DEPENDENCE OF ACTIVITY OF LYSOSOMAL PROTEINASES (CATHEPSINS B AND L) ON QUALITY GROUP OF MEAT, ITS THERMAL STATE AND TIME OF AGEING

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The process of natural proteolysis of meat proteins during meat ageing is the base of its natural tenderization. Hence, it is important to study this process for different quality groups of meat. This process is regulated by natural catalysts-enzymes: lysosomal cathepsins and calpains. A large number of works of the past decade indicated a major role of calpains during proteolysis (Barnieri et al., 1993). However, the rate of degradation of muscle proteins and hence, the extent of tenderization do not go in parallel with the changes in calpains activity which points to the fact that it is not this single group of proteins that is responsible for the tenderization process (Geesink, 1993). Among the cathepsins the amount of aspartatproteinase-cathepsin D is prevailing. However, according to literature data, other cathepsins, primarily cathepsins B and L have a large influence on the proteolysis in a muscle cell, which, as contradictory literature data indicate, affect both the muscle and connective tissue. The content of this cystein proteinases in the cells is less than that of cathepsin D and calpains. However, as investigations show, their role in tenderization of meat is rather significant (Barnnieri et al., 1993; Penny, 1980; Goll et al., 1983; Valin, 1985; Beltran et al., 1992; Kas et al., 1983).

Having this is mind, we considered it necessary to study the activity of these proteinases in meat, belonging to different quality groups, as far as its natural tenderization is concerned.

Materials and Methods

The object of the investigations was M. longissimus dorsi of PSE and normal (N) pork. The grading of PSE and [1] meat groups was done within the first hour post mortem by the pH value directly in the carcasses. The group of PSE meat included the samples having $pH_1<5.8$ and the group of N meat - those with $6.2 \leq pH1 \geq 5.8$. The samples for investigations were taken after 6, 24, 48, 72 and 96 hours of meat ageing. The chilled raw materials were stored at 2-4°C. Freezing was carried out at -12-14°C. Before analysis the samples were defrosted at domestic refrigerator, and their enzymic activity was determined. The activity of cathepsins was determined according to (Kirschke et al., 1983). A synthetic fluorescing L-Phe-Arg-N Mec (Company "Bechem", Germany) was used as a substrate. The fluorescent investigations were carried out on spectrofluorometer "Signe-4" at a wavelength of light excitation 330 nm and fluorescence 390 nm. All the measurements were carried out as related to control. The activity of cathepsins B + L was determined on the meat from which only the fat tissue was trimmed, and on the samples from which the intramuscular connective tissue was additionsly trimmed. The activity of cathepsins B + L of intramuscular connective tissue was determined as a difference between activities of untrimmed and trimmed samples. The activity was expressed as the number of micromoles of the substrate as hydrolysed during 1 min. g of muscle.

Results and Discussion

The graphs (Fig. 1) of total activity of cathepsins B + L plotted against the time of meat ageing (during strage at 2-4°C) indicate that the activity of these enzymes in M long. dorsi of PSE pork with untrimmed intramuscular connective tissue was significantly higher than that of the normal (conventional) meat. The differences are especially large after 48 and 72 hours of ageing. These differences in the cathepsins B and L activity are in no way connected with pH of meat and its change during ageing, because pH of both kinds of meat (N and PSE) in this case was within the ranges of the optimum of the effect of these enzymes (pH = 5.0-5.5). When determining the activity of cathepsins B and L of the muscle from which the intramuscular connective tissue was trimmed, one can observe another picture. First of all, in boch kinds of meat the activity of cathepsins decreases for each period of ageing up to 96 hours (Fig. 1). In the case of PSE meat, initially it doesn't change

(up to 72 hours), and then decreases. In the case of N meat one can observe a decrease by 48 hours, then an increase - by 72hours and subsequent decrease. It is very important that although in the case of trimming the intramuscular tissue from the muscle the activity of cathepsins B + L decreases as compared to the untrimmed muscle its general level is sufficitntly high (20×10^4 substrate/min. · g). The activity of cathepsins B + L of intramuscular connective tissue is well expressed and changes significantly during ageing (Fig. 2). First of all, in the case of PSE meat after 42-72 hours of ageing it is 20-25% higher, than in the case of N meat; in both cases the peak of activity of cathepsins is at 48 hours of ageing, then a decrease is observed. At 72 hours of ageing the enzymic activity of PSE meat remains sufficiently high and falls upon subsequent storage, whereas in the case of N meat the fall of activity is distinctly expressed immediately after 48 hours. These data agree well with the results of the investigations of thermal stability of collagen of intramuscular connective tissue of PSE meat that had been carried out by us earlier (Borisova et al., 1992). It was shown that the collagen structure of PSE meat is less stable than that of N meat and this is followed by the shift of the maximum of its thermal denaturation to low temperatures and decrease of Δ H structural transitions in that case. It is not improbable that a decrease of collagen structure stability of PSE meat as compared to N meat, is to some extent influenced by a greater activity of cathepsins B + L as collagenolytic agents. Freezing and subsequent defrosting have an essential influence on the activity of cathepsins B + L of PSE and N meat. In PSE meat the general activity of proteases decreases on average by 20-25% as compared with the meat after chilling. After freezing and defrosting the difference in the activity of cathepsins in N and PSE meats practically disappears.

The muscles trimmed from intramuscular connective tissue, after freezing and defrosting had a lower activity of cathepsins B + L, as compared to the intact muscles. The difference in the activity of cathepsins B + L of N and PSE muscles (trimmed) is insignificant. In the intramuscular connective tissue of N and PSE meat after freezing the activity of both proteases in the course of ageing changes in a manner similar to chilled meat. Thus, our experiments have shown that cathepsins B and L have proteolytic activity both in relation to myofibrillar proteins and to collagen of intramuscular connective tissue. The behavior of these enzymes in PSE and N meat is to a large extent different. In the case of PSE meat their activity is 20% higher than in the case of N meat (first of all, this is true for 48 hours of meat ageing). Since, in accordance with our procedure of determination of the cathepsins B and L activity, we determined the free activity of these enzymes, its greater value in the case of PSE meat may be accounted for by the specific nature of this meat, having a large amount of free Ca^{2+} in sarcoplasm, low pH value at a high temperature of the muscle already at the first hour after slaughter and as a consequence of this - disturbance of the integrity of cellular and subcellular membranes. Obviously, all these factors lead to release of cathepsins from lysosoms, their going out into sarcoplasm and increase of their free activity.

A rather important finding was obtained in our investigations, that is the clearly marked changes of activity of cathepsins B + L during muscle ageing up to 96 hours characterized only their collagenolytic activity. Having in general almost a 6-fold less level of activity of these enzymes it undergoes essential changes during meat ageing with the maximum at 48 hours. At the same time in the trimmed myofibrillar part of the muscle, the activity of cathepsins B + L during ageing up to 72 h changes insignificantly. Probably, the conflicting data in literature concerning the changes of activity of cathepsins B and L during ageing can be explained by the fact that the authors worked with different objects: with the homogenates of the intact muscle, with myofibrillar fraction or with the soluble or aged collagen. Hence, the conflicting statements of one authors pointing to complete absence of the activity change of these cathepsins during ageing and of the others, pointing to changes of activity with maximums at 42-72 hours of ageing.

A decrease in activity of cathepsins B and L after freezing and defrosting of these kinds of meat and practical smoothing off the differences in this value in N and PSE meat, being essential in chilled meat, in our opinion, is not a result of physical and chemical influence of freezing itself (disturbance of cellular and subcellular membranes, mechanical damages of molecular and above-molecular formations of protein by intra- and intercellular ice, etc) but by chemical influence of proteinases inhibitors present in muscle cells and primarily of cystatine. It is probably that in freezing and subsequent defrosting it acquires more activity, decreasing the activity of studied by us lysosomal proteinases (cathepsins B and L), showing especially high activity at such periods of meat ageing, when their activity in chilled meat is expressed to a maximum extent.

Conclusions

Cathepsins B and L have proteolytic activity in relation both to myofibrillar fraction of muscles and to intramuscular connective tissue;

- in meat ageing at 0-(+4°C) the activity of cathepsins undergoes great changes with maximums at 48-72 hours after slaughter, depending upon the group of raw materials; the changes in collagenolytic activity being expressed most markedly;
- in the whole investigated period of ageing (from 2 to 96 hours) the activity of these enzymes in the case of PSE meat is significantly higher than in the case of N meat; this is markedly expressed at 48-72 hours of ageing;
- after freezing and subsequent defrosting of meat the general activity of cathepsins B and L in the period from 2 to 72 hours falls, and differences between PSE and N meat smooth off; in contrast to dynamics of the change of activity of these enzymes in chilled meat, after freezing and defrosting the meat belonging to both quality groups after 96 hours of ageing showed a growth of activity.

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Fig. 1. Cathepsin B + L activity as plotted against duration of autolysis for different quality groups of pork (M. long. dorsi)(chilled).

Fig. 2. Cathepsin B + L activity as plotted against duration autolysis for different quality groups of chilled pork (M. long. dorsi, intramuscular connective tissue).