Effect of a Proteolytic Enzyme Preparation on the Morphological Structure of the Filling Mass of Cooked-Smoked Non-Perishable Sausage

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SUMMARY: This is a study of the microstructural changes in cooked-smoked non-perishable sausages treated with a protease with the aim to intensify the ageing process involved in the technology of this type meat products. The meat for the test sausages has been treated with 0.01% and 0.02% Mezenterin 11-11 enzyme preparation. The studies have been carried out using a transmission electron microscope. In the non-treated sausages there have been observed optically thick parts in the Z-line zone. In the sausages prepared with 0.01% and 0.02% enzyme the protease has hydrolysed the myofibrils and as a result the sarcomeres have lost their individual contours and have formed a net of fine granulated myofibrillar proteins. There are certain changes in the collagen fibers as well, and there are sections where the cross furrows have disappeared. The changes established have improved the sausage texture in the cases where the meat has been protease-treated.

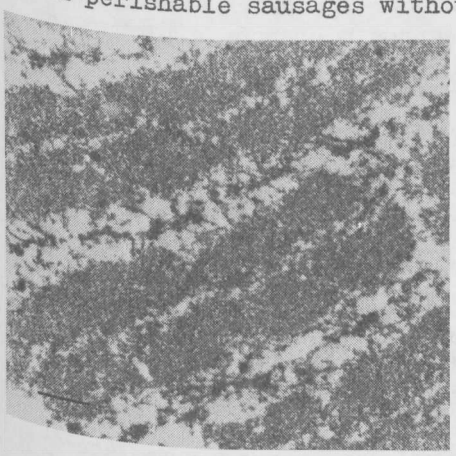
INTRODUCTION: Texture is an important organoleptic characteristic for all meat products whereby the consumers appreciate their quality. The technological processing of meat for cooked-smoked non-perishable sausages largely depends on the progress of the long autolytic changes during the postmortal ageing period (Bandman et al. 1988). It is well-known that the tenderizing of meat post mortem is assisted by the proteolytic processes which are dependent on the degree of activity of the lysosomic enzymes (cathepsins) and the calcium-dependent enzymes present in meat (Koohmaraie et al. 1988). Therefore, it is expedient to use proper enzyme preparations in order to intensify the proteolytic processes. Many authors point out that the changes in the muscles treated with proteolytic enzymes (tripsin, ficin, papain, etc.) are analogical to the changes occurring during meat ageing (Vassilyev et al. 1985).

The objective of the present work is to identify the microstructural changes in the filling mass of cooked-smoked non-perishable sausages treated with a bacterial proteolytic enzyme preparation.

MATERIALS and METHODS: The microbial protease Mezenterin 11-11 was obtained from Bacillus mesentericus, with a proteolytic activity of 210 PU/g enzyme, 55-60°C temperature optimum, 6.5 pH optimum, and was used in our study. The enzyme prepararion was added in cooked-smoked non-perishable sausages which had the following composition: 30 kg veal, 40 kg semifat pork, 30 kg non-fat pork, 2.2 kg salt, 0.007 kg sodium nitrite, 0.1 kg sugar, 0.3 kg pepper, 0.1 kg garlic. The veal was cooled to 0°-4°C and minced on a 4 mm plate. After adding salt, spices and enzyme (0.01% or 0.02%, depending on the filling mass quantity), the meat was mixed for another 3-4 min. Then the pork cuts were added and the grinding continued until 4-5 mm meat particles. The filling mass was stuffed into cutisine casings with 50-60 mm diameter. The sausages were shaped and then treated according to the technology established for this

kind of meat products. Control samples were prepared in exactly the same way without enzyme addition. Samples of the test and control sausages were sliced on a ultramicrotom LKB 8800. After being contrasted according to Reynolds (1963), the meat sections were studied on a transmission electron microscope Tesla BS-613 at 80 kV. Finally, the sausages were organoleptically evaluated by a 9-member panel using the 9-grade scale of the Higher Institute of Meat Technology Research in Moscow. The results from the evaluation were analysed according to the methods of mathematical statistics (Georgieva et al. 1987).

RESULTS and DISCUSSION: The microstructural changes which have occurred in the cooked-smoked non-perishable sausages without enzyme can be seen on Fig.1.



It is obvious from the picture that the heat treatment has denaturated the myofibrillar proteins, and, as a result, the myofibrils have lost their fibrillar nature (Fig.1). In the Z-line zone there are light bands among which are the optically thick parts of the Z-lines and some very fine grains which outline the sarcomeres clearly.

Fig.1. Electron microscopic picture of the longitudinal section of cooked-smoked non-perishable sausage without enzyme.
BAR = 0.07 µm.

In the middle of the anisotropic sections there are clear traces of the H-zones and M-lines (Fig.1). The electron microscopic picture of the sausages with added Mezenterin 11-11 enzyme (0.01% or 0.02%) is different from the one described above. It can be seen that the enzyme has hydrolysed the myofibrils to a different extent, and as a result the contours of the sarcomeres have been obliterated, and a net of fine-grain myofibrillar proteins has been formed (Figures 2-4). At some places there are light spatial bands of hydrolysed and extracted myofibrillar proteins. We suppose that the proteolytic enzyme preparation has assisted the degradation of the major proteins localized in the Z-lines (desmin and α-actinin) as well as the gap-filaments that are built up of the proteins titin and nebulin. This is also probably the reason for the microstructural changes established, namely the degradation of the Z-lines and the loss of the sarcomere contours (Fig.5). We accept that these changes impart less density to the above-mentioned fine net of myofibrillar proteins. The electron microscopic picture of the collagen fibers of the enzyme-treated sausages is a typical one. (Fig.3). Along the length of single collagen fibers there are sections of well-preserved cross furrows and others where they have disappeared, and the fibers are frayed. At some places there can be seen some destruction and swelling of the collagen fibers. The changes in the myofibrills and those in the collagen fibers are probably responsible for the better sausage texture found at the organoleptic analysis (Table 1).

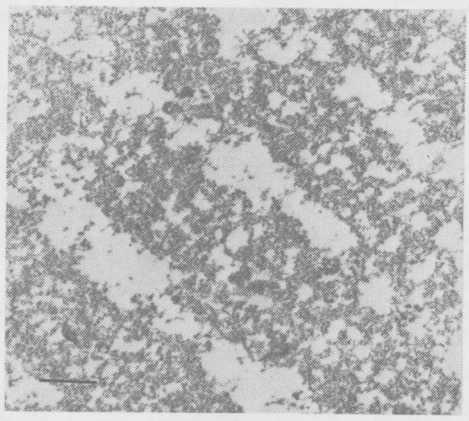


Fig.2. Electron microscopic picture of cooked-smoked non-perishable sausage, treated with 0.01% enzyme. BAR=0.10 µm.

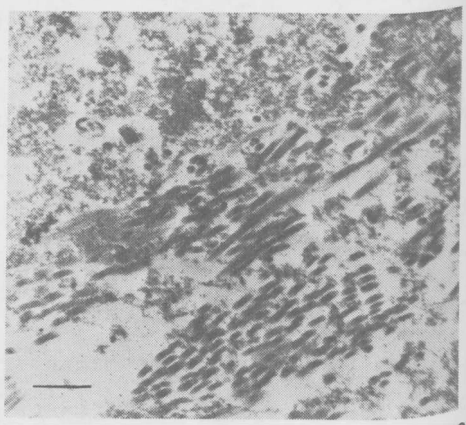


Fig.3. Electron microscopic picture of cooked-smoked non-perishable sausage, treated with 0.01% enzyme. BAR=0.05 µm.

The results from the organoleptic evaluation given in Table 1 show that Mezenterin 11-11 has improved the organoleptic properties of the cooked-smoked non-perishable sausages. The highest grade is seen for the sausages with 0.01% enzyme addition (8.82) as compared to the controls (7.20). This is expressed best in their outer appearance, texture and juiciness.

CONCLUSIONS: 1. The microstructural changes observed indicate that the enzyme preparation Mezenterin 11-11 accelerates protein proteolysis in meat. These processes are accompanied by a reduced density of the myofibrillar structures of the muscle fibers and improved texture of the finished product. 2. When added in a concentration of 0.01% in relation to the filling mass, the proteolytic enzyme improves the organoleptic properties of the cooked-smoked non-perishable sausages.

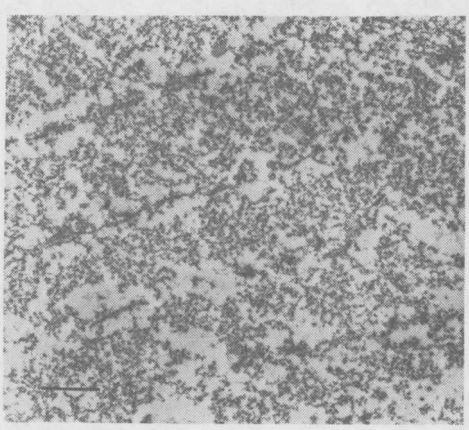


Fig.4. Electron microscopic picture of cooked-smoked non-perishable sausage, treated with 0.02% enzyme. BAR=0.10 µm.

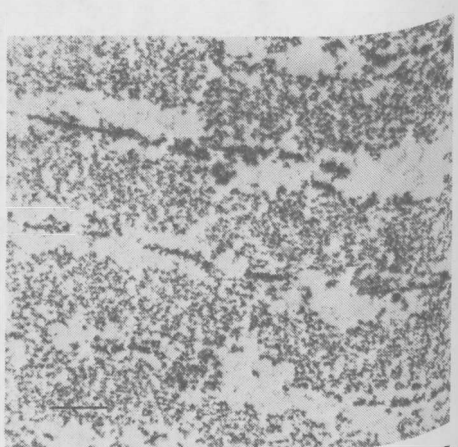


Fig.5. Electron microscopic picture of cooked-smoked non-perishable sausage, treated with 0.02% enzyme. BAR=0.07 µm.

Table 1. Organoleptic evaluation of cooked-smoked non-perishable sausages prepared with different additions of Mezenterin 11-11 enzyme preparation.

Characteristic	Control	Enzyme concentration, %	
		0.01	0.02
Texture	6.43±0.27	8.86 ± 0.10	8.25 ± 0.28
Juiciness	6.00±0.20	8.91 ± 0.09	8.17 ± 0.27
Flavour	6.72±0.28	8.10 ± 0.21	7.98 ± 0.30
Taste	7.34±0.29	8.57 ± 0.31	8.00 ± 0.31
Outer appearance	8.80±0.20	8.97 ± 0.30	8.41 ± 0.28
Colour	8.13±0.31	8.79 ± 0.17	8.60 ± 0.23
Overall grade	7.20±0.19	8.82 ± 0.17	8.41 ± 0.26

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