ANALYSIS AND COMPARISON OF CALPAIN IN MUTTON WITH DIFFERENT LEVELS OF TENDERNESS

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Abstract – The objective of this study was to investigate the activation process and the autolysis rate of Calpain-1 during postmortem in mutton with different levels of tenderness in order to provide theoretical basis for regulating tenderness through calpain activity. The *longissimus lumborum* muscles from Ujimqin Fat Tailed sheep were divided into tender group and tough group based on myofibrillar fragmentation index. The existential state of Calpain-1 large subunit and the content of native Calpain-1 and Calpain-2 during post-mortem were analyzed. The muscle samples in tender group had a higher initial Calpain-1 content compared with those in tough group. The autolysis and degradation rate of Calpain-1 was faster in tender group than in tough group, leading to higher autolysis and degradation degree in tender group. The results indicated that meat tenderness during postmortem was affected by Calpain-1 expression quantity and autolysis. In addition, changes of Calpain-1 during 24 h postmortem, especially 12 h, had the most impact on mutton tenderness.

Key Words – mutton, muscle, Calpain-1, aging

I. INTRODUCTION

Tenderness is one of the most significant characters of meat. According to presented studies, enzyme of the Calpain system, especially Calpain-1, plays a role in muscle aging in which period main tenderization occurs. Taylor *et al.* [1] found that Calpain-1 was activated in early postmortem phase while a number of vital myofibrillar proteins were degraded at the same time. Liu *et al.* [2] demonstrated positive relationship between tenderness and activation level of calpains. There were two subunits in Calpain-1 with molecular weights of 80 kDa and 28 kDa respectively. Autolysis was taken place when Calpain-1 hydrolyzed other proteins after 80 kDa subunit degraded. Autolysis was a significant reason for the activation of calpain and the degradation of myofibrillar proteins [3]. The time calpains autolyzed was positive related to the degradation of desmin and connectin [4]. Research about the relationship between Calpain-1 and tenderness mostly focused on the correlation indirectly or studied in vitro. However, whether the changes of activity and autolysis of Calpain-1 is consistent in muscle with different tenderness remains unknown. So, this study illustrated this problem by investigating the variance of calpains in mutton with different tenderness.

II. MATERIALS AND METHODS

2.1 Sample preparation

Fifty Ujimqin sheep were involved in this study. The *longissimus lumborum* (LL) muscles from both sides of the carcass were collected and taken to refrigeration room immediately after slaughtered. Connective tissue and fat on the surface were removed within 15 min. Muscles were collected at 30 min, 1 h, 2 h, 6 h, 12 h, 24 h, 48 h, 72 h, 5 d and 7 d postmortem. The samples were quick-frozen and stored at -80°C.

2.2 Mymofibril fragmentation index (MFI)

The MFI was determined using samples taken at 24 h and 48 h postmortem as described by Culler et al. [5]. Protein concentration of the suspension of myofibrils was determined by the biuret method. The absorbance was measured at 540 nm using a UV-visible spectrophotometer (T6 New Century, Beijing, China), and then multiplied by 200 to obtain the MFI value.

2.3 Degradation of Calpian-1

The muscle samples for SDS-PAGE were prepared by the method of Veiseth et al. [6]. An 8% polyacrylamide separating gel and 5% polyacrylamide stacking gel was used to detect Calpain-1 autolysis. Gels were loaded with 40μg protein per lane. Western blotting was done as described by Lomiwes et al. [7]. Primary antibody was anti-Calpain-1 (Sigma, C0355;

diluted 1:1000) and secondary antibody was goat anti-mouse IgG (H+L) HRP (Sigma, A9044; diluted 1:5000). Secondary antibody bound to the membrane was detected with a Clarity Western ECL Substrate kit (Bio-Rad, 1705061).

2.4 Detection of unautolyzed Calpian-1 and Calpain 2

The Calpain activities were determined by casein zymography using the methods of Veiseth et al. [6]. The relative activities of Calpains were expressed as volume of band which was analyzed by Totallab Quant (version 12.5, Nature Gene Corp., USA).

2.5 Statistical analysis

All statistical analyses were performed using IBM SPSS 17.0. An analysis of variance (one-way ANOVA by Duncan) was used to assess the differences between the treatments. Reported *P*-values were evaluated at a 5% significance level.

III. RESULTS AND DISCUSSION

3.1 Meat tenderness evaluation

There were four samples in the tough group and tender group, respectively. Tender meat was characterized by significantly higher MFI ($P < 0.05$) at 24 h and 48 h postmortem compared to tough meat (Table 1). It was reported that MFI is an appropriate indicator of the extent of myofibrillar protein degradation during postmortem [8], could also reflect the weakening of myofibril linkages and the degradation of key structural proteins in the I-band of the myofibril. MFI positively correlates with meat tenderness. So the MFI showed in Table1 indicated that there was a significant difference of tenderness between the two groups.

Table 1 Results of the analysis of MFI

Postmortem time	Tender group (Average ± SD)	Tough Group (Average ± SD)
24 h	58.38 ± 2.73a	30.08 ± 5.32b
48 h	62.56 ± 3.00a	44.54 ± 8.71b

3.2 Differences of Calpain-1 and Calpain-2 between tender and tough group

The 80 kDa subunit of Calpain-1 degraded gradually during postmortem (Fig.1 and 2). As Calpain-1 was activated and exerted its activity, the 80 kDa subunit degraded to 78 kDa and finally degraded to 76 kDa in the 7 days of post mortem. The content of 80 kDa subunit of Calpain-1 in tender group was significantly higher than tough group ($P < 0.05$) at 30 min postmortem. Calpain-1 in tender group started degrading at 6 h postmortem, while Calpain-1 in tough group started degrading at 2 h but remain unchanged during 2-24 h. The 80 kDa subunit of Calpain-1 in tender group degraded completely at 48 h postmortem, while tough group did not finish this process until 48 h. The degradation of Calpain-1 in mutton mainly happened at 2-48 h postmortem.

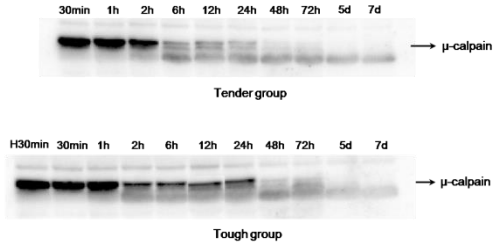

Figure. 1 Western blots profiles showing the state of Calpain-1 at post-mortem in tender/tough groups H30min represents the muscle sample at 30min postmortem in tender group. The same as below

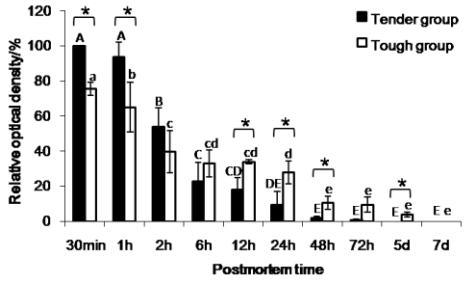

Figure. 2 Changes of 80 kDa large subunit of Calpain-1 at post-mortem in tender/tough groups * shows significant difference in the results between tender group and tough group at the same time of post-mortem ($P<0.05$). The same as below.

Research has shown that earlier autolysis of Calpain-1 was associated with earlier activation of Calpain-1 and protein degradation at postmortem [3]. Meanwhile, most protein degraded and the tenderization process was caused by the activation of Calpain-1. The faster the Calpain-1 autolyzed, the earlier Calpain-1

was activated and the faster it lost activity. Brad Kim et al. [9] observed that autolysis and activation level of Calpain-1 was related to proteolysis. The greater the degree of Calpain-1 autolysis, the greater the degree of myofibrillar protein degradation. Cruzen et al. [10] found that muscle with lower proteolysis degree had a higher existence of integrated 80 kDa subunit of Calpain-1 always showed poor tenderness. Our research determined the relationship between the activation degree or activity of Calpain-1 and mutton tenderness by contrasting the changes of Calpain-1 80 kDa subunit in tender group with tough group. The results demonstrated that Calpain-1 in tender group had a higher primary 80 kDa subunit content and a faster degraded speed than tough group. Calpain-1 in tender group had apparently 78 and 76 kDa degraded band at 6 h postmortem and appeared to show significantly lower content of 80 kDa subunit at 12 h postmortem, which illustrated that both activation degree and activity of Calpain-1 was positively related to meat tenderness.

The detection of Calpain-1 and Calpain-2 in muscle samples by casein zymography used 4 mmol·L^{-1} Ca^{2+} when incubating. Under this Ca^{2+} concentration, all of Calpain-1 and Calpain-2 in gels can be activated and degrade casein at their band position. Thus, in our experiments, the content of detected Calpain-1 and Calpain-2 remained unautolyzed state or had not played a role in proteolysis yet. Based on the results, the content of unautolyzed Calpain-1 in both groups was gradually decreased during postmortem (Fig.3 and 4). The unautolyzed Calpain-1 in tender group decreased faster before 12 h postmortem but changed slowly later, while the content of unautolyzed Calpain-1 in tough group was still high at 12 h postmortem. The caseinolytic activity of Calpain-2 in both groups did not change obviously. The Calpain-2 did not autolyze in 7 days postmortem. The results showed that the tender group had higher primary content of unautolyzed Calpain-1, but the Calpain-1 autolyzed and lost its activity fast resulted in a more powerful proteolytic ability. The Calpain-1 mainly played a role in tenderization at 2-24 h postmortem via the gradually autolysis.

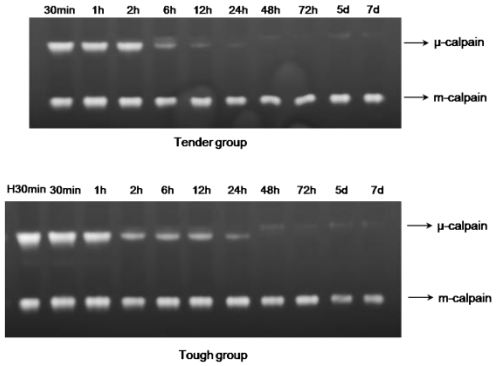

Figure. 3 Casein zymography profiles presenting the Calpain-1 and Calpain-2 at post-mortem in tender and tough groups

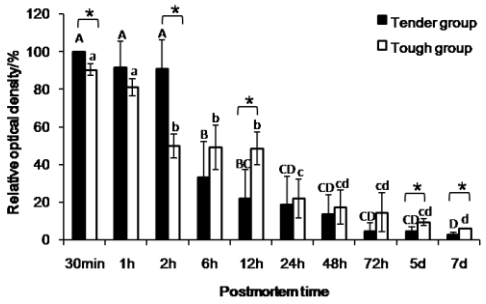

Figure. 4 Changes of the content of native Calpain-1 at post-mortem in tender/tough groups

It was reported that Calpain-1 was activated because of the autolysis, could mainly function for meat tenderization in the first three days after slaughter. After Calpain-1 exhibiting the proteolytic ability, it would lose the activity. However, the concentration of Ca^{2+} in muscle tissues at postmortem could not meet the need to activating Calpain-2, so Calpain-2 may did not work in this process [11]. O'Halloran et al. [12] documented the tender muscle had a lower activity of Calpain-1 at 3 h postmortem. In that case, the degradation degree of skeletal muscle protein was greater. So muscle tenderness was better when Calpain-1 in it has a quickly autolysis speed. As shown in Fig.3 and Fig.4, Calpain-1 autolyzed faster in tender group, which was consistent with the results of western blotting part. The activity of Calpain-2 did not change in both groups at 7 d postmortem, which indicated that Calpain-2 did not work on tenderness in this research.

IV. CONCLUSION

Tender group had higher primary 80 kDa subunit content of Calpain-1 with a faster degradation speed. At 6 h and 12 h postmortem, the differences of Calpain-1 between two groups were most significant. This study confirmed the differences of the existence and activation degree of calpain between mutton with different tenderness. The changes of activity and autolysis of Calpain-1 can reflect the ability of Calpain-1 on tenderization in the same way at postmortem. Calpain-1 had a greater influence on mutton tenderness in 24 h postmortem.

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REFERENCES

[1] Taylor R G, Geesink G H, Thompson V F, Koohmaraie M, Goll D E. (1995). Is Z-disk degradation responsible for postmortem tenderization? Journal of Animal Science 73(5): 1351-1367.

[2] Liu J, Ruusunen M, Puolanne E, Ertbjerg P. (2014). Effect of pre-rigor temperature incubation on sarcoplasmic protein solubility, calpain activity and meat properties in porcine muscle. LWT-Food Science and Technology 55(2): 483-489.

[3] Melody J L, Lonergan S M, Rowe L J, Huiatt T W, Mayes M S, Huff-Lonergan E. (2004). Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. Journal of Animal Science 82(4): 1195-1205.

[4] Zhang W G, Lonergan S M, Gardner M A, Huff-Lonergan E. (2006). Contribution of postmortem changes of integrin, desmin and l-calpain to variation in water holding capacity of pork. Meat Science 74(3): 578-585.

[5] Culler R A, Parrish F C, Smith G C, Cross H R. (1978). Relationship of MFI to certain chemical, physical, and sensory characteristics of bovine LD muscle. Journal of Food Science 43: 1177-1180.

[6] Veiseth E, Shackelford S D, Wheeler T L, Koohmaraie M. (2001). Effect of postmortem storage on mu-calpain and m-calpain in ovine skeletal muscle. Journal of Animal Science 79(6): 1502-1508.

[7] Lomiwes D, Farouk M M, Wu G, Young O A. (2014). The development of meat tenderness is likely to be compartmentalized by ultimate pH. Meat Science 96(1): 646-651.

[8] Li K, Zhang Y, Mao Y, Cornforth D, Dong P, Wang R, Zhu H, Luo X. (2012). Effect of very fast chilling and ageing time on ultra-structure andmeat quality characteristics of Chinese yellow cattle M. longissimus lumborum. Meat Science 92(4): 795-804.

[9] Brad Kim Y H, Luc G, Rosenvold K. (2013). Pre rigor processing, ageing and freezing on tenderness and colour stability of lamb loins. Meat Science 95(2): 412-418.

[10] Cruzen S M, Pedro V R P, Lonergan S M, Huff-Lonergan E. (2014). Postmortem proteolysis in three muscles from growing and mature beef cattle. Meat Science 96 (2): 854-861.

[11] Koohmaraie M, Geesink G H. (2006) Contribution of postmortem muscle biochemistry to the delivery of consistent meat quality with particular focus on the calpain system. Meat Science 74(1): 34-43.

[12] O'Halloran G R, Troy D R, Buckley D J, Reville W J. (1997). The role of endogenous proteases in the tenderization of fast glycolysing muscle. Meat Science 47(3):187-200.