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N.A. Zolotarev[1], O.B. Fedotova[1],
E.Yu. Agarkova[1], A.G. Akhremko[2], O.V. Sokolova[3]

[1]All-Russian Dairy Research Institute, Moscow, Russia;
[2]V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, Moscow, Russia;
[3]IP Sokolova OV, Moscow, Russia.
E-mail: nicki.zolotaryov@yandex.ru,o_fedotova@vnimi.org,
e_agarkova@vnimi.org, a.akhremko@fncps.ru, ol.moloko@mail.ru

DIRECTIONAL PROTEOLYSIS OF SECONDARY RAW MATERIALS

Abstract. For the food industry, technologies for processing secondary raw materials are of interest. Curd whey is a typical complex secondary bioproduct. It has a high acidity, so it is difficult to recycle. The scope of use of curd whey is limited.

There are technologies for processing whey from cheese. In the manufacture of rennet cheese, whey is formed, which is successfully processed. The technology for processing whey from cheese can only partially be applied for processing whey from cottage cheese. In particular, the use of ultrafiltration can be used for the concentration of curd whey protein. The whey protein concentrate from cottage cheese can be hydrolyzed. Curd whey after proteolytic biocatalysis has a higher potential for use in the food industry

The use of hydrolyzed whey rather than native is promising. According to studies of domestic and foreign scientists, peptides of medium length (3-10 kDa) have the highest biological value. However, during hydrolysis, a proteolytic process occurs, the consequence of which is the appearance of a bitter taste due to the formation of bitter amino acids. The aim of the study was to obtain a whey protein hydrolyzate with minimally altered sensory characteristics.

The problem with whey protein hydrolysis is that a bitter taste appears during hydrolysis. The aim of the study was to obtain a whey protein hydrolyzate of curd whey. The resulting hydrolyzis should not have a bitter taste, and the length of its peptides should be medium.

To obtain curd whey hydrolysates with harmonized sensory characteristics, an enzyme preparation from the group of fungal proteases produced by Aspergillus oryzae was selected. The experimental data made it possible to optimize the parameters of the hydrolysis process.

The results of the study and analysis confirm that the whey protein hydrolyzate has minimal changes in organoleptic characteristics compared to native serum. In the hydrolyzate there is no bitterness in the taste and aftertaste. It has been proven that the resulting peptides are of medium size.

Key words: hydrolysates, curd whey, enzymes, peptides, secondary raw materials.

Summary. Whey is a by-product of high-protein dairy products, such as cheese and cottage cheese. Whey from cheese and whey from cottage cheese have a rich chemical composition, but the use of whey in other dairy products is limited.

As a result of comprehensive studies, the process of biocatalytic conversion of curd whey ultrafiltrate was developed and optimized. A whey hydrolyzate with a high potential for dairy products was obtained. The hydrolyzate has a high biological value and a pleasant organoleptic range. It can be used to enrich other dairy products, such as cottage cheese desserts.

Introduction. The world entered the 21st century under the auspices of progressive biotechnology. Biotechnological approaches open up a space of opportunities in the modern world, in particular in the food sector. Many approaches and methods allow solving complex problems. Such a problem is, for example, the processing of whey. Among secondary dairy resources, whey processing is the most difficult.

Numerous studies of whey treatment options are based on extracting the maximum amount of benefical components from whey [1,2]. Research in this area in Russia was carried out by famous scientists: A.G., Gavrilov GB, Evdokimov I.A., Ostroumov L.A., Prosekov A.Yu., Kharitonov V.D. ect.

In their research, they expressed the prospect of using whey proteins as ingredients for other dairy products. Serum hydrolysates can be used to create preventative and functional products [3-6].

Currently, there are approaches and technologies for processing cheese whey. But these technologies cannot be used to process curd whey. Due to the fact that the acidity of curd whey is very high, it is much more difficult to process. It is difficult to use curd whey in the technology of other products [7].

A promising direction for the processing of curd whey is ultrafiltration, after which hydrolytic bioconversion occurs [8-10]

In connection with the above, the current research area is the development of a biocatalytic conversion process using Russian hydrolytic enzymes suitable for biocatalysis of curd whey. The resulting hydrolyzate can be used to enrich curd products.

The aim of our study was to develop an optimized process for the preparation of curd whey hydrolyzate with confirmed biological value and minimally altered organoleptic characteristics. The hydrolyzate should have the potential of application as an enrichment for curd products [11].

Methods. To study the molecular weight distribution of the ultrafiltration concentrate and protein hydrolyzate of curd whey, the method of high performance liquid chromatography was used.

Chromatographic analysis was performed on a Gilson high performance liquid chromatograph (Gilson Medical Electronics, Villiers le Bel, France), and a Gilson 118 variable wavelength spectrophotometric detector was also used. Detection was carried out at a wavelength of 214 nm. In the obtained chromatograms, the relative content of the high molecular weight protein fraction larger than 10 kDa, the average molecular fraction from 3.5 to 5 kDa and from 5 to 10 kDa, as well as free amino acids less than 3.5 kDa were identified.

As the main proteomic technology, two-dimensional electrophoresis (DEF) according to O'Farrell with isoelectric focusing in ampholine (IEF-PAGE) was used. The detection of proteins on two-dimensional electrophoregrams was performed by staining with Coomassie blue R-250 (CBB R-250) and then sequentially with silver nitrate. For computer densitometry, two-dimensional electrophoregrams were used, which were in a wet state. Their complete digital images and / or images of individual fragments were obtained by scanning on an Epson Expression 1680 scanner. Mass spectra ("peptide fingerprints") were decrypted using traditional bioinformation technologies. The analysis of the obtained mass spectra of tryptic peptides was performed using the Mascot program, option Peptide Fingerprint (Matrix Science, USA), with an accuracy of determining the mass of MH + equal to 0.01%.

Organoleptic studies were carried out in accordance with GOST ISO 6658-2016 and GOST ISO 10399-2015.

Results. Curd whey contains a relatively low mass fraction of protein, on average 0.5%. With such a low protein content, the efficiency of proteolysis decreases, so the ultrafiltration method was chosen to concentrate the protein fraction. Using this method, a concentration coefficient of 3.6 was achieved. Thus, the mass fraction of protein ultrafiltrate averaged 3.3%.

At the stage of choosing the hydrolytic enzyme, we evaluated the proteolytic activity declared by the manufacturer against milk proteins. The selected enzyme should hydrolyze the whey protein ultrafiltrate. Enzymes produced by Aspergillus niger and Aspergillus oryzae cultures were selected for the study. These enzymes are proteins characterized as aspergillopepsin, aspartate pepA protease.

When using the enzyme preparation Aspergillus niger, a significant intensification of the process was noted in comparison with the process carried out under the same conditions, but using the enzyme preparation Aspergillus oryzae.

So, when using the first enzyme, with a minimum enzyme-substrate ratio of (0.5), the degree of hydrolysis was 1.2%, and the maximum degree of hydrolysis was observed with an enzyme-substrate ratio of 8.5 and amounted to 8.43%. Whereas, with a minimal activity of the enzyme in the second case, the degree of hydrolysis was 7.03%, and with a maximum of 13.8%.

The duration of the hydrolysis process was varied to determine the conditions for achieving the maximum degree of hydrolysis from 1 to 5 hours. After hydrolysis, the samples were pasteurized at a temperature of 80 ° C with an exposure of 15 minutes to inactivate the enzyme.

When choosing an enzyme preparation, the most important requirement was to obtain a curd whey hydrolyzate with minimal changes in sensory characteristics. To select an enzyme preparation, a mandatory organoleptic assessment was carried out in parallel with other studies. Hydrolysates obtained under various conditions of the hydrolysis process were subjected to organoleptic analysis.

Sensory characteristics studies have shown that when using the enzyme preparation Aspergillus niger, the first signs of damage were noted with an enzyme-substrate position of 3.5 and a degree of hydrolysis of 4.5%. Aspergillus oryzae mountain taste sensation with enzyme activity of 7.5 u / cm^3 and increased hydrolysis. Comparative sensory characteristics evaluation (table 1) allowed us to choose an enzyme preparation. As a control sample, native curd whey was used to evaluate changes in the organoleptic gamut. According to the results, an enzyme preparation produced by Aspergillus oryzae was selected.

Table 1 - Sensory evaluation of the curd whey protein hydrolyzate

Indicator name	Sensory characteristic of native curd whey	Sensory characteristic of curd whey hydrolyzate obtained using enzyme preparation	
		by Aspergillus oryzae	by Aspergillus niger
Appearance and consistency	Homogeneous translucent liquid with insignificant protein precipitate	Homogeneous opaque liquid with suspended protein particles	Homogeneous opaque liquid with suspended protein particles
Colour	Pale green	Pale green with a cream tint	Pale green with a cream tint
Taste	Typical characteristic of curd whey	Typical characteristic of curd whey	Bitter, acid, uncharacterist ic for curd whey
Smell	Sour-milk, whey, characteristic without extraneous tones	Peculiar to curd whey, without extraneous smacks and odors	Uncharacteristic for curd whey, with extraneous tones

When analyzing expert assessments of sensory characteristics, the concordance coefficient was 1, which confirms the unity of opinion of the expert commission.

According to the results of a set of studies, the process of hydrolytic bioconversion is optimized. As a result of process optimization [12], the following conditions were determined: temperature 46.4°C; the duration of the process is 180 minutes; enzyme-substrate ratio of 9.5. These parameters make it possible to achieve a maximum degree of hydrolysis of 13.2%.

The selected process conditions allow targeted biocatalysis of curd whey proteins. As a result, we obtain a hydrolyzate with the desired sensory characteristics.

According to published data, the preparation of hydrolysates with a degree of hydrolysis of 5–20% with a high content of medium-length peptides (3–10 kDa), which can be used in specialized and functional nutrition, is of practical interest [13, 14].

To determine the molecular mass distribution of peptide fractions, they were studied using high performance liquid chromatography and two-dimensional electrophoresis (figure 1).

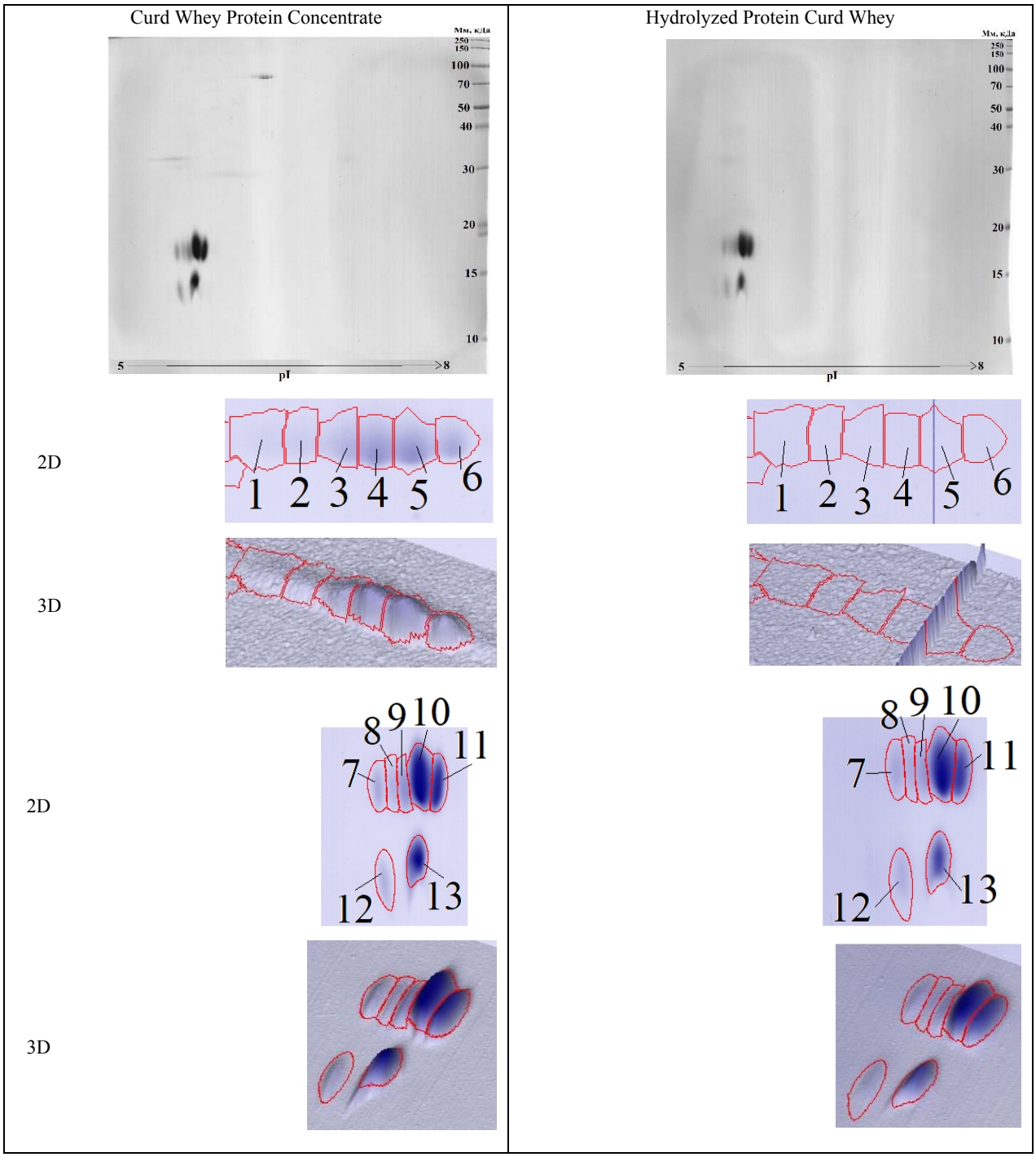

Figure 1 - Electrophoregram of the concentrate and hydrolyzed protein curd whey

Summarizing the obtained experimental results, we concluded that the content of high molecular weight peptide compositions in the obtained hydrolyzed protein of curd whey approximately halved compared to the concentrate (ultrafiltrate). The content of peptides with a molecular weight of 3.5 - 5 kDa increased by almost 10 times. The content of components with a molecular weight of less than 3.5 kDa increased by about 1.5 times. Visualization of the results is presented as a graphical interpretation of the molecular weight distribution of peptide compositions in the concentrate and hydrolyzate of curd whey proteins (figure 2).

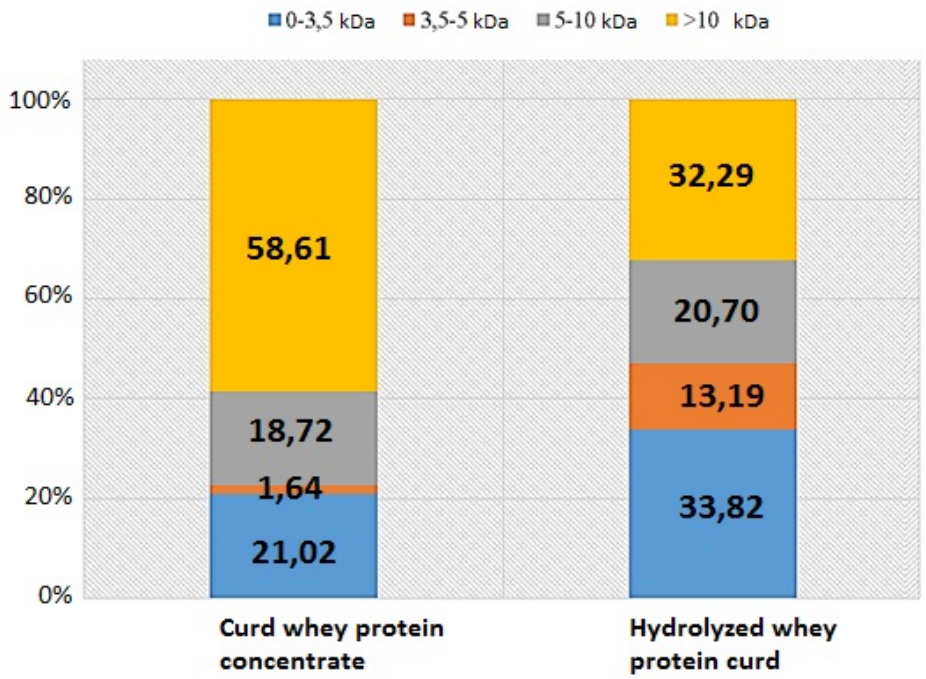

Figure 2 - Molecular mass distribution of peptide compositions
in a concentrate and hydrolyzed protein of curd whey

The obtained results of electrophoretic analysis and molecular mass distribution prove that as a result of targeted biocatalysis using the enzyme preparation Aspergillus oryzae, a degree of hydrolysis is achieved, which provides medium-length peptides with predictable functional properties.

Conclusion. The biocatalysis of curd whey ultrafiltrate was carried out using an enzyme preparation produced by Aspergillus oryzae. As a result of the work, it was proved that the obtained hydrolyzate possesses the necessary sensory characteristics. Namely, it lacks bitterness in taste and aftertaste. Peptides of medium length (3-10 kDa) prevail in the obtained hydrolyzate. This indicates the feasibility of using the hydrolyzate as an enriching agent in the production of specialized nutrition and functional products.

Discussion. Curd whey contains more than 250 compounds with unconditional biological and nutritional value [15]. According to A.G. Khramtsov and I.A. Evdokimov deep processing of whey will solve several problems. Among them are the synthesis of new composites that can be used to enrich other dairy products. Curd whey can be used biologically active or enriching component [16,17].

The use of serum concentrations is not innovative. To date, technologies have been developed for processing whey from cheese. These technologies include various types of concentration. Using concentration and separation, you can get by-products from whey from cheese [18].

Membrane filtration is applied to cheese whey to obtain demineralized whey and its subsequent drying [19].

Ultrafiltration allows you to save most of these substances. According to G. B. Gavrilov and A. G. Khramtsov, the use of membrane separation of serum fractions is the most promising industry [20].

The use of hydrolytic bioconversion is the most sparing method of biotransformation of whey proteins. In addition, targeted limited proteolysis yields medium-sized peptides. They have minimally altered organoleptic characteristics. Therefore, the resulting hydrolyzate has a high potential for use both in the dairy industry and in other food industries.

Н.А. Золотарев[1], О.Б. Федотова[1],
Е.Ю. Агаркова[1], А.Г. Ахремко[2], О.В. Соколова[3]

[1]«Бүкілресейлік сүт өнеркәсібі ғылыми-зерттеу институты» ФМАҒМ, Мәскеу, Ресей;
[2]В.М. Горбатов атындағы тағамдық жүйелердің федералдық ғылыми орталығы, РҒА, Мәскеу, Ресей;
[3]Соколов О.В. ЖК, Мәскеу, Ресей

ЕКІНШІЛІК ШИКІЗАТ РЕСУРСТАРЫНДАҒЫ БАҒЫТТЫ ПРОТЕОЛИЗ

Аннотация. Тамақ өнеркәсібі үшін екіншілік шикізат ресурстарын қайта өңдеу үлкен қызығушылық тудырады және бүкіл тамақ өнеркәсібі үшін жоғары әлеуетке ие. Сүт сарысуы – бірқатар жоғары ақуызды сүт өнімдерін өндірудің жанама өнімі, мысалы, ірімшік (ірімшік сарысуы) немесе сүзбе (сүзбе сарысуы). Сарысудың құрамы химиялық бай болғанымен, оны сүт өнімдер технологиясында қолдану шектеулі. Ірімшік сарысуы – жоғары қышқылды болғандықтан, шектеулі қолданылуына байланысты өңдеуге арналған күрделі биологиялық жүйе.

Ірімшік сарысуын өңдеудің қолданыстағы тәсілдері (мәйекті ірімшік өндіру кезінде пайда болған қайталама өнім) сүзбе сарысуын өңдеу технологиясында жартылай ғана қолданылады. Атап айтқанда, ақуыз компонентінің шоғырлануы үшін фракциялардың мембраналық бөлінуін және одан кейінгі биокатализді ірімшік сарысуына қолданады және ірімшік сарысын өңдеуге қолдану мүмкіндігі бар.

Кешенді зерттеулер нәтижесінде ультрафильтрацияланған сүзбе сарысуы концентратын биокаталитикалық конверсиялау үрдісі жасалды және оңтайландырылды, нәтижесінде жоғары биологиялық құндылығы мен қанағаттанарлық органолептикалық диапазонына байланысты сүт өнімдеріне байытатын ингредиент ретінде қолдануға жоғары әлеуеті бар гидролизат алынды. Сүзбе сарысуынан ақуыз концентратына қол жеткізілді.

Табиғи емес гидролизденген сарысуды қолдану перспективті деп саналады. Отандық және шетелдік ғалымдардың зерттеулері бойынша орташа ұзындықтағы пептидтер (3-10 кДа) биологиялық құндылыққа ие. Алайда гидролиз кезінде протеолитикалық үрдіс жүреді, ащы аминқышқылдары пайда болатындықтан ащы дәмнің болуы салдарына жатады. Зерттеу мақсаты – аз мөлшерде өзгертілген органолептикалық қасиеттері бар сарысу ақуызының гидролизатын алу.

Үйлесімді органолептикалық сипаттамалары бар сұрыпталған ірімшік сарысуы гидролизатын алу үшін *Aspergillus oryzae* бөліп шығаратын саңырауқұлақ протеазалар тобынан ферментті препарат таңдалды. Гидролиз үрдісі оңтайландырылды және сүзбе сарысуы ақуызының гидролизаты концентратының қажетті қасиеттерінің жиынтығына қол жеткізілетін параметрлер анықталды.

Алынған гидролизаттың органолептикалық сипаттамалары аз өзгеретіні, ащы дәмі әрі дәмсіздігі тәжірибе жүзінде расталды. Молекулалық салмақтың таралуын зерттеу орта ұзындықтағы пептидтердің гидролиз нәтижесінде түзілетінін дәлелдейді.

Гидролитикалық биоконверсияны қолдану сарысуы бар ақуыздарды биотрансформациялаудың ең тиімді әдісі болып саналады. Сонымен қатар, бағытталған шектеулі протеолиз құрамы органолептикалық профилі аз өзгеретін орташа мөлшерлі пептидтерді алуға мүмкіндік береді. Сондықтан оны сүт өнеркәсібінде де, басқа тамақ өнеркәсібінде де қолданудың мүмкіндігі жоғары.

Түйін сөздер: гидролизаттар, сүзбе сарысуы, ферменттер, пептидтер, екіншілік шикізат.

Н.А. Золотарев[1], О.Б. Федотова[1],
Е.Ю. Агаркова[1], А.Г.Ахремко[2], О.В. Соколова[3]

[1]ФГАНУ «ВНИМИ», Москва, Россия;
[2]ФГБНУ «ФНЦ пищевых систем им. В.М. Горбатова» РАН, Москва, Россия;
[3]ИП Соколова О.В., Москва, Россия

НАПРАВЛЕННЫЙ ПРОТЕОЛИЗ ВТОРИЧНЫХ СЫРЬЕВЫХ РЕСУРСОВ

Аннотация. Для пищевой промышленности переработка вторичных сырьевых ресурсов представляет большой интерес и обладает высоким потенциалом для всей пищевой отрасли. Молочная сыворотка представляет собой побочный продукт производства ряда высокобелковых молочных продуктов, например, сыра (подсырная сыворотка) или творога (творожная сыворотка). Сыворотка имеет богатый химический состав, однако её применение в технологии молочных продуктов ограничено. Творожная сыворотка является сложной для переработки биологической системой в связи с тем, что обладает высокой кислотностью, и, следовательно, ограниченным применением.

Существующие подходы переработки подсырной сыворотки (вторичного продукта, образующегося при выработке сычужных сыров) лишь отчасти могут быть применены в технологии переработки творожной сыворотки. В частности, применение мембранного разделения фракций для концентрирования белковой составляющей и последующий биокатализ применяются для подсырной сыворотки и имеют потенциал применения для переработки творожной сыворотки.

В результате проведенных комплексных исследований разработан и оптимизирован процесс биокаталитической конверсии ультрафильтрационного концентрата творожной сыворотки, в результате которого получен гидролизат, обладающий высоким потенциалом применения как обогащающего ингредиента для молочных продуктов за счет подтвержденной высокой биологической ценности и удовлетворительной органолептической гаммы. Получен концентрат белков нативной творожной сыворотки.

Перспективно использование не нативной, а гидролизованной сыворотки. Согласно исследованиям отечественных и зарубежных ученых, наибольшую биологическую ценность имеют пептиды средней длины (3-10 кДа). Однако при гидролизе происходит протеолитический процесс, последствием которого является появление горького вкуса вследствие образования горьких аминокислот. Целью исследования являлось получение гидролизата сывороточных белков с минимально измененными органолептическими свойствами.

Для получения гидролизатов творожной сыворотки с гармонизированными органолептическими показателями выбран ферментный препарат из группы грибных протеаз, продуцируемый Aspergillus oryzae. Оптимизирован процесс гидролиза и определены параметры, при которых достигается комбинация требуемых свойств гидролизата концентрата белков творожной сыворотки.

Экспериментально подтверждено, что полученный гидролизат имеет минимально измененные органолептические показатели, не обладает горьким вкусом и послевкусием. Исследование молекулярно-массового распределения доказывает, что в результате гидролиза образуются пептиды средней длины.

Применение гидролитической биоконверсии является наиболее щадящим методом биотрансформации сывороточных белков. Кроме того, направленный ограниченный протеолиз позволяет получить пептиды среднего размера, композиции которых обладают минимально измененным органолептическим профилем. Следовательно, имеет высокий потенциал использования как в молочной отрасли, так и в других пищевых отраслях.

Ключевые слова: гидролизаты, творожная сыворотка, ферменты, пептиды, вторичное сырье.

Information about authors:

[1]Zolotarev Nikita A. graduate student of All-Russian Scientific Research Institute of Dairy Industry (FSANO VNIMI), Moscow, Russia; E-mail: nicki.zolotaryov@yandex.ru, https://orcid.org/ 0000-0003-3992-7041;

[1]Fedotova Olga B., Doctor of technical sciences, scientific secretary of All-Russian Scientific Research Institute of Dairy Industry (FSANO VNIMI), Moscow, Russia; E-mail: o_fedotova@vnimi.org, https://orcid.org/ 0000-0002-7348-6019;

[1]Agarkova Evgeniya Yu., Candidate of technical sciences, Head of laboratory of All-Russian Scientific Research Institute of Dairy Industry (FSANO VNIMI), Moscow, Russia; E-mail:e_agarkova@vnimi.org, https://orcid.org/0000-0001-8967-7074;

[2]Akhremko Anastasya G., junior science assistance of V.M. Gorbatov Federal Research Center for Food Systems of Russian Academy of Sciences, Moscow, Russia; E-mail: a.akhremko@fncps.ru, https://orcid.org/ 0000-0002-0211-8171;

[3]Sokolova Olga V., Candidate of technical sciences, E-mail: ol.moloko@mail.ru, https://orcid.org/0000-0001-9516-6123

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