrolysis - arginine (22,1%), leucine (10,6%) and phenylalanine (12,9%) [20].

Of interest are studies aimed at studying the immunomodulatory protein hydrolysates properties.

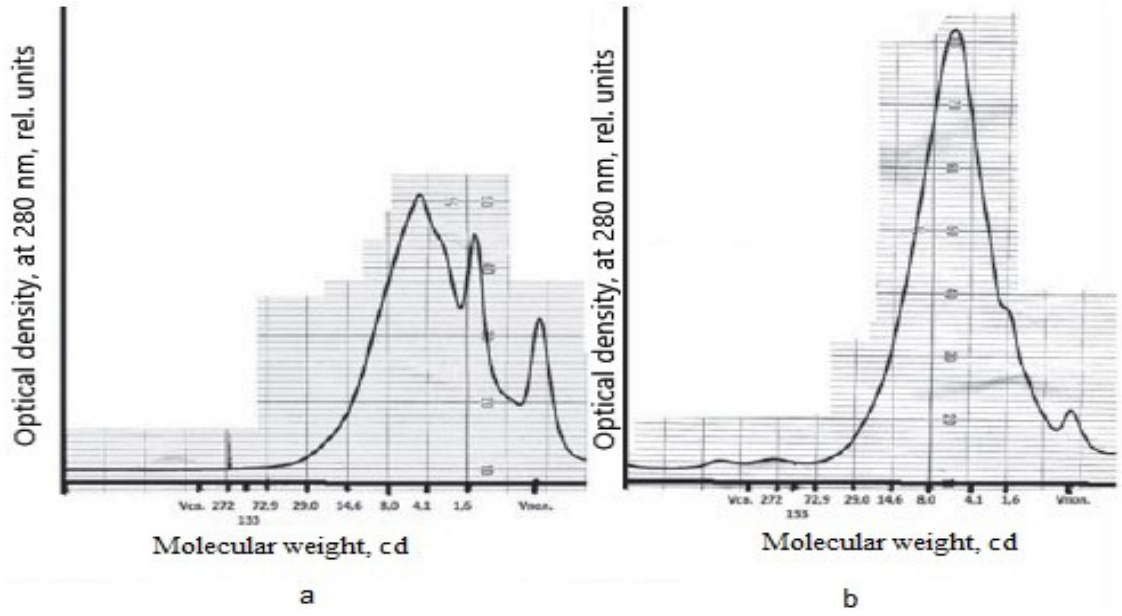
In particular, in the study of soy protein hydrolysates with a degree of hydrolysis from 42% to 87%, obtained using such enzymes as Alcalase, Flavourzyme, Trypsin, Papain, Protease A and Peptidase R, it was shown that the maximum immunomodulatory activity is positively charged peptides [21]. Such peptide mixtures are promising for use in specialized products with high immunomodulatory activity.

The research carried out in this direction is complemented by the joint work of specialists of the Federal Research Center for Nutrition and Biotechnology and All-Russian Scientific Research Institute of Dairy Industry on the soy protein isolate hydrolysis by the pancreatin enzyme in a protein/enzyme ratio of 50:1 and 20:1 (by mass proportion of dry matter). Fermentation was carried out at a temperature of (50±1 °C) with constant stirring, the pH of the reaction medium was maintained in the range of 7,4-7,6 by potassium and sodium hydroxide solutions titration in a ratio of 2:1 for 5 hours, and sampling for chromatographic analysis was carried out after 1,3 and 5 hours. The enzyme was inactivated by heating the mixture to 75 °C for 15 minutes. Then the mixture was centrifuged for clarification [21].

The obtained results served as the basis for the development of a soy protein hydrolysate pilot batch in order to develop technological modes in semi-industrial conditions.

For hydrolysis, as well as in laboratory studies, a 5% solution of soy protein isolate was used. The hydrolysis was carried out in a circulating mode using a setup equipped with a reservoir with a stirrer and a thermal shirt, as well as a dispersant with a built-in centrifugal pump. The process was carried out by dissolving soy protein isolate in water to obtain a solution of 5% concentration. The enzyme pancreatin was added to the soy protein isolate solution in a protein/enzyme ratio of 50:1. Hydrolysis was carried out at a temperature of (51±1 °C) for 3 hours, after which the enzyme was inactivated at a temperature of (75±1 °C) in 20 minutes. The hydrolyzate was dried on a spray dryer Niro-Atomizer (Denmark) with a capacity of 20 kg of evaporated moisture per hour at temperatures of incoming air in the range of 160-165 °C, leaving 80-85 °C.

The resulting product is a finely dispersed powder of light cream color, with a mass fraction of moisture of 9.5%, readily soluble in water, with a slight bitter-salty taste and a characteristic odor. The osmolality of the reduced 1% hydrolysate solution was 32 mol/kg. In laboratory conditions, experiments were carried out to reduce the bitterness and osmolality of the obtained hydrolyzate using a nanofiltration unit, which made it possible to reduce the hydrolyzate osmolality to 26 mmol/kg and practically eliminate the bitterness. The obtained results are confirmed by the data of exclusion chromatograms of the produced hydrolyzate before and after nanofiltration (figure).



Soy protein hydrolyzate size exclusion chromatography
Size exclusion chromatography of soy protein enzymatic hydrolyzate before and after nanofiltration:
a -Enzymatic hydrolyzate of soy protein isolate-Supro (EHSPI-Supro) before nanofiltration;
b - Enzymatic hydrolyzate of soy protein isolate-Supro (EHSPI-Supro) after nanofiltration

Conclusion and deductions. It can be noted that the above review materials and the obtained experimental data indicate the prospects for further technologies development and improvement for the biotransformation of plant protein-containing raw materials based on their hydrolysis by enzymes and bacterial starters in order to obtain products with improved functional properties, including those aimed at reducing anti-nutritional factors. At the same time, for a specific application, it is required to expand the range of use of protein-containing sources of plant raw materials, further search for the most effective types of enzyme preparations and bacterial starters, development and optimization of resource-saving technological parameters of raw material processing, as well as improving the objective finished products testing depending on their final destination.

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КОМПОНЕНТТЕР ҚАСИЕТІН ГИДРОЛИТИКАЛЫҚ РЕТТЕУ

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ГИДРОЛИТИЧЕСКОЕ РЕГУЛИРОВАНИЕ СВОЙСТВ КОМПОНЕНТОВ

Аннотация. В статье приведена оценка потребности молочного животноводства Российской Федерации и Республики Беларусь в кормах для телят. Рассмотрены основные мировые тенденции, направленные на обеспечение молодняка качественными кормовыми средствами.

Анализируются различные варианты направленного гидролиза белковых компонентов заменителей цельного молока (ЗЦМ), предназначенных для выпойки молодняка в первые месяцы жизни.

Описаны характерные способы ферментации компонентов сои, которые имеют свои преимущества и недостатки. В то же время разнообразие подходов в целях обеспечения эффективной биотрансформации компонентов сои путем их ферментации свидетельствует о том, что достижение действительно оптимальных решений в этой области еще впереди и требует дальнейших исследований.

Обсуждены возможности снижения антигенной активности соевых белков, широко использующихся в настоящее время в рецептурах ЗЦМ для питания молодняка сельскохозяйственных животных.

Приведены результаты совместных работ специалистов ФГБУН «ФИЦ питания и биотехнологии» и ФГАНУ «ВНИМИ» по проведению гидролиза изолята соевых белков ферментом панкреатин, полученные результаты послужили основанием для выработки опытной партии гидролизата соевого белка в целях отработки технологических режимов в полупромышленных условиях.

Приведены результаты экспериментов в лабораторных условиях по снижению горечи и осмоляльности гидролизата из соевых белков с использованием нанофильтрационной установки, что позволило снизить осмоляльность гидролизата и практически ликвидировать горечь. Результаты подтверждены данными эксклюзионных хроматограмм выработанного гидролизата до и после нанофильтрации.

Приведены результаты экспериментальных работ и патентов, в которых описаны наиболее широко используемые подходы к получению ферментативных гидролизатов белков с заданными свойствами, а также оценки их биологической активности и иммунохимических свойств. Для получения белково-углеводных растворов применены различные смеси ферментных препаратов, обладающие как протеолитической, так и целлюлазной или амилазной активностями и получение растворимых (не образовывающих коллоидных масс) фракций белков и углеводов путем последовательного гидролиза белков и углеводов.

Предложены различные способы приготовления заменителя молока из растительного белка и углеводных источников с использованием фермента, имеющего множественные карбогидразные активности.

Приведены результаты исследований, которые подтверждают, что стадия мембранной ультрафильтрации способствует снижению относительной антигенности гидролизатов до минимальных значений, используемых в дальнейшем в составе гипоаллергенных пищевых продуктов лечебного назначения

Рассмотрены вопросы использования различных ферментных препаратов бактериального, грибкового и животного происхождения для гидролиза белков молозива и растительных источников белкового сырья для производства ЗЦМ.

Установлено, что расширение спектра использования белоксодержащих источников растительного сырья требует дальнейший поиск наиболее эффективных видов ферментных препаратов и бактериальных заквасок, отработка и оптимизация ресурсосберегающих технологических параметров обработки сырья, а также совершенствования объективного тестирования готовых продуктов в зависимости от их конечного назначения.

Ключевые слова: соевые белки; способы гидролиза; антиалиментарные вещества; антигенность; иммуноглобулины; ферменты; бактериальные закваски.

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