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Using casein and gluten protein fractions to obtain functional ingredients

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Abstract:
Today, the food industry widely uses both animal and plant proteins. Animal proteins have a balanced amino acid composition, while plant proteins have more pronounced functional properties. However, both types of proteins can act as allergens, which limits their practical application. Therefore, we aimed to select optimal conditions for obtaining hypoallergenic mixtures based on casein hydrolysates and gluten proteins, which have good functional properties and a balanced amino acid composition.
We used wheat flour (Makfa, Russia) with 12.6% of crude protein and 69.4% of starch, as well as rennet casein (Atletic Food, Russia) with 90% of protein. The methods included the Lowry method, the Anson method, Laemmli electrophoresis, ion-exchange chromatography, and the enzyme-linked immunosorbent assay.
Protex 6L was an optimal enzyme preparation for the hydrolysis of gliadin, while chymotrypsin was optimal for the hydrolysis of glutenin and casein. The optimal amount for all the enzymes was 40 units/g of substrate. We analyzed the effect of casein, glutenin, and gliadin enzymolysis time on the functional properties of the hydrolysates and found that the latter had relatively low water- and fat-holding capacities. The highest foaming capacity was observed in gliadin hydrolysates, while the highest emulsifying capacity was registered in casein and glutenin hydrolysates. Further, protein enzymolysis significantly decreased allergenicity, so the hydrolysates can be used to obtain functional additives for hypoallergenic products. Finally, the mixtures of casein hydrolysate and gliadin or glutenin hydrolysates had a balanced amino acid composition and a high amino acid score. Also, they retained high emulsifying and foaming capacities.
The study proved the need for mixtures based on wheat protein and casein hydrolysates, which have good functional properties and hypoallergenicity.

Keywords: Gliadin, glutenin, casein, allergenicity, functional foods, enzymatic hydrolysis, amino acid score

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INTRODUCTION

Russia prioritizes the quality of food and seeks to improve it by developing healthy foods, including functional products [1]. The modern food industry widely uses proteins of both animal and plant origin. This is meant to make up the deficiency of protein caused by a rapid population growth, the shortage of cultivated areas, and unfavorable environmental conditions. The quality of food could be effectively improved by 20–30% in highly nutritional products, including healthy foods rich in protein, essential amino acids, vitamins, as well as micro- and macronutrients [2, 3].

Today, the main sources of plant protein are soybeans, wheat, nuts, oilseeds, and legumes. Soy protein is the most common meat substitute, with a low cost and high crop yield. It accounts for 20 to 40% of the human diet [4].

However, the role of cereal proteins, primarily wheat, is currently on the rise. The nutritional value of plant proteins is primarily determined by their fractional and amino acid composition. Cereal protein fractions are classified on the basis of solubility. Globulins and albumins are extracted by treating flour with a 5% sodium chloride solution and water. Prolamins and glutelins are extracted by treating flour with 60% alcohol and a 0.1% sodium hydroxide solution [5]. Cereal proteins also contain scleroproteins that perform a structural function [6, 7].

Wheat protein, gluten, is one of the most common in Russia. Due to its low cost, gluten is used in a variety

of food products, including functional foods. In Europe and the USA, flour is often enriched with gluten to give bakery products a marketable appearance and make them less friable. Functional additives based on gluten and its components are also used as emulsifiers and foaming agents.

One of the disadvantages of gluten is that some people have an intolerance, or allergy, to it called "celiac disease". This allergy is usually caused by an IgE-mediated reaction to ω-5 gliadin, one of gluten components (gliadin and/or glutenin in wheat) [8–10]. It is the main allergen that causes anaphylactic reaction to gluten. Also allergenic are proteins related to serine proteases (α-amylase/trypsin inhibitor – Tri a 15-AAI monomer, Tri a 39-serine protease inhibitor), agglutenins, peroxidase, nonspecific proteins (lipid carriers), and other components of gliadin [7, 11, 12].

In order to make gluten less allergic, it can be modified by enzymatic or acid hydrolysis, with its most allergenic fraction (gliadin) removed from it.

Enzymatic hydrolysis of wheat gluten is complicated by the fact that it consists of two fractions – gliadin and glutenin. These fractions are most effectively hydrolyzed by different enzymes, so multi-enzymatic compositions or alcalase need to be used. The use of alcalase requires a two-stage process with a product removal, since protein cleavage is inhibited by the reaction products. This produces a mixture of incompletely hydrolyzed but water-soluble proteins, peptides, and amino acids, as well as enhances the surfactant properties of the hydrolysate. The final result of enzymolysis significantly depends on the reaction time, which affects the composition and functional properties of hydrolysates [13]. Enzymatic hydrolysis of wheat protein is also used to reduce its allergenicity caused by wheat prolamins. Finally, gluten can be hydrolyzed by microbial proteases that destroy peptide bonds in the region of proline residues, abundant in gliadin [14].

Casein is of great practical importance among proteins of animal origin. It is the main protein of milk, cheese, cottage cheese, and other dairy products. Casein contains physiologically available calcium and phosphorus, which determine its nutritional value [15, 16].

Due to its structure, casein is easily broken down by proteolytic enzymes during digestion, even without prior denaturation [17, 18].

Abundant in nutrients and functional components, milk proteins are widely used in many food formulations (dairy desserts, nutritional drinks, ice cream, yogurt, meat products, confectionery, and baked goods). Milk proteins perform various key functions, including emulsification, thickening, gelling, and foaming. A wide range of products based on milk proteins includes caseins and caseinates, whey protein concentrates, isolates and hydrolysates, as well as milk protein concentrates [19–21].

Allergenicity is the main obstacle to using casein in food products [22, 23]. Casein fraction is represented by four types: αS1-casein, αS2-casein, β-casein, and κ-casein, with αS1-casein being the main allergen [24]. To date, there are no effective methods of treatment or drugs for the allergy to milk components that do not cause noticeable side effects. Previously, dairy products were simply excluded from the diet of people with the allergy. However, this caused a deficiency of important nutrients contained in dairy products [24].

Modern food scientists have developed a number of milk processing methods to reduce casein allergenicity. The main ones are heat treatment, enzymatic hydrolysis, and glycation [25]. Enzymatic hydrolysis seems quite promising, since casein fractions are resistant to heat. Enzyme preparations can be specially selected to ensure a desirable product (including products with good sensory properties), as well as to optimize the technology for processing milk-protein raw materials [26, 27]. The degree of casein hydrolysis determines the taste and aromatic qualities, as well as the functional properties of hydrolysates, such as emulsifying, gelling, and foaming abilities, as well as hygroscopicity. The higher the degree, the larger the amount of free amino acids in the hydrolysate, which improves its biological value [27, 28]. Also, the degree of hydrolysis can affect the solubility of the hydrolysate: the deeper the hydrolysis, the better the solubility. Denatured and dried proteins dissolve better even with incomplete hydrolysis. However, deep hydrolysis of milk proteins can worsen their sensory properties, especially the smell and the taste.

According to previous studies, chymotrypsin and thermolysin, as well as a temperature of 50°C, are optimal for the enzymatic hydrolysis of milk proteins. These conditions preserve the amino acid composition of the final hydrolysate, improve its nutritional value, and greatly reduce or completely eliminate its antigenic activity [29, 30]. Milk proteins are also hydrolyzed by proteolytic systems of lactic acid bacteria [30].

Milk casein is fundamentally different from wheat flour proteins in the fractional composition and the content of individual amino acids. Wheat proteins are high in valine and phenylalanine but low in tryptophan, lysine, and methionine, while casein is rich in leucine, valine, lysine, methionine, and tryptophan [31]. Thus, products with a balanced amino acid composition can be obtained by mixing casein and wheat proteins.

We aimed to select the conditions for obtaining hypoallergenic mixtures based on casein and gluten hydrolysates which have functional properties and a balanced amino acid composition.

STUDY OBJECTS AND METHODS

Study objects. We studied the hydrolysates of casein, gliadin, and glutenin, as well as their combinations: casein + gliadin at proportions of 0.5:1, 1:1, and 2:1 and casein + glutenin at proportions of 0.5:1, 1:1 and 2:1.

Materials. We used wheat flour of the highest grade (Makfa, Russia) with 12.6% of crude protein and 69.4% of starch, as well as rennet casein (Atletic Food, Russia) with 90% of protein. Enzymatic protein hydrolysates were obtained by using enzyme preparations with specific proteolytic activity measured by the Anson method (State Standard 20264.2-88). They included chymotrypsin (Samson-Med; 1900 u/g protein), Protex 6L (Genencor; 2100 u/g protein), pancreatin (Biosintez; 177 u/g protein), trypsin (Diaem; 1800 u/g protein), and beef pepsin (Moscow Rennet Plant; 7500 u/g protein).

Enzymatic hydrolysis of proteins. Proteins (10 g/L of a solution) were hydrolyzed for 2 h, with the enzyme preparation activity of 40 u/g of substrate, under optimal temperature and pH conditions. A modified Lowry method was used to measure the concentration of hydrolysis products in the supernatant [32]. The degree of protein hydrolysis was determined as a ratio of the low-molecular-weight protein fraction in the hydrolysate to the initial protein concentration.

Enzyme preparations. Three enzyme preparations were used, namely: Protex 6L, pancreatin, and chymotrypsin. Hydrolysis was carried out at a substrate concentration of 10 g/L, proteolytic activity of 20–60 u/g substrate, at 40°C and pH 7.6–8.2 for 2 h. The hydrolysates were analyzed for the content of the low-molecular-weight protein fraction by the modified Lowry method and the degree of protein hydrolysis was determined as described above [32].

Gliadin extraction. The protein fraction of wheat gliadin was extracted from wheat gluten with a 40% ethanol solution (1:9) at 40°C for 2 h. Then, gliadin was precipitated with acetone at an ethanol extract:acetone ratio of 1:5. Its resulting fraction contained 72% of protein.

Glutenin preparation. After the extraction of gliadin, the insoluble residue was treated with a 2% sodium hydroxide solution for 2 h, followed by precipitation from an aqueous solution at pH 5–6. The resulting glutenin fraction contained 68% of protein.

The effect of enzymatic hydrolysis time on the functional properties of hydrolysates. Hydrolysates were prepared from gliadin, glutenin, and casein by enzymolysis varying in time. For this, 1 g of each hydrolysate was mixed with water (1:25) and then an enzyme preparation was added until the proteolytic activity in the solution reached 40 u/g of protein. Hydrolysis was carried out at the temperature and pH optimal for the selected enzyme preparation for 15, 30, 60, 90, and 120 min. Heating protein hydrolysates may produce an unpleasant odor due to side chemical reactions, for example, the Maillard and Strecker reactions [34]. Therefore, the enzymes were inactivated by cooling to –10°C. The hydrolysate samples were dried at 50°C, after which their functional properties were studied.

Protein content. The content of protein in the hydrolysate samples was measured by the modified Lowry method, with a separate determination of high- and low-molecular-weight protein fractions (HMF and NMF, respectively) by a preliminary precipitation of HMF with 50% trichloroacetic acid.

Total proteolytic activity. The total proteolytic activity was measured by a modified Anson method (State Standard 20264.2-88). A unit of proteolytic activity was understood as the enzyme's ability to convert sodium caseinate into a form unprecipitable by trichloroacetic acid in an amount corresponding to 1 μmoL of tyrosine in 1 min at 30°C.

The allergenicity of gliadin and glutenin hydrolysates was quantified by enzyme immunoassay according to ALINORM Standard 08/31/26 for food products (Methodological Guidelines 4.1.2880-11 4.1). We took into account the specific interaction between the allergenic protein in the test sample and the antibodies to it contained in the test solution (Siemens, Germany). For the immunoassay, we placed 100 μL of a 1% solution of the test sample into a well of the plate and added 100 μL of a conjugate test solution containing antibodies to gliadin. The optical density was measured at 450 nm and then recalculated for gliadin (one unit of optical density corresponded to 40 μg/mL of gliadin). Allergenicity was considered low if the gluten content was under 20 mg/kg and moderate if it amounted to 20–100 mg/kg of the end product.

Amino acid composition. The amino acid composition of the hydrolysate samples and their mixtures was determined by ion exchange chromatography on an ARACUS amino acid analyzer (MembraPure GmbH, Bodenheim, Germany) equipped with a C18 column and a refractive index detector. High-performance liquid chromatography was supplemented with mass spectrometry, with electrospray ionization for separation of amino acids followed by ninhydrin reaction and photometric detection [34].

Electrophoresis by the Laemmli method. The molecular weight of casein hydrolysate components was determined by electrophoresis using the Laemmli method in polyacrylamide gel with 12.5% SDS. For comparison, we used a marker that included 11 standards of certain molecular weights, namely 250, 150, 100, 70, 50, 40, 30, 20, 15, 10, and 5 kDa (Fermentas, Lithuania). Electrophoresis was carried out in a VE-10 chamber (Helicon, Crenshaw, Alabama, USA) at room temperature without additional cooling at 60 V for the first 30 min and then at 120 V until the samples reached the lower edge of the gel. The resulting electropherograms were stained with Coomassie Brilliant Blue G 250, followed by washing with acetic acid [35].

Fat-holding capacity. 0.5 g of the test sample was placed in glass centrifuge tubes and 0.125 to 0.625 mL of vegetable oil was added with an interval of 0.125 mL. The contents of the tubes were stirred for 10 min, then the samples were kept under stirring for 15 min, cooled to room temperature, and centrifuged at 1500 rpm for 15 min. The fat-holding capacity was determined as the

maximum amount of added oil at which no separation of the oil phase was observed during the test, expressed in terms of 1 g of preparation [36].

Water-holding capacity. 0.5 g of the test sample was placed in glass centrifuge tubes and 1.5 to 2.5 mL of water was added with an interval of 0.25 mL. The experiment continued as described above (for fat-holding capacity). The water-holding capacity was determined as the maximum amount of added water, at which no separation of the aqueous phase was observed during the test, expressed in terms of 1 g of preparation [36].

Emulsifying capacity. 1 g of the test sample was placed in glass centrifuge tubes and mixed with 5 mL of water and 5 mL of oil. The contents of the test tubes were stirred for 10 min, followed by the procedures described above. The emulsifying capacity was determined as a percentage ratio between the aqueous and oil phases separated from the emulsion [36].

Foaming capacity. 0.25 g of the test sample was placed in 50 mL conical flasks and mixed with 25 mL of water. The resulting solution was shaken with a shaker for 30 s. Then it was poured into a measuring cylinder to measure the height of the foam column [37].

Statistical analysis was carried out using the Statistics 2020 programs.

RESULTS AND DISCUSSION

According to literature, hydrolysates of casein, gliadin, and glutenin are not allergenic, unlike the original proteins. In addition, they have much better functional properties (foaming, fat- and water-holding, emulsifying) than the original proteins [38].

The functional properties of protein hydrolysates are affected by the enzymolysis time and the choice of an enzyme preparation. We evaluated the efficiency of the most common protease enzyme preparations, namely chymotrypsin (1917 u/g), pancreatin (1501 u/g), and Protex 6L (2156 u/g).

The enzymatic hydrolysis of the gliadin and glutenin samples obtained by the above methods, as well as an industrial sample of casein, was carried out at the temperature and pH conditions optimal for each enzyme preparation with the activity of 40 u/g of substrate (Table 1).

According to Table 1, Protex 6L was selected for the hydrolysis of gliadin, while chymotrypsin was selected for casein and glutenin. Further, the best activities of these enzyme preparations were selected to ensure the maximum degree of protein hydrolysis (Fig. 1).

Table 1 Selection of an enzyme preparation for casein, gliadin, and glutenin hydrolysis

Enzyme preparation	Degree of hydrolysis, %		
	Gliadin	Glutenin	Casein
Chymotrypsin	25.4	34.1	48.1
Pancreatin	51.1	6.51	36.1
Protex 6L	60.5	13.0	40.1

As can be seen, an increase in the activity of proteases above 40 u/g of substrate did not lead to a noticeable increase in the degree of hydrolysis. Therefore, this activity value was taken as recommended.

At the next stage, we determined the functional properties of the hydrolysates (Fig. 2). As we know, the functional properties (water-holding, fat-holding, emulsifying, and foaming ones) depend on the degree of protein hydrolysis, which, in its turn, depends on the hydrolysis time.

According to Fig. 2, the water-holding capacity decreased in the course of hydrolysis in all the cases. Its slight increase during the first 15 min of glutenin hydrolysis might be explained by a larger specific surface area of the hydrolyzed substrate. This is due to the fact that during hydrolysis, proteins lose their ability to maintain a water-retaining structure. Longer hydrolysis increases the degree of protein degradation. The resulting peptides have a high solubility, which reduces their water-holding capacity. The lower water-holding capacity is also likely to decrease the hygroscopicity of hydrolysates and their gelling ability.

The fat-holding capacity showed a similar trend (Fig. 2). This capacity depends on fat retention in the native structure of the protein, which is destroyed during enzymatic hydrolysis. Protein hydrolysis results in low-molecular-weight peptides with a lower hydrophobicity than that of the original protein. This decreases the fat-holding capacity.

During short hydrolysis, the emulsifying and foaming capacities increased for glutenin and casein, respectively (Fig. 2). This might due to the fact that short protein hydrolysis produces peptides with surfactant properties that promote foaming and emulsification. However, longer hydrolysis leads to the destruction of these peptides and to a decrease in emulsifying and foaming capacities. Plant protein hydrolysates, which have significant surfactant properties, can become a more cost-effective substitute for animal protein hydrolysates in the products that

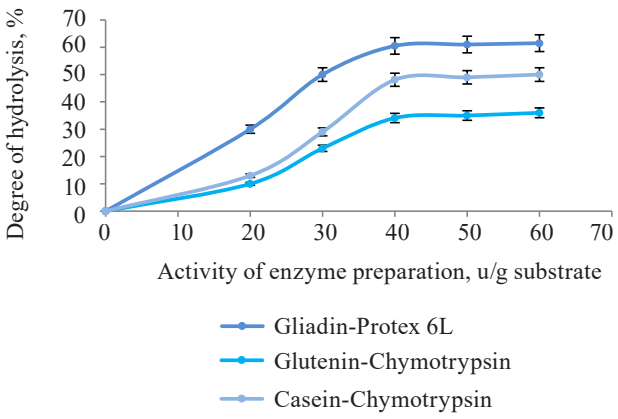

Figure 1 Effect of enzyme preparation activity on the degree of casein, gliadin, and glutenin hydrolysis

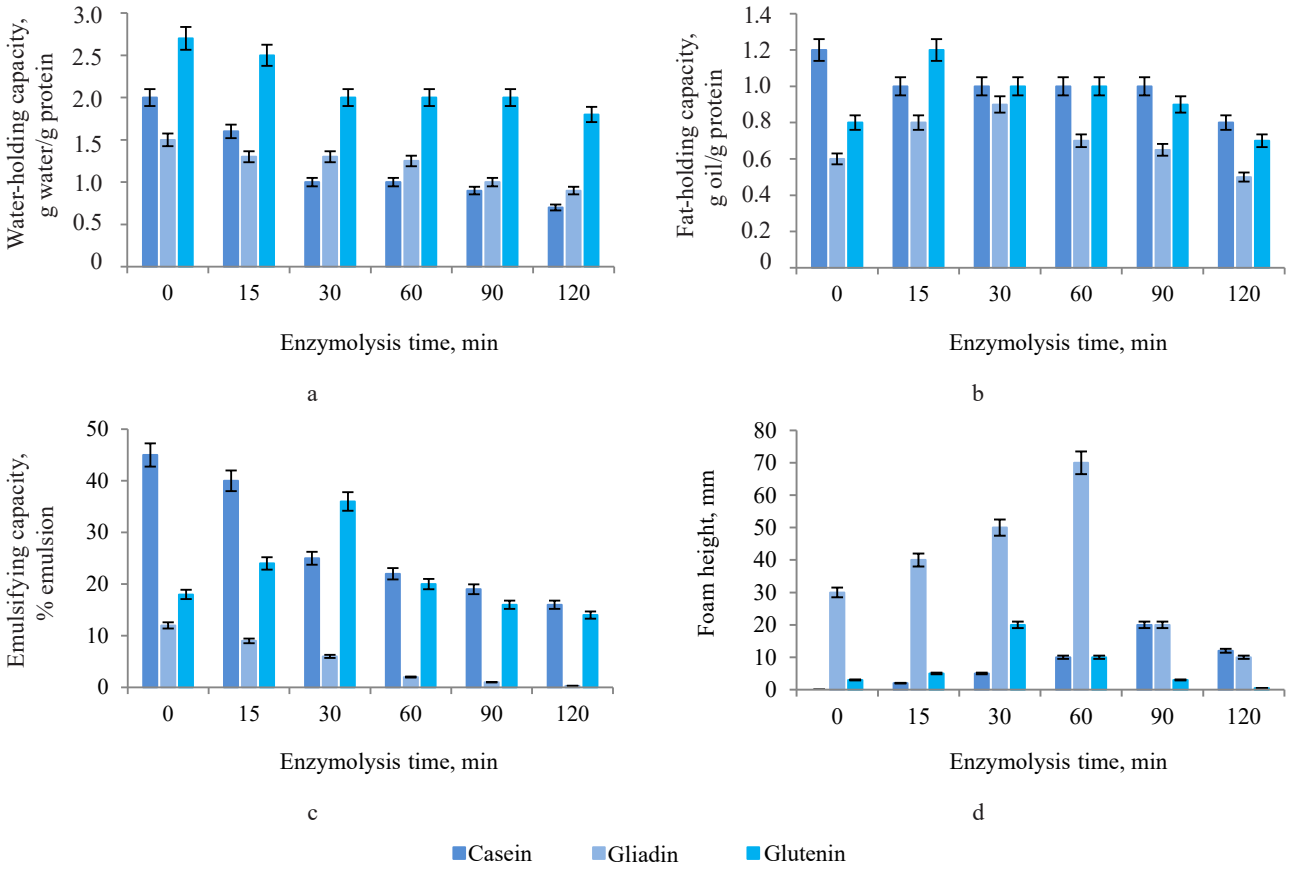

Figure 2 Effect of casein, gliadin, and glutenin enzymolysis time on the functional properties of hydrolysates: (a) water-holding capacity; (b) fat-holding capacity; (c) emulsifying capacity; and (d) foaming capacity

Table 2 Allergenicity of gliadin and glutenin hydrolysates

Protein sample	Enzymolysis time, min	Allergenicity determination		
		Degree of hydrolysis, %	Allergen concentration in hydrolysate, μg/g	Allergenicity level
Gliadin	0	7.0 ± 1.0	181.0 ± 9.0	High
	15	24.0 ± 1.0	127.0 ± 7.0	High
	30	37.0 ± 2.0	76.0 ± 5.0	Moderate
	60	46.0 ± 2.0	16.4 ± 3.0	Moderate
	90	54.0 ± 3.0	15.0 ± 1.0	Low
	120	60.0 ± 3.0	12.0 ± 1.0	Low
Glutenin	0	5.6 ± 0.3	115.0 ± 6.0	High
	15	14.0 ± 1.0	63.0 ± 4.0	Moderate
	30	18.0 ± 1.0	18.0 ± 2.0	Low
	60	25.0 ± 1.0	15.0 ± 1.0	Low
	90	30.0 ± 2.0	10.0 ± 1.0	Low
	120	34.0 ± 2.0	7.0 ± 1.0	Low

need good emulsifying and foaming properties, such as yoghurts and shakes.

At the next stage, we determined the allergenicity of wheat protein and casein enzyme lysates (Table 2).

As can be seen in Table 2, the original proteins had a high level of allergenicity, which decreased after 90 min of enzymolysis for gliadin and 60 min of enzymolysis for glutenin.

According to literature, the allergenicity of casein hydrolysates significantly decreases if they have a minimal content of proteins with molecular weights over 25 kDa and a predominance of peptides with molecular weights of 10–15 kDa. The main allergens of milk are casein (25–98 kDa), *β*-lactoglobulin (18.4 kDa), and *α*-lactalbumin (15 kDa). Therefore, low allergenicity can be provided by the predominance of peptide fractions with molecular weights below 15 kDa [39, 40]. In our study, we determined the molecular weights of hydrolysate components by Laemmli electrophoresis in polyacrylamide gel (Table 3) [35].

Table 3 Molecular weight distribution of protein fractions in casein hydrolysates depending on enzymolysis time

Enzymolysis time, min	Degree of protein hydrolysis, %	Molecular weight range, kDa			
		25–30	20–25	15–20	10–15
0	8.1 ± 1.2	47.0 ± 4.0	28.0 ± 3.0	22.0 ± 2.0	3.0 ± 0.3
15	17.0 ± 1.0	34.0 ± 3.0	37.0 ± 3.0	22.0 ± 2.0	7.0 ± 1.2
30	24.0 ± 1.0	25.0 ± 3.0	20.0 ± 2.0	40.0 ± 3.0	15.0 ± 1.0
60	37.0 ± 2.0	14.0 ± 1.0	15.0 ± 1.0	45.0 ± 4.0	26.0 ± 2.0
90	45.0 ± 2.0	8.0 ± 1.0	10.0 ± 1.0	41.0 ± 3.0	41.0 ± 3.0
120	48.0 ± 2.0	2.0 ± 0.3	3.0 ± 0.5	42.0 ± 4.0	53.0 ± 5.0

Table 4 Amino acid composition of casein, gliadin, and glutenin hydrolysates and their mixtures

Amino acid	Hydrolysate								
	Casein, 90 min	Gliadin, 60 min	Glutenin, 30 min	Casein, 90 min +					
				Gliadin 60 min, casein:gliadin ratio			Glutenin 30 min, casein:glutenin ratio		
				0.5:1	1:1	2:1	0.5:1	1:1	1:2
Gly	2.7 ± 0.1	1.9 ± 0.1	3.4 ± 0.2	2.2 ± 0.2	2.3 ± 0.1	2.5 ± 0.1	2.4 ± 0.1	2.9 ± 0.1	3.2 ± 0.2
Ala	3.0 ± 0.2	2.5 ± 0.1	2.5 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	2.7 ± 0.1
Val	7.2 ± 0.4	5.2 ± 0.3	4.5 ± 0.2	5.9 ± 0.3	6.2 ± 0.3	5.9 ± 0.3	6.5 ± 0.3	6.3 ± 0.3	5.4 ± 0.3
Ile	6.1 ± 0.3	5.1 ± 0.3	3.7 ± 0.2	5.4 ± 0.3	5.6 ± 0.3	4.9 ± 0.2	5.8 ± 0.3	5.3 ± 0.3	4.5 ± 0.2
Leu	9.2 ± 0.5	8.4 ± 0.4	7.3 ± 0.4	8.7 ± 0.4	8.8 ± 0.4	8.3 ± 0.4	8.9 ± 0,4	8.6 ± 0.4	7.9 ± 0.4
Pro	11.0 ± 1.0	18.0 ± 1.0	17.0 ± 1.0	16.0 ± 1.0	15.0 ± 1.0	14.0 ± 1.0	14.0 ± 1.0	13.0 ± 1.0	15.0 ± 1.0
Ser	6.3 ± 0.3	4.1 ± 0.2	3.3 ± 0.2	4.8 ± 0.2	5.2 ± 0.3	4.8 ± 0.2	5.6 ± 0.3	5.3 ± 0.3	4.3 ± 0.2
Thr	4.9 ± 0.2	2.2 ± 0.1	2.7 ± 0.1	3.1 ± 0.2	3.6 ± 0.2	3.8 ± 0.2	4.0 ± 0.2	4.2 ± 0.2	3.4 ± 0.2
Cys	0.30 ± 0.02	5.1 ± 0.3	5.7 ± 0.3	3.5 ± 0.2	2.7 ± 0.1	3.0 ± 0.2	1.9 ± 0.1	2.1 ± 0.1	3.9 ± 0.2
Met	2.8 ± 0.1	1.6 ± 0.1	1.8 ± 0.1	2.0 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	2.5 ± 0.1	2.1 ± 0.1
Asp	7.1 ± 0.4	2.8 ± 0.1	2.7 ± 0.1	4.2 ± 0.2	5.0 ± 0.3	4.9 ± 0.2	5.7 ± 0.3	5.7 ± 0.3	4.2 ± 0.2
Glu	22.4 ± 1.1	51.0 ± 3.0	49.0 ± 2.0	41.0 ± 2.0	37.0 ± 2.0	36.0 ± 2.0	32.0 ± 2.0	32.0 ± 2.0	41.0 ± 2.0
Lys	8.2 ± 0.4	0.73 ± 0.04	1.2 ± 0.1	3.2 ± 0.2	4.5 ± 0.2	4.7 ± 0.2	5.7 ± 0.3	5.9 ± 0.3	3.5 ± 0.2
Arg	4.1 ± 0.2	2.9 ± 0.2	2.9 ± 0.1	3.3 ± 0.2	3.5 ± 0.2	3.5 ± 0.2	3.7 ± 0.2	3.7 ± 0.2	3.3 ± 0.2
His	3.1 ± 0.2	1.8 ± 0.1	1.5 ± 0.1	2.2 ± 0.1	2.5 ± 0.1	2.3 ± 0.1	2.7 ± 0.1	2.6 ± 0.1	2.0 ± 0.1
Phe	5.0 ± 0.3	6.6 ± 0.3	6.0 ± 0.3	6.1 ± 0.3	5.8 ± 0.3	5.5 ± 0.3	5.5 ± 0.3	5.3 ± 0.3	5.7 ± 0.3
Trp	1.2 ± 0.1	0.10 ± 0.01	1.1 ± 0.1	0.54 ± 0.03	0.73 ± 0.04	1.1 ± 0.1	0.82 ± 0.04	1.1 ± 0.1	1.1 ± 0.1
Tyr	6.3 ± 0.3	3.0 ± 0.2	3.5 ± 0.2	4.1 ± 0.2	4.7 ± 0.2	4.9 ± 0.2	5.2 ± 0.3	5.4 ± 0.3	4.4 ± 0.2

Table 5 Functional properties of mixed hydrolysates of wheat proteins and casein

Mixtures of protein hydrolysates	Water-holding, g H_2O/g protein	Fat-holding, g oil/g protein	Emulsifying, % emulsion	Foaming, mm
Casein 90 min + Gliadin 60 min (0.5:1)	1.67 ± 0.08	1.16 ± 0.06	8.0 ± 1.0	35.0 ± 2.0
Casein 90 min + Gliadin 60 min (1:1)	1.25 ± 0.06	0.75 ± 0.04	14.0 ± 1.0	42.0 ± 3.0
Casein 90 min + Gliadin 60 min (2:1)	0.95 ± 0.03	0.68 ± 0.03	20.0 ± 1.0	56.0 ± 3.0
Casein 90 min + Glutenin 30 min (0.5:1)	2.05 ± 0.10	1.15 ± 0.06	18.0 ± 1.0	10.0 ± 1.0
Casein 90 min + Glutenin 30 min (1:1)	1.87 ± 0.09	0.93 ± 0.05	20.0 ± 1.0	18.0 ± 1.0
Casein 90 min + Glutenin 30 min (2:1)	1.34 ± 0.07	0.28 ± 0.01	24.0 ± 1.0	29.0 ± 2.0

As can be seen, shorter enzymolysis increased the proportion of low-molecular-weight fractions (under 15 kDa) in casein hydrolysates. After 90 min, the proportion of high-molecular allergenic fractions (over 25 kDa) was less than 20%. Therefore, this time is sufficient to obtain hypoallergenic casein hydrolysates. According to literature [41], deep hydrolysis of casein results in the formation of bitter peptides. In our study, the degree of protein hydrolysis did not exceed 45%. Therefore, we can expect a minimal amount of bitter peptides.

For further tests, we selected the samples of three hydrolysates (one for each protein), namely:
– casein hydrolysate (90 min);
– glutenin hydrolysate (30 min); and
– gliadin hydrolysate (60 min).

The hydrolysates of casein and glutenin had a high emulsifying capacity, while the gliadin hydrolysate had a high foaming capacity. All of them had a low level of allergenicity.

One of the significant disadvantages of wheat proteins is their low content of certain amino acids.

Table 6 Amino acid score of hydrolysates

Amino acid	Amino acid score, %								
	Hydrolysates								
	Casein, 90 min	Gliadin, 60 min	Glutenin, 30 min	Casein, 90 min +					
				Gliadin 60 min, casein:gliadin ratio			Glutenin 30 min, casein:glutenin ratio		
				0.5:1	1:1	2:1	1:2	1:1	2:1
Lys	149 ± 7	13 ± 1	22 ± 1	58 ± 3	82 ± 4	85 ± 4	64 ± 3	107 ± 5	104 ± 5
Met+Cys	89 ± 4	191 ± 10	136 ± 7	157 ± 8	140 ± 7	151 ± 8	171 ± 9	131 ± 7	123 ± 6
Ile	153 ± 8	128 ± 6	93 ± 5	135 ± 7	140 ± 7	123 ± 6	113 ± 6	133 ± 7	145 ± 7
Leu	131 ± 7	120 ± 6	104 ± 5	124 ± 6	126 ± 6	119 ± 6	113 ± 6	123 ± 6	127 ± 6
Thr	123 ± 6	55 ± 3	68 ± 3	78 ± 4	90 ± 5	95 ± 5	85 ± 4	105 ± 5	100 ± 5
Phe+Tyr	188 ± 9	110 ± 6	100 ± 5	102 ± 5	97 ± 5	92 ± 5	95 ± 5	88 ± 4	92 ± 5
Trp	120 ± 6	100 ± 5	108 ± 5	54 ± 3	73 ± 4	110 ± 6	110 ± 6	110 ± 6	82 ± 4
Val	144 ± 7	104 ± 5	90 ± 5	118 ± 6	124 ± 6	118 ± 6	108 ± 5	126 ± 6	130 ± 7

Particularly, gliadin is low in lysine, threonine, tryptophan, arginine, histidine, cystine, and methionine, whereas glutenin is low in lysine, although its content is higher than in gliadin. Therefore, mixtures of casein hydrolysates and wheat protein hydrolysates are of particular interest due to their balanced amino acid composition and good functional properties. Such mixtures can be used as ingredients in functional food products.

Finally, we studied mixtures of casein hydrolysates with gliadin and glutenin hydrolysates in various ratios. We determined their amino acid composition and functional properties (Tables 4 and 5).

Mixing plant protein hydrolysates with casein hydrolysate enriches them with such amino acids as alanine, valine, serine, methionine, aspartic acid, lysine, and tyrosine (Table 4). At the same time, the contents of other amino acids change insignificantly, except for glutamic acid, which somewhat decreases, but remains quite high.

The mixtures of gliadin/glutenin and casein hydrolysates had better foaming and emulsifying properties, which improved with a higher proportion of casein. Thus, we found it worthwhile to mix hydrolyzed wheat proteins with casein to obtain improved functional properties and hypoallergenicity.

Table 6 shows the amino acid score for the obtained mixtures of protein hydrolysates. As can be seen, the score was high in the mixtures of casein and gliadin hydrolysates (2:1), as well as casein and glutenin (2:1). These mixtures also showed high emulsifying and foaming capacities. Therefore, they can be considered the most promising for obtaining hypoallergenic ingredients for functional food products.

CONCLUSION

The most effective enzyme preparations for the enzymolysis of casein, gliadin, and glutenin were Protex 6L for gliadin and chymotrypsin for glutenin and casein;

We recommend 40 u/g of substrate as an optimal amount of enzyme preparations to provide the maximum degree of hydrolysis;

The study of functional properties of casein, glutenin, and gliadin hydrolysates (15, 30, 60, and 90 min) showed that all the samples had relatively low water- and fat-holding capacities. The highest emulsifying capacity was observed in casein and glutenin hydrolysates, while the highest foaming capacity was found in gliadin hydrolysates;

The enzymolysis of all the proteins significantly decreased their allergenicity. Therefore, their hydrolysates can be used to obtain functional additives for hypoallergenic food products;

The mixtures of casein hydrolysate (90 min) with gliadin (60 min) or glutenin (30 min) hydrolysates had a balanced amino acid composition and a high amino acid score. In addition, they retained high emulsifying and foaming capacities; and

Our study proved the need for mixing wheat protein hydrolysates with casein hydrolysate to obtain improved functional properties and hypoallergenicity.

CONTRIBUTION

D.V. Prikhodko carried out the experiments, as well as processed and interpreted experimental data. A.A. Krasnoshtanova supervised the research, reviewed experimental data, and formulated conclusions.

CONFLICT OF INTEREST

The authors declared no conflict of interests regarding the publication of this article.

REFERENCES

1. Ashfaq A, Jahan K, Islam RU, Younis K. Protein-based functional colloids and their potential applications in food: A review. LWT. 2022;154. https://doi.org/10.1016/j.lwt.2021.112667

2. Sá AGA, Moreno YMF, Carciofi BAM. Plant proteins as high-quality nutritional source for human diet. Trends in Food Science and Technology. 2020;97:170–184. https://doi.org/10.1016/j.tifs.2020.01.011
3. Baskar N, Varadharajan S, Rameshbabu M, Ayyasamy S, Velusamy S. Development of plantbased yogurt. Foods and Raw Materials. 2022;10(2):274–282. https://doi.org/10.21603/2308-4057-2022-2-537
4. Zhang T, Dou W, Zhang X, Zhao Y, Zhang Y, Jiang L, *et al.* The development history and recent updates on soy protein-based meat alternatives. Trends in Food Science and Technology. 2021;109:702–710. https://doi.org/10.1016/j.tifs.2021.01.060
5. Bakaeva NP, Korzhavina NYu. Method for extraction of protein and its fractions from grains of winter wheat of Volga-86 variety. Vestnik of Buryat State Academy of Agriculture named after V. Philippov. 2015;40(3):7–11. (In Russ.). https://elibrary.ru/UHSJRD
6. Ortolan F, Urbano K, Netto FM, Steel CJ. Chemical and structural characteristics of proteins of non-vital and vital wheat glutens. Food Hydrocolloids. 2022;125. https://doi.org/10.1016/j.foodhyd.2021.107383
7. Kontopidis G, Holt C, Sawyer L. Invited review: *β*-lactoglobulin: Binding properties, structure, and function. Journal of Dairy Science. 2004;87(4):785–796. https://doi.org/10.3168/jds.S0022-0302(04)73222-1
8. Saadi S, Saari N, Ghazali HM, Abdulkarim SM, Hamid AA, Anwar F. Gluten proteins: Enzymatic modification, functional and therapeutic properties. Journal of Proteomics. 2022;251. https://doi.org/10.1016/j.jprot.2021.104395
9. García-García A, Madrid R, González I, García T, Martín R. A novel approach to produce phage single domain antibody fragments for the detection of gluten in foods. Food Chemistry. 2020;321. https://doi.org/10.1016/j.foodchem.2020.126685
10. Mulder CJJ, van Wanrooij RLJ, Bakker SF, Wierdsma N, Bouma G. Gluten-free diet in glutenrelated disorders. Digestive Diseases. 2013;31:57–62. https://doi.org/10.1159/000347180
11. Yao Y, Jia Y, Lu X, Li H. Release and conformational changes in allergenic proteins from wheat gluten induced by high hydrostatic pressure. Food Chemistry. 2022;368. https://doi.org/10.1016/j.foodchem.2021.130805
12. Kurbatova EI, Rimareva LV, Sokolova EN, Davydkina VE, Serba EM, Pogorzhel′skaya NS, *et al.* Reducing the allergenicity of wheat proteins based on their biocatalytic conversion. Problems of Nutrition. 2016;85(S2). (In Russ.). https://elibrary.ru/XCFEVJ
13. Gutiérrez S, Peres-Andres J, Martinez-Blanco H, Ferrero MA, Vaquero L, Vivas S, *et al.* The human digestive tract has proteases capable of gluten hydrolysis. Molecular Metabolism. 2017;6(7):693–702. https://doi.org/10.1016/j.molmet.2017.05.008
14. Heredia-Sandoval NG, Valencia-Tapia MY, de la Barca AMC, Islas-Rubio AR. Microbial proteases in baked goods: Modification of gluten and effects on immunogenicity and product quality. Foods. 2016;5(3). https://doi.org/10.3390/foods5030059
15. Wusigale, Liang L, Luo Y. Casein and pectin: Structures, interactions, and applications. Trends in Food Science and Technology. 2020;97:391–403. https://doi.org/10.1016/j.tifs.2020.01.027
16. Casanova F, Nascimento LGL, Silva NFN, de Carvalho AF, Gaucheron F. Interactions between caseins and food-derived bioactive molecules: A review. Food Chemistry. 2021;359. https://doi.org/10.1016/j.foodchem.2021.129820
17. Souza Júnior EC, Santos MPF, Sampaio VS, Ferrão SPB, Fontan RCI, Bonomo RCF, *et al.* Hydrolysis of casein from different sources by immobilized trypsin on biochar: Effect of immobilization method. Journal of Chromatography B. 2020;1146. https://doi.org/10.1016/j.jchromb.2020.122124
18. Liyanaarachchi WS, Vasiljevic T. Caseins and their interactions that modify heat aggregation of whey proteins in commercial dairy mixtures. International Dairy Journal. 2018;83:43–51. https://doi.org/10.1016/j.idairyj.2018.03.006
19. Khan IT, Nadeem M, Imran M, Ullah R, Ajmal M, Jaspal MH. Antioxidant properties of milk and dairy products: a comprehensive review of the current knowledge. Lipids in Health and Disease. 2019;18(1). https://doi.org/10.1186/s12944-019-0969-8
20. Lordan R, Tsoupras A, Mitra B, Zabetakis I. Dairy fats and cardiovascular disease: Do we really need to be concerned? Foods. 2018;7(3). https://doi.org/10.3390/foods7030029
21. Melnikova EI, Stanislavskaya EB, Bogdanova EV, Shabalova ED. Micellar casein production and application in dairy protein industry. Food Processing: Techniques and Technology. 2022;52(3):592–601. (In Russ.). https://doi.org/10.21603/2074-9414-2022-3-2389
22. Vesnina A, Prosekov A, Kozlova O, Atuchin V. Genes and eating preferences, their roles in personalized nutrition. Genes. 2020;11(4). https://doi.org/10.3390/genes11040357
23. Linhart B, Freidl R, Elisyutina O, Khaitov M, Karaulov A, Valenta R. Molecular approaches for diagnosis, therapy and prevention of cow´s milk allergy. Nutrients. 2019;11(7). https://doi.org/10.3390/nu11071492
24. Hochwallner H, Schulmeister U, Swoboda I, Spitzauer S, Valenta R. Cow's milk allergy: From allergens to new forms of diagnosis, therapy and prevention. Methods. 2014;66(1):22–33. https://doi.org/10.1016/j.ymeth.2013.08.005

25. Sun Z, Wang M, Han S, Ma S, Zou Z, Ding F, *et al*. Production of hypoallergenic milk from DNA-free beta-lactoglobulin (BLG) gene knockout cow using zinc-finger nucleases mRNA. Scientific Reports. 2018;8(1). https://doi.org/10.1038/s41598-018-32024-x
26. Kumar D, Chatli MK, Singh R, Mehta N, Kumar P. Antioxidant and antimicrobial activity of camel milk casein hydrolysates and its fractions. Small Ruminant Research. 2016;139:20–25. https://doi.org/10.1016/j.smallrumres.2016.05.002
27. Izadi A, Khedmat L, Mojtahedi SY. Nutritional and therapeutic perspectives of camel milk and its protein hydrolysates: A review on versatile biofunctional properties. Journal of Functional Foods. 2019;60. https://doi.org/10.1016/j.jff.2019.103441
28. Vorob'ev MM. Quantification of two-step proteolysis model with consecutive demasking and hydrolysis of peptide bonds using casein hydrolysis by chymotrypsin. Biochemical Engineering Journal. 2013;74:60–68. https://doi.org/10.1016/j.bej.2013.02.020
29. Du X, Jing H, Wang L, Huang X, Wang X, Wang H. Characterization of structure, physicochemical properties, and hypoglycemic activity of goat milk whey protein hydrolysate processed with different proteases. LWT. 2022;159. https://doi.org/10.1016/j.lwt.2022.113257
30. Xing Y, Giosafatto CVL, Fusco A, Dong M, Mariniello L. Combined lactic fermentation and enzymatic treatments affect the antigenicity of *β*-lactoglobulin in cow milk and soymilk-cow milk mixture. LWT. 2021;143. https://doi.org/10.1016/j.lwt.2021.111178
31. Kataria A, Sharma R, Sharma S, Singh B, Kaur G, Yakubu CM. Recent applications of bio-engineering principles to modulate the functionality of proteins in food systems. Trends in Food Science and Technology. 2021;113:54–65. https://doi.org/10.1016/j.tifs.2021.04.055
32. Prikhod′ko DV, Krasnoshtanova AA. A study of the functional properties of wheat gluten fractions. Scientific achievements of the third millennium: Collection of scientific papers on materials XIII International Scientific Conference; 2021; New York. New York: SPC LJournal; 2021. p. 161–165. (In Russ.). https://doi.org/10.18411/scienceconf-03-2021-30
33. Kurbanova MG. Enzymatic hydrolysis of milk proteins with various proteases use. Bulletin of KSAU. 2010;40(1):157–160. (In Russ.). https://elibrary.ru/LADHZX
34. Babich OO, Razumnikova IS, Poletaev AYu, Morozova AI. Keratin containing waste processing and manufacture of albuminous hydrolysates for food and fodder purposes. Food Processing: Techniques and Technology. 2011;21(2):7–11. (In Russ.). https://elibrary.ru/NYGVEB
35. Vasilevskaya ER, Aryuzina MA, Vetrova ES. Saline extraction as a method of obtaining a mixture of biologically active compounds of protein nature from a porcine pancreas. Food Systems. 2021;4(2):97–105. (In Russ.). https://doi.org/10.21323/2618-9771-2020-4-2-97-105
36. Kamilov FKh, Galimov ShN, Agletdinov EhF, Knyazeva OA, Abdullina GM, Karyagina NT, *et al.* Biochemical practicum: a manual for student classroom work (020400.62 Biology, Microbiology. Part II). Ufa: Bashkir State Medical University; 2014. 99 p. (In Russ.). https://elibrary.ru/XPDBCX
37. Evdokimova OV, Griminova EB, Tolkunova NN, Pryanishnikov VV. Functional and technological properties of protein preparations. Izvestiya Vuzov. Food Technology. 2006;291–292(2–3):73–74. (In Russ.). https://elibrary.ru/KVKZRL
38. Nasri R, Abdelhedi O, Nasri M, Jridi M. Fermented protein hydrolysates: Biological activities and applications. Current Opinion in Food Science. 2022;43:120–127. https://doi.org/10.1016/j.cofs.2021.11.006
39. Prosekov AYu, Ulrih EV, Noskova SYu, Budrik VG, Botina SG, Agarkova EYu, *et al.* The getting enzymatic whey protein hydrolyzate using proteolitic enzyme. Fundamental Research. 2013;(6–5):1089–1093. (In Russ.). https://elibrary.ru/OLLOIH
40. Golovach TN, Kurchenko VP. Allergenicity of milk proteins and ways to reduce it. Proceedings of the Belarusian State University. Series of Physiological, Biochemical and Molecular Biology Sciences. 2010;5(1):9–55. (In Russ.). https://elibrary.ru/ZDDSUH
41. Sviridenko YuYa, Myagkonosov DS, Abramov DV, Ovchinnikova EG. Theoretical and practical aspects of development technology of manufacturing protein hydrolyzates for special nutrition use. Part 2. Functional properties of protein hydrolysates that depend on the specificity of proteolytic processes. Food Industry. 2017;(6):50–53. (In Russ.). https://elibrary.ru/YRXEBF

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