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CHANGE IN ACTIVITY OF CATHEPSINS AND STRUCTURAL AND FUNCTIONAL CHARACTERISTICS OF BOVINE MUSCULAR TISSUE AFTER TREATMENT BY BACTERIAL AND ENZYME PREPARATIONS OF MICROBIAL ORIGIN

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Key words: activity, bacterial and enzyme preparation, quality, peptidases, proteinases

Resume: In this work the results of investigations of beef muscular tissue quality changes under the influence of bacterial and enzyme preparation of microbial origin introduced into the system during curing are presented. It is observed a shift of heat absorption maximum peaks and a fluorescence spectrum maximum into a long wave region at higher than 60°C, and into a short wave region at higher than 70°C, as well as changes in cathepsins activity, quantitative and qualitative compositions of free amino acids, forms of water binding, structural-mechanical and physical and chemical parameters of raw materials.

Introduction: In developing food products a great deal of attention is given to utilization of enzyme and bacterial preparations, allowing not only to improve quality attributes and enhance food and biological value of products, but also significantly influence the digestibility and assimibility of main nutrients of products.

The available information about research in this field doesn't present sufficient data about pattern of changes in meat system and its constituents enzyme activity, and structural-functional characteristics of muscular tissue during meat processing (1, 2).

The aim of this work was the study of cathepsins activity and structural and functional characteristics of beef muscular tissue treated by bacterial and enzyme preparation of microbial origin during curing.

Materials and Methods: The investigations were carried out on model systems, which were prepared as follows: M. long dorsi which was taken from beef sides after 6 days of storage, ground through a 2-3 mm plate. The obtained muscular tissue (MT) was mixed with the cure (10%) containing salt, bacterial (BP) and/or enzyme (EP) preparations. The amount of salt in each sample was 2.5%, and EP and BP - 0.013 and 0.1% to mass of meat respectively. Protosubtilin "GIOX" being a neutral protease with activity optimum at pH 6.5-7.5 and at a temperature 40-50°C was used as an enzyme preparation, and BP-RSS (dry bacterial preparation for semi-dry raw-smoked sausages), being a concentrate of Lactobacillus plantarum and denitrifying micrococcaceae, mixed with glucose with the activity optimum at pH 4.5-5.0. was used as a bacterial preparation. Muscular tissue (MT) without any additives was a control. Changes that took place in MT under the influence of EP and BP in the process of curing during 3 days were assessed by the change in peptidases and proteinases activity. As a substrate was used sodium caseinate in universal phosphate-acetate buffer with pH value 7.2 and 5.5, respectively. Optical density of filtrates was determined by a wavelength of 680 nm; structural changes of proteins of MT - by methods of differential scanning microcalorimetry and protein fluorescence; change of mass during heating - by thermogravimetric method on a Q-derivatograph by the procedure, modified by Oreshkin E.F. et al; free amino acid content - with the use of ion-exchange chromatography on automatic analyser of HITACHI-835 (Japan); pH value - with the pH-meter "pH-340"; water-binding ability - by method of Grau and Hamm, modified by VNIIMP; a degree of penetration - by means of the instrument EPM-MPEM.

Results and discussion: Activity of muscular proteinases and peptidases to a great extent depends upon a kind and a quantity of additives, as well as upon length of curing (Fig. 1). Increase in activity of muscular cathepsins is observed when salt, BP and/or EP are used. If the activity of proteinases in the control is 1, then after introduction of salt, BP, EP and BP + EP it increases after 24 h of curing by 2.6; 4.0; 2.5 and 1.2, respectively, and after 72 h - by 6.3; 4.1; 7.1 and 5.8, respectively. The pattern of changes of peptida-

ses activity is slightly different after 24 hours of curing; their ratio is 1.8:1,7:1.9:1.2 respectively, and after 72 hours - 0.9:0.5:1.1:0.8 respectively. Microcalorimetric investigations show great structural changes in MT after introduction of EP and BP (Fig.2). Changes in parameters of heat absorption allow to make a conclusion about significant disintegration of protein structures of tissue. It should be noted that EP and BP destruct the structure of MT differently. The data obtained have a good agreement with the data of fluorescent investigations (Fig. 3). Judging from the change of quantum yield value, in the control the structural changes were observed already at 60°C, and from the position of maximum of fluorescence spectrum - the shift into a long wave region at a temperature above 70°C. In the presence of BP significant differences were observed as compared to the control. Thus, temperatures at which structural changes begin (according to change of value of quantum yield) increase with the increase of curing time. In this case the shift of maximum of fluorescence spectrum into long wave region is observed at lower temperatures as compared to control. In the presence of EP, structural changes begin at higher temperatures for every length of curing, and the shift of the fluorescence spectrum maximum into long wave region occurs at a lower temperature, as compared to the sample, cured with the utilization of BP. The depth and velocity of proteolytic processes can be also judged by the amount of free amino acids. The results obtained indicate that in case of using the salt noticeable changes of amino acids occur 3 days after curing: their total content increases by a factor 1.2. Introduction into muscular tissue EP results in increase of total content of amino acids during curing process - in the first day by 9%, after 3 days - by 12%. With the utilization of BP the content of free amino acids in muscular tissue decreases in the course of curing, that may be associated with development of microorganisms in the system at the expense of consumption of amino acids. In the system with BP and EP after 24 hours of curing, a decrease in content of free amino acids by 5%, and after 72 hours by a factor of 1.6 is noticed, which indicates a a substantial influence of EP and BP on the system.

Conclusion: The results of the investigations clearly indicate a substantial destruction of muscular proteins in case of utilization of EP and/or BP. The pattern and degree of these changes depends upon the kind of introduced biological preparations, length of technological processes, as well as on the prospects of EP and BP utilization for intensification of technological processes, improving quality characteristics of raw materials and products, and development completely new kinds of food products.

References:

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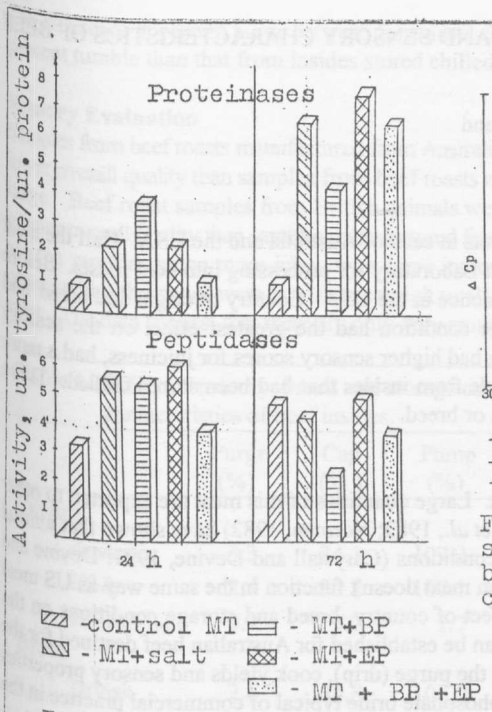

Fig 1. Change in activity of proteinases and peptidases in the course of beef curing

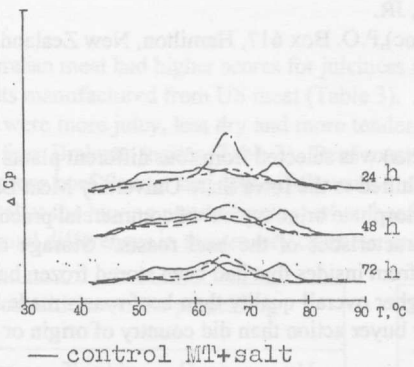

Fig. 2. Dependence of excess specific heat absorption of bovine muscular tissue during curing

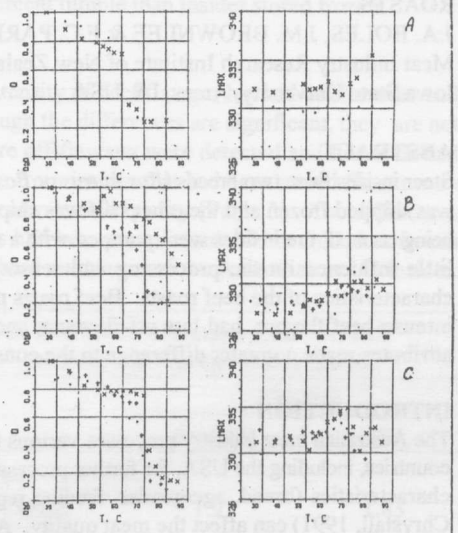

Fig. 3. Temperature dependence of area under the spectrum of fluorescence (Q) and spectrum maximum position (L_{max}) of muscular bovine tissue on storage time. A-control; B-muscular tissue in the presence of bacterial preparation; C- in the presence of enzyme (.- 24h; +-48 h; x- 72h)