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HYDROLYZATION FROM EGGSHELL MEMBRANE UNDER THE INFLUENCE OF PAPAIN ENZYME AND DETERMINATION OF TOTAL PROTEIN AMOUNT

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Annotation. Obtaining chondroprotective hydrolysates from local raw materials, i.e. eggshell waste from poultry farms, comparing hydrolysates obtained by enzymatic and non-enzymatic methods, finding the optimal temperature for the hydrolysis reaction. The object of research is eggshell membrane grown in chicken farms with local raw materials (waste). The subject of research is hydrolysates from the egg shell membrane.

Key words: eggshell membrane, hydrolysis, enzymatic hydrolysis, papain enzyme, temperature.

Introduction. Chicken eggs are one of the most widely used products in the food industry. Approximately 250,000 tons of eggshell waste are produced worldwide each year. Eggshell is divided into inner (thickness about 0.02 mm) and outer (thickness about 0.05 mm). The eggshell membrane is a network of biopolymers and consists of randomly oriented individual fibers. It has been found that about 10% of the total protein content of eggshell membranes is collagen. It has been found that about 10% of the total protein in eggshell membranes is collagen. The eggshell membrane mainly contains type I collagen, type V collagen, and type X collagen, as well as glycoproteins, phosphoproteins, and soluble/insoluble proteoglycans. Type X collagen is a cross-linked and insoluble protein that masks type I collagen [1,3]. A recent proteomic analysis revealed that eggshell contains 29 proteins. Proteomic analyzes can be used to determine the minimum amount of biologically active proteins in a tissue or fluid [3]. Zhao Q. C. et al. et al., 2019 showed that eggshell membranes have a collagen structure (Gly-Pro-Hyp) and studied the amino acid composition of type I collagen. Eggshell membrane collagen is three-dimensional and the most common is a triangular helical configuration (Fig. 1) [8].

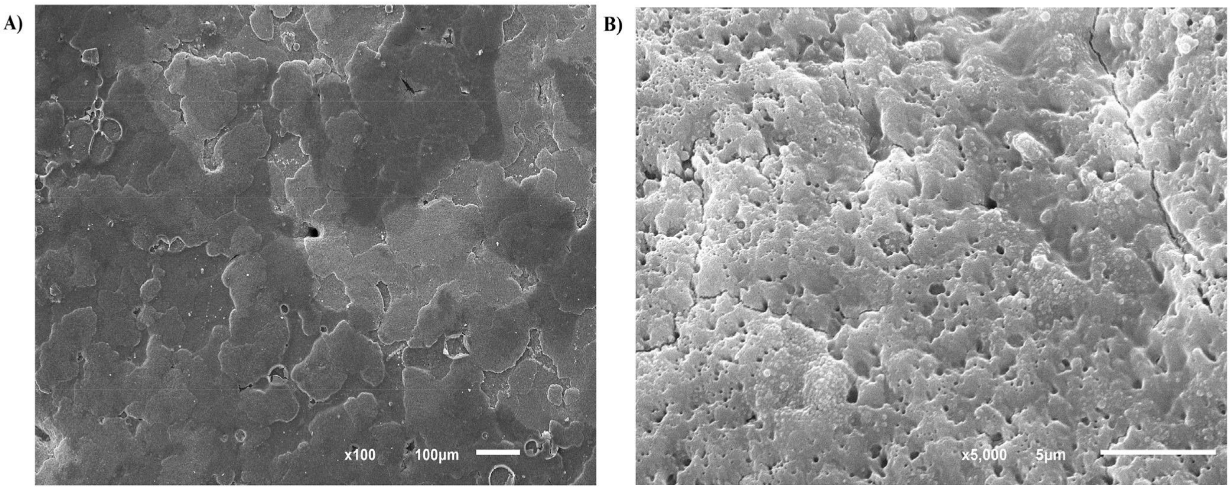

Figure 1. Microstructure of domestic chicken eggshell using scanning electron microscope (SEM). Magnification and magnification: 100x100 mm (A) and 5000x, 5 mm (V) (adapted from Hincke M. T. et al.)

Collagen is the most abundant protein in the human body. Mainly, it is found to the greatest extent in the connective tissue. Collagen solution products, that is, hydrolysates, are widely used in the cosmetic industry, pharmaceutical industry, medicine and other fields. Currently, there is a wide variety of literature on different methods of obtaining collagen. Collagen hydrolysates containing a set of amino acids such as glycine, proline, oxylazine, hydroxyproline and polypeptides are more available for absorption in the human body (Table 1) [9-11].

Table 1

Amino acid content of type I collagen isolated from eggshell membrane (%)

Amino acid content of eggshell membrane type I collagen (%)								
Asp	Thr	Ser	Glu	Gly	Ala	Cys	Val	Met
5.34	1.71	3.16	10.58	30.27	9.04	0.34	2.32	0.76
Ile	Leu	Tyr	Phe	Lys	His	Arg	Pro	Hyp
1.18	2.86	0.92	0.81	2.15	0.80	6.55	11.94	9.28

Currently, the production of collagen hydrolyzates is aimed at breaking the intermolecular bonds of insoluble collagen. Chemical and enzymatic methods are mainly used to obtain hydrolyzates. Acids, salts and alkalis are used in chemical processing. These chemicals affect collagen structure. First of all, they break interchain and internal hydrogen bonds, interact with carboxyl and amine groups of proteins, and thus form soluble collagen hydrolysates. The dissolution of collagen is affected by the concentration of acids and alkalis, the duration of exposure, as well as the temperature. When raw materials are processed by the enzymatic method, hydrolyzates of low molecular weight can be obtained, which can be used in cosmetology and pharmaceuticals. There are many literature data comparing chemical and enzymatic methods. According to statistics, the enzymatic method is better than the chemical method. But the price of the drug doubles and even triples. In addition, not all enzymes can be used, and only enzymes with a narrow substrate specificity can be used. Proteolytic enzymes are used for the enzymatic method: pepsin, trypsin, chymotrypsin, bromelain, papain (Table 2) [4-8].

Papain (lat. Papain) is a polypeptide, proteolytic plant enzyme that accelerates the interaction of substances with water, which breaks down proteins, peptides, amides and esters of basic amino acids. It is found in large quantities in the fruits of the melon tree - papaya (Carica papaya). Papain is used in food and light industry for tenderizing meat, skin treatment and cleaning drinks, as well as in pharmacology ("Karipazym", "Karipain", "Karipain-plus", "Karipain Cream", "Karipain Ultra Gel", digestive preparations "Unienzym", "Vitazym", as well as "Wobenzym", etc.) are found in the composition. Included in many toothpastes: it dissolves plaque and helps clean teeth more effectively [12].

Table 2

Properties of proteolytic enzymes

Enzyme	Proteolytic activity, units/g	Optimal pH value	Optimum temperature value, °C
Papain	270	5,0–7,2	37–40
Trypsin	223	7,8-8,5	37-40
Chymotrypsin	1320	7,0-8,5	37-40

The purpose of the study. Separation of hydrolyzates with chondroprotective properties of the eggshell membrane by enzymatic and non-enzymatic methods, study of the effect of temperature on the yield of total proteins in the obtained hydrolysates, and determination of the amount of total proteins in the hydrolysates by the biuret method.

Materials and methods of research. Hydrolysis of eggshell membrane using enzymatic and non-enzymatic methods. Application of the method of quantitative determination of total proteins in the obtained hydrolyzates by the biuret method. Materials: Eggshell membrane; 1.5% acetic acid; aqueous solution of calcium hydroxide; papain enzyme, Mindray BS-200 automated biochemical analyzer.

Experimental part. Preparation of the object. The eggshell membrane was separated mechanically, washed under running water, defatted with 70% ethyl alcohol, dried, ground in a homogenizer, and stored at -6 ℃ to -24 ℃. In the first method, 1.0048 g of crushed eggshell membrane is extracted in 30 ml of 1.5% acetic acid in a heated magnetic stirrer at a temperature of 30-90 ºC for 1 hour. Then it is treated with an ultrasound device at a frequency of 42 kHz for 30 seconds. The next step is centrifugation at 3000 rpm for 10 minutes. The supernatant is carefully separated from the precipitate. Hydrolyzate is neutralized with 1.0M Ca(ON)2 solution (rN=7.0-7.8). Then again, it is centrifuged at 3000 rpm for 3 minutes and the supernatant is separated from the sediment, in this way 7 samples are obtained. Each sample is hydrolyzed in the temperature range of 30-90 ºC. The second method is the same as the first method, but in the first step, 5 µl of 350 U papain enzyme dissolved in 10 ml of 0.9% NaCl is added. Each hydrolyzate is dialyzed to free the enzyme from the hydrolyzate. Dialysis is carried out in "Scientific research special" bags against distilled water at a temperature of 24 ℃ for 6 hours. The pores of dialysis bags are 26 kDa. In this case, we isolate hydrolyzates purified from the enzyme.

Results and discussion. Results of quantitative determination of proteins in hydrolysates by biuret method. The results of the hydrolyzates obtained by the first method in the temperature range of 30-90℃ on the Mindray BS-200 automatic biochemical analyzer (Table 3) and the graph of the obtained results (Figure 2). The results obtained by Mindray BS-200 automatic biochemical analyzer of enzyme-added hydrolysates obtained using the second method in the temperature range of 30-90 ℃ (Table 4) and the graph of the obtained results (Figure 3).

Table 3

Amount of total oxyls in hydrolysates obtained by the first method

Temperature ℃	30	40	50	60	70	80	90
Density g/l	1.4	13.1	13.4	15.05	16.08	15.35	15.78

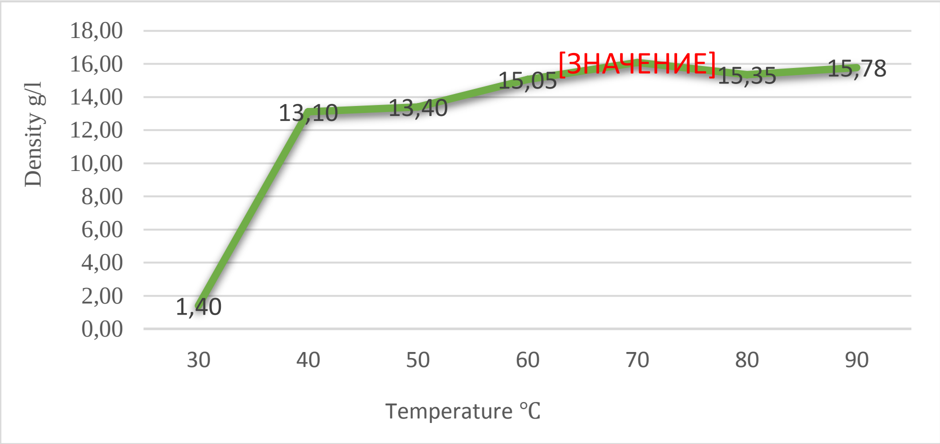

Figure 2. The ratio of the density of the hydrolysates in the first sample obtained to the temperature

Table 4

Amount of oxyls in hydrolyzates obtained by the second method

Temperature °C	30	40	50	60	70	80	90
Density g/l	1.4	11.1	11.1	14.3	17.86	18.65	18.28

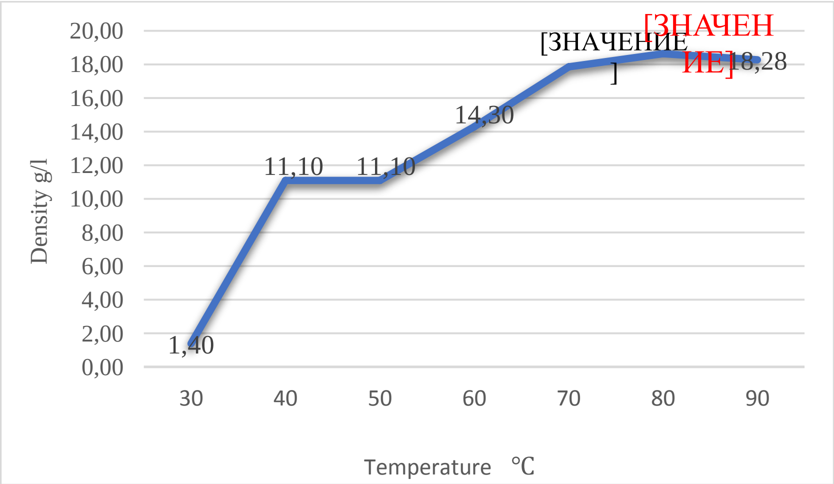

Figure 3. The ratio of the density of the hydrolysates in the second sample obtained to the temperature.

Conclusion. Mechanical separation of the eggshell membrane presented some difficulty. Two methods of extraction of acetic acid-soluble proteins were considered, mainly the effect of temperature on the extraction was studied. According to the available literature, the most effective enzyme for extraction is papain. The reason for the use of papain enzyme is that it is active in acidic, neutral and alkaline environments (pN 3-12). It also does not lose its activity over a wide temperature range of 50-80 °C. Based on the obtained results, it can be concluded that separation with the help of proteolytic enzyme during extraction increases the yield of proteins. The highest amount of oxygen in our hydrolyzates obtained by the first method was at 70 ℃, but the protein content did not decrease in the range of 0.3-0.73 g/l when the temperature of the hydrolysates was increased by 10 ℃. The highest protein content of the hydrolysates obtained by the enzymatic hydrolysis method was annealed at a temperature of 80 ℃. Comparing the first and second methods, the amount of total proteins increased in both methods starting from 60 ℃. Based on the obtained results, it can be concluded that papain hydrolyzes the peptide bonds in the eggshell membrane and accelerates the hydrolysis reaction, thereby increasing the yield of the product.

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