

Важливим джерелом біоактивних пептидів є гідролізовані продукти на основі молочної сироватки: гіпоалергенні продукти, гідролізати для дитячого харчування, продукти для спортсменів. Проте при їх виробництві використовують протеолітичні препарати різного походження. Це може відобразитися на ступені протеолізу протеїнів-попередників біологічно активних пептидів (БАП), молекулярно-масовому розподілі продуктів протеолізу і, відповідно, ймовірності утворення БАП. Було проведено порівняння ступеню протеолізу протеїнів концентрату сироваткових білків (КСБ) і встановлення молекулярно-масового розподілу продуктів протеолізу, отриманих за дії протеолітичних препаратів тваринного, рослинного і мікробіологічного походження. Для проведення протеолізу були використані наступні ензимні препарати: папаїн, нейтральна протеаза, трипсин, хімотрипсин і панкреатин. Як субстрат використовували КСБ. Перед протеолізом в ньому було охарактеризовано фракційний склад протеїнів та молекулярно-масовий розподіл протеїнів і пептидів. Протеоліз 15 % розчину КСБ проводили при температурі 37 °С, значенні рН 7,9 та співвідношенні ензим:субстрат – 1:20. Під час протеолізу періодично відбирали зразки для спектрофотометричного визначення продуктів протеолізу, розчинних в 5 % трихлороцтовій кислоті (ТХО). Молекулярно-масовий розподіл отриманих поліпептидів і пептидів встановлювали гель-фільтрацією реакційної суміші після осадження нерозщеплених протеїнів в 5 % ТХО.

В результаті проведених досліджень було встановлено, що основна кількість продуктів протеолізу утворювалася в перші 30–60 хвилин. Протеоліз в основному завершувався до 120 хвилин. Гель-фільтрацією на сефадексі G-50 було показано, що гідролізати відрізняються за молекулярно-масовим розподілом. Найвища кількість низькомолекулярних пептидів ($M < 1500$ Да) була отримана за дії нейтральної протеази (29 %) і панкреатину (25 %). Основний попередник БАП – β -лактоглобулін, за результатами електрофорезу, показав найвищу чутливість до дії нейтральної протеази (79 %), панкреатину (81 %) і трипсину (71 %)

Ключові слова: концентрат сироваткових білків, протеолітичні препарати, протеоліз, біоактивні пептиди, гель-фільтрація, електрофорез

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COMPARISON OF PRODUCTS OF WHEY PROTEINS CONCENTRATE PROTEOLYSIS, OBTAINED BY DIFFERENT PROTEOLYTIC PREPARATIONS

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1. Introduction

In recent years, the value of dairy protein products has often been associated with the biological action of the proteins themselves, as well as the products of their normal cleavage by digestive proteases – bioactive peptides. In particular, this concerns whey proteins. To date, hundreds of peptides that positively affect the functions of the nervous, cardiovascular, and digestive systems of the body have been found [1]. Some peptides play an important role in the development of the immune system, exhibit bactericidal and anticarcinogenic actions. In many cases, the conditions in which bioactive peptides are formed and the mechanism of their action have already been established [2, 3].

Proteolysis is one of the important ways of milk whey processing [4]. In particular, athletes' food products, infant formula, hypoallergenic products, and enteral nutrition products are produced with the help of proteolysis [5]. Different enzyme proteolytic preparations of animal, plant and microbiological origin are used in the technologies of these products [6]. Proteolysis is performed in optimal conditions for each of these preparations. The conditions and specificity of proteolytic action on whey proteins may significantly differ. In this context, the following questions arise: whether the positive potential laid by nature in the form of bioactive peptides is lost, and whether bioactive peptides can be formed in case of using different proteolytic preparations? Therefore, it is actual to compare the character of whey pro-

teins proteolysis with proteolytic preparations of different origin.

2. Literature review and problem statement

In the process of milk whey proteins proteolysis, a large number of different bioactive peptides (BAP) are formed in the gastrointestinal tract. This has been proven in many *in vitro* and *in vivo* studies. β -lactoglobulin (β -LG), α -lactalbumin (α -LA) and lactoferrin (LF) are the main precursors of BAP among whey proteins [7]. Most (about 75 % of known BAP) were obtained as a result of proteolysis of whey protein precursors by pancreatic enzymes [2]. First of all this concerns trypsin and chymotrypsin, as well as the combined action of pancreatic proteases. Several peptides with bactericidal action are formed in the result of proteolysis of LF by enzymes of gastric juice: pepsin and chymosin. Individual peptides with antihypertensive activity were obtained by the action of neutral protease from *Bacillus subtilis*, as well as proteinase K [8].

BAP from whey proteins can be divided into four groups by biological action on the physiological systems of the body. These are BAP that affect the cardiovascular system (antihypertensives), the nervous system (opiate receptor agonists), the digestive system (regulators of intestinal motility) and the immune system (immunomodulatory and antimicrobial peptides) [7]. In addition, a large group of BAP can inactivate radicals and have antioxidant effects [9, 10]. Such new biological activities as antidiabetic activity and regulation of appetite are of great interest. The mechanism of action of antidiabetic peptides is the inhibition of dipeptidyl peptidase IV, which splits insulinotropic polypeptide [11, 12]. Complex mechanism of appetite regulation with the action of BAP from milk whey proteins is on the stage of the study [13]. The areas of the primary structure corresponding to BAP are unevenly distributed between the major whey protein fractions. Most of these sequences were found in β -LG. About 50 % of the amino acid residues of β -LG are in the composition of various BAP [2]. Less of such sequences are in the composition of α -LA (about 39 % residues) and LF (about 10 % residues). BAP also has a certain fractional specificity by the biological action. Thus, β -LG is a precursor to all known types of BAP except immunomodulatory. α -LA does not form BAP with hypocholesterolemic action and peptides affecting intestinal motility. BAP from LF have only two types of biological action: immunomodulatory and bactericidal.

Many studies have shown that BAP, which are formed as a result of normal digestion of whey proteins in the gastrointestinal tract, have a positive effect on the organism [1]. Hydrolyzed products based on milk whey (hypoallergenic products, baby hydrolysates, products for athletes) can be an important source of such BAP. In the production of such products, proteolytic preparations of different origin are used. Neutral protease produced by *Bacillus subtilis* strains, Flavoenzyme preparation from *Aspergillus oryzae* culture, and also pancreatic from pancreas can be applied to them. It has been shown that β -LG is resistant to pepsin, but is well cleaved by papain, trypsin and neutral protease in the process of hypoallergenic mixtures obtaining. Various preparations of microbiological and plant origin are also used in the production of products for athletes [14, 15]. These preparations have different specificity and activity [6]. At the same

time, most of the natural BAP are formed by the action of the proteolytic enzymes of the gastrointestinal tract. Numerous BAP have been discovered in recent decades and are currently at the stage of research [7]. Selection of proteolytic preparations for the production of whey protein hydrolyzates was carried out, as a rule, without taking into account the possibility of BAP formation. This situation is due to the fact that BAP from milk whey proteins were discovered much later than other BAP from milk [2]. By that time, most technologies that include the stage of whey proteins proteolysis had already been developed. Of course, the specificity and degree of proteolysis of BAP proteins-precursors have not been taken into account in these technologies. The main attention was turned to organoleptic and technological parameters [16]. It is obvious that the replacement of enzyme preparations will have little influence on the technological processes of hydrolysed products production and their cost price, but can significantly increase their value due to the formation of natural BAP. The possibility of the natural BAP formation is closely related to the degree of proteolysis of individual BAP proteins-precursors, as well as to the molecular weight distribution of the obtained proteolysis products [17]. The question of comparing these indicators by the action of proteolytic preparations of different origins on milk whey proteins remains unresolved.

3. The aim and objectives of the research

The aim of the research is to compare the degree of whey protein concentrate proteins proteolysis and to establish the molecular weight distribution of proteolysis products obtained by the action of proteolytic preparations of animal, plant and microbiological origin.

To achieve this goal, the following tasks were formed:

- to establish dynamics of whey protein concentrate proteolysis by the action of papain, neutral protease, trypsin, chymotrypsin and pancreatin;
- to carry out gel filtration and to establish molecular weight distribution of proteolysis products of whey protein concentrate by the action of various proteolytic preparations;
- to establish the sensitivity of different protein fractions of whey protein concentrate to the action of proteolytic preparations with the help of electrophoresis in polyacrylamide gel.

4. Materials and methods of spectrophotometric, chromatographic and electrophoretic studies of milk whey proteins proteolysis

4.1. Materials, reagents and equipment used in studies of whey protein concentrate proteolysis products

The following enzymatic preparations were used for proteolysis: neutral protease and papain from “Barret industrial limited” (UK), trypsin and chymotrypsin from “Biozym” (Germany) and pancreatin produced by PJSC “Technolog” (Ukraine). Whey protein concentrate produced at LLC “Buchach Cheese Factory” (Ukraine) according to the TU U 15.5-00419880-XX: 2011 “Whey Protein Concentrate (WPC-UF). Specifications” was used as the substrate.

Its moisture content was 7.85 % and protein content was 72.00 %. Bovine serum albumin (BSA) from “Sigma” (Germany) and cow’s milk β -LG were used to identify pro-

tein fractions on electrophoregrams. Homogeneous β -LG was obtained from milk whey by repeated gel filtration [18]. To isolate the whey of milk, the pH of fresh skim milk was adjusted by 0.1 N HCl with stirring to 4.6 achieving the isoelectric precipitation of caseins. After that, the casein aggregates were precipitated by centrifugation on an OPN-8 centrifuge (5,000 rpm, 10 minutes). The resulting whey was centrifuged twice.

Sephadex G-50 from "Pharmacia" (Sweden) company was used for gel filtration. Gel filtration was carried out in columns of "Reanal" (Hungary) company. To obtain samples for gel filtration, 3 cm³ of whey protein concentrate (WPC) hydrolyzate was mixed with 3 cm³ of 10 % TCA solution and kept for 20 min to precipitate the unsplit proteins. The resulting precipitate was filtered off and 6.5 cm³ of 5 % acetic acid solution was added to 1 cm³ of the filtrate. 2 cm³ of the prepared solution was applied on the gel filtration column. Electrophoresis in polyacrylamide gel (PAG) slabs was performed in the Stadier type apparatus. Buffer and PAG solutions for electrophoresis were prepared using "Reanal" company reagents and high purification reagents produced in Ukraine. Spectrophotometry of whey protein concentrate proteolysis products was performed by the SF-46 spectrophotometer. To determine the concentration (mg/cm³) of whey proteins and products of its proteolysis, the known absorption coefficient ($D_{1\%}$) – 12.3 has been used. An absorption coefficient of 9.6 was used to determine the β -LG concentration and 6.7 for the BSA [18].

4. 2. Spectrophotometric, chromatographic and electrophoretic methods for the research of proteolysis of whey protein concentrate

The proteolytic activity of enzyme preparations was determined spectrophotometrically by the method of V. F. Selemenev [19]. Gel filtration of the proteolysis products was performed on a column (75×1.5 cm) from the "Reanal" company liquid chromatography kit. The elution rate was set at 20 cm³/h. 5 cm³ of the eluate were collected into the fraction. The concentration of proteins and proteolysis products in the chromatographic fractions was determined spectrophotometrically by absorption at a wavelength $\lambda=280$ nm.

The fractional composition of WPC and proteolysis products was analyzed by the method of express electrophoresis in the anode system of homogeneous PAG [20]. Quantitative processing of electrophoregrams was performed by using the image reading function imread [21].

The mathematical and statistical processing of the study results was performed using Microsoft Office Excel 2007 software packages.

5. Results of whey protein concentrate proteolysis products researches

5. 1. Comparisons of the dynamics of whey protein concentrate proteolysis by the action of different proteolytic preparations

The fractional composition of proteins and the molecular weight distribution of proteins and peptides were characterized before conducting the WPC proteolysis. The results of 1 % water WPC solution gel filtration on a column with Sephadex G-50 are shown in Fig. 1, *a*. The chromatogram was divided into three sectors (I, II, III) taking into account the volume of elution. The chromatographic fractions of each sector

were combined and spectrophotometrically determined the amount of proteins and peptides. By the results of the three gel filtrations, it has been shown that the first sector contains 31 % of all WPC proteins. Their molecular weight (M) is $>30,000$ Da. The second sector (1,500 Da $< M < 30,000$ Da) contains 67 % of proteins and peptides, and the third ($M < 1,500$ Da) contains 2 %. The results of the electrophoretic analysis of the fractional composition of WPC proteins are shown in the densitogram (Fig. 1, *b*). The densitogram shows a typical distribution of whey protein fractions: β -LG, α -LA, serum albumin (BSA), immunoglobulins (IG), and some high molecular weight components of the proteose-peptone fraction (PPF).

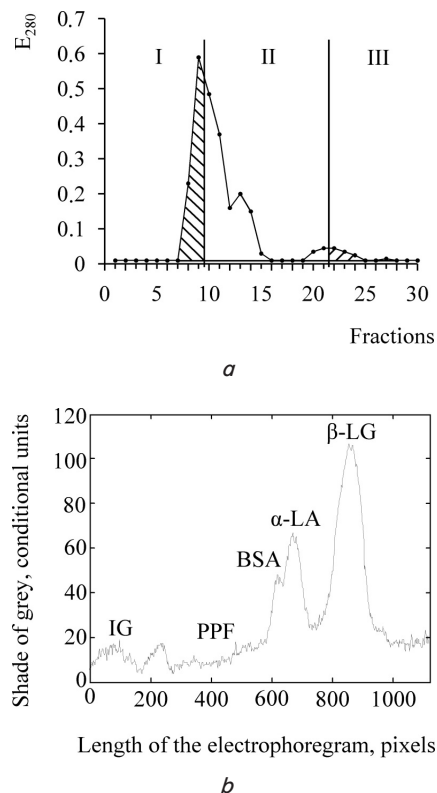


Fig. 1. Chromatogram and densitogram of whey protein concentrate electrophoregram: *a* – chromatogram of whey protein concentrate obtained by gel filtration on Sephadex G-50; *b* – densitogram of whey protein concentrate electrophoregram

Proteolysis of 15 % solution of WPC was performed at the ratio of enzyme: substrate – 1:20. Such ratios are used to obtain whey protein hydrolysates [15, 22]. Before the proteolysis, indicator b , which is proportional to the proteolytic activity of the preparation, was determined for enzymatic preparations by the method of Selemenev [19]. The ratio of the most active preparation (chymotrypsin, $b=5.67$) to the substrate was set as 1:20. The concentration of other preparations was increased according to indicator b . It was 5.55 for neutral protease, 2.67 for pancreatin, 2.02 for trypsin and 1.35 for papain.

Proteolysis was carried out at a temperature of 37 °C and a pH of 7.9. During proteolysis, samples were periodically taken off for spectrophotometric determination of proteolysis products soluble in 5 % trichloroacetic acid (TCA). In addition, samples were taken for chromatographic and electrophoretic analysis. The results of the study of the formation of TCA-soluble products of WPC proteolysis are shown in Fig. 2.

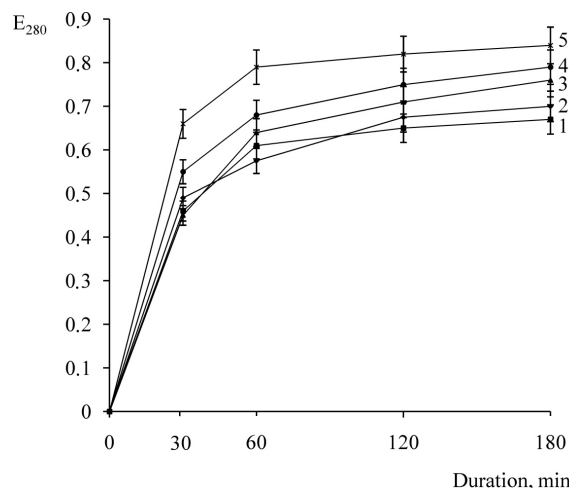


Fig. 2. Proteolysis of whey protein concentrate by proteolytic preparations: 1 – chymotrypsin; 2 – trypsin; 3 – papain; 4 – pancreatin; 5 – neutral protease

As can be seen on the plots, proteolysis passes intensively first 60 minutes and mainly completes up to 120 minutes. The highest degree of proteolysis was shown by neutral protease and pancreatin.

5.2. Gel filtration of whey protein concentrate proteolysis products on Sephadex G-50

It is important to establish the molecular weight distribution of the resulting polypeptides and peptides while conducting whey proteins proteolysis. For this purpose, gel filtration of the reaction mixture, taken off at the 60-th and 120-th minutes of proteolysis, was carried out after the TCA precipitation of unsplit proteins. The results of proteolysis products gel filtration on Sephadex G-50 are shown in Fig. 3, 4. It can be seen that the chromatographic profiles of proteolysis products can significantly differ even at close values of the proteolysis degree. This can be seen comparing the chromatograms shown in Figs. 3, *d*, 4, *d*. Also, with a slight change in the value of the proteolysis degree, the ratio of the products number differing in molecular weight can be intensely changed (Fig. 3, *c*, *d*).

All chromatograms were divided into three sectors, as was done with the gel filtration of the WPC (Fig. 1, *a*). In the combined fractions, by the results of three gel filtrations, the average content of proteolysis products from: $M < 1,500$ Da, $1,500 \text{ Da} < M < 30,000$ Da and $M > 30,000$ Da was determined (Table 1).

Table 1 shows the values in a percentage of the amount of proteolysis products soluble in 5% TCA from the total amount of proteins and pepti-desin WPC that were used for gel filtration.

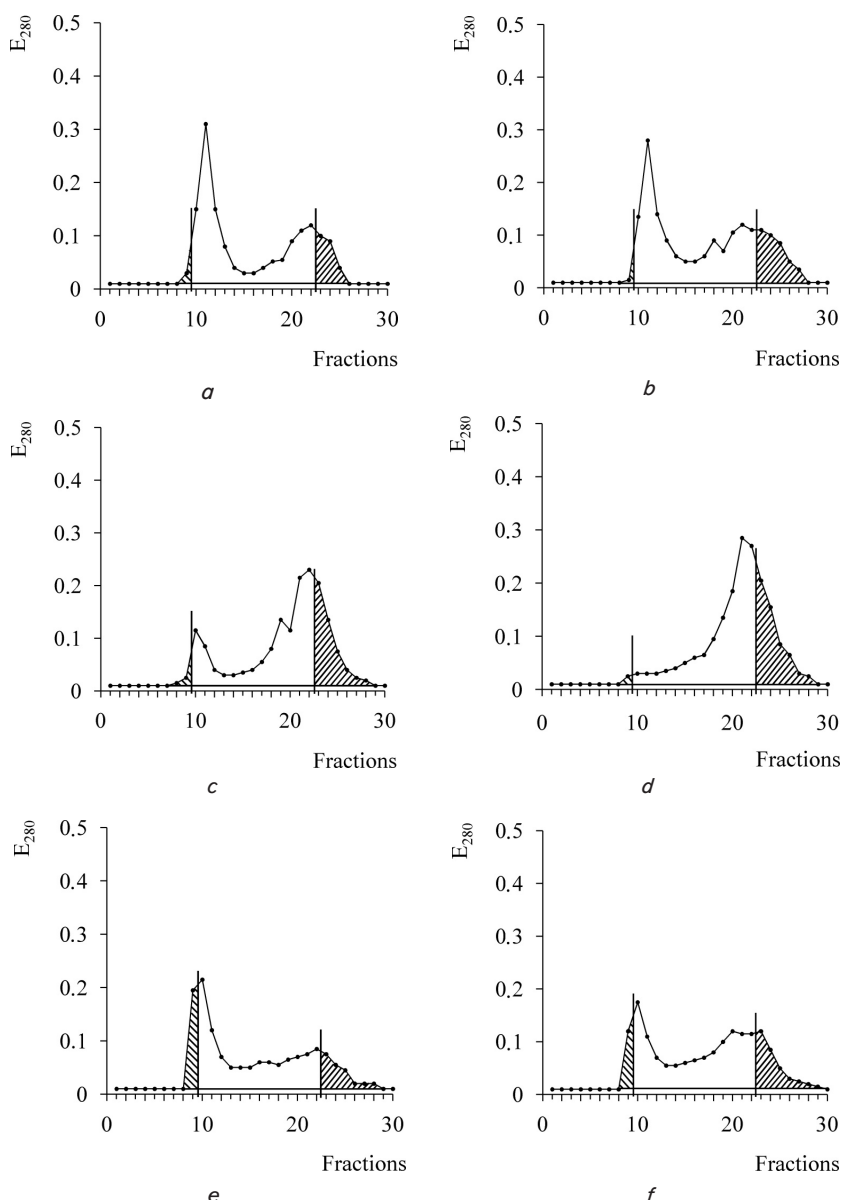


Fig. 3. Chromatograms of WPC proteolysis products obtained at the 60-th and 120-th minutes of proteolysis by the action of enzyme preparations: *a* – papain (60 min); *b* – papain (120 min); *c* – neutral protease (60 min); *d* – neutral protease (120 min); *e* – trypsin (60 min); *f* – trypsin (120 min)

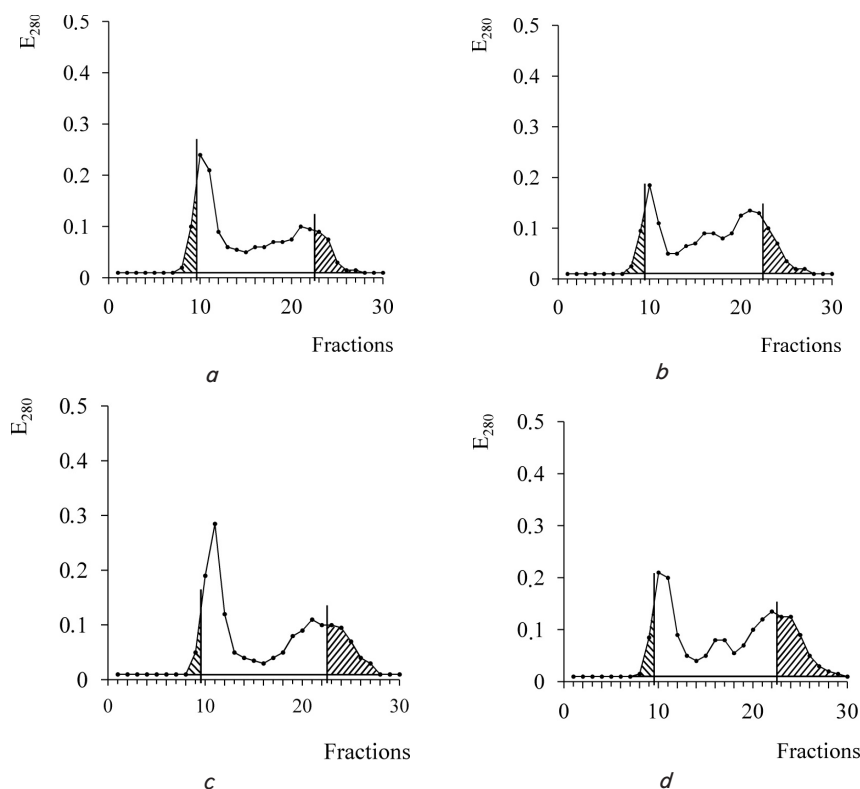


Fig. 4. Chromatograms of WPC proteolysis products obtained at the 60-th and 120-th minutes of proteolysis by the action of enzyme preparations: *a* – chymotrypsin (60 min); *b* – chymotrypsin (120 min); *c* – pancreatin (60 min); *d* – pancreatin (120 min)

Table 1

Molecular weight distribution of WPC proteolysis products by proteolytic preparations according to the gel filtration on Sephadex G-50 ($M \pm m, n=3$)

Proteolytic preparation	Proteolysis products with $M \geq 30,000$ Da		Proteolysis products with $1,500 < M < 30,000$ Da		Proteolysis products with $M \leq 1,500$ Da		The total amount of proteolysis products*	
	mg	%	mg	%	mg	%	mg	%
The duration of proteolysis 60 minutes								
Papain	4.0±0.8	3	100±5.2	82	18±1.4	15	123±7.8	57
Neutral protease	3.1±0.5	2	107±5.3	71	41±2.5	27	151±9.1	70
Trypsin	16.6±1.3	14	91±3.7	73	16±1.2	13	124±7.0	57
Chymotrypsin	11.0±0.9	8	106±7.0	79	18±2.3	13	135±7.2	63
Pancreatin	4.3±0.7	3	107±9.1	75	31±2.0	22	143±10.0	66
The duration of proteolysis 120 minutes								
Papain	2.7±0.5	2	108±6.3	75	33±3.2	23	144±6.7	67
Neutral protease	2.3±0.4	1	114±8.1	70	46±4.9	29	163±9.1	75
Trypsin	12.3±1.2	9	102±6.4	73	25.6±2.4	18	140±8.7	65
Chymotrypsin	11.3±1.1	8	110±7.3	79	18.7±2.7	13	141±10.5	65
Pancreatin	5.1±0.8	3	109±10.3	72	38.7±3.3	25	153±7.9	71

Note: * – the percentage of the protein content in the sample taken for gel filtration

5. 3. Electrophoresis of whey protein concentrates protein fractions in polyacrylamide gel

Milk whey protein fractions are characterized by different resistance to proteolytic enzymes. Therefore, to determine the origin of proteolysis products, it is important to establish the fractional composition of WPC proteins that had not been cleaved during proteolysis by various enzyme preparations. For this purpose, samples taken at different stages of proteolysis (0 min, 60 min, 120 min and 180 min) were analyzed by electrophoresis. The express method previously proposed

for the analysis of a large number of milk whey protein samples was used for analysis [20]. Densitometric analysis of the electrophoregram of the sample taken at the 120-th minute of proteolysis was also performed in all cases. The results of electrophoretic and densitometric analysis are shown in Fig. 5, 6. In all cases, as can be seen on the electrophoregrams, complete cleavage of the protein fractions does not occur during 180 minutes of proteolysis. Densitometric studies testify about differences in the degree of protein fractions cleavage by different proteolytic preparations.

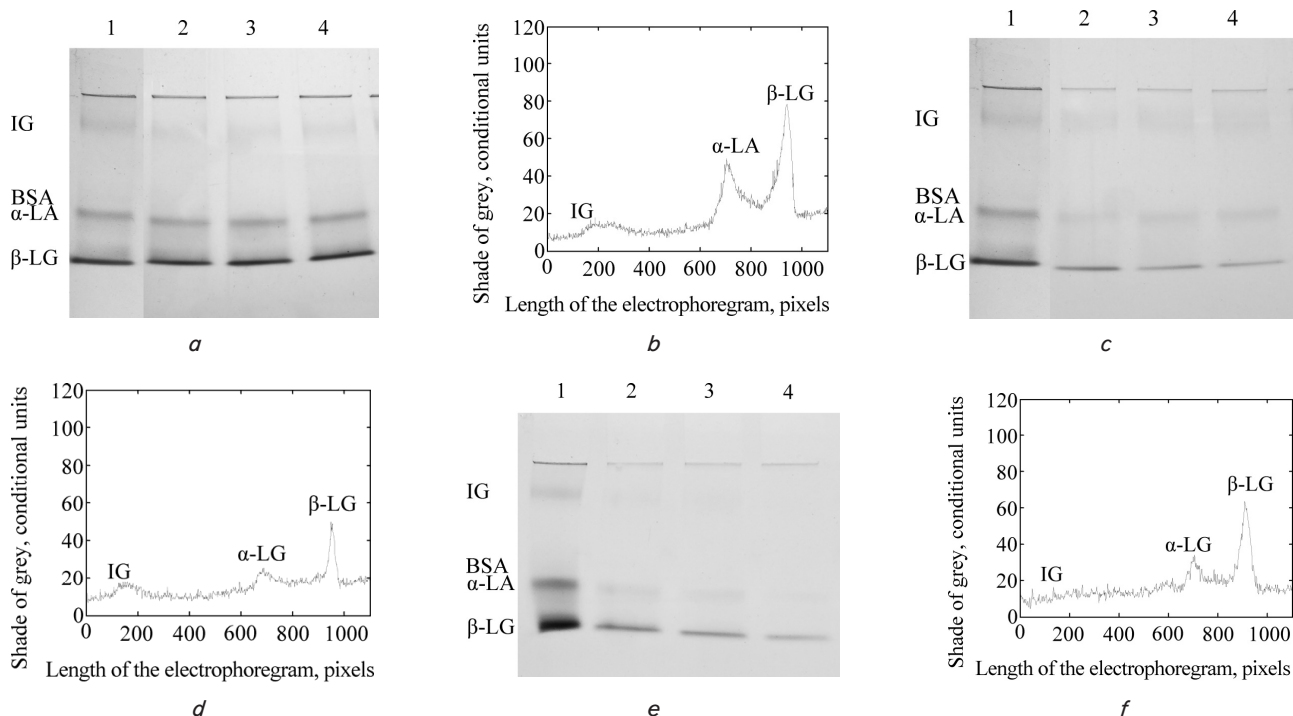


Fig. 5. Electrophoregrams (1 – 0 min; 2 – 60 min; 3 – 120 min; 4 – 180 min) and densitograms (120 min) of whey proteins proteolysis products obtained at different stages of enzyme preparation action: *a* – papain (electrophoregram); *b* – papain (densitogram); *c* – neutral protease (electrophoregram); *d* – neutral protease (densitogram); *e* – trypsin (electrophoregram); *f* – trypsin (densitogram)

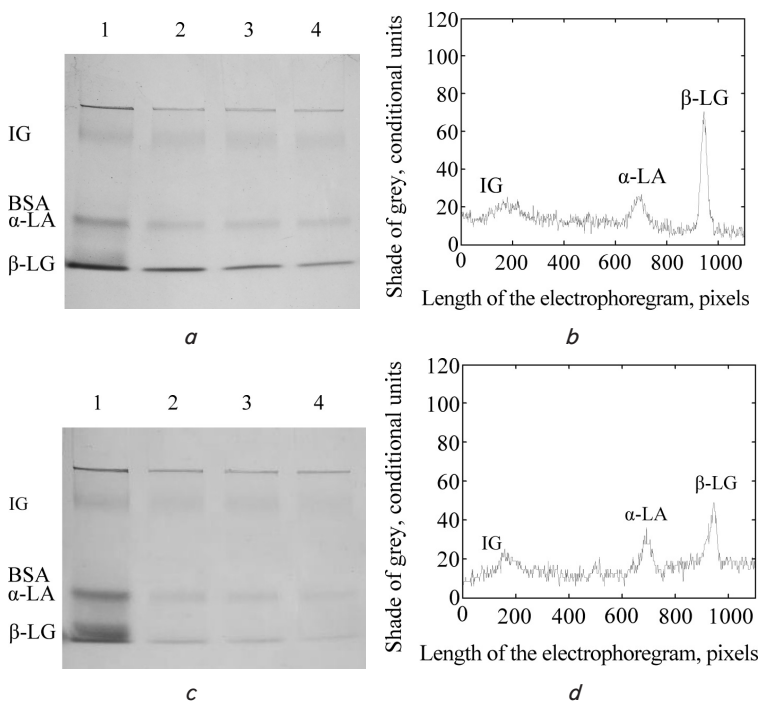


Fig. 6. Electrophoregrams (1 – 0 min; 2 – 60 min; 3 – 120 min; 4 – 180 min) and densitograms (120 min) of proteolysis products of whey proteins obtained at different stages of enzyme preparation action: *a* – chymotrypsin (electrophoregram); *b* – chymotrypsin (densitogram); *c* – pancreatin (electrophoregram); *d* – pancreatin (densitogram)

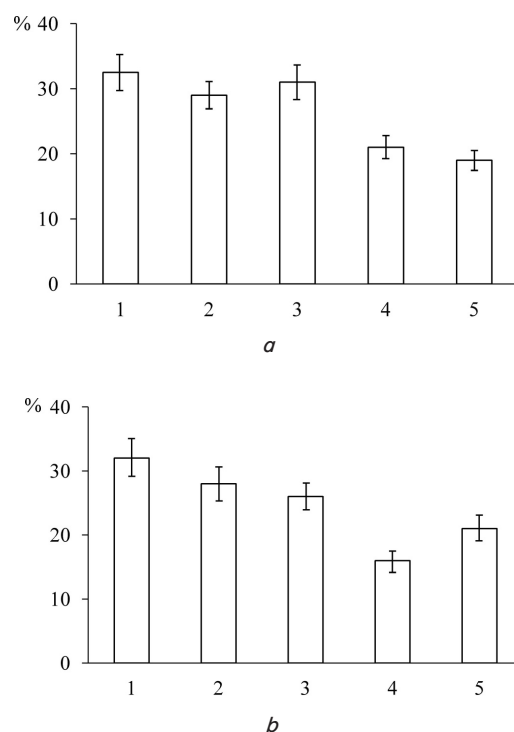


Fig. 7. Percentage of non-hydrolyzed WPC protein fractions by the action of papain (1), trypsin (2), chymotrypsin (3), neutral protease (4) and pancreatin (5): *a* – β-LG; *b* – α-LA

By the area of the corresponding peaks on the densitograms, the residual amount of unsplit β-LG and α-LA after 120 minutes of proteolysis has been calculated. The results are shown on the diagrams (Fig. 7).

About 80 % of β-LG and α-LA are cleaved by neutral protease and pancreatin. About 70 % of the proteins in these fractions hydrolyze trypsin and chymotrypsin.

6. Discussion of the results of whey protein concentrates proteolysis

The comparative characterization of the proteolytic preparations action was performed with a substrate “Whey protein concentrate”. This substrate contains all major whey proteins that are precursors of natural BAP. The content of these proteins according to the results of electrophoretic analysis (Fig. 1) is close to their content in milk whey [23].

Proteolytic preparations, which provide a high degree of proteolysis are used in the process of whey proteins hydrolysis [14, 22]. An important indicator while obtaining hypoallergenic products based on whey proteins is low molecular weight peptides content [15]. The preliminary equalization of the total proteolytic activity of five enzyme preparations of different origin gave in the result close values of the proteolysis degree (Fig. 2). At the same time, the proteolysis products obtained by the action of used enzymatic preparations may differ in molecular weight distribution, origin from different milk whey proteins fractions and, accordingly, in the primary structure. It is known that the main part of natural BAP has a molecular weight in the range from 200 to 1,500 Da [1, 2]. Gel filtration on Sephadex G-25 is often used for the molecular weight of milk whey proteins proteolysis products characterization. In this case, a part of the BAP (with $M < 1,000$ Da) is eluted with a volume equal to the full volume of the chromatographic column. The other part of the BAP is eluted with the volume between full and free volume. When using Sephadex G-100 [22], all BAP, as well as a part of large polypeptides (up to 50 amino acid residues), are eluted with full column volume. Sephadex G-50 was taken for gel filtration, which allows obtaining a group of chromatographic fractions containing almost all natural BAP. These are fractions that are eluted with a volume equal to the full volume of the column. On Fig. 3, 4, these fractions are in the third sector of chromatograms. The largest amount of proteolysis products in the fractions of this sector is formed by the action of neutral protease and pancreatin (Table 1).

Electrophoresis in PAG is rarely used for the analysis of short peptides because they are poorly fixed even in PAG with high concentration. However, electrophoresis can be used to establish proteins fractions that are not cleaved during proteolysis. It is known that the method of express electrophoresis in the anode system of homogeneous PAG allows to reliably identify the major whey proteins that are precursors of BAP [20]. Using this electrophoretic system allowed to obtain appropriate densitograms (Fig. 5, 6) and to quantitatively evaluate the splitting of individual protein fractions by the action of enzyme preparations (Fig. 7). The smallest amount of unsplit β -LG, which is the main precursor of BAP, was obtained by neutral protease and pancreatin. The second important precursor of BAP – α -LA is actively cleaved by pancreatin, neutral protease and chymotrypsin. Literature data confirm the effectiveness of trypsin and pancreatin in the production of whey proteins hydrolyzates [15]. Comparing the WPC proteolysis degree (Table 1) and the residual of the unsplit fractions, some discrepancy can be seen (Fig. 7). Obviously, part of the high molecular weight products of proteolysis is precipitated by 5% TCA, but is not fixed in the PAG. Such

polypeptides may also be fixed in the PAG but do not form clear bands on electrophoregrams.

According to literary data, almost all known natural BAP from whey proteins are formed by the action of pancreatin, as well as trypsin and chymotrypsin, which are part of its composition [2]. This is due to the specificity of the enzymes of the complex preparation. BAP from whey proteins are characterized by a certain primary structure that strictly determines their properties and biological action [7, 24, 25]. In the case of plant (papain) and microbiological (neutral protease) proteases origin, the primary structure of the peptides will differ [6]. The probability of natural BAP formation, in this case, will decrease.

The obtained results testify about the differences in molecular weight distribution and fractional origin of the proteolysis products of whey proteins obtained by the action of various proteolytic preparations with the same total proteolytic activity. However, in this research, the primary structure of peptides in proteolysis products was not determined, which is necessary to establish their compliance to known BAP from milk whey protein. This is important for a more accurate comparison of the proteolytic preparations action. But, even the results already obtained in the research prove that in order to preserve the potential of natural BAP in final products, it is important to carry out proteolytic processes in the conditions that reflect the processes of normal digestion by the action of the pancreatic proteases.

7. Conclusions

1. At proteolysis of WPC 15% solution (E:S=1:20) in physiological conditions (pH 7.9 and 37 °C) by proteolytic preparations (papain, neutral protease, trypsin, chymotrypsin and pancreatin) during 180 minutes, close concentrations of proteolysis products soluble in 5% TCA were obtained. The majority of proteolysis products were formed in the first 30-60 min. Proteolysis was mostly completed by 120 min.

2. The amount of products and the degree of WPC proteolysis at 60 and 120 minutes were determined by the gel filtration on the Sephadex G-50. They were respectively: for papain – 123 ± 7.8 mg (57%) and 144 ± 6.7 mg (67%); for neutral protease – 151 ± 9.1 mg (70%) and 163 ± 9.1 mg (75%); for trypsin – 124 ± 7.0 mg (57%) and 140 ± 8.7 mg (65%); for chymotrypsin – 135 ± 7.2 mg (63%) and 141 ± 10.5 mg (67%); for pancreatin – 143 ± 10.0 mg (66%) and 153 ± 7.9 mg (71%). The content of proteolysis products with $M < 1,500$ Da, $1,500 \text{ Da} < M < 30,000 \text{ Da}$, $M > 30,000 \text{ Da}$ was also investigated by dividing the chromatograms into sectors. The highest amount of low molecular weight peptides ($M < 1,500$ Da) was obtained by the action of neutral protease (29%) and pancreatin (25%). It is also shown that at close values of the degree of proteolysis, the ratio of products by molecular weights can be significantly different.

3. Electrophoretic studies of WPC hydrolyzates testify about differences in the accessibility of different protein fractions to proteolytic preparations. The highest degree of proteolysis (about 80%) of the β -LG and α -LA fractions was shown by pancreatin and neutral protease.

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