EFFECTS OF AGEING OF BULL LONGISSIMUS DORSI ON PROTEOLYSIS AND CALPAIN I AND II ACTIVITIES

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SUMMARY

The biochemical changes, especially proteolysis, which take place during postmortem storage of meat, are a result of the action of muscle proteases such as calpains. In this study, the degradation of muscle proteins and activities of calpains I and II have been investigated at various ageing periods of postmortem storage (0, 1, 2, 3, 4, 5, 7,9 and 12 days). The changes in meat proteins were observed by SDS - polyacrylamide gel electrophoresis (SDS - PAGE). The results indicated that cytosolic calpains I and II decreased during ageing of bull Longissimus dorsi while calpain I was not detected at the end of ageing. There was a progressive disapperence of some high molecular weight muscle proteins such as titin and nebulin on SDS - polyacrilamide gel electrophoresis. The some high and low molecular weight muscle proteins did not seem to change.

INTRODUCTION

Tenderness occurs in all muscles of all meat species is the most important quality criterion and sensorial property. The improvement in tenderness is of myofibrillar origin and has already been documented by different researches (Azanza et al., 1980; Demeyer 1990 and 1991; Etherington et al., 1987; Koohmaraie, 1988; Olson et al., 1976; Ouali et al., 1987; Ouali and Talmont, 1990; Ouali and Valin, 1981; Ötle*f*, 1992; Ötle*f* and Uytterhaegen, 1992; Zeece et al., 1986). Proteolysis of muscle proteins is considered as a primary mechanism in biochemical changes occuring postmortem muscle. Endogenous muscle proteases in the cytosolic fraction may be involved in the dinamic systems that function in protein accretion and degradation in muscle. Endogenous muscle proteases (endopeptidases) include three types grouped by their optimum pH as follows: acidic proteases (cathepsin A, B, C, D, H and L), calpains I and II (Ca^{2+} dependent neutral proteases, CDP I and II), and alkaline proteases. Calpains and cathepsins have received much attention from many scientist. At pH 5.5 calpains could be more potent content to meat tenderization than cathepsins. In the opinion of Koohmaraie (1988), calpains -in contrast to cathepsins- have all the characteristics (endogenous to skeletal muscle cells, cellular location and the ability to mimic postmortem effects on myofibrils) that a proteolytic system must have to be involved in postmortem tenderization. Calpains which located in cytosol and required Ca^{2+} for activation, has a variety of other names, including Ca^{2+} dependent neutral proteases, calcium activated factor, calcium activated proteases. Caipains (Mr of 112,000) have two equimolar subunits (Mr of 80,000 and 30,000). Both subunits of calpains affect myofibril similarly and they play an important role in meat tenderness. They have been indeed reported to be responsible for specific degradation of some low and high molecular weight muscle proteins during meat ageing process (Ouali and Talmont, 1990; Koohmaraie, 1988; Zeece et al., 1986).

The purpose of the present investigation was to study the effects of ageing of bull Longissimus dorsi on proteolysis and calpains I and II activities.

MATERIALS AND METHODS

The experiment was conducted on bull Longissimus dorsi. For the isolation of myofibrils the method of Olson et al. (1976) was used. Calpain I and II were determined by the method of Etherington et al. (1987). The changes in myofibrilar proteins were observed by SDS-polyacrylamide gel elctrophoresis (Ötle*f* and Uytterhaegen, 1992) at various ageing periods (0, 1, 2, 3, 4, 5, 7, 9 and 12 days after slaughter).

RESULTS AND DISCUSSION

Changes in calpain I and II activities

The effect of postmortem storage on calpain I and II activities are summarized in Table 1. The calpain II activity remained nearly constant throughout the first days of postmortem storage, then there was a regularly decrease during the storage. After 12 days the loss in activity was appr. 52 %. In the calpain I activity there was a progressive decrease at various ageing periods of postmortem storage. After 7 days of storage the calpain I activity was not determined. It would seem unlikely that calpain II is involved in the postmortem tenderization process because of the reasons as follows (Koohmaraie, 1988): a. calpain II is maximally active at pH 7.5, therefore would be little activity, at pH 5.5 prevalent during meat ageing; b. calpain II is maximally active at 25°C, whereas postmortem tenderization occurs at 2° to 4°C; c. the Ca^{2+} concentration requirement (1-5 mM) of calpain II for activation exceeds the Ca^{2+} levels in postmortem storage. However, calpain I has overcome these problems, and may play an important role in meat tenderness taking place during postmortem storage.

Changes in myofibrillar proteins

The results of SDS-PAGE revealed that some high and low molecular weight myofibrillar proteins degraded during the postmortem storage. Table 2 presents data on the percentage of the myofibrillar proteins and their changes during postmortem storage. As can be observed in Table 2, there were numerous changes in proteins. The main changes corresponded to titin, nebulin, C-protein and troponin T. Nebulin is degraded very fast during the first four days of ageing and slower later on. A major degradation after twelve days was found for troponin T, followed by nebulin, C-protein, titin, troponin C, troponin I, tropomyosin, filamin etc. These myofibrillar proteins lose at least 36 % to 82 %. There was a progressive increase of several degradation products with various molecular weights. However, the results demonsrated that no myosin, actin and α-actinin in degradation occured in postmortem storage. The other high and low molecular weight myofibrillar proteins did not seem to change.

CONCLUSION

Our results showed that some high and low Mr proteins such as nebulin, troponin T, C-protein of bull muscle aged for twelve days were degraded at a significant rate. It can be proposed that calpains are probably involved in meat tenderization, but they are not responsible for the postmortem decrease of all myofibrillar proteins and the improvement in our knowledge of the hydrolytic activity of calpains I and II in postmortem muscle is needed.

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