UDC: 577.112.7:577.114.7:575.222.7–022.532

OBTAINING AND CHARACTERISTICS OF MANNAN-PEPTIDE NANOHYBRID

N. Cherno, Doctor of Technical Sciences, Professor
L. Gural, Candidate of Technical Sciences, Associate Professor
K. Naumenko, Candidate of Technical Sciences, Associate Professor
Department of Food Chemistry and Expertise
Odessa National Academy of Food Technologies
112, OdessaKanatnaya Str., Ukraine, 65039

DOI: https://doi.org/ 10.15673/fst.v15i4.2251

Article history

Received 22.08.2021
Reviewed 12.09.2021
Revised 17.10.2021
Approved 01.12.2021

Correspondence:

L. Gural
E-mail: gural.onaft@gmail.com

Cite as Vancouver style citation

Cherno N, Gural L, Naumenko K. Obtaining and characteristics of mannan-peptide nanohybrid. Food science and technology. 2021;15(4):40-47. DOI: https://doi.org/ 10.15673/fst.v15i4.2251

Цитування згідно ДСТУ 8302:2015

Cherno N., Gural L., Naumenko K. Obtaining and characteristics of mannan-peptide nanohybrid // Food science and technology. 2021. Vol. 15, Issue 4. P. 40-47. DOI: https://doi.org/ 10.15673/fst.v15i4.2251

Abstract. Bioactive peptides belong to a new generation of biologically active regulators with a wide range of important physiological effects. This determines the prospects for their use in functional foods as dietary supplements and medicine. The main source of such bioactive peptides is cow's milk. It contains proteins, which include 80% caseins. Like other bioactive compounds, they are exposed to unwanted changes in technological processes and during transit along the gastrointestinal tract. In order to improve their stability and increase bioavailability, conjugates obtained by the Maillard reaction have recently been used. Currently, it is positioned as a promising way to prevent the destructive impact of external factors. This article highlights the results of studies designed to determine the feasibility of casein fragmentation with papain to obtain bioactive peptides and the subsequent creation on their basis of a nanohybrid with a mannan component by the Maillard reaction. Enzymatic hydrolysis of sodium caseinate with papain was carried out with varying the duration of the hydrolysis process (30, 60, 120, 180, 240 min). The yield of protein proteolysis products depending on the duration of the process ranged from 77.8% to 83.9%.Enzymatic hydrolysis of sodium caseinate contributed to a significant increase in the products of its fragmentation mass fraction of amine Nitrogen – from 4561.4 to 5687.5 mg/100 g (4.6–5.7%), which is 3.5–4.4 times more than the content of free amino groups in the original sodium caseinate. Among them, almost ½ arebioactive peptidescontaining up to 20 amino acid residues.The mannan-peptide nanohybrid was incubated with biopeptides with modified coffee mannan, which had the ability to dissolve in water.The reaction mixture was exposed to 60°C for 6 h. The yield of lyophilized product was 38.1%, the mass fraction of mannan reached 10.6–10.9%, and peptides, respectively, – 89.1–89.4%. The protective effect of the polysaccharide component against the peptide component was evaluated in vitro in a pepsin-trypsin system. It was found that the stability of peptides in the nanohybrid is much higher than in the original hydrolyzate. The use of the created nanohybrid is possible both to obtain protective shells of labile biologically active substances and to increase the bioavailability of peptides when they are included in dietary supplements and functional foods.

Key words: sodium caseinate, papain, bioactive peptides, mannan, Maillardreaction, mannan-peptide nanohybrids.

Introduction. Formulation of the problem

During the second half of the last and the beginning of this century, the concept of biological function of dietary proteins has changed significantly. If previously food proteins were considered only as biopolymers capable of providing amino acids to living organisms for the formation of the protein component of organs and tissues, as well as involved in the production of metabolic energy, today it is known that some proteins present in food and their fragments or peptides can participate in the overall metabolism in a much more complex way. This is due to their biological activity, which goes far beyond the nutritional functions that were discovered by the end of the first half of the last century.

It was found that certain proteins present in food are naturally biologically active and can be absorbed from the gastrointestinal tract in unchanged or slightly altered form and show specific biological activity in systemic metabolism [1] or, in addition, resist the action of digestive enzymes. It was concluded that food proteins of both plant and animal origin contain in their primary structure specific amino acid sequences that

when cleaved by proteolytic enzymes or specific chemical reagents may exhibit a variety of biological activity that was hidden in the structure of the original native protein. It is believed that the use of products of artial destruction of protein as a whole and / or their individual fractions will be given greater priority in the creation of food additives and the development of foods with specific physiological functionality. Only in the United States of North America, Canada, some European countries have developed and used functional products using bioactive peptides [1,2].

However, the problem of instability of bioactive (BP) to proteolytic digestion limits their use in biological environments due to the functional diversity and aggressiveness of endogenous proteases. Although many strategies have been developed to improve the proteolytic stability of peptides [3,4] including cyclization, and ingredients for dietary nutrition based on peptides [5] modification of the chain and functional groups, proteolytic stability (BP) still remained low.

Strategies based on nanotechnological approaches are considered to be a promising way to stabilize biologically active substances and increase their bioavailability. These include conjugation of these substances with other structures, including polysaccharides, which can protect the target substance from aggressive gastrointestinal environment. Polysaccharide nanohybrids can be obtained by a number of techniques: by electrostatic interaction, heat treatment of electrostatic nanocomplexes between proteins and polysaccharides at a temperature higher than the denaturation temperature of the protein. Unlike native proteins, native polysaccharides or electrostatic complexes without heat treatment, heat-treated nanocomplexes have shown high resistance to dissociation or ggregation when the pH, temperature or salt concentration changes. Therefore, heat-treated nanocomplexes have significant potential as means for encapsulation and stabilization of biologically active substances (BAS). A special form of nanohybridization is chemical conjugation based on the Maillard reaction. This reaction occurs naturally between the amino groups of proteins/peptides and the carbonyl group.

Analysis of recent research and publications

Bioactive peptides (BP) are defined as specific fragments present in the original sequence of the precursor protein but when released under the action of proteolytic enzymes can interact with specific receptors, positively affecting the physiological functions of the body [6-9].

Today BP is a new generation of biologically active regulators with a wide range of important physiological effects. This determines the prospects for their use in functional foods, as well as dietary supplements and medicines [10,11].

Cow's milk, cheese and dairy products are the main sources of BP and peptides derived from food [12]. Milk contains approximately 3.5% protein, of which 80% is casein and 20% is whey protein. The multifunctional properties of biologically active milk proteins are gaining more and more recognition. The activity of peptides is based on their inherent amino acid composition and sequence. The size of bioactive milk peptide sequences, which are known to have multifunctional properties, can vary from two to twenty amino acid residues [13].

The most common method of obtaining BP from milk is enzymatic hydrolysis. Digestive enzymes and combinations of various proteinases, including alkalase, chymotrypsin, pepsin and thermolysin, as well as enzymes from bacterial and fungal sources are also used to produce PD from various proteins. BP derived from milk become active after release from the precursor protein, where they are encrypted by digestion or proteolysis both in vivo and in vitro [1].

Characterization of the peptide sequence allowed to establish the path of hydrolysis of casein, which leads to the formation of small peptides. It was found that the action of the plasma-like enzyme that acts on certain lysine residues is the primary stage of casein degradation. This stage is followed by endopeptidase and / or exopeptidase-mediated cleavage of oligopeptides to form many short peptides with one or more amino acid residues [14].

The list of physiological activities of casein peptides is extremely wide. For example, it is antimicrobial, immunomodulatory activity, antihypertensive, probiotic, antioxidant and other physiological effects [15-16].

Here are some examples that illustrate the physiological effects of bioactive peptides.

Chimezine cleavage of casein yielded a peptide that exhibits antimicrobial activity against Staphylococcus spp., Sarcina spp., Bacillus subtilis, Streptococcus pyogenes. Lactoferramine, extracted as a fragment of lactoferrin, exhibits inhibitory activity against Streptococcus mutans, E. coli, B. subtilis and Pseudomonas aeruginosa [17].

Many cytochemical studies have shown that casein-derived immunomodulatory PDs are associated with the stimulation and proliferation of human lymphocytes, phagocytic activity of macrophages, antibody synthesis, and cytokine regulation [15].

Cytomodulatory peptides produced from casein can inhibit the growth of cancer cells by stimulating the activity immunocompetent cells [18].

Casein macropeptide (CMP) promotes the growth of bifidobacteria or lactobacilli that suppress intestinal infection [19].

Antioxidant peptides derived from casein consist of five to eleven hydrophobic amino acids, including proline, histidine, tyrosine. They can function by absorbing or preventing the formation of free radicals as well as inhibiting the enzymatic and non-enzymatic peroxidation of lipids [16,20].

BP like other bioactive compounds can undergo undesirable changes in technological processes and during transit along the gastrointestinal tract. In order to improve their stability and increase bioavailability conjugates derived from the Maillard reaction have recently been used which is now positioned as the latest way to prevent the destructive effects of external factors [21].

The Maillard reaction is a complex chemical condensation reaction that occurs between nucleophilic groups of amino acids, peptides or proteins with reactive carbonyl groups of reducing sugars. It ends with the formation of complex mixtures of aromatic compounds and macromolecular pigments of melanoidins [22].

In Figure 1 shows a fragment of the melanoidin molecule which illustrates the complexity of the structure of this substance which there are still discussions in the scientific community around.

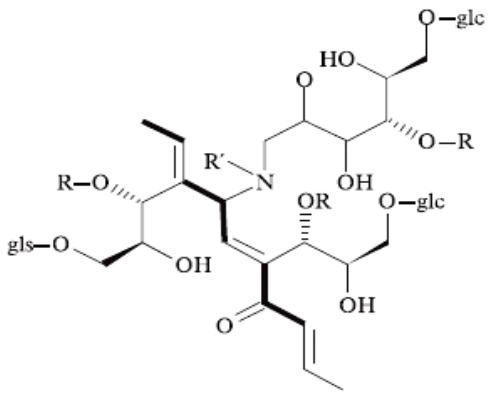

Fig. 1. The structure of the fragment of melanoidin polymer (glc -D-glucose residue) [23]

The prevailing opinion in the national literature is that melanoidins are compounds that have a negative effect on the human body. However foreign researchers emphasize the beneficial properties of the products of the Maillard reaction due to the fact that it is natural to the human body and occurs under controlled conditions, and this avoids the formation of unwanted compounds.

Beneficial properties of melanoidins include antioxidants, antimicrobials, immunomodulatory properties as well as the ability to bind heavy metal ions. It is believed that their antioxidant action is due to the presence in these compounds of conjugated double bonds in heterocyclic and quinoid units. The antimicrobial action of melanoidins has been linked to the formation of hydrogen peroxide in the Maillard reaction which inhibits the growth of Escherichia coli and Listeria innocua [24].

It is believed that coffee melanoidins reduce the risk of cancer, increase the synthesis of enzymes of the glutathione-S-transferase family which neutralize various xenobiotics [25].

Today the melanoidin reaction has become widely used in the field of creating the latest nanostructures designed to correct the technological properties of food systems. Much attention has been paid to the development of Maillard conjugates as functionally physiological ingredients and preparation. The following are examples of using the Maillard reaction in these areas.

The authors of the review [26], which examines in detail the current methods of obtaining nanosystems formed by conjugation of proteins / peptides with polysaccharides by the Maillard reaction. They noted that such nanohybrids are promising since they achieve the desired stability of the protein component due to the high stability of polysaccharides.

Nanostructures from conjugates of tare gum and α-lactalbumin were obtained by the Mayar reaction using the method of dry heating of lyophilized or spray-dried mixtures. Glycosylation was shown to be more intense in lyophilized mixtures. Nanostructures with average sizes less than 300 nm were formed at different values of temperature, pH and heating time [27].

Adi Seifert et al. [28] by combining galactooligosaccharides with lactoferrin hydrolysis products based on the Maillard reaction under mild conditions a prebiotic conjugate was obtained. It is shown that it resists digestion in the stomach. The growth rate of the model probiotic bacterium (Lactobacillus casei) in the conjugates was found to be twice as high as in the unconjugated components. The authors believe that they have proposed a new generation of prebiotics – protein-oligosaccharide conjugates because no existing prebiotic product contains protein. Yan Zhou et al. [29] used the Maillard reaction to obtain a conjugate of hydrolysis products of soy protein isolate with dextrin at dry heating, temperature of 60 °C and relative humidity of 79 %. The maximum yield of the conjugate was obtained using components each of which had a molecular weight of 40 kDa. The conjugate was then used to improve the physical stability of the nanoemulsions.

The purpose of the research was to determine the feasibility of casein fragmentation with papain to obtain BP and subsequent creation on their basis of a nanohybrid with a mannan component by the Maillard reaction.

To achieve the purpose, the following **objectives** were defined:

– substantiate the conditions for obtaining BP by enzymatic hydrolysis of sodium caseinate using papain and provide a characteristic of BP;

– to obtain a carbohydrate-peptide nanohybrid by the Maillard reaction to protect BP from degradation and during transit along the gastrointestinal tract;

– to evaluate the stability of the peptide component of the carbohydrate-peptide nanohybrid *in vitro*.

Research materials and methods

In studies to obtain bioactive casein peptides used water-soluble sodium caseinate Excellion EM 7 (Friesland Compania DMV B.V., Netherlands) and the enzyme preparation papain of plant origin with protelitic activity of 6000 units/mg ("Loba-Chemie", Austranal-Präparate, Austria). Sephadex G-100 (GE Healthcare, USA) and Molselect G-15 (Hungary) were used for liquid column chromatography. As internal standards of molecular weights for calibration of chromatographic columns we used a set of markers of dextrans with molecular weights of 9–11, 35–45, 70, 100 kDa (Pharmacia, Sweden), glucose and maltose (LLC "PHARMACTIVE", Ukraine), raffinose (LLC "KHIMPROMRESURSY-LD", Ukraine), glycine (Merck KGaA, Germany), a set of proteins with a molecular weight of 10–250 kDa Page Ruler TMP restained Protein Ladder (Fermentas, Lithuania), trypsin from the pancreas of cattle (molecular weight 22680–23800 Da, JSC "Olaine Chemical Plant BIOLAR", Latvia), lysozyme of chicken egg protein 3x crystallized (molecular weight 14500 Da, Sigma-Aldrich, USA).Pepsin from porcine gastric mucosa with an activity of 2500 units/mg protein (Sigma-Aldrich, USA) and trypsin from pancreatic gland with activity 1000–2000 units/mg of dry matter (JSC "Olainsky Chemical Plant BIOLAR", Latvia) was used in a study to model the digestion of hydrolysis products of sodium caseinate and them in the mannan-peptide conjugate.The studies also used biomodified water-soluble mannan of coffee sludge obtained at the Department of Food Chemistry and Expertise Odessa National Academy of Food Technologies (Odessa, Ukraine) [30], Folin-Ciocalteu reagent, 37% formalin solution and anthrone (CHIMLABORREAKTIV LLC, Ukraine).

In the composition of sodium caseinate, the mass fraction of amine Nitrogen in the form of free NH_2-groups was determined by the method of formal titration [31], carbonyl groups of the carbohydrate component – by the Hagedorn-Jensen method [32]. The molecular weight of the protein in aqueous solution was determined by the method of exclusive gel filtration chromatography [31] using a chromatographic column filled with Sephadex G-100 (h = 42.0 cm, Ø = 1.8 cm), which was calibrated with standard substances.1 cm^3 of aqueous sodium caseinate solution (7–10 mg / cm^3) was added to the column and elution was carried out with distilled water at a flow rate of 2 cm^3 / min, the volume of eluate was 2 cm^3.In the obtained eluates the content of the protein component was determined by a modified Lowry method with Folin-Ciocalteu reagent [31].

Enzymatic fragmentation of sodium caseinate in aqueous solution (concentration 20 mg/cm^3) was performed with papain at an ratio enzyme : substrate 1:25, pH of the reaction medium 6.5 at 37°C for 4 h [33]. Next, the enzyme was inactivated by heat treatment at a temperature of 90–100°C for 15 minutes. The supernatant with BP was separated from the precipitate by centrifugation at 12,000 rpm for 15 min and the content of casein fragmentation products was determined by the biuret method [31], then concentrated to a dry matter content of about 40%, lyophilized and dried. The degree of hydrolysis of fragmentation products of sodium caseinate in solution was determined by formal titration by changing the mass fraction of amine Nitrogen compared to the original sodium caseinate [31], and their molecular weight fractionation – by exclusive gel filtration in chromatographic columns with Sephadex G-100 (h = 42.0 cm, Ø = 1.8 cm), then with Molselect G-15 (h = 39.4 cm, Ø = 1.8 mm).Elution was performed with distilled water to obtain 2 cm^3 fractions.The content of the protein component in the obtained eluates was determined by a modified Lowry method [31].

The preparation of water-soluble manna of coffee sludge was characterized by the content of carbonyl (reducing) groups by the Hagedorn-Jensen method [32] and molecular weight distribution by gel filtration in a chromatographic column with Sephadex G-100 [32].

The conjugate based on water-soluble mannan and casein hydrolysates was obtained in aqueous medium where the mass fraction of both components was 20 mg/cm^3, and their mass ratio was 1:1. The reaction mixture was kept at 60 °C for 6 h [34]. Next the protein component which did not interact with mannan was precipitated at the isoelectric point of casein at *pH* 4.6 with hydrochloric acid solution. The conjugation process was monitored by changing the content of free amino groups of the protein component –by the method of formal titration [31], the mass fraction of carbonyl groups – by Hagedorn-Jensen method [32], by the tendency to change the molecular weight – gel filtration in a chromatographic column with Sephadex G-100 (in the eluate was determined the content of the protein component by the modified Lowry method [31], the carbohydrate component – photo-electrocolorimetric method with anthron reagent [32]). The mass fractions of the peptide [31] and carbohydrate components [32] were determined in the mannan-peptide conjugate.

Digestibility of hydrolysis products of sodium caseinate and mannan-peptide complexes was studied in the pepsin-trypsin model system by the accumulation of free amino groups [31, 35]. In this case, the mass fraction of peptides in the reaction mixture and their content in the conjugate were the same.

Results of the research and their discussion

Commercial sodium caseinate was used as a source of BP in the studies. It had a moisture content of 5.4%, contained 86.3% protein, 4.2% ash. The mass fraction of free amino groups in it is insignificant and was 1.3% (1303.9 mg/100 g). Because sodium caseinate consists of molecules of α-, β- and κ-casein, and κ-casein is known to be chemically heterogeneous and may contain salicylic acid and up to 10% carbohydrates (mainly trisaccharidescontaining galactose residues, N-acetylgalactosamine, taloses) attached to the peptide chain through the OH-groups of serine or threonine, or both) which as a result of

enzymatic hydrolysis are likely to be transferred to water-soluble glycomacropeptide and not to water-insoluble paracasein it was advisable to the content of carbonyl groups of the carbohydrate component [36] which was taken into account in subsequent experiments. It was found that the mass fraction of carbonyl groups in sodium caseinate reaches 3.4%. According to gel chromatography on columns with Sephadex G-100 sodium caseinate is heterogeneous in molecular weight (Fig. 2) and contains two dominant fractions with molecular weights of 95–89 and 70–62 kDa.

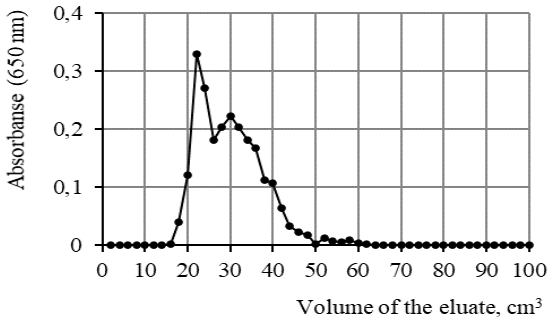

Fig. 2. The original curve of gel chromatography of sodium caseinate on Sephadex G-100

Enzymatic hydrolysis of sodium caseinate was carried out with the enzyme preparation papain which is obtained from papaya latex (Carica papaya).It is used in the food industry (to soften meat, causing significant degradation of myofibrillar and collagen proteins, to soften condiments, to lighten drinks) and in pharmacology in particular in preparations for indigestion. This enzyme belongs to cysteine endoproteases (peptidases), similar in action to pepsin, shows specificity for a wider range of protein substrates than pancreatic proteases, specifically cleaving peptide bonds formed by hydrophobic amino acids in particular has specificity for amino acids with aromatic side chains such as phenylalanine and tyrosine, also hydrolyzes amino acid esters and amides. Unlike pepsin, papain is active in a wide pH range of 3–12 (optimum 6.0–7.0) and a temperature range of up to 65°C which makes it convenient to use. Papain-derived peptides exhibit antioxidant, antidiabetic, and pancreatic lipase and α-amylase inhibitory activity and are used to control obesity [37].

Proteolysis of sodium caseinate with papain was performed by varying the duration of the hydrolysis process (30, 60, 120, 180, 240 min). The yield of proteolysis products depending on the duration of the process ranged from 77.8% to 83.9% (Table 1). Enzymatic hydrolysis of sodium caseinate contributed to a significant increase in the products of its fragmentation of the mass fraction of amine Nitrogen – from 4561.4 to 5687.5 mg/100 g (4.6–5.7%), which is 3.5–4.4 times more than the content of free amino groups in the original sodium caseinate. Protein hydrolysates obtained by proteolysis for 30 min were characterized by the highest content of free $–NH_2$-groups, and kept for 2 hours – contained 8.3% less.

The results of gel chromatographic studies of the fragmentation products of sodium caseinate on columns with Sephadex G-100 and Molselect G-15 show that with increasing duration of the fermentolysis process gradually decreased the share of high molecular weight protein fragments and increased the share of low less than 1 kDa (Fig. 3, Table 1). Gel chromatograms of casein fragmentation products obtained during the first 30 and 60 min of fermentolysis are characterized by the presence of one inhomogeneous peak in contrast to those obtained by hydrolysis for 2, 3, 4 hours, which have a wide molecular weight distribution. The molecular weight of peptides with a maximum number of amino acid residues up to 20, which are considered BP, reaches 2.4–2.2 kDa, including BP with a molecular weight of 1 kDa and less, which contain up to 9 amino acid residues. The largest share of target casein BP contains hydrolysates obtained within 30 min of hydrolysis of sodium caseinate with papain, slightly less – hydrolysates obtained by fermentolysis for 2 and 4 hours. The results of gel chromatogram studies on Molselect G-15 show that the amount of peptide fraction with an average molecular weight of less than 1 kDa gradually increases with prolonged proteolysis of sodium caseinate (Fig. 2).

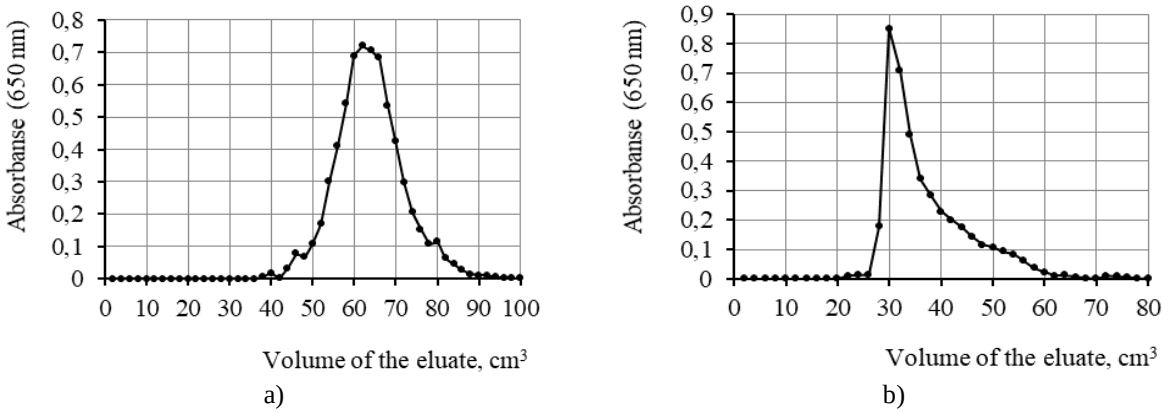

Fig. 3. The original curve of gel chromatography of casein peptides obtained during 120 min of the fermentolysis process: a – on Sephadex G-100, b – on Molselect G-15

Table 1 – Characteristics of the products of hydrolysis of sodium caseinate(n=5, P≥0.95)

Sample		Yield, %	Mass fraction of amine Nitrogen		Molecular weight of peptides, kDa	Mass fraction of peptides with molecular weight ≤ 3 kDa,%
			mg/100 g	%		
Casein peptides depending on the duration of fermentolysis with papain, min	30	78.1	5687.5	5.2	65–1, < 1	59.5
	60	77.8	4561.4	4.6	42–1, < 1	39.5
	120	84.6	5212.8	5.7	42–1, < 1	55.2
	180	83.9	4711.5	4.7	45–1, < 1	46.2
	240	77.8	5090.8	5.1	40–1, < 1	57.0

Thus the action of papain on sodium caseinate yielded casein peptides with a wide molecular weight distribution and degrees of polymerization among which almost 1/2 are BP with average molecular up to 2.4 kDa, containing up to 20 amino acid residues. Because the yield of peptides obtained by proteolysis of sodium caseinate for 2 hours was the highest and they contained more than half of BP and had the largest number of free amino groups, in subsequent studies to obtain a nanohybrid used this fermentolysate.

To obtain a nanohybrid based on casein peptides by the Maillard reaction to purposefully diversify their physiological action as the second physiologically active component used water-soluble mannan of coffee sludge [30]. An important biological activity of mannans is the activation of macrophages and the stimulation of T-cells as a result of which they are considered powerful immunostimulants against infectious diseases and tumors. Mannan with a molecular weight of up to 20 kDa has a high immunomodulatory effect.

Water-soluble coffee mannan contained 11.2% of carbonyl groups (reducing terminal groups) as reaction centers for interaction with amino groups of peptides. According to the results of gel chromatographic studies on Sephadex G-100 (Fig. 4) water-soluble mannan is heterogeneous and has a wide distribution of molecular weights from 79 to 1 kDa which is dominated by high molecular weight fractions (72–32 kDa). The content of these fractions reaches 70.2%, 2.4 times less than fractions with a molecular weight of 11–28 kDa. Such a fractional composition of the mannan preparation will contribute to the manifestation of a wide range of physiological effects.

The mannan-peptide complex was obtained under the conditions established in [34] to obtain covalently bound casein-maltodextrin conjugates exactly the incubation of both components in the reaction mixture was carried out for 6 h at 60°C. After precipitation of the protein at the isolelectric point which was not part of the conjugate and the target water-soluble nanohybrid was in the supernatant. The mass fraction of free amino groups in the nanohybrid was 1.8% or 1842.1 mg/100 g which proves their reduction by 3.2 times compared to casein peptides and the total number of carbonyl groups was 3.5% which is 42.6% less than in peptides and 3.2 times less than in mannan. The yield of neutralized and lyophilized product was 38.1%, and the mass ratio of mannan : casein peptides in it was 1 : 8.0–8.4. The mass fraction of mannan in it reached 10.6–10.9% and peptides – 89.1–89.4%. Gel chromatography profiles of the obtained product (Fig. 5) show the coincidence of peaks of carbohydrate and peptide components in the high- and low-molecular regions. In their composition the content of high molecular weight fractions of mannan was significantly reduced and in the protein component the presence of high molecular weight fraction was stated. Apparently casein peptides covalently bind to both high- and low-molecular-weight fractions of mannan. As a result there is a redistribution of molecular weights: there are fractions with average molecular weights less than 1 kDa and in the range of 82–1 kDa, with the mass fraction of low molecular weight fraction (31–1 kDa) reaches 75.1% and the quantitative content of high molecular weight fraction (81–31 kDa) is 3.0 times smaller.The resulting product is a powdery mass of dark brown color and soluble in water.

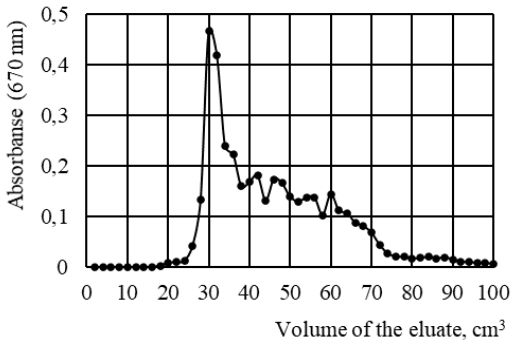

Fig. 4.The original curve of gel chromatography of water-soluble mannan from coffee sludge on Sephadex G-100

Mannan-peptide nanohybrid was obtained by thermal incubation of casein peptides and water-soluble mannan in an aqueous medium. It contained significantly fewer free amino groups of the peptide component and reducing mannan groups compared to the original peptides and polysaccharide. However the profile of the gel chromatogram of the conjugate changes significantly in comparison with its individual components: the peaks of the carbohydrate component coincide with the peaks of the peptides. The conjugate

contains 8 times more peptides than mannan although at the initial stage of the Maillard reaction the starting components were used in equal proportions. Such changes in the characteristics of the obtained product in comparison with free casein peptides and mannan indicate their interaction and obtaining of mannan-peptide nanohybrid.

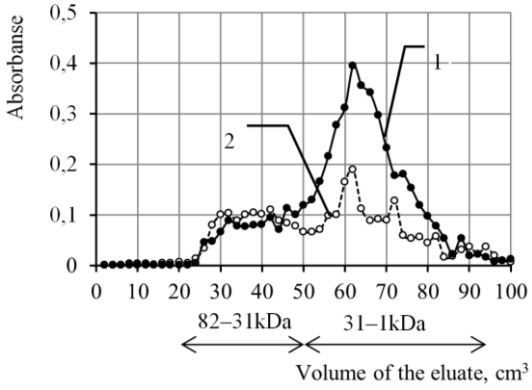

Fig. 5. The original curve of gel chromatography of mannan-peptide conjugate on Sephadex G-100: 1 – peptide component (λ = 650 nm), 2 – carbohydrate component (λ = 670 nm)

The effect of the polysaccharide component on the stability of the peptide component was evaluated in vitro in the pepsin-trypsin system. For this sample with the same content of peptide component (starting hydrolyzate and nanohybrid complex) was subjected to enzymatic influence and then compared the content of free amino groups in both studied solutions. It was found that enzyme treatment leads to a significant increase in these groups in the fermentolysate of the original model sample in comparison with such groups of the peptide component of the nanohybrid – almost 2.4 times. Thus the results of this experiment indicate that due to the combination of peptides with mannan by the Maillard reaction the stability of peptides is significantly increased. This approach can be used to obtain protective shells for labile BAS and to increase the bioavailability of peptides in dietary supplements and functional foods.

Conclusion

The expediency of using papain for fragmentation of sodium caseinate to obtain bioactive casein peptides has been established. The conditions under which the amount of BP among the fragmentation products of sodium caseinate is 50% (treatment time 120 min, sodium casein concentration 20 mg/cm^3, enzyme : substrate 1:25, pH of the reaction medium 6.5, temperature 37°C).

To increase the stability of BP in technological processes and during transit along the gastrointestinal tract is promising to create mannan-peptide nanohybrids by the Maillard reaction (mass fraction of each of the components of the conjugate in aqueous medium 20 mg/cm^3; mass ratio of mannan : BP 1:1, temperature 60°C, time 6 h). This approach can be used both to obtain protective shells of labile BAS and to increase the bioavailability of peptides when they are included in dietary supplements and functional foods.

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ОТРИМАННЯ ТА ХАРАКТЕРИСТИКА МАНАН-ПЕПТИДНОГОНАНОГІБРИДУ

Н.К. Черно, д-р техн. наук, професор, *E-mail*: cherno.onaft@gmail.com
Л.С. Гураль, канд. техн. наук, доцент, *E-mail*: gural.onaft@gmail.com
К.І. Науменко, канд. техн. наук, доцент, *E-mail*: shapkinak@gmail.com
Кафедра харчової хімії та експертизи
Одеська національна академія харчових технологій, вул. Канатна, 112, м. Одеса, Україна, 65039

Анотація. Біоактивні пептиди відносяться до нової генерації біологічно активних регуляторів, що володіють широким спектром найважливіших фізіологічних ефектів. Це визначає перспективи їх використання у складі функціональних продуктів харчування як дієтичних добавок та лікарських засобів. Головними джерелами біоактивних пептидів є коров'яче молоко, яке містить білки, 80% яких складає казеїн. Як й інші біоактивні сполуки, вони зазнають небажаних змін у технологічних процесах та під час транзиту вздовж шлунково-кишкового тракту. Із метою покращення їхньої стабільності та підвищення біодоступності останнім часом використовують кон'югати, отримані за реакцією Майяра, що сьогодні позиціонується як перспективний спосіб запобігання руйнівного впливу зовнішніх чинників. Дана стаття висвітлює результати досліджень, присвячених визначенню доцільності фрагментації казеїну папаїном для отримання біоактивних пептидів та подальшого створення на їхній основі наногібриду з манановою складовою за реакцією Майяра. Ферментативний гідроліз казеїнату натрію папаїном здійснювали з варіюванням тривалості процесу гідролізу (30, 60, 120, 180, 240 хв). Вихід продуктів протеолізу білка, залежно від тривалості процесу, коливався у межах від 77,8% до 84,9%. Ферментативний гідроліз казеїнату натрію сприяв суттєвому збільшенню в продуктах його фрагментації масової частки амінного Нітрогену – від 4561,4 до 5687,5 мг/100 г, що у 3,5–4,4 рази більше, ніж вміст вільних аміногруп у вихідному казеїнаті натрію. У результаті дії папаїну отримано казеїнові пептиди з широком розподілом молекулярних мас. Серед них майже 1/2 становлять біоактивні пептиди, які містять до 20 амінокислотних залишків. Манан-пептидний наногібрид отримували інкубацією біоактивних пептидів з модифікованим водорозчинним кавовим мананом. Реакційну суміш піддавали експозиції за температури 60°С упродовж 6 годин. Вихід ліофільно висушеного продукту складав 38,1%, масова частка манану сягала 10,6–10,9%, а пептидів, відповідно, – 89,1–89,4%. Захисну дію полісахаридної складової відносно пептидної компоненти оцінено в умовах *invitro* у системі пепсин-трипсин. Встановлено що стабільність пептидів у складі наногібриду значно вища, ніж у вихідному гідролізаті. Застосовування створеного наногібриду можливе як для отримання захисних оболонок лабільних біологічно активних речовин, так і з метою підвищення біоактивних пептидів при їх включенні у склад дієтичних добавок та харчових функціональних продуктів.

Ключові слова: казеїнат натрію, папаїн, біоактивні пептиди, манан, реакція Майяра, манан-пептиднінаногібриди.