Effect of lactic or acetic acid on degradation of myofibrillar proteins in post-mortem goose (*Anser anser*) breast muscle

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Abstract: The effects of lactic acid (LA) and acetic acid (AA) on changes in myofibrillar proteins of post-mortem goose breast muscle marinated for 24 h at 5 °C were studied. Purified myofibrils were prepared from 0.1 M LA or AA samples and controls (non-marinated samples) after 0, 1, 3, 7 or 14 days of storage at 5 °C. The changes in myofibrillar proteins of goose muscle were examined by SDS-PAGE. Goose breast muscle marinated in LA and AA exhibited degradation of myosin heavy chains. The appearance of ~95 and ~27 kDa components and the disappearance of titin and nebulin were also more rapid than for control muscle. These results suggest that acid marination enhanced the post-mortem proteolysis of goose breast muscle.

Keywords: acid marination; post-mortem proteolysis; myofibrillar proteins; goose muscle

INTRODUCTION

Goose meat is a popular poultry red meat source in local markets; however, it is generally tougher than broiler meat. As a tenderising agent, organic acid can decrease the shear force of connective tissue[1] and improve meat tenderness[2] in bovine muscle. However, little information is available regarding the effects of acid marination on goose muscle. The purpose of this study, therefore, is to examine the effect of 0.1 M lactic or acetic acid on changes in myofibrillar proteins of post-mortem goose breast muscle marinated for 24 h at 5 °C.

MATERIALS AND METHODS

White Roman geese (*Anser anser*; ~100 days old with an average live weight of 4.4 kg) were obtained and slaughtered in the poultry farm of the National Chia-Yi Institute of Technology. Both pectoralis major muscles (~$10 \times 6 \times 2\,cm^3$) were excised from each bird 6–8 h post-mortem. One pectoralis major muscle was immediately marinated in 1 l of 0.1 M acetic or lactic acid solution at 5 °C for 24 h. After marination, whole muscle samples were quickly rinsed twice with doubly distilled water to remove excess acid on the muscle surface, then blot-dried with filter papers. Muscle specimens were subsequently vacuum-packed and kept at 5 °C for 0, 1, 3, 7 or 14 days of storage. This experiment was done in three replicates. Thirty geese were randomly selected per replicate, with duplicate samples for each time period. Marinated and control (non-marinated) breast muscles from two individual geese were randomly selected per time period. The samples were then finely ground, evenly mixed and divided into equal parts for pH measurement and for myofibril purification. This sample preparation excludes bias of sampling and the potential effect caused by the pH gradient probably occurring in muscle specimens during acid marination.

The pH of samples after 24 h acid marination and after 14 days of storage at 5 °C (final pH) was measured by the method of Farouk and Swan.[3] Breast myofibrils were purified via the method of Goll *et al.*[4] The myofibril samples for SDS-PAGE (sodium dodecyl sulphate polyacrylamide electrophoresis) were prepared by the method of Wang *et al.*[5] The SDS-PAGE was performed on $18\,g\,kg^{-1}$ polyacrylamide gels (weight ratio of acrylamide to methylenebisacrylamide was 100:1) according to the method of Laemmli[6] and Ho *et al.*[7] The same amount of protein (100 μg) from each samples was loaded on gels. The electrophoresis of titin and nebulin was performed on $8\,g\,kg^{-1}$ polyacrylamide gels (weight ratio of acrylamide to methylenebisacrylamide was 200:1) by the method of Wang *et al.*[5] The protein amount from each sample loaded on titin and nebulin gels was 120 μg.

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The protein concentration was determined using a modified biuret method.[8]

All gels were run with a current of 15 mA at room temperature, and SE 400 slab gel electrophoresis units (Hoefer Scientific Instrument, San Francisco, CA, USA) were 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 and 7.5% (v/v) acetic acid. Molecular weight markers ranging from 42.7 to 200 kDa (BDH Laboratory Supplies, UK) were used as protein standards. Data reported in this paper are representative results from three repeat experiments with duplicate samples in each storage period (total of 90 geese).

RESULTS AND DISCUSSION

The effects of 0.1 M lactic acid (LA) and acetic acid (AA) on post-mortem changes in goose breast muscle marinated for 24 h at 5 °C are examined. The SDS-PAGE results (Fig 1) show four major differences between acid-marinated samples and control (CON) samples. The most notable difference in proteins migrating below myosin heavy chains (MHCs) is that the MHC (molecular weight ~200 kDa) band see-

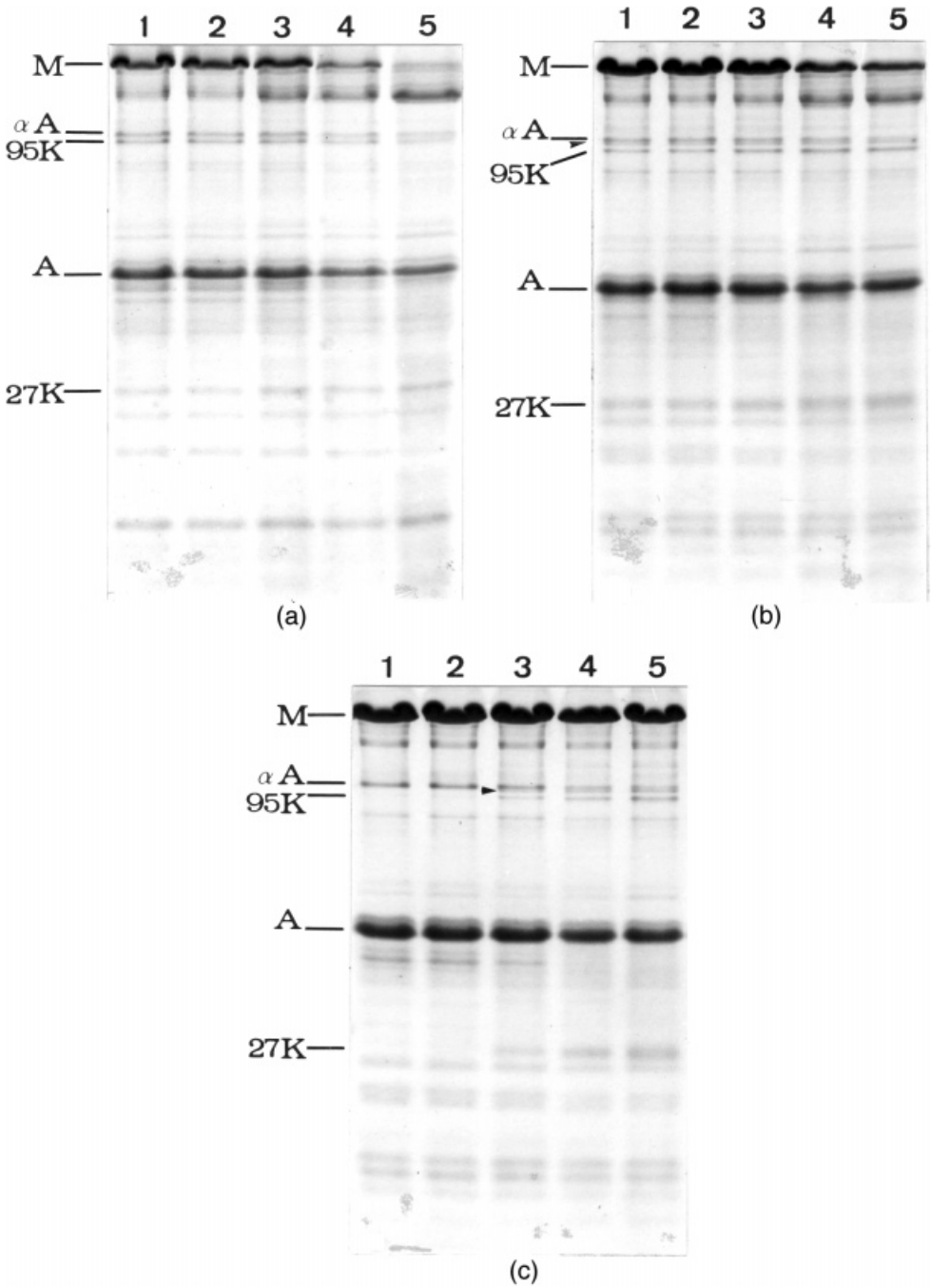

Figure 1. SDS gels showing changes in myofibrillar proteins of goose breast muscles marinated in 0.1 M lactic or acetic acid at 5 °C for 24 h: (a) lactic acid samples; (b) acetic acid samples; (c) control samples (0 days, lane 1; 1 day, lane 2; 3 days, lane 3; 7 days, lane 4; 14 days, lane 5). Abbreviations: M, myosin heavy chain; αA, α-actinin; A, actin; arrowhead, 98 kDa component.

mingly begins to decrease in 3 day LA samples and obviously reduces after 7 days (Fig 1(a)). In AA samples, only some degradation of MHCs is observed after 7 and 14 days (Fig 1(b)). MHCs, however, remain unchanged in CON samples (Fig 1(c)). These results may indicate that MHCs could be degraded in LA and AA samples. MHC degradation is also more extensive in LA samples than in AA samples. This difference could be related to the pH difference of samples after 24 h marination in LA (4.68 ± 0.07, $n = 6$) and in AA (5.25 ± 0.08, $n = 6$). After 14 days of storage at 5 °C, this pH difference remains between LA (4.65 ± 0.06, $n = 6$) and AA (5.28 ± 0.07, $n = 6$) samples.

It has been known for years that MHCs, one of the major contractile elements, do not change in bovine muscle during post-mortem aging at 5 °C.[9,10] Our SDS-PAGE results indicate, however, that MHCs are degraded in LA and AA samples (Fig 1). This is consistent with observations from duck muscle incubated in acid solution.[11] Previous studies[12] suggest that isolated myofibrils in acidic environments may result in protein degradation by cathepsins (lysosomal enzymes). It has also been shown that degradation of MHCs occurs after ovine myofibrils are incubated with lysosomal enzymes.[13] Moreover, both cathepsins B[14] and D[15,16] degrade MHCs.

Several review papers[17,18] indicate that calpains (calcium-dependent neutral proteases), non-lysosomal proteases, are critical in post-mortem proteolysis of muscles stored at 5 °C. It has been demonstrated that purified calpain from either bovine[19] or amphibian[20] skeletal muscle degrades MHCs in purified myosin molecules, but not those in myosin thick filaments. This result could be explained by the difference in the conformation of myosin molecules *in situ* and in the purified form.[20] The immunoblotting results of Bandman and Zdanis[10] have also confirmed that MHCs do not degrade in post-mortem bovine muscle during a prolonged aging period (4 weeks) at 5 °C. In addition, the activity of calpains would be limited in an acidic environment.

Another non-lysosomal protease system, proteasome (multicatalytic proteinase complex), can degrade MHCs in purified rabbit myofibrils with the addition of SDS as an activator; however, in the absence of SDS no specific effect on the degradation of MHCs was observed.[21] Previous results from Koohmaraie[22] have also shown that proteasome is not capable of degrading MHCs in post-mortem ovine muscle.

Collectively, these results imply that cathepsins (lysosomal proteases) play a major role in degrading myofibrillar proteins of goose breast muscle marinated in LA or AA. Apart from the possible action of cathepsins, however, our results cannot exclude the possibility that an acidic environment may also cause the changes in myofibrillar proteins. In the presence of acid the ionic strength of post-mortem muscle may increase. This increase destabilises the myofibrils[23] and may influence the breakdown of myofibrillar proteins. On the other hand, acid marination (below pH 5) not only produces rapid swelling of bovine myofibrils, but also affects the extraction of myosin.[24] This phenomenon can be noticed by comparing control samples (Figs 1(c) and 2(c)) and acid-marinated samples (Figs 1(a), 1(b), 2(a) and 2(b)). More experimental evidence is therefore required to understand the precise effect of acid marination on post-mortem muscle.

Secondly, the α-actinin band in CON samples begins to migrate as a doublet (arrowhead pointing to the lower band of the doublet) at day 3 and becomes more prominent after 7 days (Fig 1(c)). The appearance of this α-actinin doublet has been observed consistently in chicken[25] and duck[26] breast muscle during the late stages of aging. On the other hand, this α-actinin doublet appears earlier in AA samples at day 0 (Fig 1(b)). This could indicate that AA marination accelerates the appearance of the α-actinin doublet. The doublet, however, is absent in LA samples (Fig 1(a)), confirming the results of Chou *et al.*[11] The difference in the appearance of the α-actinin doublet could be due to the difference in pH between AA and LA samples. LA samples with lower pH may have more extensive proteolysis or a different mechanism from AA samples.[11]

Thirdly, a ~95 kDa degradation component appears at day 0 in both LA and AA samples (Figs 1(a) and 1(b)). However, it does not appear until day 3 in CON samples (Fig 1(c)). This indicates that LA and AA marination could accelerate the appearance of the ~95 kDa component. In addition, this ~95 kDa component increases gradually in both AA and CON samples, but decreases slightly in LA samples. This further implies that the proteolysis in LA samples with lower pH could be more extensive than that in CON and AA samples. This 95 kDa polypeptide was first identified in post-mortem bovine muscles by Koohmaraie *et al*[27] and consistently noted in animal muscles from various species afterwards.[26,28,29] The origin of the 95 kDa polypeptide remains unclear, though Koohmaraie *et al*[27] have suggested that it may originate from any of the myofibrillar proteins which have subunit molecular weights larger than 95 kDa.

Fourthly, a ~27 kDa polypeptide is observed earlier in LA and AA samples (at day 0) than in CON samples (at day 3). This indicates that LA and AA marination could accelerate the appearance of the ~27 kDa component. Additionally, the ~27 kDa polypeptide accumulates gradually in all samples. The appearance of a ~30 kDa component in aged muscle has been reported to be typical in animal muscles from many species.[7,25,26] The accumulation of this component correlates with an improvement in post-mortem meat tenderness.[30] However, the appearance of the ~30 kDa component may be simply considered as an indicator of post-mortem proteolysis rather than an indicator of tenderness.[31] Our results therefore indicate that marination of goose muscle in LA and AA may enhance the degradation of myofibrillar proteins

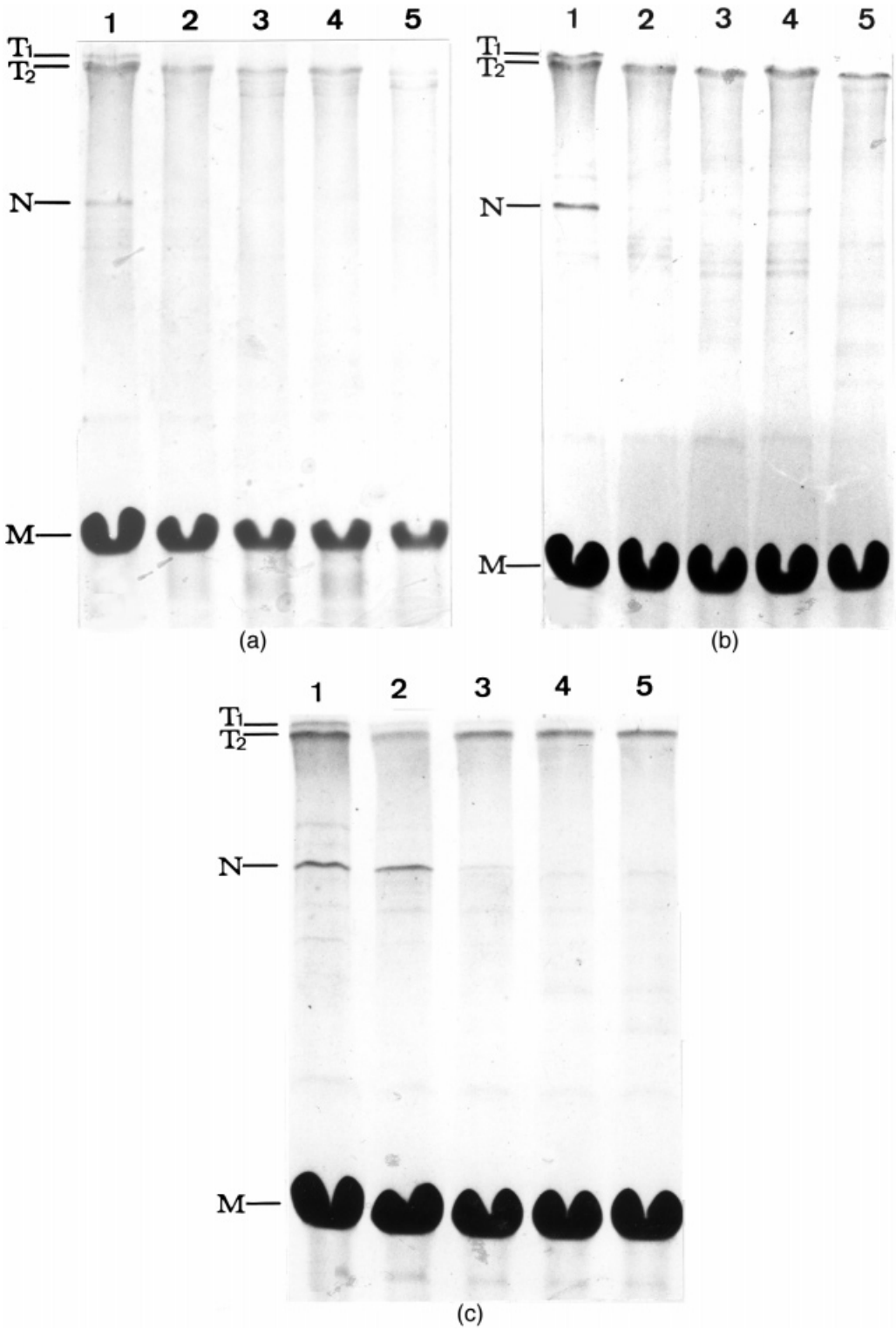

Figure 2. SDS gels showing changes in titin and nebulin of goose breast muscles marinated in 0.1 M lactic or acetic acid at 5 °C for 24 h: (a) lactic acid samples; (b) acetic acid samples; (c) control samples (0 days, lane 1; 1 day, lane 2; 3 days, lane 3; 7 days, lane 4; 14 days, lane 5). Abbreviations: T1, titin 1; T2, titin 2; N, nebulin; M, myosin heavy chain.

and result in the early appearance of the ~30 kDa component.

Based on our SDS-PAGE analysis, the migration of titin and nebulin reveals that the 0 day CON sample (lane 1 in Fig 2(c)) compares favourably with the typical electrophoretic patterns described by Wang *et al.* [32] Titin migrates near the top of the gels as a closely spaced protein doublet (T1 and T2), and nebulin is clearly visible below the titin doublet. Our results indicate that the disappearance of the T1 (intact titin) band occurs most rapidly in LA samples and slowest in CON samples. The T1 band in LA samples (Fig 2(a)) is visible at day 0 and disappears by day 1. In AA samples (Fig 2(b)), on the other hand, the T1 band

appears at day 0 and disappears by day 3. The T1 band in CON samples, however, is visible at day 0, faintly visible by days 1 and 3, barely noticeable by day 7 and absent by day 14 (Fig 2(c)). Moreover, the T2 band (a major degradation product of titin) in LA samples becomes faintly coloured at day 14, but it remains visible in AA and CON samples throughout the 14 days of storage.

A similar result is found in the rapid disappearance of the nebulin band in LA and AA samples (Fig 2). The nebulin band is clearly visible in CON samples at days 0 and 1, very faintly visible by day 3 and absent after day 7 (Fig. 2(c)). In AA samples, on the other hand, the nebulin band is visible at day 0, faintly coloured by day 1 and absent by day 3 (Fig 2(b)). The nebulin band in LA samples (Fig 2(a)), however, is only visible at day 0.

Titin and nebulin are two huge proteins in striated muscles.[33] Titin comprises a set of elastic filaments that maintain thick filaments in register during muscle contraction.[33] Nebulin, on the other hand, is considered as a candidate for the length-regulating template of thin filaments in skeletal muscle.[34,35] These two proteins in bovine muscle are very susceptible to post-mortem degradation[36] and calpain[37] and cathepsin[16] hydrolysis. Current studies have indicated that both LA and AA marination of post-mortem goose breast muscle may speed up the disappearance of titin and nebulin. Similar to the degradation of MHCs (Fig 1), titin and nebulin are degraded more extensively in LA than in AA samples. This difference may be due to the lower pH found in LA samples. The rapid degradation of titin and nebulin may alter the integrity of muscle cells[38] and decrease the myofibrillar strength.[33]

In summary, goose breast muscle marinated in 0.1 M LA and AA for 24 h at 5 °C exhibits degradation of MHCs. The appearance of ~95 and ~27 kDa components and the disappearance of titin and nebulin are also faster than in control muscle. These results suggest that 0.1 M LA and AA marination may enhance the post-mortem proteolysis of goose breast muscle.

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