Additives based on hydrolysates from animal raw materials for food psycho-emotional products

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Abstract. The process of enzymatic hydrolysis of meat and bone raw materials of animal origin to obtain a protein product with a high content of individual essential amino acids that affect the psycho-emotional state is described. The amino acid and fractional composition of the product is presented, which is obtained due to the high dispersion of the feedstock. The developed methodology will contribute to the involvement of little-used waste of food raw materials in the target turnover.

1 Introduction

Protein substances are the most important components of all food systems consumed by living organisms both at the level of microobjects, animals and humans [1, 2].

A complete protein diet consists in the consumption of food proteins through the intermediate mechanism of their degradation by the digestive system of a living organism to amino acids, which, in fact, are the target substances for building organs and tissues of the consumer organism [3]. When eating food, a living organism must receive all natural amino acids in the optimal ratio. An excess or deficiency of amino acids in this case can lead to a change in the biochemical processes of a living organism [4, 5, 6].

A number of protein components used in the form of food raw materials have an unbalanced amino acid composition compared to the balance of amino acids in the most favorable proteins. Egg albumin and human milk casein can be considered as the most favorable proteins for humans. An imbalance in the amino acid composition sometimes predetermines the inferiority of protein nutrition [7, 8].

Table 1 presents the typical amino acid composition of some of the main types of products used in food systems for human nutrition [6, 8].

In the main natural proteins, all twenty L-amino acids are present in certain ratios, which predetermine the usefulness of protein food [9].

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Table 1. The most important amino acids of the components of food systems, g\100g of protein (n = 6)

Amino acid	Fish protein	Animal protein	Plant protein	Yeast protein
Ile	1.94	4.93	4.85	3.62
Leu	6.63	8.57	6.98	6.64
Lys	8.10	10.65	5.70	5.23
Met	0.81	3.35	1.21	0.82
Cys	0.27	1.18	1.61	0.03
Phe	1.01	4.55	4.47	4.14
Tyr	1.12	3.98	2.89	3.25
Thr	2.63	5.91	2.18	4.31
Trp	0.6	1.32	1.25	0.77
Val	4.07	5.56	4.76	5.60
Ala	6.39	3.63	3.71	6.90
Arg	2.25	7.80	6.53	6.46
Asp	11.47	7.98	10.95	9.53
His	1.47	3.46	2.15	3.49
Gly	11.74	2.24	3.76	4.50
Glu	21.81	16.4	16.58	14.52
Pro	5.89	3.10	0.9	3.01
Ser	3.49	1.59	6.08	3.92
Protein in raw material, %	19.1	22.7	18.1	9.8

Significant protein resources are practically unavailable for use as components of food systems. These are rigid collagen and keratin proteins of bone, feather-down and fish origin. Such macromolecular proteins are not digested in the body. For their use, it is necessary to carry out the process of degradation to small peptides and amino acids. The resulting protein-peptide mixture can increase the biological value of the product [10].

A possible way to degrade rigid scaffold proteins is to use acid hydrolysis. In this case, the protein-containing components of the raw material are cleaved, however, a significant part of the released amino acids is destroyed and their partial racemization occurs [3, 10, 11].

Another possible way to cleave rigid protein structures is enzymatic lysis in the presence of hydrolytic enzymes. Fermentolysis avoids the breakdown of such labile amino acids as tryptophan, methionine, cystine and other amino acids released from the protein structure. Multiple attempts to carry out enzymatic cleavage of bone collagen, as well as keratin from bird feathers, did not lead to acceptable results due to low yield [12, 13].

To obtain food products with a psycho-emotional effect based on animal raw materials, there is a need to form a complete protein product with a high content of essential amino acids, which can be, for example, the simplest amino acid with a sweet taste - glycine, which actively promotes sedative sleep tryptophan, as well as a stabilizer of radical activity L-lysine [14, 15].

The aim of the work was to obtain a hydrolyzate with a high content of essential amino acids by enzymatic cleavage of animal bone tissue.

2 Materials and Methods

The object of the study was meat processing waste – a meat and bone mixture containing 94 % bone tissue and 6 % pulp tissue. Before fermentolysis, the raw material was subjected to double grinding on a Buchi apparatus (Switzerland) followed by grinding on a high-speed disperser ULTRA-TURRAX T 25 (IKA, Germany). The finely ground product was subjected to enzymatic treatment under the action of the Protepsin polyenzyme preparation (Endocrine Enzyme Plant, Russia) with a proteolytic activity of 150 U/g.

Processing conditions: hydromodule 1:4; the amount of enzyme is 0.3 % by weight of the raw material; time – 6 hours; pH 6.0; temperature 40 ℃. After treatment, the resulting slurry was spray dried.

The amino acid composition was determined on a Biotronic LC3000 analyzer (Germany) using a Winpeak Eppendorf-Biotronic integrating system (Germany) against a standard mixture of amino acids containing 2.5 μmol of each amino acid in 1 ml of solution.

The fractional composition of the protein was studied by SDS electrophoresis in 12 % polyacrylamide gel. The chemical composition was analyzed according to standard procedures [8, 10].

The degree of destruction of bone tissue in the processed raw material was assessed gravimetrically by separating the remains of bone particles from the suspension by centrifugation at 4000 g.

Statistical processing of the results was carried out by standard methods [10].

3 Results and Discussion

As is known, enzymatic processing of natural raw materials containing rigid collagen proteins does not lead to significant degradation of the protein [10, 16]. The reason for this is the fairly strong molecular framework of the natural protein bound into stable macrocomplexes. For intensive degradation of such macrocomplexes, it is expedient to use the principle of mechanochemical treatment. In particular, a significant dispersion of the raw mass is sufficient to implement the protease activity of the enzyme.

Two-stage grinding of the meat and bone mixture made it possible to obtain a protein system, mainly consisting of particles with an average diameter of 20-50 μm. Such a colloidal system had a significant, more than a day, temporary stability under hydromodule conditions from 1:2 to 1:10.

Table 2. Main fractions of meat and bone fermentolysate (n = 3)

Molecular mass, kDa	Feedstock, %	Enzymatic hydrolyzate, %
> 200	95	1
50 – 120	Not detected	6
30 – 50	1*	5
20 – 30	Not detected	6
15 – 20	1*	3
10 – 15	1*	75
< 10	2*	4

* – from meat proteins

To carry out the enzymatic treatment under these conditions, a hydromodule 1:4 was used, the choice of which was made for technological reasons.

The enzyme lysate obtained under the described conditions is a protein product consisting of cleaved protein fragments and free amino acids. The content of free amino acids in the product, depending on the conditions, was up to 65 %, the rest was fragmented proteins. The main amount of it is shown in Table 2. The molecular weight distribution of the residual protein fraction, which accounted for about a third of the product mass in the enzymatic lysate, shows that most of the completely nonhydrolyzed portion of the protein contains peptides with a molecular mass of 15-120 kDa. This is the most favorable range for the efficient functioning of the digestive system of mammals and humans.

Enzymatic processing of animal raw materials with proteases is a fairly mild bioprocess, during which free amino acids are released from the protein.

Table 3 shows the amino acid composition of the obtained fermentolisate.

Table 3. Free amino acids in the product, obtained by enzymatic cleavage of meat and bone raw materials in the presence of Protepsin, g/100 g of protein

Amino acid	Content	Amino acid	Content
Ala	6.45	His	1.92
Arg	5.55	Gly	17.44
Asp	5.34	Glu	10.44
Val	3.11	Ile	1.85
Leu	4.21	Ser	3.78
Lys	9.89	Tyr	2.85
Met	4.23	Thr	3.05
Pro	8.17	Trp	4.85
Phe	3.45	Cys	2.76

For the prevention of psycho-emotional disorders, the optimal level of glucose in the blood is important, which largely depends on the balance of nutrients in the diet, primarily the presence of essential amino acids. Human emotions are associated with physical activity, which is limited during protein starvation. Individual amino acids act as neurotransmitters, determining the activity of certain areas of the brain, regulating reflection and predetermining the perception of what is happening around a person.

In the resulting enzyme lysate, an increased content of tryptophan, lysine and glycine can be noted, even in comparison with conventional meat or fish products. Glycine, as the first amino acid with the simplest chemical structure, determines the quality of brain nutrition. Gly in the form of a pure pharmacopoeial preparation is used in medicine, especially for the elderly.

An increased content of lysine in the enzyme lysate can contribute to a significant increase in the stabilization of radical activity in a living organism and a decrease in psychochemical reactions when used as an additive in the food system.

Of particular interest may be an increased content of tryptophan in the resulting product. Amino acid Trp can act as a suppressor of active mental reactions of a living organism. Thus, during the slaughter of livestock in contaminated animals, a sharp increase in the content of tryptophan in muscle and brain tissues is observed, which, apparently, is a natural protective reaction with a sedative function.

The increase in the content of the three noted amino acids is apparently associated in this case with the use of bone tissue, which is usually not used for food purposes, as a raw material.

Processing of the described dispersions with Protepsin made it possible to obtain dry hydrolysates for food systems with the following properties: protein content, including free

amino acids – up to 65 %, fat – up to 10 %, ash – up to 20 %, carbohydrates – up to 5 %, pH of 1 % solution was 5.6-5.8.

A product that was not technically processed before the drying stage could contain dispersed remains of bone tissue with particles with an average diameter of 150 μm, which makes it possible to use such a product in animal feed.

4 Conclusion

Thus, the conducted studies made it possible to carry out the process of enzymatic processing of bone tissue into products with potential psycho-emotional characteristics.

The conditions found for enzymatic hydrolysis of bone tissue of animal origin make it possible to obtain enzyme lysates with a high content of essential amino acids, which expands the search for a rational methodology for processing the resulting bone waste.

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