EFFECTS OF ENDOGENOUS PROTEASES EFFECTORS ON THE DEGRADATION OF TROPONIN-T AND DESMIN OF CHICKEN BRISKET MUSCLE DURING POSTMORTEM AGING

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Introduction

A number of investigations have indicated that consumers considered tenderness as the most important eating quality characteristics of meat. In practice, storage of meat, especially, beef, lamb and pork for a given time is a prerequisite for the development of tenderness. To date, it has been extensively studied and well established that postmortem degradation of myofibrillar proteins has close relationship with the structural destruction and improved tenderness of meat during refrigerated storage. However, currently, the mechanism underlying postmortem myofibrillar protein degradation is still an unresolved question. Ji et al. (2006) reported that meat postmortem tenderization and myofibrillar protein degradation was caused by the elevated concentration of free calcium in muscle fiber sarcoplasm. While, others believed that meat tenderizing process is mainly enzymatic in nature (Ouali et al., 2006). The most studied proteolytic systems include calpains, cathepsins and proteasome, among which the role of calpains especially, μ-calpain are highlighted in meat aging, whereas, this statement has never been definitely proven and far from being fully understood. Hence, it is justifiable that, in addition to the proteases cited above, other proteases should not be excluded and are possibly involved in meat postmortem aging.

The term of apoptosis was first introduced in 1972 by John Kerr to designate the common morphologic features of programmed cell death in tissue remodeling, and was studied extensively thereafter. The cell death through apoptosis can lead to the activation of death executioner proteases, which consisting of caspase 3, 6, and 7, especially caspase 3, are responsible for most of the cleavage events observed during apoptosis. After animal bleeding, all the muscle fiber will die, but little is known about the role of these caspases in postmortem muscle myofibrillar protein degradation.

The objectives of the present study was to investigate the influence of meat endogenous protease effectors on the muscle troponin-T and desmin degradation during postmortem aging.

Materials and Methods

Chicken breast muscles were obtained immediately after slaughter, and assigned to different treatments. One group marinated in solution containing 100 mM NaCl and 2 mM NaN_3 was designated as control, the others were soaked in solutions as control but containing 30 mM EGTA, 20 mM $CaCl_2$, protease inhibitor cocktail (10 ml/tablet), 100 μM caspase 3 inhibitor (DEVD-CHO), and 20 mM $CaCl_2$ plus protease inhibitor cocktail, respectively in the ratio 1:1(W/V) (meat : solution), then stored at 4 □ for 1, 3, 7 d.

Myofibrils were purified at 2 □ according to the myofibril preparation procedure of Etlinger et al. (1976). In the last step, the myofibrils were suspended in Tris-EDTA buffer (10 mM Tris, 5 mM EDTA pH 8.0) and immediately removed and mixed with treatment buffer (125 mM Tris, 4% SDS, 20% glycerol), the samples were heated at 50 □for 20 min, after centrifugation protein concentration was determined with BCA Protein Assay Kit, and diluted to 3.5 mg/ml using treatment buffer containing 10% MCE and 0.001% bromophenol blue. Samples were well mixed and heated at 50 □ for 10 min, and then stored at -80 □ for subsequent use. The SDS-PAGE and western blotting analysis of troponin-T and desmin was performed following the protocols described by Wheeler et al. (1999).

Results and Discussion

Troponin-T. The postmortem degradation of troponin-T and the appearance of bands migrating at approximately 30 kDa has been well documented, many reports have related the presence and amount of this 30 kDa polypeptide to an indication of the rate and extent of postmortem proteolysis and meat tenderization, although there was no direct evidence concerning its contribution to postmortem myofibril destruction. In the present study, different treatments had significant influence on the degradation of troponin-T and the appearance of 30 kDa polypeptides. After 1 d, the 30 kDa fragments appeared in calcium chloride treated samples, the 30 kDa bands also appeared at 3 d in control and EGTA treated samples although the latter is fainter than the former. Until 7 d, a faint 30 kDa band appeared in DEVD-CHO treated samples, but no 30 kDa fragment appeared in

treatments with proteases inhibitor cocktail, or calcium plus proteases inhibitor cocktail (figure 1. A, B, C).These results indicated that meat endogenous proteases, possibly including caspase 3 are responsible for the PM degradation of troponin-T, while calcium alone could not cleave troponin-T.

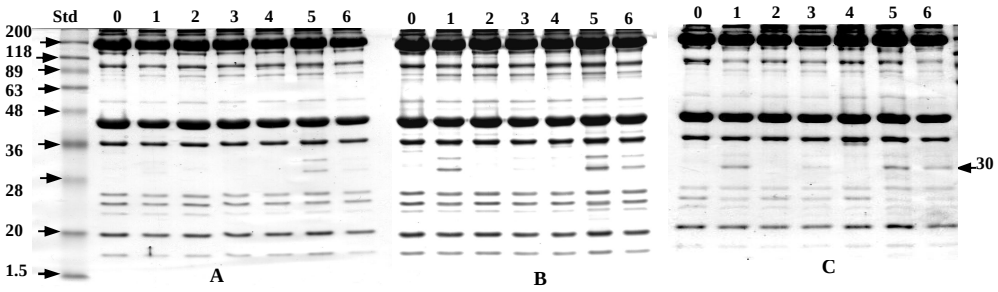

Figure 1. Representative SDS-PAGE (12.5%) profiles showing generation of 30 kDa fragment in different treated samples at 1 d (A), 3 d (B), and 7 d (C) postmortem. 0: at death ; 1: control; 2: proteases inhibitor cocktail; 3: DEVD-CHO; 4: calcium chloride plus proteases inhibitor cocktail ; 5: calcium chloride; 6: EGTA. The total protein loaded each well was 15 µg.

Desmin. As one of the major intermediate filament proteins, desmin plays an important role in maintaining the lateral alignment of adjacent myofibrils and also links the peripheral layer of myofibrils to the cell membrane skeleton. Hence, it is conceivable that desmin degradation may influence the structural integrity of muscle cells. The positive correlation between desmin degradation and meat tenderness has been reported by some researchers (Huff-Lonergan et al., 1996). Figure 2 (A, B, C) shows that even after 7 d postmortem storage, the intact desmin bands were still apparent in samples treated with proteases inhibitor cocktail and DEVD-CHO (lane 2, 3, 4). Compared to control, EGTA did not block the degradation of desmin, whereas the desmin degradation was accelerated in calcium treated samples without proteases inhibitor, suggesting that the postmortem desmin degradation is caused by muscle endogenous proteases.

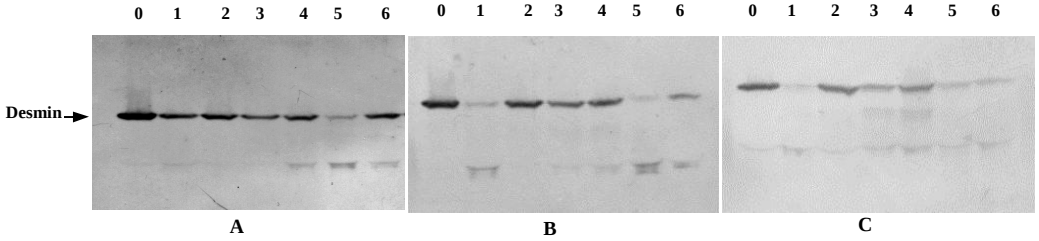

Figure 2. Representative western blotting profiles showing the degradation of desmin with different treatments at 1 d (A), 3 d (B), and 7 d (C) postmortem. 0: at death; 1: control; 2: protease inhibitor cocktail; 3: DEVD-CHO; 4: calcium chloride plus proteases inhibitor cocktail; 5: calcium chloride; 6: EGTA.The protein loaded each well was 40 µg.

Conclusions

The findings substantiated the comparatively more widely accepted hypothesis that endogenous proteases play major role in meat protein degradation, while calpains alone can not account for the majority of muscle protein changes occurred during postmortem aging, and the apoptosis effector caspase 3 is possibly a new member involved in meat tenderization.

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