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ENRICHED PROTEIN PRODUCTS OF MARINE ORIGIN LIKE THE NEW COMPONENTS OF THE DIET FOR PEOPLE WITH PHYSICAL LOAD

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Abstract: The possibility of the use of the native and enzyme modified shrimp biomass (*Pandalus borealis*) for the development of functional foods that compensate for the lack of protein in people engaged in various sports (cyclic, speed-strength, competitive, complex coordination, technically complex, and combat sports) is scientifically substantiated and experimentally confirmed.

For the first time, the technology is developed for the production of combined high-protein structured food systems (pate) using the native and enzyme modified shrimp biomass.

Organoleptic, physico-chemical, and microbiological indicators of quality of high-protein structured food systems (pate) have been analyzed.

In order to prove the biological effect and activity, amino acid and fatty acid comparative compositions of the native and enzyme modified shrimp biomass for the development of functional sport food products have been investigated.

For the first time, fatty acid compositions of pate with and without the addition of an antioxidant have been examined. The storability of these products has been analyzed.

The purpose of the paper is the development of the technology for the production of combined high-protein structured food systems (pate) using the native and enzyme modified shrimp biomass and the comparative analysis of their biological value.

Keywords: native shrimp biomass, enzyme modified shrimp biomass, functional product, sports nutrition, proteins, amino acids, northern shrimp, soy peptides, polyunsaturated fatty acids

INTRODUCTION

According to forecasts of the global supply of the population of the Earth with protein substances in this century the protein balance in products can only be achieved by the combination of vegetable and animal proteins [1].

In this regard, research on expansion of the range of safe and quality food products based on marine fauna and terrestrial plant material has become increasingly important [2].

The Far East is rich in biological resources of plant and animal origin. Northern shrimp (*Pandalus borealis*) which is common in the north of the Far East, the Sea of Japan, and the Gulf of Peter the Great, one of the most accessible for the population of the Far East, is considered as raw material for the production of functional foods. It is the species of cold-water shrimp. This is a small but most valuable species since it contains many useful substances (antioxidant, astaxanthin vitamins, enzymes and hormones, calcium, potassium, magnesium, manganese, copper, molybdenum, phosphorus, iron, amino acids leucine, lysine, aspartic and glutamic acids, and glycine). It was

taken as a basis for the development of the shrimp biomass [3].

According to the Institute of Nutrition of the Russian Academy of Medical Sciences, soy protein is easily digestible, high-value, reasonably balanced in terms of amino acid composition, comparable in biological value with the protein of milk, fish, and beef. The soybean and derived products occupy a special place among legumes because of the high content of valuable protein (up to 40% or more) and fat (20% or more). It is the most common legume in the world.

The importance of the source of soy protein should be noted, since various soy products (soy flour, soy concentrate, and isolated soybean protein) have a different coefficient of digestibility. They are unequal in their medical and biological properties which is also associated with various manufacturing methods. Only soy protein products manufactured with the most modern technology, i.e., by aqueous extraction, have the highest digestibility coefficient and retain most of the natural properties of soy. On average soybean seeds contain about 35% of protein, while isolated soy proteins (isolates) contain up to 90% of protein. In this

context, *isolated* denotes *highly purified protein derived from soybeans* [4].

The reason for the increased use of the shrimp biomass and soy peptides in the production of high-protein functional foods is the unique amino acid composition of shrimp biomass and soy protein, complementarity of soy proteins with muscle proteins which increases the overall biological value of the protein composition of the finished product, neutral flavor characteristics of soy proteins and their compatibility with various types of raw materials in product formulas [5].

These properties make it possible to introduce protein ingredients in formulas of high-protein foods and obtain end products that are not inferior to traditional products in terms of the biological value and most importantly to efficiently use expensive raw materials of animal origin [6]. Products developed with the addition of soy protein are more affordable to the mass consumer in terms of price and make it possible to partially cover the deficit of the protein in the diet [7, 8].

High-protein diet is necessary for people involved in strength sports (cyclic, speed-strength, competitive, complex coordination, technically complex, and combat sports) [9, 10].

The course of food containing protein can be assigned when physical activity is an integral part in maintaining muscle mass and in stimulating muscle hypertrophy, ensuring proper recovery after physical exercises, and maintaining optimal immune function of the human body [11, 12].

SUBJECTS AND METHODS OF RESEARCH

The following subjects of research were used in this paper

1. Fresh-frozen northern shrimp (*Pandalus borealis*).
2. Soybean peptides from the Soybean National Center, Changchun, China.
3. Pate with the native shrimp biomass "Naslazhdenie."
4. Pate with the enzyme-modified shrimp biomass "Bodrost'."

Materials

1) A high concentration of bioflavonoid complex Flavio, TU 9197-030-026999613-2007.

2) Fresh-frozen northern shrimp (*Pandalus borealis*), GOST (State Standard) 20845-2002 Frozen shrimp. Specifications.

3) Fresh food garden carrots for retail, GOST (State Standard) R 51782-2001 Fresh food garden carrots for retail. Specifications.

4) Fresh food pumpkins, GOST (State Standard) 7975-68 Fresh food pumpkins. Specifications.

5) Cream butter, GOST (State Standard) R 52969-2008 Butter. Specifications.

6) Whey dairy, GOST (State Standard) R 53438-2009 Whey dairy. Specifications.

7) Food common salt, GOST (State Standard) R 51574-2000 Food Common Salt. Specifications.

8) Soy peptides, GOST (State Standard) 17109-88 Soy. Requirements for purchases and deliveries.

Methods

1. Determination of the fatty acids content. The fatty acid content was determined by the Folch method [13].

2. Determination of the amino acids content. The amino acid content was determined using the Moore method [14].

3. Determination of the protein content. The total protein content was determined by the Lowry method [15].

Research experimental work was carried out in the laboratories of the Department of Biotechnology of Products from Animal Raw Materials and Functional Foods at the School of Biomedicine of the Far Eastern Federal University.

An original taste of the shrimp is determined by the composition of amino acids, minerals, lipid fraction, and nucleotides. The main contribution is made by free amino acids, i.e., alanine, arginine, glutamic acid, and glycine which give a distinctive sweet taste to shrimp meat. Table 1 shows the amino acid composition of raw northern shrimp (*Pandalus borealis*).

Table 1. Amino acid composition of raw northern shrimp (*Pandalus borealis*), g/100 g of protein [16]

Indicators	Amino acids content
Essential amino acids	
Valine	5.19
Phenylalanine	4.91
Methionine	1.21
Isoleucine	4.69
Leucine	7.26
Threonine	3.88
Lysine	7.84
Sum	31.98
Nonessential amino acids	
Aspartic acid	9.3
Serine	4.29
Glutamic acid	10.76
Proline	5.07
Glycine	12.96
Alanine	9.48
Cystine	0.43
Tyrosine	2.24
Histidine	2.02
Arginine	8.47
Sum	64.82

From Table 1, basic acids with comparable content can be identified. Among essential amino acids there are leucine and lysine. Among nonessential amino acids there are aspartic and glutamic acids as well as glycine. The only vegetable protein featuring a unique amino acid composition and equivalent to proteins of animal origin in this regard is soy protein. Soy protein is the most digestible vegetable protein, and in terms of nutritional value it is identical to the proteins of meat, fish, milk, and eggs.

Table 2 shows the amino acid composition of soy protein.

In accordance with the data presented in Table 2, it can be concluded that the basic amino acids, whose content exceeds the content of the other amino acids, are

leucine and lysine (among essential amino acids) and glutamic and aspartic acids (among nonessential amino acids).

Table 2. Amino acid composition of soy protein, g/100 g of protein

Indicators	Content	
Essential amino acids		
Isoleucine	1.81	0.66
Leucine	2.67	1.95
Methionine	0.52	-
Threonine	1.39	0.84
Phenylalanine	1.61	0.87
Valine	2.09	0.84
Lysine	2.09	1.24
Sum	26.91	12.8
Nonessential amino acids		
Serine	2.07	1.12
Glutamic acid	6.05	4.23
Proline	1.86	0.96
Glycine	1.42	0.79
Alanine	1.47	0.80
Citrulline	-	0.26
Aspartic acid	4.4	2.12
Cysteine	0.55	0.06
Tyrosine	1.06	0.60
Ornithine	-	0.08
Sum	18.98	10.72
Total	45.89	23.52

The process flow diagram for the production of the native biomass comprises the following steps: thawing, blanching, cooling, cutting, homogenization, preparation of cans, packing, packaging, labeling, and storage.

Fresh-frozen shrimp are subjected to blanching at 100°C for 4 minutes in a low (4%) saline solution. Before lowering the shrimp in the boiler the water is boiled. After that it should boil again (not later than after 2–3 minutes). Boiled shrimp are taken out and immediately cooled in the refrigerator at 0°C for 10–15 min. Next, boiled shrimp are cut, rinsed, and homogenized for 3 minutes.

The enzyme-modified shrimp biomass is produced by the hydrolysis of the frozen northern shrimp (*Pandalus borealis*) using chymotrypsin.

Chymotrypsin cleaves peptide bonds which are formed by carboxyl groups of aromatic amino acids. Furthermore, bonds formed by leucine, valine, methionine, and asparagine can be hydrolyzed with chymotrypsin.

Chymotrypsin hydrolysis is carried out at 37°C in an alkaline medium (pH 8.0–8.6). The ratio of enzyme to protein is 1:100 (weight ratio). In the case of the prolonged hydrolysis the enzyme is added to the substrate in two or three portions. The nature of the buffer used for the hydrolysis depends on the nature of the subsequent operation. In separation of hydrolyzate peptides by chromatographic and electrophoretic techniques on paper or on a thin layer it is convenient to use “volatile” buffers such as 0.5% solution of NH₄HCO₃ (pH 8.2). In hydrolysis with chymotrypsin relatively small peptides are obtained.

The process flow diagram for the production of the enzyme-modified shrimp biomass comprises the following steps: thawing, blanching, cooling, cutting, homogenization, enzymatic hydrolysis, preparation of cans, packing, packaging, labeling, and storage.

We have developed six samples for the research. For the manufacture of pate the native or enzyme-modified shrimp biomass, which is the main source of proteins (18.3%), was used as the main raw material. For the long-term storage the antioxidant Flavio was used.

Types of samples are given in Table 3.

Table 3. Samples

Sample number	Specification
Sample 1	Native shrimp biomass
Sample 2	Native shrimp biomass + antioxidant
Sample 3	Native shrimp biomass + soy peptides
Sample 4	Native shrimp biomass + soy peptides + antioxidant
Sample 5	Enzyme-modified shrimp biomass + soy peptides
Sample 6	Enzyme-modified shrimp biomass + soy peptides + antioxidant

In order to prepare the pate mass, vegetable products, carrots and pumpkin which contain a large amount of β -carotene (3–9 mg) were added to the main raw materials. Whey and soy peptides have been used that enhance the nutritional value of the product and butter which makes it possible to give a specific taste and aroma to the finished product.

Table 4. Amino acid composition of shrimp, g/100 g of protein

Indicators	Native shrimp biomass	Enzyme-modified shrimp biomass
	Content	
Essential amino acids		
Methionine	0.08	0.55
Isoleucine	2.65	2.28
Leucine	5.50	7.98
Valine	3.11	2.59
Phenylalanine	2.03	2.62
Lysine	5.15	7.62
Threonine	4.07	3.64
Sum	22.59	25.38
Nonessential amino acids		
Taurine	0.90	0.25
Aspartic	4.02	7.53
Serine	3.36	3.42
Glutamic	8.59	13.85
Oxoproline	0.49	0.14
Proline	2.22	3.02
Glycine	4.17	7.00
Alanine	3.26	4.12
Citrulline	0.77	4.42
Cysteine	0.15	0.27
Tyrosine	1.62	2.26
Ornithine	0.67	7.78
Histidine	1.38	1.45
Arginine	3.21	3.66
Sum	34.81	56.87
Total	57.4	82.25

The content of amino acids in the native and enzyme-modified shrimp biomasses was analyzed. The results are shown in Table 4.

According to the data presented in Table 4, it can be concluded that in terms of the total protein content the enzyme-modified shrimp biomass exceeds the native one. But it is also important to note that the content of aspartic and glutamic amino acids as well as leucine and glycine necessary for a human body is greater than the content of other amino acids.

Based on the guidelines of the Russian Academy of Medical Sciences and the National Standard for Functional Foods we have calculated the highest content of amino acids that are most important for human life.

The calculation of glutamic acid. If the protein content in 100 g of shrimp is known, it is possible to calculate the protein content of 50 g of shrimp, x:

$$= \frac{?}{?} = 9 \text{ g of protein} \quad (1)$$

Given that in the formula 100 g of pate includes 1 g of soy peptides, the total amount of protein will be 10 g.

Knowing that 100 g of protein contain 11.85 g of glutamic amino acids, we define the amount of glutamic amino acid in 9 g protein of shrimp, β :

$$= \frac{?}{?} = 1.25 \text{ g of glutamic acid} \quad (2)$$

The content of the glutamic amino acid in 1 g of soy peptides is 0.04 g. Let us find the total amount of glutamic amino acid in 100 g of pate, γ :

$$= 1.25 + 0.04 = 1.29 \text{ g of glutamic acid} \quad (3)$$

The recommended daily requirement of glutamic acid is 13.6 g.

Let us determine the percentage of the daily requirement of glutamic acid, δ :

$$= \frac{?}{?} \% = 9.6\% \quad (4)$$

Knowing that 100 grams of protein contain 6.62 g of lysine, we determine the amount of lysine in 9 g of the shrimp protein.

$$= \frac{?}{?} = 0.69 \text{ g of lysine} \quad (5)$$

The lysine content in 1 g of soy peptides is 0.012 g. Let us find the total lysine content in 100 grams of pate

$$= 0.69 + 0.012 = 0.702 \text{ g of lysine} \quad (6)$$

The recommended daily requirement lysine is 4.1 g. Determine the percentage of the daily requirement.

$$= \frac{?}{?} \% = 17.12\%, \quad (7)$$

Knowing that 100 grams of protein contain 7.08 g of leucine, we determine the amount of leucine in 9 g protein shrimp.

$$= \frac{?}{?} = 0.72 \text{ g of leucine} \quad (8)$$

The leucine content in 1 g of soy peptides is 0.02 g. Let us find the total amount of leucine in 100 g of pate

$$= 0.72 + 0.02 = 0.74 \text{ g of leucine} \quad (9)$$

The recommended daily requirement of leucine is 4.6 g.

Determine the percentage of the daily requirement of leucine.

$$= \frac{?}{?} \% = 16.1\% \quad (10)$$

Figure 1 shows the comparison scale of the recommended daily requirement and the percentage of the daily requirement.

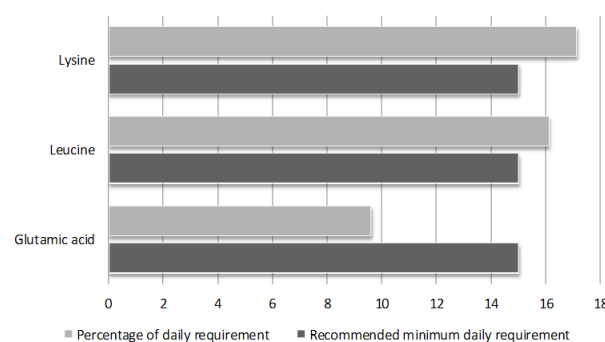


Fig. 1. The comparison scale of the recommended daily requirement and the percentage of the daily requirement.

The results of determining the percentage of the daily requirement show that as a result of the use of this high protein structured system the human body receives the daily requirement of leucine and lysine in accordance with the recommendations of the FAO/WHO. The content of glutamic acid is much lower than the recommended amount of daily requirement.

Based on the above calculations of the greatest amino acid content high-protein structured systems (pate "Nalazhdenie" and "Bodorst") are enriched functional food obtained by adding one or more physiologically functional food ingredients in conventional food products in order to prevent or correct the existing in humans nutrient deficiency, since the amount of functional food ingredients of vegetable and (or) animal raw material in one portion of the product is more than 15% of the daily requirement.

The investigation has been conducted on the content of fatty acids in the pate. Table 5 shows the fatty acid composition of pate with the native shrimp biomass without the addition of an antioxidant (Sample 1 is the nonhydrolyzed shrimp biomass) and antioxidant (Sample 2 is the hydrolyzed biomass of shrimp + antioxidant).

According to the results shown in Table 5, it can be concluded that the fatty acid composition of the Sample 1 without the addition of an antioxidant is higher than in the Sample 2. But some fatty acids contain slightly less

of fatty acids. These are caprylic acid, oleic acid, lauric acid, stearic acid, and arachidonic acid.

Table 5. Fatty acid composition of pate, g/100 g of the product

Acid	Formula	Sample 1	Sample 2
		Content	
Saturated fatty acids			
Myristic	C _{14:0}	5.4	4.85
Palmitic	C _{16:0}	34.32	30.02
Stearic	C _{18:0}	4.12	4.69
Caprylic	C _{8:0}	0.24	0.25
Lauric	C _{12:0}	6.08	6.81
Decanoic	C _{10:0}	2.5	0.6
Pentadecanoic	C _{15:0}	0.27	0.23
Amount		52.93	47.45
Unsaturated fatty acids			
Palmitoleic	C _{16:1}	0.48	0.44
Oleic	C _{18:1}	26.66	27.32
Sum		27.14	27.76
Polyunsaturated fatty acids			
Linoleic	C _{18:2}	14.93	14.53
Linolenic	C _{18:3}	5.22	3.77
Arachidonic	C _{20:4}	0.37	0.55
Eicosapentaenoic	C _{20:5}	0.22	0.22
Sum		20.74	19.07
Total		99.81	94.28

RESULTS AND DISCUSSION

1) The technology for obtaining the native shrimp biomass (*Pandalus borealis*) has been developed. The main operations are thawing, blanching, cooling, cutting, homogenization, preparation of cans, packing, packaging, labeling, and storage.

2) The biotechnology for obtaining the enzyme-modified shrimp biomass (*Pandalus borealis*) has been developed. The basic operations include thawing, blanching, cooling, cutting, homogenization, enzymatic hydrolysis, preparation of cans, packing, packaging, labeling, and storage.

3) Formulas of high-protein combined pates have been developed. Their main components are the native or enzyme-modified shrimp biomass, 50%, and soy peptides, 0.1%.

4) The analysis of the fatty acid composition have shown that the content of unsaturated fatty acids exceeds the amount of saturated acids by 26%.

5) The determination of the composition of protein and amino acids of the shrimp biomass have shown that the content of protein of the enzyme-modified shrimp biomass is higher than that of the native one. However, it is also important to note that asparagine and glutamic acids, leucine and glycine necessary for the human body predominate compared to the other amino acids.

The developed products are functionally orientated since the content of essential amino acids such as leucine and lysine in relation to the daily requirement is more than 15%.

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