Protein hydrolysates from *Mytilus edulis L.* mussel: physicochemical and antioxidant properties

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Abstract. Meat of marine molluscs is a promising protein-containing raw material for obtaining protein hydrolysate and biologically active peptides. The purpose of this research was to study the physicochemical properties and antioxidant properties of protein hydrolysate obtained from the mussel *Mytilus edulis L.* The hydrolysates were obtained by acidic hydrolysis and enzymatic hydrolysis using protozyme as an enzyme. Gel permeation chromatography was used as experimental method in this study to characterise the molecular weight distribution of the protein hydrolysates. Chemical analysis was used for determine the composition and antioxidant activity of hydrolysates. It is shown that the content of low-molecular fractions - biologically active peptides with a molecular weight of less than 5 kDa is almost three times higher in the enzymatic hydrolysate compared to the acidic one. Accordingly, the enzymatic hydrolysate demonstrates high antioxidant activity, which determines its prospects as a component for functional food products and biomedical drugs.

1 Introduction

Shellfish cultivation of is a rapidly developing and highly profitable area of the global aquaculture industry [1, 2]. Mussel meat is a dietary product, rich in proteins, polyunsaturated fatty acids, vitamins and microelements [3, 4]. However, during industrial processing, about 30% of mussels are rejected due to non-compliance with size criteria. So, a large number of shells remain as a by-product of processing. One of the promising ways to use the resulting by-product is to obtain protein hydrolysates [5].

Protein hydrolysates have high nutritional value, are rich in essential amino acids; they are an excellent source of biologically active peptides and are easily digestible. Protein hydrolysates are widely used in the food industry as a protein component in functional products with high biological and nutritional value [2, 3, 6]. The traditional method for obtaining protein hydrolysates is protein hydrolysis in an acidic or alkaline medium (using acids or alkalis). Acid hydrolysis of various protein-containing raw materials is most often used [7].

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Recently, the method of protein hydrolysis and obtaining biologically active peptides under the action of proteolytic enzymes has become widespread. The advantages of enzymatic hydrolysis are associated with the mild conditions of the reaction medium affecting the protein, with the possibility of using a proteolytic enzyme with high selectivity [8, 9]. The physicochemical properties, as well as the functional, technological and organoleptic properties of protein hydrolysates depend on many factors, including the type of enzyme, its concentration, pH, temperature and duration of hydrolysis. The analysis of the influence of various factors on the properties of the obtained protein hydrolysates is carried out in reviews [10, 11]. Additionally, the type and properties of the original protein-containing raw material have a great influence on the quality and properties of the final product [12]. Protein hydrolysates from marine mollusks are an excellent source of biologically active peptides [13], which are used in the composition of functional foods and pharmaceuticals [14, 15].

The relevance of the work is associated with the high potential for the development of aquaculture of bivalve mollusks - mussels in the Murmansk region in small settlements located on the coast of the Barents Sea [16]. They are found everywhere in the littoral zone; their biomass can reach up to 2.5 kg or more per 1 m^2. However, to date, work on the use of non-commercial (small) sized mussels has been virtually non-existent. At the same time, they are an excellent protein-containing raw material for obtaining protein hydrolysates and, therefore, a source of biologically active peptides that exhibit antioxidant and anti-inflammatory activity, which can be used to enrich food products to reduce the risk of chronic diseases.

The work investigated the influence of the method (acid and enzymatic hydrolysis) of obtaining on the physicochemical and functional properties of protein hydrolysates from marine mussels *Mytilus edulis L*. Protozyme was used as an enzyme when carrying out enzymatic hydrolysis.

2 Materials and methods

2.1 Protein-containing raw material

The protein-containing raw material was mussel meat (*Mytilus edulis L.*). The mussels were collected on the coast of the Barents Sea. The mussels were cut up, the soft tissues (the body of the mollusc) were washed with cold water to remove excess salts and then crushed.

2.2 Obtaining protein hydrolysate

To carry out enzymatic hydrolysis, the enzyme preparation of microbial origin protozyme was used. The protein hydrolysate was obtained using standard technology [17]. The crushed protein-containing raw material was mixed with distilled water in a weight ratio of 1:1, the parameters of the reaction medium were brought to the conditions necessary for the effective operation of the enzyme (pH=8.2±0.3, temperature 60±1 °C), and the calculated amount of the enzyme preparation was introduced (C_{EP}=4 g/kg of raw material). Fermentolysis was carried out for 2 hours with constant stirring. Then the reaction mixture was heated to a temperature of 90–95 °C, non-hydrolysed raw materials were removed by filtration, the filtrate (hydrolysate solution) was dried in a freeze dryer. Acid hydrolysis was carried out at pH=2.0±0.2 for 6 hours at a temperature of (60±1) °C and constant stirring. A 5M hydrochloric acid solution was used to regulate the pH of the aqueous phase. After hydrolysis, the reaction mixture was neutralized to pH 5.5–6.0, then heated to 100 °C, filtered and dried.

The resulting hydrolysate samples were homogeneous finely dispersed powders of beige and yellow color, respectively, well soluble in water.

The degree of hydrolysis (DH, %), characterizing the efficiency of protein breakdown, was determined by the formula:

$$DH = \frac{N_{AA}}{N_{OA}} \cdot 100\%, \quad (1)$$

where N_{AN} is the mass fraction of amino nitrogen (%), N_{TN} is the mass fraction of total nitrogen (%).

The yield of the product (protein hydrolysate) (B, %) was calculated using the formula:

$$B = (m / M) \cdot 100\%, \quad (2)$$

where *m* is the mass of dry protein hydrolysate (g), *M* is the mass of the original wet protein-containing raw material (g).

2.3 Chemical analysis

Chemical analysis of hydrolysates was carried out using standard methods [18]. Moisture content was determined by drying samples at 105±5 ºC to constant weight; fat content was determined by the Soxhlet method (solvent extraction); protein content – by the Kjeldahl method, amine nitrogen – by formol titration, mineral substances – by burning samples in a muffle furnace at 550±10 °C.

2.4 Molecular weight distribution

The molecular weight distribution of the protein hydrolysates was determined by gel permeation chromatography. Separation conditions: eluent 0.5 M acetic acid solution, flow rate 0.8 ml/min, T=30 °C, ELSD detector (low-temperature evaporative light scattering detector). Column: TSK-GEL G3000SWXL, 7.8 mm ID × 30.0 cm L, 5 μm.

2.5 Antioxidant properties

Antioxidant properties (biologically active properties) were assessed by the ability of hydrolysate solutions to interact with the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) [19]. A reaction mixture containing 3 cm^3 of 0.3 mM DPPH in ethanol, 1 cm^3 of 50 mM Tris buffer solution with pH 7.4 and 1 cm3 of an aqueous solution of hydrolysate (the concentration of the hydrolysate solution varied from 0.5 to 3.0 wt.%) at 20.0±0.1 °C for 40 min was used. Then the mixture was centrifuged and the optical density (at 518 nm) was recorded using a T-70 UV-Vis spectrophotometer. Antioxidant properties were assessed by radical-binding activity (RSA, %) and effective concentration of the substance (IC50, mg/ml), at which 50% of DPPH free radicals are reduced. Radical-binding activity was calculated by the formula:

$$RBA = \frac{A_0 - A_1}{A_0} \times 100, \quad (3)$$

where A_0 is the optical density of the control solution; A_1 is the optical density of the system after adding the hydrolysate. The effective concentration of the substance (IC_{50}, mg/ml) was determined from the graph of the dependence of the optical density of the system on the concentration of the hydrolysate solution.

3 Results and discussions

The physicochemical properties and yield of protein hydrolysates obtained by acid hydrolysis and enzymatic hydrolysis are presented in Table 1. Protein hydrolysates are characterized by good organoleptic properties; the samples are powders of yellow and beige color, respectively, without foreign odor.

Table 1. Physicochemical properties and yield of protein hydrolysates obtained by acid and enzymatic hydrolysis methods.

Characteristics	Protein-containing raw material (mussel meat)	Acid hydrolysate	Enzymatic hydrolysate
Moisture, %wt.	85.9±0.5	7.1±0.5	8.5±0.5
Ash, % масс.	0.7±0.1	19.9±0.5	7.8±0.7
Protein*, %wt.	11.9±0.6	73.1±1.8	85.6±1.6
N_{TN}, %wt.	1.9±0.1	11.7±0.4	13.7±0.7
N_{AN}, %wt		1.7±0.6	2.3±0.3
pH**		5.6±0.2	6.8±0.2
DH, %		13,4±1.1	18.8±1.2
B, %		5.6±0.7	8.7±0.8

* Protein content was determined as N_{NT}×6.25
** pH was determined in 1% protein hydrolysate solution.

The analysis of the data presented in Table 1 shows that the enzymatic hydrolysate is characterized by a higher content of protein substances of 85.6 %wt. and a reduced ash content of 7.8 %wt., compared to the acid hydrolysate, in which the protein and ash content are equal to 73.1 %wt. and 19.9 %wt., respectively. Apparently, in the process of acid hydrolysis, an increase in the number of mineral salts occurs at the neutralization stage when obtaining hydrochloric acid hydrolysate. The yield of the enzymatic hydrolysate was 8.7 %, while the yield of the acid hydrolysate was 1.5 times lower – 5.6%. All hydrolysates are fat-free, the amount of moisture does not exceed 7.1 %wt. The obtained data indicate the advantages of enzymatic hydrolysis in the processing of marine mussel meat.

The degree of hydrolysis is one of the main characteristics of hydrolysates. The degree of hydrolysis corresponds to the proportion (%) of peptide bonds split during hydrolysis from the total number of peptide bonds in the protein. However, determining the degree of hydrolysis is not enough to fully characterize the composition of the protein hydrolysate. For a detailed description of the composition of the hydrolysate, including polypeptide protein fragments, the molecular weight distribution profile is used.

Molecular weight distribution is an important characteristic of protein hydrolysates. Protein fragments (peptides) with a molecular weight of less than 5 kDa have bioactive properties, they have antioxidant, antimicrobial, antitumor properties, anti-inflammatory action, etc. [20, 21, 22]. Hydrolysates from marine molluscs containing bioactive peptides are used to obtain therapeutic and prophylactic drugs [23].

The molecular weight distribution of protein hydrolysates obtained as a result of acid and enzymatic hydrolysis is shown in Fig. 1 and Table 2.

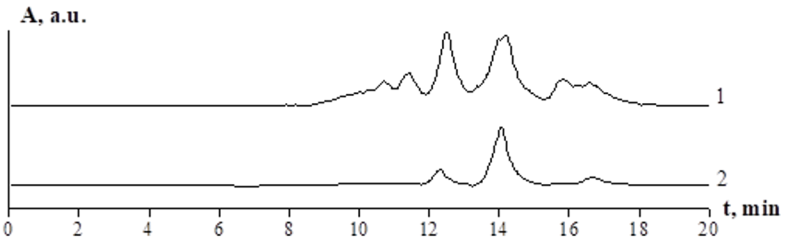

Fig. 1. Molecular weight distribution of protein hydrolysates from mussel meat: 1 – enzymatic hydrolysate; 2 – hydrochloric acid hydrolysate.

Table 2. Molecular weight distribution of protein hydrolysates from mussel meat obtained by enzymatic and acid hydrolysis methods

Protein hydrolysate	Molecular weight of fraction, M_{wf}, kDa	Fraction share, ω, %	Molecular weight, M_w^*, kDa
Enzymatic	46.2	14.4	16.1
	27.9	9.7	
	21.6	22.8	
	10.9	33.9	
	1.9	8.0	
	≤ 0.4	11.1	
Hydrochloric acid	113.1	52.1	64.7
	28.5	1.8	
	22.3	7.9	
	10.8	31.3	
	2.2	0.9	
	≤ 0.6	6.0	

* Mw (kDa) calculated according to additivity rule.

Data analysis showed that the hydrolysates have a wide molecular weight distribution and contain at least 6 protein fractions. The average molecular weight calculated by the additivity rule was 16.1 kDa for the enzymatic hydrolysate and 64.7 kDa for the hydrochloric acid hydrolysate. Thus, the content of low-molecular fractions - biologically active peptides with a molecular weight of less than 5 kDa is almost three times higher in the enzymatic hydrolysate compared to the acidic one (see Table 2). Determination of the antioxidant properties of the obtained hydrolysates confirmed the higher biological activity of the enzymatic hydrolysate.

Radical-binding activity and effective concentration of the substance, characterizing the antioxidant properties of the two types of hydrolysates, are given in Table 3. Analysis of the obtained data shows that radical-binding activity of the enzymatic protein hydrolysate is more than three times higher than the similar characteristic of the acid hydrolysate.

Table 3. Antioxidant properties of protein hydrolysates from mussel meat.

Protein hydrolysate	Radical-binding activity, RBA, %	Effective concentration of the substance, IC_{50}, mg/ml
Enzymatic	44.6±0.6	0.23±0.8
Hydrochloric acid	14.6±0.4	0.48±0.5

4 Conclusion

Cultivation of mussels in coastal regions is an intensively developing area of aquaculture. Mussels' meat, rich in protein, is a dietary product in the diet. Meat of substandard mussels, which are not in demand in the food industry, is a promising protein-containing raw material for obtaining protein hydrolysates and bioactive peptides with a molecular weight of less than 5 kDa.

In this research, it is shown that chemical (acidic) and enzymatic hydrolysis of mussel processing by-product can be used to obtain bioactive peptides; protozyme was used as an enzyme in the work. In this case, the yield of enzymatic hydrolysate is 8.7 %, which is significantly higher in comparison with acidic hydrolysate, the yield of which is 5.6%. The advantages of enzymatic hydrolysate have been shown, which is characterized by a low average molecular weight of 16.1 kDa, a high content of proteins of 85.6 %wt. The enzymatic hydrolysate contains a large number of low-molecular protein fractions – biologically active peptides more than 19%; the acid hydrolysate contains bioactive peptides less than 7 %. Accordingly, the enzymatic hydrolysate demonstrates high antioxidant properties, which determines its prospects for use in functional food products.

Acknowledgements

This research was funded the Russian Science Foundation (project no. 22-16-20046), and the Ministry of Education and Science of the Murmansk Region (agreement no. 103).

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