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THE CHANGE OF CATHEPSIN ACTIVITY AND STRUCTURO-FUNCTIONAL CHARACTERISTICS OF BEEF MUSCLE TISSUE TREATED BY BACTERIAL AND ENZYMIC PREPARATION

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ABSTRACT

In this paper it is shown that utilization of bacterial and enzymic preparation for beef curing modifies significantly qualitative characteristics of muscle tissue. Maximum absorption and fluorescence peaks are shifted starting from 70 to 80°C and into the long wave region after 60°C and into the shortwave region after 70°C. These preparations modify cathepsin activity, qualitative and quantitative composition of free amino acids, patterns of water-bindings, mechanical and other physical and chemical properties.

Enzymic and bacterial preparations have found broad use lately, due to development of new meat products. They make it possible to improve the quality of products, to raise their biological and nutritive value, to improve digestion and assimilation of basic nutrients, and to solve a number of technological processes. The available data inadequately elucidates the pattern of change of enzymic activity of meat and its composition, including structural, and functional characteristics of muscle tissue during meat processing.

INTRODUCTION

Enzymic and bacterial preparations have found broad use lately, due to development of new meat products. They make it possible to improve the quality of products, to raise their biological and nutritive value, to improve digestion and assimilation of basic nutrients, and to solve a number of technological processes such as ways to utilize raw meat in the best way to intensify technological processes. The available data inadequately elucidates the pattern of change of enzymic activity of meat and its composition, including structural, and functional characteristics of muscle tissue during meat processing.

The object of the study was to investigate cathepsin activity, structural and functional changes of proteins of beef muscle tissue treated with bacterial and enzymic preparations during curing.

MATERIALS AND METHODS

The study has been conducted on model systems. M.Longissimus dorsi was excised from beef carcasses after six days of storage, trimmed of visible fatty and connective tissue and minced through a 2mm to 3mm plate. The resultant muscle tissue (MT) was mixed carefully with brine (10%) containing salt, bacterial and/or enzymic preparations. In all experiments, salt concentration was 2.5%, enzymatic and bacterial preparations were 0.013% and 0.1% respectively to the weight of the meat. Protosubtheline G 10 H, which is a neutral protease with activity optimum at pH6.5 to pH7.5 and temperature range from 40°C to 50°C, was used as an enzymic preparation (EP). The bacterial preparation (BP) PB-SK is a concentrate of lactic acid bacteria Lactobacillus plantarum and of denitrified micrococci mixed with glucose,

having activity optimum at pH from 4.5 to 5.0. Muscle tissue without additives served as control.

Changes in MT caused by EP and BP during three days of curing have been assessed by changes in peptidase and proteinase activity. Sodium Caseinate was used as a substrate in a universal phosphate-acetate buffer with pH 7.2 and 5.5 respectively. Optical density was measured at 680nm wavelength (Kas *et al.*, 1982). Structural change of proteins were determined using the differential scanning calorimetry and protein fluorescence methods (Oreshkin *et al.*, ND). Thermogravimetric method using a Q-derivationgraph by procedure modified by Oreshkin was used to measure weight changes (Oreshkin *et al.*, 1986). Content of free amino-acid was analyzed using the ion exchange chromotography on automatic analyzer Hitachi-835 (Japan). pH in muscle was determined by a pH-meter, type pH-150. Water-holding capacity was determined by filter paper press method of Grau and Hamm as modified by NVIIMP (Oreshkin and Borisova, 1989). Salt generation was measured using a EPM-MPEM device developed by VNIIMP (Oreshkin and Borisova, 1986).

RESULTS AND DISCUSSION

The activity of muscle proteinases and peptidases depends on the type and amount of introduced additives and time of curing (Figure 1). The increase of muscle cathepsins activity is observed when salt, BP and/or EP are used. If the proteinase activity in the control sample is one unit, then after 24 hours of curing, the proteinase activity increases by 2.6, 4.0, 2.5, and 1.2 respectively when salt, BP, EP, and BP and EP are added. After 72 hours of curing, it increases by 6.3, 4.1, 7.1, and 5.8 respectively. The change of peptidase activity after curing shows a somewhat different behaviour. After 24 hours of curing, their ratio is equal to 1.8, 1.7, 9.1, and 2.0 respectively. After 72 hours of curing it is equal to 0.9, 0.5, 1.1, and 0.8 respectively.

The increase of proteolytic activity of muscle tissue cathepsins when salt, BP and/or EP are added naturally affects other qualitative characteristics of beef: water-holding capacity increases, heat losses reduce, and penetration rate increases. However, the level of the mentioned change isn't substantial and is evident mostly during the first 24 hours of curing. Temperature at which weight losses are observed depends on the type of additives and duration of curing. After 24 hours of salt curing it reaches 48.5°C; with EP, 35°C; with BO, 43°C; and with both BP and EP, 48°C. After 72 hours of curing, temperature of all samples fall by 5°C on the average. When MT is heated to 75°C, the least weight losses are observed in samples containing EP and BP, and after 72 hours, in samples containing BP.

The results of the microcalorimetric studies are indicative of significant of significant structural change of MT when BP and EP are introduced (Figure 2). On the basis of changes of heat absorption variables, we can conclude that protein tissue structures are significantly disintegrated. EP and BP destroy the structure of MT in a different way. The obtained data are in good accordance with results of fluorescent studies (Figure 3). Transition in the control sample (judging by the change in yield) is observed in the range of 60°C by position of peak at temperatures above 70°C. In samples into which BP is added, significant differences are observed as compared to controls. Thus, transition temperatures by yield increase as storage time increases, while transition temperatures of the long wavelength shift showing a tendency to fall. In the presence of EP, transition temperature increases at all stages of storage compared to the control sample. However, the central temperature point of the long wave shift is lower than in the presence of BP. When the temperature is above 70°C, protein structures tend to coagulate.

The rate and intensity of proteolytic processes can be judged by the amount of free amino acids. The obtained results show that significant changes of free amino acids occur after three days of beef curing with salt utilization. Their total content increases 1.2-fold. The content of the following amino acids (the most important ones for flavour of finished products): threonine, serine, proline and histidine all increase the most intensely, from 1.3 to 1.4-fold; leucine, isoleucine, alanine, glycine and phenylalanine increase by 1.5 to 1.7-fold. The introduction of EP into connective tissue will cause the increase of the total content of free amino acids by 9% during the first 24 hours of curing and by 12% by the third day. After the introduction of EP, the content of isoleucine and leucine increase considerably in comparison with model systems with salt: after 24 hours, isoleucine content increases 1.7-fold, after 72 hours, 2.5-fold; and leucine increases 1.5-fold and 2.3-fold respectively. Tyrosine increases 1.6-fold by 72 hours while phenylalanine increases 1.4-fold by 24 hours and 2-fold by 72 hours. The observed phenomena testify that enzymic activity of MT cathepsins

increases, which causes acceleration of protein denaturation. When BP is added in brine, the amino acid content decreases, that apparently being connected with the development of microorganisms at the expense of amino acid consumption. But it is necessary to conduct further microbiological studies to confirm this hypothesis. In meat with BP and EP, after 24 hours of curing, a 5% drop in amino acid content is observed and 1.6-fold drop by 72 hours. This phenomenon indicates that EP and BP complex significantly affects meat.

CONCLUSION

The obtained results testify that proteins of muscle tissue are destroyed under the impact of introduced BP and EP. The rate and pattern of these changes depend on type and manner of biological preparations, introduction and duration of technical processes. The introduction of EP and BP can speed up the rate of technological processes, improve the qualitative characteristics of raw materials and products, including biological value, and development of new products.

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