CALPAIN ACTIVITY AND PROTEOLYTIC EFFECTS ON *LONGISSIMUS LUMBORUM* MUSCLE FROM HANWOO COWS AND HEIFERS

D.E. Aguayo[1], D. Dashdorj[1,2], M.N. Uddin[1], Ch. Ochirbat[1], J.S. Lee[1], Duhak Yoon[3] and I.H. Hwang[1*]

[1]Department of Animal Science, Chonbuk National University, Republic of Korea

[2]Department of Livestock Production, Mongolian University of Life Sciences, Ulaanbaatar, Mongolia

[3] Department of Animal Science, Kyungpook National University, Daegu 41566, Republic of Korea

[*]Corresponding author email: inho.hwang@jbnu.ac.kr

Abstract- Calpain activity has been categorized as the most important factor causing proteolysis on structural muscle proteins. Casein Zymogramy is a good technique to study calpain activity with no need to separate calpain and calpastatin from meat samples. Enzymatic activity between cows and heifers did not differ significantly between the two age groups during aging. Decline activities as normally expected were obtained from μ-calpain (P<0.001) and m-calpain (P<0.05). Regarding to μ-calpain, several events mediated by its activity (autolysis) were evident allowing to measure μ-calpain activity through its products of degradation. Meat from cow and heifer animals significantly differed (P<0.001) due to aging effects and at 14 d cow's meat samples were tougher (P<0.01) than heifers' samples. Lightness and yellowness of meat significantly increased (P<0.01), as well as cooking loss percent (P<0.01). The degradation of troponin-t bands (37 kDa) also suggested robust μ-calpain activity on myofibrillar muscle proteins along the storage period.

Key words; Calpains, Hanwoo beef, tenderness

I. INTRODUCTION

Tenderness has become a complex but at the same time an interesting meat quality trait to study. Today, among the enzymatic systems present in the muscle cell the calcium-dependent protease system is the main responsible in producing meat tenderization [1]. The softening of the muscle has a multifactorial origin involving not only proteolysis but also amounts of connective tissue and sarcomere lengths [2]. Most of this tenderization is believed to rely on the breakdown of myofibril structures in muscle cells during postmortem aging [3]. The degradation of muscle proteins and the disruption of the skeletal muscle architecture influences postmortem tenderization [4]. Both μ and m-calpain are active at micromolar and milimolar levels of Ca^{2+} [2]. These two proteases combined are responsible for the 85% of meat postmortem tenderization [5]. Studies done up today strongly suggest that the calpains cleave key skeletal muscle proteins such us desmin, titin and troponin-t [6]. As a result, considerable rate of breakdown is generated in the muscle fiber enhancing meat tenderness [4]. In addition to this, most of the variability of tenderness in LD muscle is attributed to the rate of proteolysis caused by the calpains [7]. Therefore, the aim of this study is to analyze postmortem changes on muscle proteins (troponin-t) and meat quality traits governed by μ and m-calpain in beef from Hanwoo Cows and Heifers at different chiller conditions.

II. MATERIALS AND METHODS

Longissimus lumborum muscles from a total of 20 animals between Hanwoo cows and heifers were separated, proceesed, and aged at 3 and 14 d (4°C).

- Meat color: Minolta Spectrophotometer CM-2500 d with D65 illuminat and 10° observers
- pH and Warner-Bratzler Shear Force: Seven compact, Mettler Toledo and Instron Universal Testing Machine ø 0.5inch, 400 mm/min, and 40 kgf.
- Casein Zymography: calpain activity was investigated according to the procedure of Raser [7].
- SDS-PAGE: muscle proteins were extracted and run by following the protocol of Laemmli [8]
- Statistical analysis: SAS version 9.4 (Statistical Analysis System)

III. RESULTS AND DISCUSSION

Evidence of self-proteolysis was detected indicating earlier μ-calpain activation. At 14 d of conditioning, two upper white bands

belonging to native and autolyzed calpain suggests remaining μ-calpain activity (Fig. 1). The calpain active isoform (78 kDa) decreased significantly (P<0.001) from 3 d to 14 d in both age groups (Table 1). Cows and Heifers did not differ statistically in their μ-calpain activity. M-calpain activity showed weak significant difference in calpain activity between the two aging times (P<0.05) indicating a decreased activity along the storage time. And there was no statistic difference between cows and heifers.

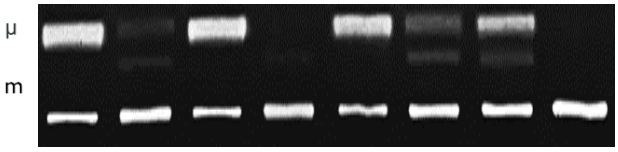

Fig. 1 Clear zones appearing at the top of the gel inducate u-calpain activity and at the bottom of the gel m-calpain activity at different aging times.

Table 1. Relative calpain activity from *LL* muscle by using Casein Zymography (% of band density)

Age Group	μ-Calpain				m-Calpain	
	78 kDa		76 kDa			
	3d	14d	3d	14d	3d	14d
Cow	12.4^{aX}	4.0^{aY}	2.7^{aY}	6.5^{aX}	13.4^{aX}	12.3^{aY}
Heifer	11.8^{aX}	3.8^{aY}	3.5^{aY}	5.3^{aX}	12.8^{aX}	11.5^{aY}
SEM	0.43	0.43	0.45	0.45	0.36	0.36
F-value						
Aging df 1/39	178.06***		19.10***		5.61*	
Age df 1/19	0.31	0.06	1.62	1.17	0.63	1.69

^{X,Y} means within each column with different superscripts in grouping animal sections are significantly different.
df, degrees of freedom; *** P<0.001, ** P<0.01, * P<0.05.

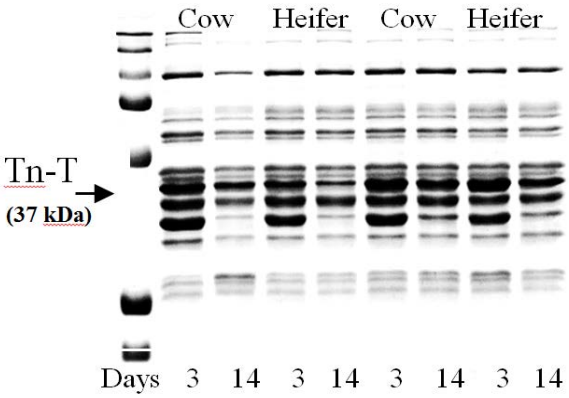

Fig 2. SDS-PAGE degradation of proteins

CONCLUSION

The μ-calpain showed activity as early as the 3rd day, and at 14 d autolyzed μ-calpain products were detected indicating loss of enzymatic activity; m-calpain appeared at the aging stages. Intense degradation of Tn-T by the end of conditioning indicated considerable postmortem changes contributing to meat tenderization. Heifer tenderness scores were significantly lower than the cows' values by the end of the storage period.

ACKNOWLEDGEMENTS

It should be acknowledged that this work was carried out with the support of Next-Generation BioGreen 21 Program (Project PJ011102), RDA, Republic of Korea.

REFERENCES

1. Nowak, D. (2011). Enzymes in Tenderization of Meat - The System of Calpains and Other Systems-a Review. Polish J. Food and Nutr. Sci. 61 (4): 231-237.
2. Koohmaraie, M. (1996). Biochemical factors regulating the toughening and tenderization processes of meat. Meat Science 43: S193-S201.
3. Rhee, M. S., Wheeler, T. L., Shackelford, S. D., & Koohmaraie, M. (2004). Variation in palatability and biochemical traits within and among eleven beef muscles. Journal of Animal Science 82: 534-550.
4. Huff-Lonergan, E., Zhang, W., & Lonergan, S., M. (2010). Biochemistry of postmortem muscle . Meat Science 86: 184-195.
5. Bowker B. C., Eastridge, J. S., Paroczay, E. W., Callahan, J. A., & Solomon, M. B. (2010). Aging/tenderization Mechanisms. Handbook of meat processing 1st Ed (pp87-99). Blackwell Press.
6. Takahashi, K. (1999). Mechanism of meat tenderization during post- mortem ageing: Calcium theory. Proceedings of the 45th International Congress of Meat Science and Technology, Yokohama, Japan. pp 230-235.
7. Veiseth, E., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2001). Effect of postmortem storage on μ-calpain and m-calpain in ovine skeletal muscle. Journal of Animal Science, 79, 1502-1508.
8. Raser, K. Posner, J & Wang, K. K. (1995). Casein zymography: a method to study mu- calpain, m-calpain, and their inhibitory agents. Archives of Biochemistry and Biophysics.
9. Laemmli U., K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.