PROPERTIES AND PERSPECTIVES OF A NEW PROTEOLYTIC PREPARATION FROM HYDROBIONTS IN TECHNOLOGICAL PROCESSING OF THE MEAT RAW MATERIALS

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Introduction

Practical realization of methods of industrial enzymology in technologies of meat products is often limited due to the lack of scientifically based recommendations on their application.

Experience of usage of fermentative preparations shows that /1/, not all ferments having high proteolytic activity, at meat treatment give a desirable effect. Some of them, catalyzing hydrolysis of proteins of muscle fibers, influence weakly upon proteins of a connective tissue imparting toughness to meat, and limits the application of raw materials with the high contents of a collagen in the technology of meat products. Besides, for meat treatment, is very important the optimal ratio of proteins components, degree of their autolytic transformation and condition of a medium according to varied parameters (temperature, pH, presence of activators and inhibitors).The predetermined influence of ferments on protein systems of meat, on the based through study of their physico-chemical and biochemical properties, allows to realize effectively biotechologies of meat products.

Lately, there appeared some data about collagenaza from organs of hydrobionts and about the perspective of its use in the technology of meat products /1/.

On this aspect, the considerable interest represents a through study of properties of collagenolytic proteinase from Kamchatka crab liver.

Objects and methods

The object of the study was the fermentative preparation of collagenolytic proteinase (PCP) from the liver of Kamchatka crab Paralithodes camtshatica (producer PLC "Bioprogress", Russia, Schelkovo, Moscow region). Determination of total proteolytic activity has been carried out by modified method of Anson /2/ with the application of casein according to Hammersten at the pH 7.2 as a substrate. The rate of collagen destruction was estimated according to the quantity of the formed tyrosine, which was determined according to reaction with the "biuret" reactive /3/. Determination of collagenolytic activity has been carried out according to /4/. Collagen masses (produced – plant "Belcosine", Russia, Luga, Leningrad region) and by-products from beef trimming (veins, tendons, fasciae), purified at laboratory conditions according to recommendations /5/ have been used at substrates. Treatment of PCP by impulse magnetic field (IMF) has been carried out on the experimental installation of the nuclear physics department of the Voronezh State University.

For evaluation of PCP influence upon hysto-morphologycal, functional and technological, structural and mechanical properties of meat we used trimmed beef of second grade according to ГОСТ 779-87 (Russian standard), model minced meat on its base and non-fatty pork according to ГОСТ 7724-74.

The additive P-27 (USA) was used in the composition of brine. Coloring of ultra-thin cuts has been carried out according to the method of Van-Gison /6/.

Waterholding capacity (WHC) and waterbinding capacity (WBC) has been determined according to the method /7/, adhesion properties of model minced meats – according to method /8/.

Results and discussion

Dependence of proteolytic activity of PCP from temperature is expressed by a typical "bell-like curve", on which the zone of optimal temperatures 37 – 38 °C is pronounced. Dependence from pH-value has two expressed maximums at 7,0 and 7,6. The obtained data allow to assume presence of heterogeneous system of enzymes. The character of the dependence gives the possibility to control the dynamics of the process of the fermentative treatment of the meat raw materials that is important for depth of conversion of protein of meat and consequently for industrial use of a preparation.

Production technology of meat products is connected with the use of curing salt, that makes to obligatory the study of its effect upon the PCP activity. Analysis of the experimental data showed that depending of the mass contents of the curing salt an both an activating and an inhibiting take place. Maximum activating being observed at the 0.75 % content of curing salt. The reason for this is obviously the conformational changes of ferment molecules, connected with the degree of their hydration.

Study of regularities of IMF effect on the fermentative systems is one of the discussed question of biophysics. In order to study IMF influence on proteolytic activity of PCP, we carried out the treatment of the latter by series of triangular impulses with the varied amplitude of intensity.

Experimental data show that, proteolytic activity of PCP in 5 h after radiation, treatment with the intensity amplitude 150 mTl decreased on 20 %, in 22 h – on 10 % more in comparison with the control sample. When increasing the duration the back front with the intention amplitude up to 250 Mt, ferment activity decreased on 45 % in 5 h, but increased up to 95 % from the initial level by 22 h.

It is worth mentioning, that alongside with the pronounced decrease of the total proteolytic activity of preparation increased. The effect of IMF action was noted during 36 h. The explanation of molecular mechanism of this influence has not been found yet. We may suggest that such effect is achieved by the way of changing conformational conditions, caused by mutual turning of fragments of macromolecules connected by weak interaction of electrons being present on the outer orbits. Under the influence of IMF, the singlet-triplet transition may take place as it takes place in radical pairs /9, 10/. Arising of intermediate /9/ excited

conditions with non-equilibrium settlement of triplet levels lead to the weakening of chemical bonds. It may promote rotations of molecular fragments. The reason of the irreversible or reversible changes of the fermentative activity of collagenaze preparation may be disintegration of the ferment molecules on inactive sub-units in the first case, and reversible change of conformation – in the second. The given results show that IMF may be used for certain change of activity of fermentative preparations and systems, gives meat raw material particular functional ant technological properties.

The results of the experiments, on determination of substrate peculiarity of PCP allow to speak that preparation reveals high collagenolytic activity, affinity with native tendons being higher than with collagen mass.

The effect of action of collagenaze preparation is confirmed on the microstructural level of hysto-morphological study of fermented samples of the second grade beef. Fermentative treatment was carried out together with curing. Samples of uncured beef, from the same batch, and samples subjected to curing without fermentation, have used as the objects of composition.

The results of the experiment have shown that fermentative treatment lead to positive change of functional and technological properties of model minced meats. It has been noted, that the largest influence take place at the level of WBC, the level of WBC increasing faster and achieving the 161 % in a sample with the addition of salt. Such effect may be explained by absorbtion of salt ions on surface of protein molecules. This leads to the increase of charged centres, on which the joining of water dipoles take place /11/. The level of WHC and adhesion changes slightly.

The analysis of data, obtained at the study of structure and mechanical properties of the fermented beef showed that, with the increase of time of treatment by ferment preparation, the effort of cutting decreases, this process being faster in the system with the addition of P-27. It show that P-27 and the same additions do not inhibiting PCP. It has been established that, the treatment of both model minced meats and pieces of beef by preparation of collagenaze, results in increase of the output, that is connected with the increase of WBC. Addition of the P-27 to the curing mixture promotes output increase.

Thus, experimental data testify to the pronounced collagenolytic effect revealed by the preparation.

The character of morphological changes of muscle tissue allow to suggest a positive influence of the fermentative treatment on organoleptic indices of meat raw materials. It is very important when the low-grade meat is used.

The results of the study allow to suggest the following perspective trends of usage of the collagenaze preparation in industry:

- as the biologically active additive for tenderizing low grade raw materials in the production of curing-cooked meat products, meat half-finished products, fast frozen cooked dishes, canned meat and canned-vegetables made of low grade raw materials;
- in the production of protein enricher for the development of pastes, emulsions, hydrolyzates;
- for the production of purified collagen products, in the production of edible sausage casings, food dispersions, edible film coverings, functional ingredients, biomaterials on the basis of controlled biocatalytic process.

Generalization of the obtained data gave the possibility to motivate and suggest modified technological schemes of production of canned meats and canned meat-vegetables, fast-frozen dishes and cured-cooked meat products envisaging the aim biomodification of low-grade meat raw material through applying fermentative treatment during the stage of curing, to develop recommendations on conditions of application of collagenaze preparation in special technologies of meat products.

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