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Abstract – Biotechnological methods of processing and purposeful transformation of structure, chemical composition, physico-chemical and functional properties of biopolymer systems are the new approach to the solution to the problem of human health protection and nutrition.
Minced samples of collagen-containing raw material were used as the object of research, including cattle tendons, the mixture of cattle tendons and fat and muscle tissue (meat of II category), cattle subcutaneous layer (trimmings), pig cutaneous integument (hide), collagenase proteolytic complex from Kamchatka Crab (collagenase was obtained from The Knipovich Polar Research Institute of Marine Fisheries and Oceanography (PINRO)), and samples of microbial collagenase from *Serratia proteamaculans*-94 (VNIIMP). The enzyme from hepatopancreas of Kamchatka Crab had the proteolytic activity of 620 tyr units/g and the collagenase activity of 1.1 mU/mg with optimum pH 6.5-7. It showed the activity over the temperature range of 4-50^{0}C.
The digestibility of collagen treated in the presence of 0.1% of crab enzyme (40^{0}C, 6 h, pH 7.4, water duty 1:4) was > 35-45% vs. 7% of protein.
Based on the results of the investigation we can recommend the use of the enzyme from the Kamchatka Crab collagenase to improve the quality of low-grade meat raw material according to the following processing conditions: beef of II category as raw material, temperature 6^{0}C, brine 1% of raw material weight, NaCl containing 0.1% of collagenase (on raw material weight basis), added to the minced meat raw material in the ratio of 1 part : 5 parts of minced meat, curing time 3 days.
Therefore, the differentiated approach to raw material biomodification allows producers to make the best use of secondary protein resources of meat industry for the functional protein ingredient production. Application of enzyme preparations with required specifity gives the opportunity to regulate hydrolysate final composition. The obtained fermented products have improved properties and can be used as additives in meat and meat-vegetable canned food formulations in order to improve functional- technological properties of food protein systems.

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I. Introduction

Biotechnological methods of processing and purposeful transformation of structure, chemical composition, physico-chemical and functional properties of biopolymer systems are the new approach to the solution to the problem of human health protection and nutrition.

In meat industry a problem of enzyme modification of proteins and protein systems with strengthened structure is of particular importance. The solution to this problem is linked with the increase in biological and technological functionality of collagen-containing raw material, the possibility of partial or complete conventional raw material replacement, product property and yield improvement through protein conversion and transformation of complex biosystem properties.

One of the possible approaches to such improvement can be new combined functional meat products on the basis of conventional meat raw material with biotransformed compositional ingredients. It is appropriate to use products of biochemical transformation of collagen-containing raw material as such ingredients. The obtained results enable us to address more reasonably the problem of new functional product development using biotransformation.

II. Materials and methods

The aim of this work was to substantiate the conditions of the biomodification of the strengthened structure protein (collagen) using engineering enzymology in order to develop a product range with required degree of destruction and regulated technological and physiological functionality.

Minced samples of collagen-containing raw material were used as the object of research, including cattle tendons, the mixture of cattle tendons and fat and muscle tissue (meat of II category), cattle subcutaneous layer (trimmings), pig cutaneous integument (hide), collagenase proteolytic complex from Kamchatka Crab (collagenase was obtained from The Knipovich Polar Research Institute of Marine Fisheries and Oceanography (PINRO)), and samples of microbial collagenase from *Serratia proteamaculans*-94 (VNIIMP). The enzymic activity and amino acid composition were analyzed by standard methods [1].

III. Results and discussion

One of the possible ways of meat products protein (14-22%) processing is the enzymic biodegradation in the presence of specific enzymes. Various proteinases (proteases), such as trypsin, pancreatin, collagenase and other enzymes with proteinase activity [2-5] can be used giving meat products completely different consumer properties.

For fermentative hydrolysis of protein-containing raw material we have chosen proteinase complex from hepatopancreas of Kamchatka Crab, produced in accordance with the PINRO technology [4] compared to microbial proteinase from *Serratia proteamaculans*-94 [5].

The tests of enzyme from hepatopancreas of Kamchatka Crab showed that this enzyme has the proteolytic activity of 620 tyr units/g and the collagenase activity of 1.1 mU/mg, and can be referred formally to neutral proteinases with optimum pH approaching 6.5-7. Maximum enzyme activity (both proteinase and collagenese) was shown at 37^0C, and at temperatures higher than 50^0C the enzyme lost its activity, which is in concordance with literature data about low resistance to temperature of fermentative complex from hepatopancreas of Kamchatka Crab. This fact can be used in future in meat product technology as a method of deactivation of used enzyme residues during thermal processing of meat products.

The impact of fermentative preparations on animal protein substrates leads to their degradation and formation of free amino acids and oligopeptides. Such products treated *in vitro* with enzymes having proteinase and collagenase activities in addition to some new properties should have higher bioavailability *in vivo* for digestive tract enzymes in a mammalian stomach. In other words, the digestibility of fermented collagen-containing products should increase. Thus, the digestibility of collagen from young cattle tendons assessed by standard methods in the presence of pepsin and trypsin was 7% (relative to albumin). The treatment of the same collagen in the presence of 0.1% of crab enzyme (40^0C, 6 h, pH 7.4, water duty 1:4) led to production of animal collagen dispersion with the digestibility level of >35%, that not only proved indirectly the presence of the preparation collagenase activity affecting directly the animal connective tissue proteins, but also enabled biological and nutritional value of food compositions including fermented collagen to be enhanced. In the similar conditions the treatment of animal collagen with the microbial enzyme with the collagenase activity from *Serratia proteamaculans*- 94 led to production of collagen mass with the digestibility level of 35-45%.

The investigation of the impact of Kamchatka Crab collagenase on low grade meat raw material (trimmings, tendons, hide, beef of II category) showed that this enzyme with proteolytic and collagenase activities causes the change in fractional composition of protein and more than threefold increase in free amino acids content. The typical protein fractional composition for beef of II category showed the following main fractions content (kDa, (%)): > 400-600 (0.5), 230-400 (0.5), 170-230 (4.5), 100-170 (7.0), 40-100 (26.7), 20-40 (30.0), 10-20 (30.8). In the raw material treated with the crab collagenase the main fractions (kDa, (%)) were 100-170 (9.0), 40-100 (21.2), 20-40 (35.40), 10-20 (34.4).

In this study the fermentative decomposition process was conducted as follows. The meat raw material was dispersed in water 1:10, the enzyme was added in the amount of 10% to the meat raw material weight, and the mixture was kept under continuous agitation at 20-50^0C for 0-4 h. The process was

controlled by the accumulation of free amino acids determined by formol titration [1].

The results of the microstructure investigations of animal tissue (beef) samples showed the changes due to enzymic degradation. Cross striation typical of beef was observed distinctly in the control meat tissue samples. Boundaries between fibers were seen clearly. Fiber nuclei had rod-like form with graininess of chromatin. Destructive changes were determined in individual fibers as microfractions and cross- fissured disturbance of regular structure. As a result of the enzymic treatment, a part of meat fibers became less swollen, and the main part of fibers was in the state of multiple fragmentation with granular degradation areas. The connective tissue areas were characterized by separation, fragmentation and partial lysis of collagen fibers. Thus, the destructive process development linked mainly with collagen break-up under the enzyme action was observed in the connective tissue, which is the main component determinating meat and meat produce toughness.

The enzymic treatment of some kinds of collagen stuff (trimmings, tendons) did not lead to obtaining products with satisfactory water and fat binding properties preventing the use of this enzyme for effective nutrient material production from these kinds of raw material.

Biodegradation process can be carried out in the presence of the enzyme, as well as in the process of meat raw material curing with NaCl.

Based on the results of the investigation we can recommend the use of the enzyme from the Kamchatka Crab collagenase to improve the quality of low-grade meat raw material according to the following processing conditions: beef of II category as raw material, temperature 6^0C, brine 1% of raw material weight, NaCl containing 0.1% of collagenase (on raw material weight basis), added to minced meat raw material in the ratio of 1 part : 5 parts of minced meat, curing time 3 days.

IV. Conclusions

Thus, the differentiated approach to raw material biomodification allows producers to make the best use of secondary protein resources of meat industry that are ignored or used restrictedly for food purpose. Application of enzyme preparations with required specifity gives the opportunity to regulate hydrolysate final composition. The obtained fermented products have improved properties and can be used as additives in meat and meat-vegetable canned food formulations in order to improve functional-technological properties of food protein systems.

REFERENCES

[1] A.B. Lisitsyn, A.N. Ivankin, A.D. Nekludov Methods of practical biotechnology. Analysis of components and microscopic amounts of admixtures in meat and other food products. – M. VNIIMP. 2002. – 408 pp.

[2] A.N. Ivankin, A.B. German, A.A. Osotov, V.V. Telezhkin Practice on the fundamentals of biotechnology. – M. Moscow State University of Forest, 1996. 100 pp.

[3] A.D. Neklyudov, A.B. Berdutina, A.N. Ivankin, S.I. Mitaleva, E.A. Evstaf'eva Collagen fractions obtained by water-salt extraction from raw materials of animal origin // Prikl. Biokhim. Mikrobiol., 2003, vol. 39, no. 4, pp. 426–430.

[4] V.A.Muhin, V.Yu. Novikov, L.L. Konstantinova, A.N. Ivankin, A.B. Berdutina Enzyme preparation from king crab as a facilitator of maturation of hydrobionts at ambassador//Hranenie & pererabotka selhoz sirja (rus), 2002, no.8, pp. 41–44.

[5] A.D. Neklyudov, A.N. Ivankin, A.B. Berdutina Bases of biochemical processings of an animal and combined raw material – M. VNIIMP. 2008. – 104 pp.

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