CALPAIN PROTEOLYTIC EFFECTS IN DIFFERENT MUSCLE TYPES FROM HANWOO CATTLE AND THEIR RELASHIONSHIP WITH MEAT SOFTENING TRAITS

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Abstract-The process of tenderization is mostly attributed to the calcium-dependent proteases. *TB, ST, DP* and presented μ-calpain activity by 21 d, m-calpain did not present self-proteolysis either among muscle types or aging times. Activity of μ-calpain (78 kDa) found in *LT, LL, SS, BF, ST, DP, TB* differed significantly (P<0.001) from *SM, GM,* and *PM* muscles. These values were not fully in accordance with WBSF values (r=0.20; P<0.05). Calpain activity had a similar tendency with tensile strength (r=0.31; P<0.001), springiness (r=0.28; P<0.001) but different with TPA deformation (r=-0.21; P<0.05). Troponin-t degradation was markedly visible in muscles like *PM, LT* or *SM* (21d). Results suggest that the development of tendering traits is not only influenced by calpain activity and that more studies related to proteolysis are needed.

Key words; Calpains, Hanwoo beef, muscle types

I. INTRODUCTION

The beef-producing industry has identified the variability of meat tenderness as the main problem related to meat quality [1]. Factors such as animal age, slaughter conditions and muscle type can affect tenderness [2]. Indeed, beef tenderness variation among different muscle types has been found even within the same animal [3; 4]. It is known that differences in the rate of structural muscle proteins via proteolysis contribute to obtain less or more ultimate tenderness [5]. A plenty of research about beef proteolysis has been done, particularly in LD muscle, however it is little known about these proteolytic events in different muscle types during aging [6]. However, the calpain system, especially μ-calpain, plays the most important role causing postmortem tenderization through myofibrilar breakdown [7]. Therefore, the aim of this experiment was to investigate the relationship of calpain activity from ten muscle types and meat softening traits of Hanwoo steers at two different chiller times.

II. MATERIALS AND METHODS

Ten different muscle types from a total of 15 Hanwoo steers (25 to 28 months) were used: Psoas major (*PM*), Longissimus thoracis (*LT*), Longissimus lumborum (*LL*), Gluteus medius (*GM*), Semimembranosus (*SM*), Semitendinosus (*ST*), Biceps femoris (*BF*), Triceps brachii (*TB*), Supraspinatus (*SS*) and Diaphragm (*DP*). Ageing conditions were at 3 and 21 days at 4°C.

- pH and Warner-Bratzler Shear Force
- Casein Zymography, according to the procedure of Raser [8].
- SDS-PAGE by following the protocol of Laemmli [9]
- Statistical analysis: SAS version 9.4 (Statistical Analysis System)

III. RESULTS AND DISCUSSION

The activity of μ-calpain in *TB, ST* and *DP* muscles significantly (P<0.001) reduced by 21 d PM (Table1), while the rest of the muscle only presented enzymatic activity up to 3d (Fig 1). *SM, GM,* and *PM* had lower calpain activity (78 kDa) than the rest of the muscles (P<0.001). Contrary to μ-calpain, m-calpain presents no evidence of fragments caused by autolysis in each muscle and is present at both aging stages.

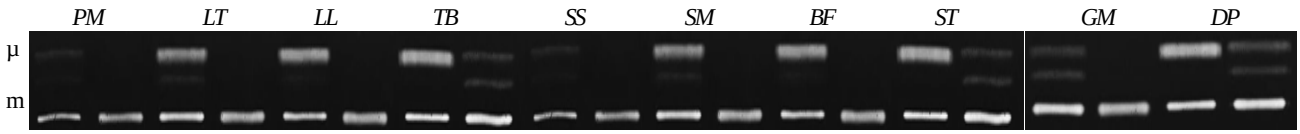


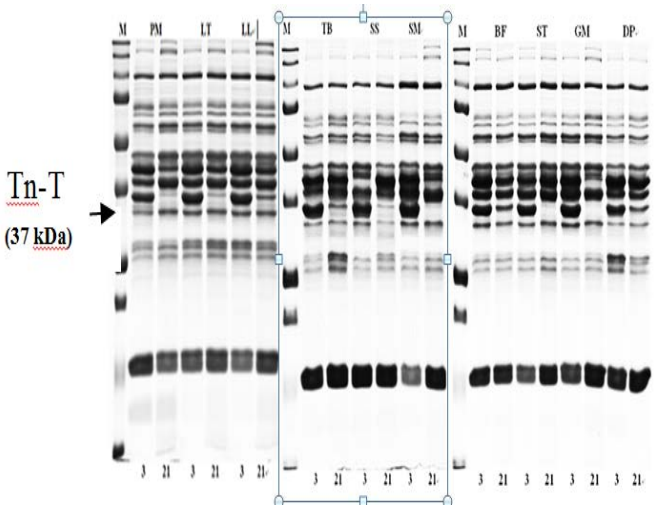
Fig 1. Calpain activity of ten different muscles from Hanwoo beef.

Table 1. Relative calpain activity from diferent Hanwoo musces (density, % of band area)

Muscles	μ-Calpain				m-Calpain	
	78 kDa		76 kDa			
	3d	21d	3d	21d	3d	21d
PM	9.4^{b}	.	6.5^{cde}	.	12.0^{cX}	10.7^{bY}
LT	14.6^{a}	.	7.6^{bcd}	.	13.5^{abX}	11.0^{abY}
LL	15.2^{a}	.	6.8^{cde}	.	14.2^{aX}	11.2^{abY}
GM	9.6^{b}	.	9.0^{bc}	.	13.6^{abX}	10.7^{bY}
SM	9.7^{b}	.	12.8^{a}	.	12.8^{bcX}	10.9^{abY}
SS	16.6^{aX}	7.4^{Y}	7.8^{bcY}	8.8^{X}	13.2^{abX}	10.6^{bY}
BF	17.5^{a}	.	8.2^{bc}	.	14.0^{abX}	11.0^{abY}
ST	14.6^{a}	.	9.3^{b}	.	13.2^{abX}	11.2^{abY}
DP	17.3^{aX}	10.6^{Y}	5.2^{deY}	8.6^{X}	13.8^{abX}	11.9^{abY}
TB	18.8^{aX}	9.8^{Y}	4.7^{eY}	6.9^{X}	14.1^{abX}	12.3^{aY}
SEM	1.3	1.16	0.82	1.1	0.40	0.44
			F-value			
Muscle df 9/149	7.4***	0.22	7.7***	1.2	2.7***	1.5
Aging df 1/29	15.5***		0.01		147.1***	

^{a,e}, means within each column with different superscripts in muscle type sections are significantly different.
^{X,Y,} means within each row with different superscripts in aging days sections are significantly different.;
df, degrees of freedom; *** P<0.001, ** P<0.01, * P<0.05.

Fig. 2 Representative SDS-PAGE of sarcoplasmic and myofibrillar proteins from different muscle types.



Complete absence of the 37 kDa troponin-t was observed in *PM*, *LT*, *SM*, *ST* and *GM* during the last stage of aging. However, reduced band intensity was observed in *LL*, *TB*, and *SS* while *BF* and *DP* showed slight protein degradation.

IV. CONCLUSION

At 3 d, calpains were detected in all muscles and only *TB*, *ST*, and *DP* showed residual μ-calpain activity by 21 d; m-calpain did not present signs of autolysis. Correlation data showed that calpain activity is not directly related to tenderness and textures scores. This suggests that other variables than the calpains can improve the development of these of these meat traits.

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