Comparison of Postmortem Proteolysis between Breast and Leg Muscles in Chiayi Native Chickens

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ABSTRACT : Postmortem Proteolysis of breast (BM) and leg (LM) muscles in Chiayi native chickens at 5°C were compared. Myofibrils were purified from BM and LM samples that were randomly taken from carcasses after 0, 1, 3, 7 and 14 days of storage at 5°C. Fragmentation of myofibrils were determined, and degradation of myofibrillar proteins were analyzed by the SDS-PAGE and western blots. The results showed that myofibril fragmentation index (MFI) was significantly ($p<0.05$) higher in BM than in LM samples. Disappearance of titin and nebulin and appearance of the 30 kDa component were more rapidly as seen on SDS-PAGE in BM than in LM samples. Western blots labeled with a monoclonal antibody to desmin also demonstrated that desmin degraded more quickly in BM samples. Our data suggested that postmortem proteolysis occurred more rapidly in breast muscles in Chiayi native chickens. ***(Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 5 : 721-724)***

Key Words : Postmortem Proteolysis, Myofibrillar Protein, Breast Muscle, Leg Muscle

INTRODUCTION

It is generally accepted that meat tenderness can be improved by postmortem storage of carcasses at refrigerated temperature. Several review papers (Goll, 1991; Koohmaraie, 1994; Robson et al., 1997) have agreed that the major changes occurred in postmortem muscles are degradation of Z-lines and myofibrillar proteins. Chiayi native chickens are settled in the central area of Taiwan island and have been saved as a close population. One of the major characteristics of this unique strain is small in size and takes 5-6 months to reach ~1.5 kg of the market weight. The postmortem change in broiler has been studied very extensively (Samejima et al., 1976; Chou et al., 1994). Little information, however, is available regarding postmortem changes in the muscles of this strain. The purpose of this study, therefore, was to compare the postmortem proteolysis of breast and leg muscles in Chiayi native chickens. The changes in myofibril fragmentation index and degradation of myofibrillar proteins were examined.

MATERIALS AND METHODS

Sample preparation

Chiayi native chickens (~22 weeks old with an average live weight of 1.5 kg) were slaughtered by using normal commercial practices. The 0 day breast and leg muscle samples were taken from carcasses after 2-3 h postmortem. The rest of carcasses were vacuum-packed and stored at 5°C for 1, 3, 7, and 14 days of storage. This experiment was done in three replicates. Fifteen chickens were randomly selected per replicate with triplicate samples for each time period. After sampling, breast and leg muscles were ground through a 3 mm plate, evenly mixed and divided into equal parts for myofibril purification. Breast and leg myofibrils were purified via the method of Goll et al. (1974). Myofibril fragmentation index (MFI) was measured by the method of Olson et al. (1976). The MFI data were statistically analyzed by the split-plot design (SAS Institute Inc., 1986).

SDS-PAGE analysis

The myofibril samples for SDS-PAGE were prepared by the method of Wang et al. (1988) and routinely analyzed in a 12% tris-glycine slab gel (acrylamide: methylenebisacrylamide was 37.5:1, w/w) for proteins migrating below the myosin heavy chains according to the method of Laemmli (1970). Degradation of titin and nebulin was examined in an 8% tris-glycine slab gel (acrylamide:methylenebisacrylamide was 200:1, w/w) (Wang et al., 1988). The same amount of protein (150 μg) from each sample was loaded into each well of the 12% and 8% gels. The protein concentration was determined using a modified biuret method (Robson et al., 1968).

All gels were run at 15 mA at 25°C. A SE 400 slab gel electrophoresis unit (Hoefer Scientific Instrument, San Francisco, CA) was used. Gels were stained in a solution of 0.05% (w/v) Coomassie blue R-250, 45% (v/v) methanol and 9.2% (v/v) acetic acid for 4 h and destained in 10% (v/v) methanol, 7.5% (v/v) acetic acid. Molecular weight markers ranging from 42,700-200,000 (BDH Laboratory Supplies, Poole, England) were used as protein standards.

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Received July 24, 2001; Accepted October 24, 2001

Western blot analysis

Proteins were transferred from a 12% slab gel (acrylamide:methylenebisacrylamide was 37.5:1) to a nitrocellulose membrane by the method of Towbin et al. (1979). After transfer, the membrane was incubated in a 5% bovine serum albumin-phosphate buffer solution (BSA-PBS) for 30 min at 37°C and then was washed three times in a 0.1% BSA-PBS solution for 5 min at room temperature. A monoclonal antibody (mAb) to desmin (D-1033) (Sigma, St. Louis, MO, USA) was used as a primary antibody. The membrane was incubated with the primary antibody for 2 h at room temperature, washed three times in 0.1% BSA-PBS for 5 min, incubated with immunogold-labeled secondary antibody for 2 h at room temperature, washed twice in 0.1% BSA-PBS solution for 5 min each and twice in deionized water for 1 min each. The gold label was enhanced by silver staining (Moeremans et al., 1989).

RESULTS AND DISCUSSION

The MFI assay is a measure of the degree of myofibril fragmentation during postmortem storage (MΦller et al., 1973). Our results (figure 1) indicated that MFI values increased ($p<0.05$) with storage time in both breast muscle (BM) samples and leg (LM) samples. A greater value ($p<0.05$) in MFI, however, was observed in BM samples (figure 1). For instance, the MFI value of 3 day BM samples was similar to that of 14 day LM samples. This meant that fragmentation of myofibrils was very greater in BM samples than in LM samples. It has been thought that MFI is directly related to meat tenderness (Culler et al., 1978). Stromer et al. (1974) suggested that calpain activity played a major role in the fragmentation of myofibrils during postmortem storage although whether calpain (Koohmaraie et al., 1987) or m-calpain (Boehm et al., 1998) was responsible remained unclear.

According to the SDS-PAGE analysis, the disappearance of the titin 1 (T1) and nebulin bands occurred more rapidly in BM samples than in LM samples (figures 2a,b). The T1 and nebulin bands were visible at day 0 and disappeared by day 1 in BM samples (figure 2a). In LM samples, on the other hand, the T1 and nebulin bands appeared at day 0 and day 1 but disappeared by day 3 (figure 2b). This difference in titin and nebulin degradation may be due to the difference in the endogenous enzyme activity or in the structures of titin and nebulin molecules in

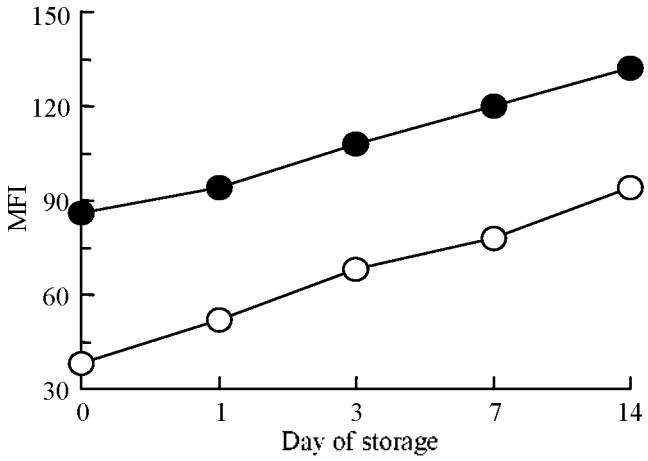

Figure 1. Changes in myofibril fragmentation index (MFI) of breast muscles and leg muscles with day of storage in Chiayi native chickens
●, Breast muscles; ○, Leg muscles

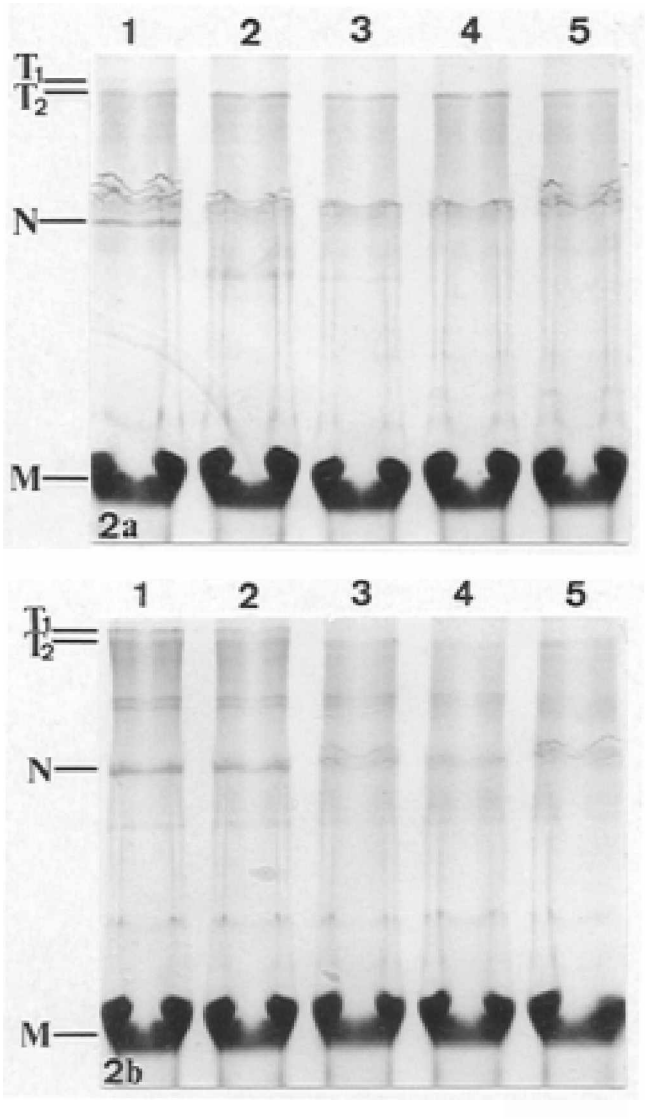

Figure 2. Changes in titin and nebulin of (a) breast muscles and (b) leg muscles of Chiayi native chickens. 0-day=lane 1; 1-day=lane 2; 3-day=lane 3; 7-day=lane 4; 14-day=lane 5.
T1, titin 1; T2, titin 2; N, nebulin; M, myosin heavy chain.

BM and LM samples (Chou et al., 1994). Previous reports also showed that titin and nebulin were very susceptible to postmortem degradation (Fritz and Greaser, 1991), and degradation of these proteins coincided with increased tenderness (Anderson and Parrish, 1989).

Our SDS-PAGE results (figure 3) also showed that the 30 kDa band was present by day 1 and became more prominent after day 3 in BM samples. On the other hand, the 30 kDa band appeared very lightly in LM samples (figure 3). Our result and other studies (Ho et al., 1994; Chou et al., 1996; Hwang et al., 2000) showed that the appearance of the 30 kDa component during postmortem was a typical change in animal muscles from many species. Previous report (Ouali, 1984) suggested that the accumulation of the 30 kDa component was associated with increasing in postmortem meat tenderness. Farouk et al. (1992) further stated that the appearance of the 30 kDa component might be also used as an index of the rate of proteolysis. Therefore, our result implied that the postmortem proteolysis at 5°C was more rapidly in BM samples than in LM samples, which was consist with other reports (Samejima et al., 1976; Chou et al., 1994).

Desmin was known as a major component of desmin-containing intermediate filaments (IFs) in muscle cells (Lazarides, 1982). Western blots labeled with a mAb (D-1033) to desmin (figure 4a) demonstrated that intact desmin in BM samples could hardly be seen at 0 day and was completely disappeared by day 1. In LM samples (figure 4b), however, desmin seemed to remain intact throughout the entire 14 days of postmortem storage. This result confirmed that the postmortem proteolysis at 5°C was more rapidly in BM than in LM samples. A faster degradation of desmin might correlate with an improvement in meat tenderness (Robson et al., 1997).

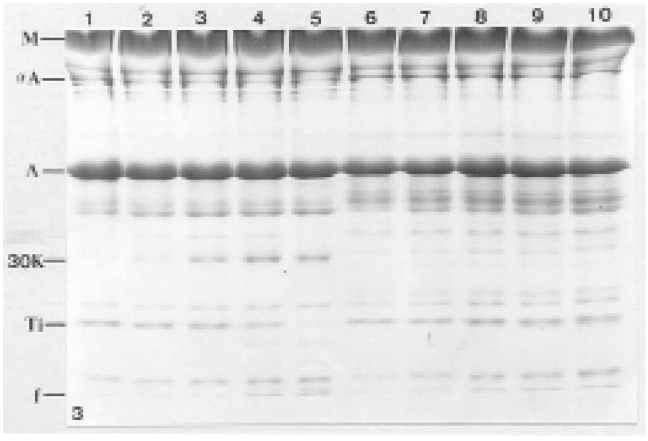

Figure 3. Changes in myofibrillar proteins of breast muscles and leg muscles of Chiayi native chickens. Breast muscle samples=lanes 1-5; Leg muscle samples=lanes 6-10; 0 day=lanes 1 and 6; 1 day=lanes 2 and 7; 3 day=lanes 3 and 8; 7 day=lanes 4 and 9; 14 day=lanes 5 and 10. M, myosin heavy chain; αA, α-actinin; A, actin; Ti, Troponin-I; 30 K, 30 kDa component; f, dye front.

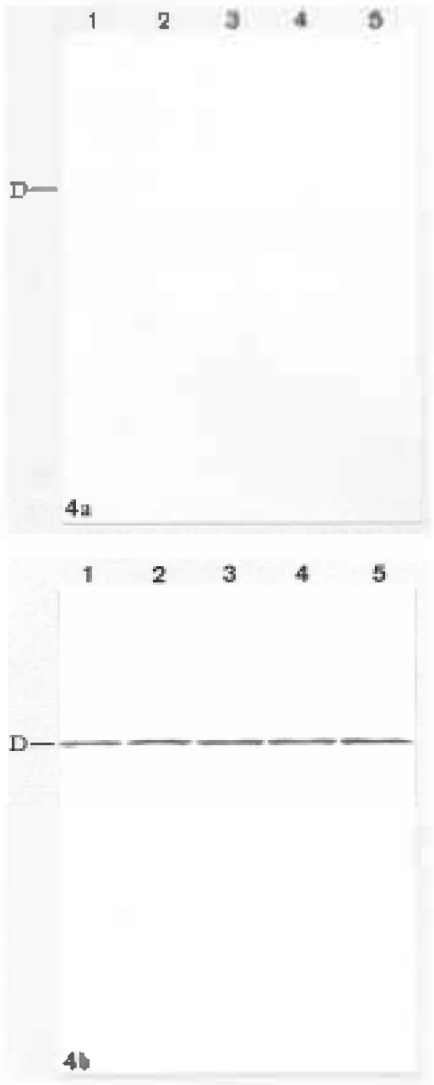

Figure 4. Western blots prepared from 12% gels of (a) breast muscle and (b) leg and thigh muscle samples were labeled with desmin monoclonal antibody. 0-day=lanes 1; 1 day=lane 2; 3 day=lane 3; 7 day=lane 4; 14 day=lane 5.

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