THE INFLUENCE OF EXOGENIC CA^{2+} ON THE MICROSTRUCTURE OF MUSCULAR TISSUE DURING POST—MORTEM AUTOLYSIS

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Background

One of the most important problems in meat industry up to the present time is the development of effective meat tenderization technology with the aim of reduction of its toughness. In spite of a more than a half-century history of the problem it is still urgent.
Till the recent times there was an opinion that the main mechanism of myofibrillar structures degradation was the proteolysis of connectin and nebulin under the action of muscular tissue own proteases – cathepsins and calpains (Pavlovsky P.E., 1960; Solovyov V.I.,1966; Robson R.M. et al., 1991; King N.L. et al., 1981; Bandman E., 1992). These ideas still predominate in studying the meat tenderization processes. However, by the present time some results have been published about molecular mechanism of fragmentation of muscular tissue and subsequent degradation of cytoskeletal proteins of connectin and nebulin under the action of calcium ions (Takahashi et al., 1992; Tatsumi R., Takahashi K., 1992; Tatsumi R. et al., 1992; Shimada K.-I et al., 1988; Tatsumi R. et al., 1999; Takahashi K., 1999).
All this can be the basis for the development of non-enzymatic methods of raw materials treatment with the aim of acceleration of the autolytic processes during their post-mortem aging.

Objective

The objective of the present work was the determination of the influence of exogenic calcium ions on microstructure of muscular tissue.

Materials and methods

The object of the investigation was the beef Longissiumus dorsi muscle. Fresh meat 1 – 1.5 hours after slaughter of the animal was trimmed with the separation of visible fat and connective tissue. Then the muscular tissue was minced in a chopper under cold.
In conducting the microstructure investigations a mincemeat not treated by phosphate buffer and incubated at 4 ±2° C at different stages of post-mortem autolysis was used as a control. As the experimental samples – the mincemeat incubated under the same conditions after treatment with buffer solutions with pH 5.8 of the following compositions:

1 - phosphate buffer (containing 0.01 M KH_2PO_4, 0.075M KCL, 0.02% NaN_3) + 1mM EDTA
2 –phosphate buffer + 0.1mM $CaCl_2$
3 –phosphate buffer + 0.1 mM $CaCl_2$ + 10 mM iodoacetamide (proteases inhibitor).

The original samples of control and experimental mincemeat, as well as the specimens after incubation at 4±2° C during 20, 40 and 60 hours were fixed during 48 hours in a 15% solution of formalin at ambient temperature. After fixation the samples were washed with a tap water and impregnated with 12.5% and 25% solutions of gelatin at 37 ± 2°C in a thermostat during 6 and 12 hours, respectively. The sections with the thickness 10 - 12 μm were produced with the help of microtome-cryostat MK-25. To differentiate structural elements of tissues and cells the sections were stained with hematoxylin of Ehrlich with additional staining with 0.5% solution of eosin.
The stained sections were studied under the light microscope "Jenoval" (Germany) with 400 x magnification.

Results and discussion

The investigations have revealed that beginning from 20 hours, in the muscular tissue of the control and experimental samples of all the series of experiments there developed a rigor mortis process about which one could judge from the contraction and dehydration of fibers, increasing of the longitudinal and progressive weakening of the cross striation.
At 40 hours, in the control and experimental samples of mincemeat in the absence of calcium ions (+EDTA) and also in case of incorporation of calcium ions into the incubation medium, in the background of proteases inhibitors, the rigor contraction of muscle fibers is expressed moderately and is in the process of development, while in case of incorporation of calcium ions only, it reaches maximum.
At 60 hours of storage at 4 ± 2° C the control samples of mincemeat are characterized with weakening of cross striation in the main mass of muscle fibers and with increasing of longitudinal striation. The nuclei of fibers had a round shape and were homogenous. The muscle fibers in the cross sections have a polygonal shape, lie freely in relation to each other. Destructive changes were not revealed (Fig. 2).
The microstructure of muscle bundles fibers of the experimental sample of the mincemeat incubated in phosphate buffer + EDTA after 60 hours had an increase in longitudinal striation in the most of distorted fibers and sharp weakening of cross striation. The nuclei of fibers had a round shape. The frontiers between fibers were clearly distinguishable. Destructive changes of muscle fibers were not revealed (Fig. 3).
The investigations of microstructure of experimental samples of mincemeat, as treated with calcium ions, after 60 hours of incubation at 4±2°C have shown that muscle fibers feature a restoration of cross striation in individual fibers and formation of single microfractures; the process of resolution of rigor contraction occurs asynchronously and hence the degree of expression of longitudinal and cross striation in different fibers is not similar. Muscle fibers lie porously in relation to each other, have different configuration – from straightly lying to zigzag. The nuclei of the fibers are homogenous (Fig. 4).
In the experimental samples of the mincemeat treated with calcium ions in the presence of the tissue SH-proteases after 60 hours of incubation at 4 ± 2°C the muscle fibers in the bundles are distorted. They lie porously in relation to each other, the cross striation is drawn close together, it is shallow, sometimes not revealed, and the longitudinal striation is well pronounced. Destructive changes are not revealed (Fig. 5).
After analysis of the data obtained it becomes evident that during storage up to 60 hours the muscular tissue of both the control and experimental samples had changes of structure, characteristic of the development of processes of rigor mortis and their subsequent resolution. But the dynamics of changes characterizing these processes differs in different series of experiments.
After 60 hours, in the series of experiments in the presence of calcium ions in the incubation medium, the muscular tissue featured the resolution of rigor mortis, which was evidenced by partial recovery of cross striation and the beginning of the destruction of myofibrils which was moderate. At the same time in the case of complete absence of calcium ions in the incubation medium as well as in the case of their introduction in the background of the inhibitor of calcium-activated and SH-proteases of iodoacetamide there were no resolution of rigor mortis and destruction of muscle fibers (Figs. 1, 2, 3,4,5).

Conclusions

Thus the investigations have shown that the introduction of calcium ions into the meat system in quantities exceeding the physiological concentration leads to deeper autolytic changes of muscle fibers characterized with more pronounced destruction of myofibrillar structures.

Pertinent literature

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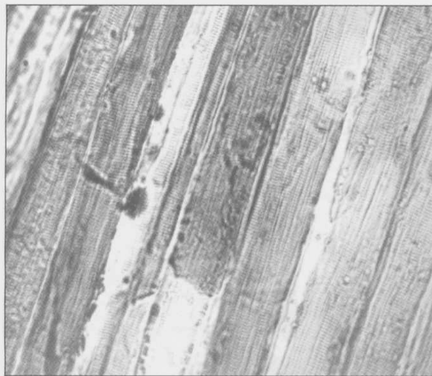

Fig. 1. Microstructure of the mincemeat control sample

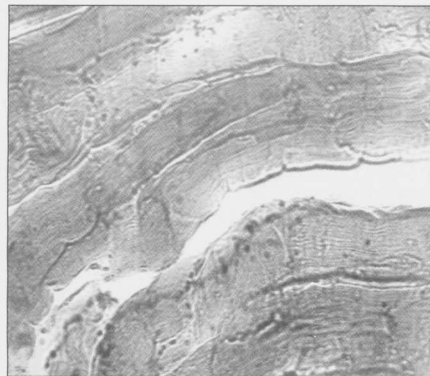

Fig. 2 Microstructure of the mincemeat control sample after 60 hrs storage period

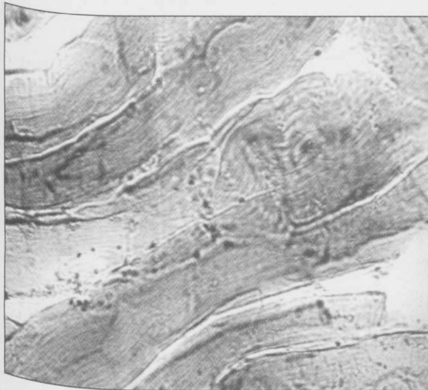

Fig. 3 Microstructure of the mincemeat experimental sample after 60 hrs incubation in buffer phosphate +EDTA

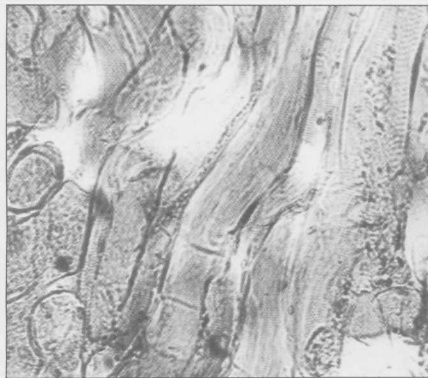

Fig. 4 Microstructure of the mincemeat experimental sample treated with calcium ions, after 60 hrs incubation

Fig. 5 Microstructure of the mincemeat experimental sample after treatment with calcium ions in the presence of proteases inhibitor, after 60 hours incubation