EFFECT OF CHILLER AGEING AND MUSCLE TYPE ON COLLAGEN CHARACTERISTCIS OF HANWOO BEEF

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Abstract – The study was conducted to investigate the collagen properties of 10 major beef muscles from 15 Hanwoo steer at 3 and 21 days of ageing. The muscles were *Psoas major* (PM, tenderloin), *Longissimus thoracis* (LT, ribeye), Longissimus lumborum (LL, *striploin*), *Gluteus medius* (GM, top-sirloin butt), *Semimembranosus* (SM, top inside round), *Semitendinosus* (ST, eye of round), *Biceps femoris* (BF, outside flat round), *Triceps brachii* (TB, chuck roll), *Supraspinatus* (SS, chuck tender), and *Diaphragm* (DP, outside skirt). The total collagen, insoluble collagen and collagen solubility differ significantly among the muscles during the ageing period. The SS, BF and TB muscle contain significantly higher (P<0.001) amount of total collagen than the PM muscle. The type I and III collagen content were significantly higher in BF muscle and lowest in PM muscle (P<0.001). The type III collagen content significantly decreased at 21 day ageing. The ratio of collagen type I and III significantly increased at 21 days ageing in PM muscle. No significant ageing effect was found for collagen type I, but collagen type III exhibit significant ageing effect. Therefore, the muscle effect on collagen properties used as a differential indicator of tenderness variation among muscle.

Key Words – Collagen types, Post mortem ageing, Tenderness.

I. INTRODUCTION

Tenderness of meat is determined by the characteristics of myofibril and the intramuscular collagen. Collagen, the major connective tissue protein, is an integral constituent of muscle, and mainly contributing in meat tenderness. Collagen is present in 19 different forms, each having a different role in biological systems [1]. The degree to which connective tissues affect tenderness is determined by the type and amount of collagen content. The amount of collagen present in muscle tissue is important in understanding the effect on meat quality parameters; however, the type of collagen is a more direct measure of the tenderness and consumer acceptability. Every single muscle can respond in a different manner in a degree to which their tenderness increase after postmortem ageing periods due to divergences in connective tissue [2]. Chiller ageing of meat is a practice in which meat toughness by rigor mortis is naturally tenderized. The mechanical integrity of myofibrils alterations during postmortem ageing, results in tenderness of aged beef [3]. In the course of ageing muscles undergo a sequential physical and biochemical alterations regarding the weakening of Z-disk and the myofibrillar proteins [4]. The structural weakening of the endomysium and perimysium is strictly associated with breakdown of proteoglycans, which connect with collagen fibrils and stabilize collagen fibers. The collagen fibrillar structure remains as it is up to ten (10) days post-mortem, but the advancement of ageing structural consistency changes noticeably observable after 14 days of post-mortem. The prolonged time ageing produced satisfactory tenderized meat [4]. Therefore, the objective of present study aimed to evaluate the effect of ageing and muscle types on collagen characteristics of Hanwoo beef.

II. MATERIALS AND METHODS

Muscle sample preparation. Ten muscles were collected from fifteen 25 -30 months age of Hanwoo steer. The animals were randomly selected. After slaughter, the right side of each carcass was hung by the tendon Achilles and cooled at 4º C. The following ten muscles were vacuum-packaged and stored 4º C for 3 and 21 days of ageing. The ten muscles include, *Psoas major* (PM, tenderloin), *Longissimus thoracis* (LT, ribeye), *Longissimus lumborum (LL,* striploin*), Gluteus medius* (GM, top-sirloin butt), *Semimembranosus* (SM, top inside round), *Semitendinosus* (ST, eye of round), *Biceps femoris* (BF, outside flat round), *Triceps brachii (TB, chuck roll), Supraspinatus* (SS*, chuck tender),* and *Diaphragm* (DP, outside skirt). The ageing muscle samples were used to estimate the collagen characteristics.

Estimation of total collagen, Heat soluble collagen and Insoluble collagen: The total collagen content present in different samples were determined after 16 h hydrolysis of 2 g of meat with 7 N H_2SO_4 at 105^0C using modified colorimetric method of Kolar [5]. Hydrolysate was diluted with 500 mL distilled water (3D) in Erlenmeyer flask. Diluted filtrate (2 mL) was taken and mixed with chloramine T solution in a test tube and left for 20 min at room temperature. After adding 4-dimethyl-aminobenzaldehyde solution, the mixture was heated at 60^oC for 15 min. The absorbance of samples and hydroxyproline standards were determined at 558 nm using a spectrophotometer. For heat stable (or insoluble) collagen content, homogenized meat sample was heated in a 77^oC water bath for 70 min in a 3 times dilution of Ringer's solution (Hill, 1966), followed by centrifugation, residual fractions were hydrolyzed in 7N H_2SO_4 for 16 h at 105^oC. After neutralization, the hydroxyproline content of hydrolyzate was determined according to the procedure outlined by Kolar [5]. The amount of hydroxyproline content was determined from a standard curve and converted to the collagen content by a factor of 7.14. The soluble collagen was calculated from the differences between the total and insoluble collagen contents. Collagen solubility was expressed as the percentage of heat soluble collagen to total collagen.

Collagen Extraction from muscles of Hanwoo beef: Extraction was performed by the methods of Muralidharan et al., [6] Sato et al., [7] with slight modification. Thirty grams of fresh deboned, fatless meat were cut into pieces of 5 mm in size and washed three times with sodium chloride at a ratio of 1:6 for 10 min. Then three times washed with cold distilled water after that treated with sodium hydroxide at a ratio of 1:10 for continuous magnetic stirrer for three days and finally washed with cold distilled water. Samples were homogenized at 10000rpm two times for 15s with tris maleate and KCl. For gradual dissolution added 0.5 M acetic acid with 5 mM EDTA at a ratio of 1:15 to homogenized and stirred by magnetic stirrer for 48 h, then pepsin was added and the homogenate was centrifuged. The supernatant was salt out with 4 M sodium chloride and centrifuged at 13000g for 15 min. The precipitate was collected and dissolved in minimum volume of 1 M acetic acid. Finally the precipitate solutions were dialyzed for 48h against 0.02M Na_2HPO_4 with daily changes of solution.

Detection of collagen types I and III by SDS-PAGE

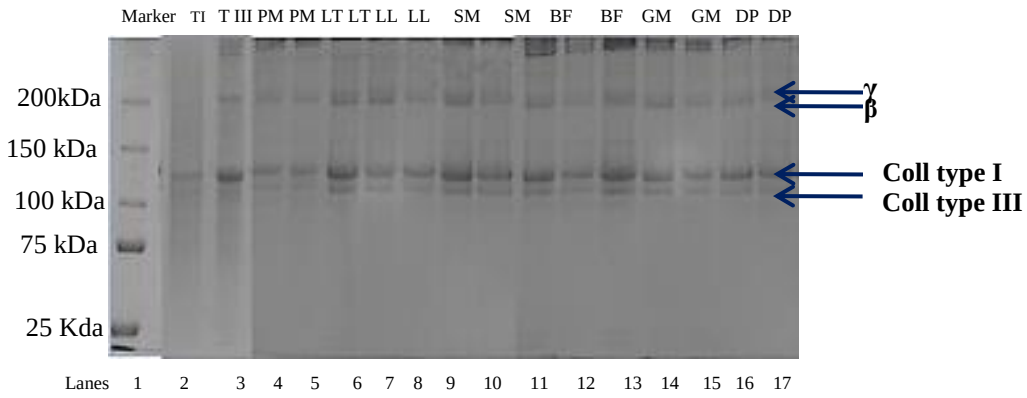
SDS-PAGE was performed according to the method of Laemmli [8] with a slight modification, using 6% separating gel and 4% stacking gel. Following BSA protein standard extracted collagen was dissolved in 1 ml sample buffer (Tris-HCl, pH 6.8 containing 2-mercaptoethanol, bromophenol blue, 10% SDS) and heated at 50^0C for 10 minutes. After that 25 µl of the sample was loaded in each well along with high molecular weight protein markers (5µl, Precision Plus protein TM dual color Standards, Bio-Rad laboratories, USA). Electrophoresis was done at constant 100 Voltage for 2 hours 10 minutes. After electrophoresis the gel was stained with Commassie Brilliant Blue R-250 in 5% (v/v) methanol and 7.5% (v/v) acetic acid for overnight and destained using 7.5% (v/v) of acetic acid and 25% (v/v) methanol for about 5 hours with a change of solution every hour. The image of SDS-PAGE gel was taken by the Versadoc Imaging system model 3000 Bio –Rad with Quantity One software. The densitometry for collagen protein specific bands was done on Gel Documentation System (Alpha Innotech, USA) with the help of AlphaEase™ FC Stand Alone V.4.0 software.

Statistical analysis: Data were analyzed using the GLM procedure of SAS Version 9.3 (SAS Institute, Cary, NC, USA) for model with treatments (muscle, aging & their interaction) as the main effects. The significant F-values were obtained and significance of the differences between means was tested by Duncan's multiple range tests

III. RESULTS AND DISCUSSION

The total collagen, insoluble collagen and collagen solubility, type I and III collagen contents of 10 muscles from Hanwoo steer were shown in table 1. The total collagen content of *supraspinatus* muscle, *biceps femoris, triceps brachii* was significantly higher ($p<0.001$) than that of *Psoas major* muscles. No significant difference in total collagen content was found among the other six muscles during 3 days chiller ageing, whereas BF muscle content significantly higher amount of total collagen than the of PM muscle in 21 days ageing period. Meanwhile, total collagen content increased significantly ($p<0.001$) in all muscles except SM muscle in 21 days chiller ageing. The variation in total collagen content result in tenderness variation

Fig SDS-PAGE pattern of collagen



PM, *Psoas major;* LT, *Longissimus thoracis*; LL, *Longissimus lumborum;* GM, *Gluteus medius*; SM, *Semimembranosus*; ST, *Semitendinosus;* BF, *Biceps femoris*; TB, *triceps brachii,* SS, *Supraspinatus* and DP, *Diaphragm muscles*

Table 1 Effects of muscle type and Post mortem ageing on collagen characteristics

Muscles	Total collagen, g/100g		Insoluble collagen, g/100g		Collagen solubility %		Type I collagen		Type III Collagen		Ratio (I:III)	
	3d	21d	3d	21d	3d	21d	3d	21d	3d	21d	3d	21d
Psoas major	0.19^{cy}	0.24^{cX}	0.12^{Yd}	0.15^{cX}	36.84	33.33	10.83^{j}	11.23^{g}	2.21^{iX}	2.05^{gY}	4.90^{aY}	5.48^{aX}
Longissimus thoracis	0.31^{b}	0.31^{bc}	0.20^{c}	0.18^{c}	35.48	41.94	11.34^{i}	12.53^{f}	2.50^{hX}	2.41^{fY}	4.51^{bY}	5.18^{bX}
Longissimus lumborum	0.32^{b}	0.36^{b}	0.21^{c}	0.22^{c}	34.4	38.88	12.55^{h}	13.54^{e}	2.80^{gX}	2.60^{eY}	4.47^{bY}	5.19^{bX}
Gluteus medius	0.38^{b}	0.48^{a}	0.24^{bc}	0.30^{b}	36.84	35.41	15.42^{f}	15.80^{c}	3.90^{eX}	3.71^{cY}	3.95^{eY}	4.25^{dX}
Semimembranosus	0.37^{b}	0.35^{b}	0.24^{bc}	0.22^{c}	32.43	37.14	13.59^{g}	14.48^{d}	3.21^{fX}	3.03^{dY}	4.24^{cY}	4.80^{cX}
Biceps femoris	0.47^{aY}	0.54^{aX}	0.33^{a}	0.37^{a}	29.8	31	17.58^{a}	18.44^{a}	5.03^{aX}	4.71^{aY}	3.49^{iY}	3.91^{eX}
Diaphragm	0.45^{a}	0.49^{a}	0.33^{a}	0.30^{b}	26.70^{Y}	38.80^{X}	16.39^{d}	16.97^{b}	4.59^{bX}	4.48^{bY}	3.57^{hY}	3.79^{fX}
Semitendinosus	0.46^{a}		0.28^{ab}		39.96		16.25^{e}		4.33^{c}		3.77^{g}	
Supraspinatus	0.49^{a}		0.31^{a}		36.73		16.71^{c}		4.35^{c}		3.89^{f}	
Triceps brachii	0.47^{a}		0.31^{a}		34.04		16.99^{b}		4.21^{d}		4.06^{d}	
SEM	0.03	0.03	0.02	0.05	0.01	0.01	0.04	0.01	0.01	0.04	0.02	0.01
					F value							
Muscle df 9/254	14.8^{***}	10.9^{***}	10.1^{***}	10.5^{***}	1.1	1.8	3829^{***}	4580^{***}	6183^{***}	1251^{***}	557^{***}	157^{***}
Aging df 1/254	6.6*		1		6.9^{**}		0.03		12.8^{***}		73.9^{***}	
Muscle* Aging	0.8		1.3		23.4^{***}		31.01***		22.6^{***}		52.9^{***}	

$^{a-f,}$ 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

SEM, Standard error of mean; df, degrees of freedom; *** P<0.001, ** P<0.01, * p<0.05

in beef, but that may be due to differences in structural composition and location of muscles in the animal body. Similarly, the meat scientist Seggem et al [9] stated that collagen content differed significantly in different muscles by its location and function. Total collagen content increases in 21 days chiller ageing period due to breakdown of perimysium collagen and extracellular matrix by metalloproteinase (MMPs) enzyme which helps in yielding the higher amount of hydroxyproline [10] (McCormick, 2009). The PM muscle contain a significantly lower amount of insoluble collagen whereas BE muscle contain the significantly higher amount of insoluble collagen (P<0.001) in both ageing periods. Most of the tender muscle contain lower amounts of insoluble collagen [11] that is fitted with our result in order to lowest amounts of insoluble collagen in PM, LT and LL muscle. The amount of insoluble collagen content in beef muscle mostly associated with palatability. In terms of collagen solubility there are no significant muscle effects on collagen solubility but there is a significant (P<0.01) ageing effect on collagen solubility. The Diaphragm muscle contains the lowest amount of soluble collagen and PM muscle contains the highest amount of soluble collagen except Semitendinosus muscle in 3 days ageing. On the other hand, in 21 days

ageing LT muscle contains the highest amount of soluble collagen and BF muscle contains the lowest amount of soluble collagen. Collagen solubility increased in farm animal muscles during the ageing period, due to collagen weakening by cathepsins enzyme [12]. The increase in collagen solubility associated with increased tenderness in muscles.

The SDS-PAGE gel quantification result (Fig 1) indicates that, In terms of collagen type I there is no significant ageing effect but a significant muscle effect is found. The highest amount of quantified SDS-PAGE gel value in BF muscle and lowest in PM at both ageing conditions. During the ageing period the type I collagen increased, but not at a significant level. Type I collagen mostly associated with meat tenderness. Collagen type III significantly higher in BF muscle and lowest in PM muscle at 3 days ageing whereas collagen type III significantly decrease in all muscle due to type III collagen most preferably destroyed during the ageing period, BF muscle contain the highest amount of collagen type III at 3 and 21 days of ageing whereas, PM muscle contain the lowest amount of collagen type III. The ratio of Collagen type I and III significantly higher in PM muscle and lower in ST muscle. The high amount of collagen type I and III in muscle indicate the toughness of the meat, whereas the lower amount of collagen type III in muscle with high ratio of collagen type I indicate the tenderness of meat.

IV. CONCLUSION

Our current result suggested that meat tenderness or toughness of different muscle beef can be evaluated by collagen type III, solubility and its ratio.

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