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OPTIMIZATION OF PARAMETERS OF FERMENTOLYSIS OF PROTEINS IN THE COMPOSITION OF SERUM-PROTEIN CONCENTRATE

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Abstract. The food industry is a strategic industry that works quite steadily even during periods of economic crises, providing food security to any state, and is a source of raw material for other industries with a high potential for development, for example, for the production of cosmetics. The modern cosmetics market is represented by various cosmetic products, often expensive, but not always made from natural ingredients. Therefore, the search for the newest ingredients for the production of natural cosmetics on the basis of domestic raw materials is an urgent task of the present. Ingredients from milk serum can be used for the production of natural cosmetics that in large quantities is obtained in milk processing enterprises and often remains unprocessed. Whey protein concentrations can be a source of short-chain peptides and free amino acids for the production of various cosmetic products. The process of fermentolysis of serum proteins in nanofiltration concentrate KSB-65 with the content of dry matter of 20% using neutral peptidase C from the domestic producer at a temperature of 40 ºC with the duration of the process varying from 1 to 5 hours, the content of peptidase – from 0,5 to 2,0 U/g. It is established that the optimal parameters of the fermentolysis of serum proteins in KSB-65 are as follows: temperature 40 º C, neutral peptidase C content – 0.78 U/g, duration of fermentolysis – 3.17 hours. With optimal parameters of the fermentolysis process, the hydrolyzate of the nanofiltration concentrate KSB-65 contains the maximum amount of short chain peptides (57.03 mg/cm^3) and a high concentration of free amino acids (54.66 μg/cm^3). Recommendations for the further use of serum protein hydrolyzate obtained using the recommended optimal parameters of the enzyme production process from the nanofiltration concentrate KSB-65, in the manufacture of cosmetic products, including with anti-age effect, and hydrolyzates of proteins enriched with probiotic cultures of lactam bifidobacteria or their lysates.

Key words: food industry, cosmetics, short chain peptide, free amino acid, serum protein concentrate, peptidase, fermentolysis, optimization, response surface.

ОПТИМІЗАЦІЯ ПАРАМЕТРІВ ПРОЦЕСУ ФЕРМЕНТОЛІЗУ БІЛКІВ У СКЛАДІ СИРОВАТКОВО-БІЛКОВОГО КОНЦЕНТРАТУ

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Анотація. Харчова промисловість є стратегічною галуззю, яка достатньо стабільно працює, навіть у періоди економічних криз, забезпечуючи продовольчу безпеку будь-якої держави, а також є джерелом сировинних ресурсів для інших галузей промисловості, які мають достатньо великий потенціал для розвитку, наприклад, для виробництва косметичних засобів. Для виробництва натуральної косметики можуть бути застосовані інгредієнти із молочної сироватки, яка у значних кількостях отримується на молокопереробних підприємствах і часто залишається не переробленою. Концентрати сироваткових білків можуть бути джерелом коротколанцюгових пептидів та вільних амінокислот для виробництва різних косметичних продуктів. Проведено процес ферментолізу сироваткових білків у нанофільтраційному концентраті КСБ-65 із вмістом сухих речовин 20 % з використанням нейтральної пептидази С вітчизняного виробника за температури 40ºС при варіюванні тривалості процесу від 1 до 5 годин, вмісту пептидази – від 0,5 до 2,0 од/г. Встановлено, що оптимальними параметрами процесу ферментолізу сироваткових білків у КСБ-65 є наступні: температура 40 ºС, вміст нейтральної пептидази С – 0,78 од./г, тривалість ферментолізу – 3,17 год. За оптимальних параметрів процесу ферментолізу гідролізат нанофільтраційного концентрату КСБ-65 містить максимальну кількість коротколанцюгових пептидів (57,03 мг/см^3) та високу концентрацію вільних амінокислот (54,66 мкг/см^3). Надано рекомендації щодо подальшого використання гідролізату сироваткових білків, отриманого за використання рекомендованих оптимальних параметрів процесу ферментолізу із нанофільтраційного концентрату КСБ-65, у виробництві косметичних засобів, у т.ч. з anti-age ефектом, та гідролізатів білків, збагачених пробіотичними культурами лакто- та біфідобактерій або їхніми лізатами.

Ключові слова: харчова промисловість, косметика, коротколанцюговий пептид, вільна амінокислота, концентрат сироваткових білків, пептидаза, ферментоліз, оптимізація, поверхня відклику.

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Introduction. Formulation of the problem

The modern cosmetics market is represented by various cosmetic products, often expensive, but not always effective and very rarely made of natural ingredients. In today's world, natural and organic cosmetics are considered to be the best. Scientists from different countries have forecast the growth of demand for natural cosmetics in the beginning of the XXI century. Today one can affirm that their prognosis has been confirmed. Specialists of cosmetic industry state annual increase of consumer interest in natural cosmetics. Therefore, the search for the newest ingredients for the production of natural and organic cosmetic products on the basis of domestic raw materials is an actual task of the present [1-2].

For the production of natural cosmetics, ingredients derived from milk whey, which in large quantities are obtained in dairy processing plants, and often remains unprocessed, can be used. The most valuable component of whey from the point of view of biological value are proteins that do not contain the limited amino acids [3]. Whey proteins are a valuable source of arginine, histidine, methionine, tryptophan and leucine, as well as short chain peptides that have a positive effect on the condition of the skin, hair and nails [3-6].

It is recommended to use milk products hydrolyzate in cosmetics for better effect of the serum and its proteins on the skin and hair [5-6].

Therefore, the substantiation of the parameters of obtaining hydrolysates of serum proteins with high content of short-chain peptides on the basis of concentrates produced by domestic dairy processing enterprises is an actual task.

Analysis of recent research and publications

Analysis of statistical data showed that the production of food products in Ukraine has a tendency to increase. If in 2011 compared to 2010 in Odesa region production was reduced by 16.7%, then in the period 2016-2017, the growth of production volumes to the previous year was 15.3% and 24.6% respectively, but the production of goods by enterprises in other industries, including the production of cosmetics, has a reverse trend [7].

Milk whey is a product of milk processing, which is obtained in the production of cheese, casein, sour cheese [3-4]. Studies show that the production of dairy products for the period 2013-2017 in Odesa region is quite stable: the production of fresh unfermented cheese, grated cheese, blue cheese and other not melted cheese increased in 2016 by 19.4%, in 2017 – by 17.5% compared to the previous year [7]. Thus, whey, as a product of milk processing, is an additional kind of dairy raw material, which can be used for the production of various goods, including cosmetic products [8]. Milk serum is now widely used for cosmetic purposes; It improves skin and hair condition. Today you can find in stores a huge number of cosmetics, which includes this raw material [4-6, 9]. The production and marketing of cosmetic products based on whey will increase the efficiency of the activity and ensure the efficiency of the company's operation, will ensure the competitiveness of the company in implementing a moderate and soft transformation of the enterprise [8].

So, milk proteins and milk whey – the main components of milk – are known for their softening, moisturizing, restorative, anti-allergenic and anti-inflammatory properties. Due to the presence of biologically valuable ingredients, milk proteins restore and soften sensitive skin, protect it from the negative effects of the environment and promote equal pigmentation. Dairy proteins also contribute to the active development of collagen, keeping the skin fresh, smooth and young. In addition, milk proteins have a good effect on the condition of the hair, strengthening and restoring the hair shaft, and its outer layer, which fills the hair with vital energy, enhances smoothness and shine [4-6,9].

Hydrolyzate of milk proteins is a biologically active ingredient derived from milk proteins by the method of protein fermentolysis. Hydrolyzed protein consists of short chains of amino acids, the molecular weight of which is 1.4–1.6 Da, which allows penetration into the deep layers of the skin. Milk hydrolyzate is a true healing elixir for irritated, damaged, dry skin [9]. This hypoallergenic product is suitable for sensitive and infant skin, and high bioavailability allows to use it in instant healing tools, moisturizing skin. Milk protein activates the growth of new cells, contributing to the renewal of the epidermis [4-6,9].

At the present stage of the development of the milk processing industry, concentrates of serum proteins are produced, which differ in protein content and the level of demineralization. By content of protein they are divided into KSB-45, KSB-50 and KSB-65; By the level of demineralization, concentrates with 30%, 70% and 90% of demineralization level are distinguished.

Today, domestic cheese-making enterprises produce ultrafiltration and nanofiltration concentrates of serum proteins [1]. The level of demineralization in concentrates of serum proteins produced by modern cheese-making enterprises is 30 or 70%. For the production of hydrolyzates it is advisable to use nanofiltration concentrates with a maximum content of protein – KSB-65 with a demineralization level of 70% [1,9].

Proteases, (proteinases, proteolytic enzymes) are enzymes of the class of hydrolases that break down the peptide bond between amino acids in proteins [3]. Proteases are one of the most important industrial enzymes. Proteases can be divided into two main groups: exopeptidases

(cleavage of amino acids from the end of the peptide) and endopeptidases (splitting peptide bonds within the peptide chain). Endopeptidases have found a wider industrial application than exopeptidases. Also, peptidases are classified according to the optimum pH of the enzyme (sour, alkaline or neutral), according to the substrate specificity (collagenase, keratinase, elastase, etc.), according to their homology with well-studied proteins (trypsin-like, pepsin-like).

Depending on the type of protease, the scope of their application is different. In the food industry, neutral protease is used for the hydrolysis of milk, meat and soy proteins [3].

Enzyme products for the foreign-made food industry of such enterprises as Palma Group S.A. (Switzerland); Novozymes (Denmark); Alland & Robert (France) are represented in the Ukrainian market. The cost of enzyme products of these manufacturers varies from 102 to 650 UAH/100 g. The proteolytic enzymes of the domestic producer – State Enterprise "Enzym" (Ladyzhyn city, Ukraine) – are divided into alkaline, sour and neutral; the cost of domestic proteases varies from 35 to 42 UAH/100 g.

Taking into account the cost of enzyme products, it is expedient to use the neutral peptidase of the domestic manufacturer for the fermentation of the nanofiltration concentrate of serum proteins with a 70% level of demineralization (KSB-65).

The purpose of the study is to optimize the parameters of fermentolysis of serum proteins in nanofiltration concentrate KSB-65 using neutral peptidase.

Objectives of the study:

- to carry out the process of fermentolysis of serum proteins in a nanofiltration concentrate KSB-65 using neutral peptidase for various process parameters;
- to determine the content of short-chain peptides and free amino acids in samples KSB-65 after fermentolysis;
- to determine the optimal parameters of the process of fermentolysis of serum proteins in the nanofiltration concentrate KSB-65 by neutral peptidase;
- provide recommendations for the further use of hydrolyzate of whey proteins obtained using the recommended optimal parameters of the fermentolysis process.

Research materials and methods

The following raw ingredients and materials were used for research: the concentrate of serum proteins KSB-65, which is produced at Additional Liability Partnership "Zolotoniskyy Maslorobnyy Kombinat" ("Zolotonosha oil plant") (Zolotonosha town, Ukraine); Neutral peptidase C, manufactured by "Enzym" (Lviv city, Ukraine).

To optimize the regimen of fermentolysis of serum protein concentrate with neutral peptidase, the methodology of the surface response was used [10-12]. The indicated method is a set of mathematical and statistical methods that are aimed at modeling technological processes and finding the relationships of experimental series of predictors in order to optimize the response function $\hat{y}(x, b)$, which in general is described by such a polynomial:

$$\hat{y}(x,b) = b_0 + \sum_{l=1}^{n} b_l x_l + \sum_{k=1}^{n} b_k x_k^{\,2} + \sum_{i=1}^{n-1}\sum_{j=i+1}^{n} b_{ij} x_i x_j \,, \quad (1)$$

where $x \in R^n$ – vector of variables, b – vector of parameters.

Simulation and processing of experimental data were performed in the software package *Statistica 10 (StatSoft, Inc.*, USA).

In the course of fermentolysis, KSB-65 varied enzyme content in the reaction mixture (from 0.5 to 2.0 U/g) and the duration of fermentation (from 1 to 5 hours); The temperature of fermentation was accepted constant on recommendations of the manufacturer – 40ºC. For fermentolysis of serum proteins in a nanofiltration concentrate KSB-65, it was reduced to a mass fraction of dry matter of 20% (such a mass fraction of dry matter corresponds to their content in a freshly prepared concentrate) in pasteurized water at a temperature of 35–40°C and a solution of an enzyme with activity of 50 U/g. The renewed KSB-65 was heated to a temperature of 40°C in a water bath, the required amount of the enzyme (according to the research plan) was added, fermentolysis at 40°C for a predetermined time was carried out.

Upon completion of the fermentation, the enzyme was inactivated by heat treatment of the samples in a water bath at a temperature of 57°C for 2 min. Experimental specimens were cooled to a temperature of 4–6 °C and stored for further investigation in a cooled state at a temperature of 4–6°C for no more than 24 hours. The content of short-chain peptides (CP, mg/100 g) was determined in the experimental samples according to the procedure given in [13-15], and the free amino acids (BA, μg/100 g) according to [16].

Results of the research and their discussion

The criteria for optimizing the parameters of fermentolysis of serum proteins in the nanofiltration concentrate KSB-65 were selected from the content of short-chain peptides (CP, mg/cm^3) and free amino acids (BA, μg/cm^3). Independent factors that varied in the experiment were the concentration of the enzyme (C_e, U/g) and the duration of fermentolysis (DF, year). To simulate and optimize the parameters of fermentolysis, a function of response which has the form of a polynomial of the second degree was selected:

$$C_{CP} = b_0 + b_1 \cdot C_e + b_{11} \cdot C_e^{\,2} + b_2 \cdot DF + b_{22} \cdot DF^2 + b_{12} \cdot C_f \cdot DF, \quad (2)$$

$$C_{BA} = b_0 + b_1 \cdot C_e + b_{11} \cdot C_e^{\,2} + b_2 \cdot DF + b_{22} \cdot DF^2 + b_{12} \cdot C_f \cdot DF, \quad (3)$$

where b_0 – constant; C_e – concentration of the enzyme; DF – duration of fermentolysis; b_1, b_{11}, b_2, b_{22}, b_{12} – coefficients for each element of the polynomial.

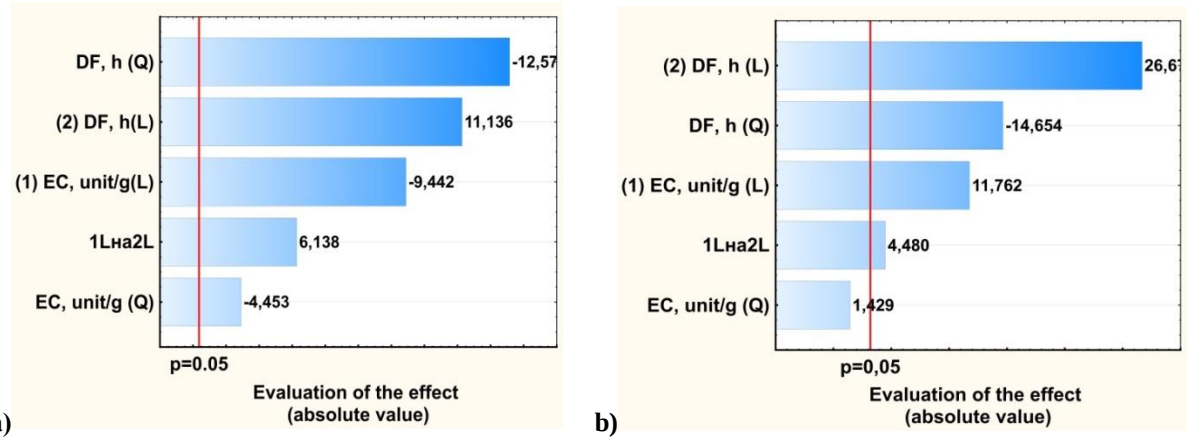
The research used a central composite rotatable plan [10]. The choice of levels and intervals for variation of factors was carried out according to the recommendations of the manufacturer of the enzyme; the concentration of the enzyme varied within the range of 0.5–2.0 units/g; time of fermentolysis – from 1 to 5 hours.

The matrix of planning and the experimental values of the response functions are presented in Table 1. To reduce the influence of systematic errors caused by external conditions, the sequence of experiments was randomized.

To verify the significance of the regression coefficients (2), (3), Pareto charts were constructed, which are presented in Fig. 1 (L – linear effect, Q – quadratic effect).

Table 1 – Planning Matrix and Response Functions

Experiment No	Mass fraction of enzyme		Duration of fermentation		The content of short chain peptides in the sample (CP), mg/cm^3	The content of free amino acids in the sample (VA), μg/cm^3
	coded value	U/g	coded value	hours		
1	–1	0.72	–1	1.6	54.7	28.5
2	–√2	0.50	0	3.0	57.0	66.0
3	–1	0.72	+1	4.4	56.2	49.5
4	0	1.25	+√2	5.0	54.4	58.5
5	+1	1.78	+1	4.4	56.3	57.0
6	+√2	2.00	0	3.0	53.7	72.0
7	+1	1.78	–1	1.6	51.0	34.5
8	0	1.25	–√2	1.0	52.3	57.0
9	0	1.25	0	3.0	56.7	60.0
10	0	1.25	0	3.0	56.5	55.5
11	0	1.25	0	3.0	56.6	56.25
12	0	1.25	0	3.0	56.1	56.25



a) b)

Fig. 1. Pareto chart to verify the significance of the coefficients: a – regression (2); b – regression (3)

In the indicated Pareto charts (Figure 1), standardized coefficients are sorted by absolute values. Analysis of data in Fig. 1a shows that all coefficients in regression (2) are significant, since the rating columns cross the vertical line, which is a 95% confidence probability. In regression (3), the quadratic content of the enzyme is insignificant, since the column of the indicated effect does not cross the vertical line, which is a 95% confidence probability (Fig. 1b), therefore the specified regression member was eliminated from regression (3). The obtained equations with calculated coefficients have the form:

$$C_{CP} = 51{,}174 - 0{,}940 \cdot C_e - 1{,}940 \cdot C_e^2 + 3{,}905 \cdot DF - 0{,}773 \cdot DF^2 + 1{,}280 \cdot C_e \cdot DF, \quad (4)$$

$$C_{BA} = 28{,}141 - 0{,}758 \cdot C_e + 12{,}964 \cdot DF - 1{,}856 \cdot DF^2 + 1{,}887 \cdot C_e \cdot DF, \quad (5)$$

The adequacy of the developed models (4) and (5) was verified by the method of dispersion analysis. Its results, in particular, significance of the determination coefficients is close to one (for model (4) $R^2 = 0.943$ and $R^2adj = 0.896$, for model (5) $R^2 = 0.979$ and $R^2adj = 0.967$), and the lack of consistency (for all models the significance level of this index $p > 0.05$) indicate that the models adequately describe the experiment.

The described polynomials (4) and (5) combined effects of the enzyme concentration (C_e, U/g) and the duration of fermentation (DF, h) on the content of short chain peptides (CP, mg/cm^3) and free amino acids (BA, μg/cm^3) in graphic form are presented in Fig. 2 and Fig. 3, respectively.

With an increase in the enzyme content in the reaction mixture from 0.50 to 0.78 U/g and with an increase in the duration of fermentolysis from 1.0 to 3.17 hours the mass concentration of short-chain peptides with a molecular weight of 1.4–1.6 Da is increased from 54.02 to 57.03 mg/cm^3 (Fig. 2). Further increase in the content of peptidase and the extension of the duration of fermentolysis does not contribute to increasing the mass concen-

tration of short chain peptides, which is probably due to their subsequent hydrolysis to free amino acids.

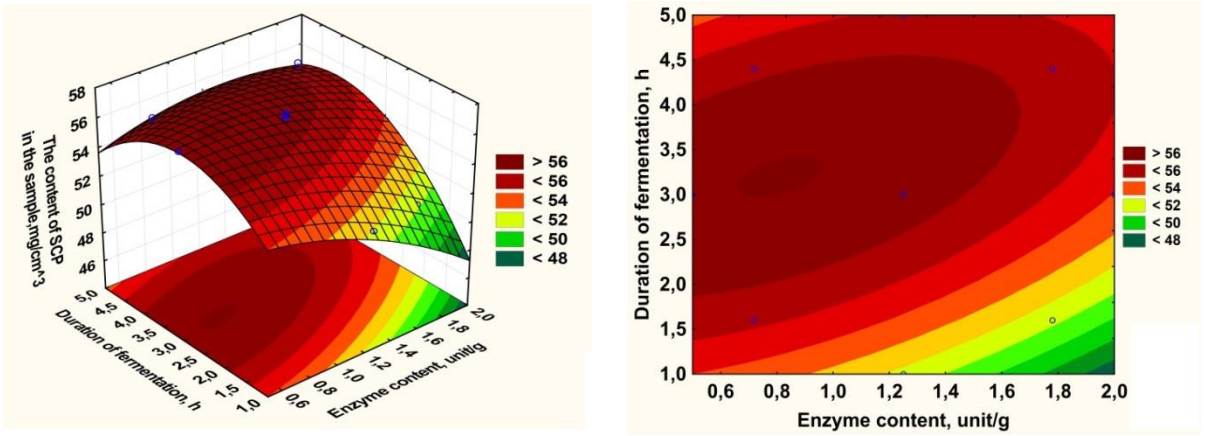
The minimum content of short-chain peptides (46.51 mg/cm^3) is noted at the maximum concentration of peptidase in the reaction mixture (2.0 U/g) and the minimum duration of the fermentolysis process (1.0 hour). Even the extension of the duration of fermentolysis to 5.0 hours at a maximum concentration of peptidase, provides a mass concentration of short chain peptides of 54.53 mg/cm^3 (Fig. 2), which is less than the maximum value.

According to the parameters for which we note the maximum mass concentration of short-chain peptides, the content of free amino acids is 54.66 μg/cm^3 (Fig. 3), which is less than their maximum content under the chosen conditions of the experiment. However, short-chain peptides, which are the source of histidine and lysine, can penetrate deeply into the epidermis and provide these effects [17], are more important for activating the production of collagen and mucopolysaccharides of the skin, increasing its elasticity, activating fibroblasts, strengthening the skin and the disappearance of deep wrinkles [17].

Therefore, the optimum parameters of the process of fermentation of proteins in nanofiltration serum concentrate KSB-65 are as follows: temperature 40 ºC, neutral peptidase C content – 0.78 U/g, duration of fermentolysis – 3.17 hours.

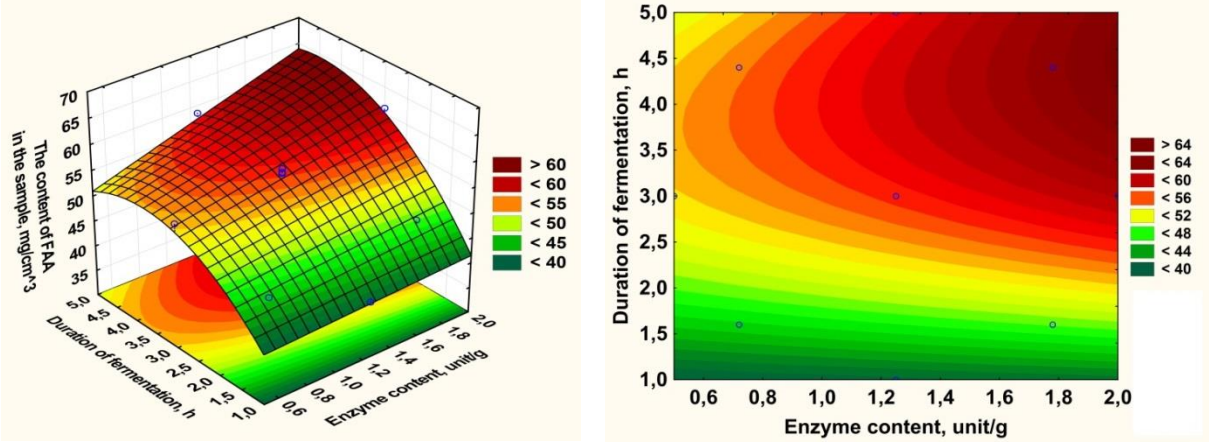
The hydrolysate of whey proteins obtained using the recommended optimal parameters of the fermentolysis process can be used directly for the production of cosmetics (anti-age cream, serums, masks, scrubs, shampoos, body lotions and hair, body gels, etc. [1]) subject to receiving it at a cosmetic company.

If the indicated hydrolyzate of whey proteins is expected to be obtained in a dairy processing plant, it will become a semi-finished product for obtaining dry hydrolysates of whey proteins, obtained by lyophilic drying followed by gamma sterilization in the container. In addition, the resulting hydrolyzate can be a raw material for subsequent hydrolysis of proteins using fermentation compositions from cultures of lactose bifidobacteria, incl. probiotic, in order to obtain dry concentrates of short-chain peptides, free amino acids enriched with viable cultures of probiotics obtained by lyophilic drying, or their lysates (using gamma sterilization) [18-20].



a) b)

Fig. 2. Dependence of the content of short-chain peptides (CP, mg/cm^3) on the mass fraction of the enzyme (C_e, U/g) and the duration of fermentation (DF, hours)



a) b)

Fig. 3. Dependence of the content of free amino acids (BA, μg/cm^3) of the mass fraction of the enzyme (C_e, U/g) and duration of fermentation (DF, h)

The mass concentration of free amino acids in the reaction mixture increases with an increase in the content of peptidase from 0.5 to 2.0 U/g and an extension of the duration of fermentolysis from 1.0 to 5.0 hours from 39.81 to 63.92 μg/cm^3 (Fig. 3). The maximum concentration of free amino acids is achieved with maximum content in the reaction mixture of the enzyme and the duration of the fermentolysis for 5 hours.

Increasing content of free amino acids with increasing enzyme concentration and lengthening the duration of fermentolysis is probably due to the fact that parallel to the cleavage of free amino acids from the serum proteins, the breakdown of the newly formed short chain peptides in the reaction mixture with the formation of free amino acids is evident, as evidenced by a decrease in the content of CP at the content of the enzyme more than 0.78 U/g and duration of fermentolysis over 3.17 hours (Fig. 2).

Conclusions

1. The research of manufacture of the food industry products, including dairy products, has been conducted, and it is substantiated that whey, as a product of milk processing, is an additional kind of dairy raw material, which can be used for the manufacture of various products, including cosmetics, the production and sale of which will contribute to ensuring the efficiency of the functioning and competitiveness of the enterprise, including by the transformation of its activities.

2. The process of fermentolysis of serum proteins in a nanofiltration concentrate KSB-65 with a dry matter content of 20% using neutral peptidase C at a temperature of 40°C with a variation in the duration of the process from 1 to 5 hours, the content of peptidase – from 0.5 to 2.0 U/g.

3. The content of short-chain peptides and free amino acids in samples of KSB-65 nanofiltration concentrate after fermentolysis according to selected parameters was determined. It is concluded that the optimum parameters of the fermentolysis of serum proteins in the nanofiltration concentrate KSB-65 by neutral peptidase C are as follows: temperature 40°C, neutral peptidase C content – 0.78 U/g, duration of fermentolysis – 3.17 hours. With optimal parameters of the fermentolysis process, the hydrolyzate of the nanofiltration concentrate KSB-65 contains the maximum amount of short chain peptides (57.03 mg/cm^3) and a high concentration of free amino acids (54.66 μg/cm^3).

4. Recommendations for the further use of serum protein hydrolyzate obtained using the recommended optimal parameters of the fermentolysis process from the KSB-65 nanofiltration concentrate, in the production of cosmetics and hydrolysates of proteins enriched with probiotic cultures of lactose bifidobacteria or their lysates were given.

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