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The Effect of Quality Grade and Muscle on Collagen Contents and Tenderness of Intramuscular Connective Tissue and Myofibrillar Protein for Hanwoo Beef

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ABSTRACT : Six muscles were seamed out randomly from Hanwoo carcasses (n = 12) of each quality grade (quality grades 1, 2 and 3). Samples were analysed for their total and soluble collagen contents, IMCT (intramuscular connective tissue) and Warner-Bratzler shear force (WBSF). Simple correlation (n = 21) was determined for WBSF among major muscles. For LT (*longissimus thoracis*), total collagen content was significantly higher (p<0.05) for quality grade 3 than those for quality grades 1 and 2. For *semitendinosus* (ST), s*emimembranosus* (SM), *psoas major* (PM) and *serratus ventralis* (SV), total collagen content of quality grade 1 was lowest (p<0.05) of all quality grades. IMCT shear force for *gluteus medius* (GM) decreased (p<0.05) with better quality grade, and those for other muscles, with the exception of GM, were higher (p<0.05) for quality grade 3 than for quality grades 1 and 2. WBSF values showed GM and LT to be decreased (p<0.05) with better quality grade, and PM to be higher (p<0.05) for quality grade 3 than those for quality grades 1 and 2. SM, ST and SV from quality grade 1 had lower (p<0.05) WBSF value than those from quality grades 2 and 3. Total collagen content of ST was highest (p<0.05) of all muscles, whereas that of PM was lowest (p<0.05). Soluble collagen contents of LT and SV from quality grades 1 and 2 were, in general, higher (p<0.05) than other muscles, but that of SM was lowest (p<0.05). ST and SM had higher (p<0.05) WBSF values for three quality grades when compared to other muscles, whereas PM was lowest (p<0.05). LT had the strongest simple correlation with SV (r = 0.78) and GM (r = 0.77), and SM had the strongest correlation with ST (r = 0.73) and LT (r = 0.73). Also, PM had the strongest correlation with SV (r = 0.62). (**Key Words :** Hanwoo Beef, Quality Grade, Collagen, IMCT, WBSF)

INTRODUCTION

The quality grade is primarily determined by the marbling score and additionally adjusted by other carcass traits such as meat colour, fat colour, texture and maturity when there is a particular defect in these traits (USDA, 1989; APGS, 1995). Better quality grades have a heavier carcass weight with a higher marbling score, redder meat colour and whiter fat colour (Lorenzen et al., 1993; Vergara et al., 1999; Moon et al., 2003). Consumers judge meat quality on the basis of the quality grade, and they are willing to pay a premium for a better quality grade (Savell et al., 1989; Neely et al., 1998; Moon et al., 2003). Carcass and retail cut price are largely determined by the quality grade, so consequently breeders and producers have forced the improvement of the quality grade (Savell et al., 1989; Park et al., 2002; Moon et al., 2003).

Tenderness is affected by intramuscular connective tissue, myofibrillar proteins and their interactions (Sacks et al., 1988). Although the connective tissue is comprised of collagen and elastin, collagen is the main component of intramuscular connective tissue and forms the structural framework for muscle and adipose tissue (Flint and Pickering, 1984). It therefore greatly influences meat toughness. Collagen has been known to contribute to the so-called “background” toughness of meat. Soluble collagen, particularly, is significantly linked to toughness and tenderness and it differs between muscles from various anatomical locations (Cross et al., 1973). Intramuscular fat is deposited between fasiculi, and it disrupts the structure of endomysium and separates and thins perimysial fibers, therefore it could improve beef tenderness (Nishimura et al., 1999).

Much research has been focused on the prediction of longissimus tenderness, because the longissimus comprises a higher proportion of total carcass value as compared to any other muscles (Shackelford et al., 1995, 1997). Under the circumstances of the Korean meat market, longissimus within the same quality grade is estimated at the highest price compared with other muscles (APGS, 2003). Furthermore, it might be desirable if longissimus could be

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used to predict the tenderness of other major muscles. The relationship between the tenderness of the longissimus and other muscles has been reported to be moderate (Slanger et al., 1985; Shackelford et al., 1995). However, the relationship between the tenderness of major muscles within a wide marbling range of Hanwoo beef, which is highly marbled, has not been evaluated.

Therefore, the main objective of this study was to determine the effect of the quality grade and major muscles on the collagen content and shear force of Hanwoo beef. It also determined the relationship of the tenderness between longissimus and other major muscles in cooked beef.

MATERIALS AND METHODS

The left sides of Hanwoo bull carcasses (n = 12) were selected to analyse the total collagen and soluble collagen content, IMCT (intramuscular connective tissue) and WBSF (Warner-Bratzler shear force). Nine of carcasses (total = 21) were additionally selected, because twelve of carcasses were not enough for simple correlation analysis, from a commercial slaughterhouse to determine the relationship of Warner-Bratzler shear force between major muscles. Carcasses were assigned into three quality grades, which are quality grades 1, 2 and 3, based on marbling, meat colour, fat colour, texture and overall maturity score as decided by meat graders (Park et al., 2002; Moon et al., 2003).

Six muscles were anatomically seamed out from the carcasses in each quality grade. The muscles removed were the *longissimus thoracis* (LT; loin), s*emimembranosus* (SM; top round), *gluteus medius* (GM; top sirloin), *serratus ventralis* (SV; jacobs ladder), *psoas major* (PM; tenderloin), *semitendinosus* (ST; eye of round). The muscles were selected by considering a location (hindquarter and forequarter) and the amount of muscle enough to analyse. Steaks were cut up from central portions of each muscle. All steaks were then identified and vacuum packed, and evaluated.

Total and soluble collagen contents

The total collagen content was determined according to the method of Bergman and Loxley (1963) with a slight modification by Reich (1970), and the hydroxyproline content was converted to the collagen content with a factor of 7.25 (Goll et al., 1963).

The soluble collagen content was determined by Hill's method (1966) with slight modifications. Two grams of the sample was homogenised for 1 min at 10,000 rpm with a fourfold volume of 1/4-strength Ringer's solution containing 0.86% NaCl, 0.03% KCl, and 0.033% $CaCl_2$. The resulting homogenate was heated for 70 min at 77°C and then centrifuged for 30 min at 1,000 g. The supernatant solution was decanted, and the pellet was suspended in the same solution and re-centrifuged. The supernatant solutions were combined, and hydrolyzed in 6 N HCl for 24 h at 110°C. After removing HCl, the amount of hydroxyprolin was determined by the method of Bergman and Loxley (1963). The amount of soluble collagen was determined by the difference between the total collagen and insoluble collagen content.

IMCT shear force

The intramuscular connective tissue (IMCT) was prepared using the method of Nishimura et al. (1996). The shear force of IMCT preparations embedded in acrylamide gels was measured according to the method of Nishimura et al. (1998), and using a Rhoemeter (NRM-2002J, Fudo, Co) with a straight-edged blade (0.35 mm thickness) and a crosshead speed of 6 cm/min.

Warner-Bratzler shear force

Steaks for Warner Bratzler shear force measurement were weighed, placed in open plastic bags and cooked in a water bath at 72°C to an internal temperature of 70°C. The same steaks were chilled overnight at 2°C. Cores were cut from the steaks, and allowed to reach room temperature prior to shearing on an Instron Universal Testing Machine (Instron Corporation, MA, USA) at a crosshead speed of 5 cm/min. Seven cores were taken in total from each steak and results were averaged. Cores were of 15mm diameter and sheared perpendicular to meat fibre direction.

Statistical analysis

The data obtained was analysed by ANOVA using SAS (SAS Inst. Inc., Cary, NC). We compared mean values between six muscles in the combined quality grade, and quality grades in each muscle. Tukey's multiple range comparison was used to compare differences between mean values at a 5% level of significance. The PROC CORR procedure of SAS was also used for a simple correlation.

RESULTS AND DISCUSSION

Effect of quality grade on carcass traits

The quality grade 1 had a heavier carcass weight ($p<0.05$), higher marbling score ($p<0.05$) and better texture ($p<0.05$) when compared with quality grades 2 and 3, but no significant differences were found in the *longissimus* area, fat and meat colour and overall maturity between quality grade 1 and any of quality grades 2 and 3 (Table 1).

Effect of six muscles on physical traits in the combined quality grade

The total collagen content of ST was the highest ($p<0.05$) of six muscles, whereas PM was lowest ($p<0.05$)

Table 1. Carcass traits among quality grades of Hanwoo beef

Carcass traits	Quality grades		
	1	2	3
Carcass weight (kg)	338.00±8.54^{x}	317.33±23.69^{xy}	287.67±17.17^{y}
Back fat thickness (mm)	8.00±1.00	6.33±2.31	6.00±1.00
M. Longissimus area	82.67±4.16	83.67±4.73	79.00±8.72
Marbling score[1]	12.33±1.16^{x}	7.00±1.00^{y}	1.67±0.58^{z}
Fat colour score[2]	2.67±0.58	3.00±0.00	3.00±0.00
Meat colour score[3]	4.33±0.58	5.00±0.00	5.00±0.00
Texture score[4]	2.67±0.58^{y}	4.00±0.00^{x}	4.00±0.00^{x}
Overall maturity[5]	2.00±0.00	2.00±0.00	2.00±0.00

$^{x-z}$ Means in the same column with different letters are significantly different (p<0.05).
[1] 1 = trace, 19 = very abundant. [2] 1 = white, 7 = yellow.
[3] 1 = very light cherry red, 7 = very dark red. [4] 1 = very fine, 7 = very coarse. [5] 1 = young, 9 = old.

Table 2. Effect of six major muscles on total and soluble collagen contents, IMCT and Warner-Bratzler shear force

Items	Total collagen (mg/g)	Soluble collagen (mg/g)	IMCT shear force (g/cm^2)	Warner-Bratzler Shear force (kg/cm^2)
Gluteus medius	4.25±0.15^{y}	1.34±0.11^{xy}	52.2±7.2^{yz}	5.2±0.6^{x}
Longissimus thoracis	4.52±0.26^{y}	1.57±0.17^{x}	58.3±8.1^{y}	5.4±0.6^{x}
Semimembranosus	5.53±0.23^{xy}	0.64±0.12^{y}	62.2±8.5^{y}	6.6±0.7^{wx}
Semitendinosus	6.85±0.28^{x}	1.26±0.16^{xy}	95.8±6.1^{x}	7.2±0.8^{w}
Psoas major	3.71±0.22^{z}	0.78±0.14^{y}	35.7±3.8^{z}	3.4±0.5^{z}
Serratus ventralis	4.32±1.19^{y}	1.46±0.15^{x}	43.7±6.2^{z}	4.7±0.6^{y}

$^{w-z}$ Means in the same column with different letters are significantly different (p<0.05).

(Table 2). The SV also tended to have a higher (p<0.05) total collagen content compared with other muscles, with the exception of ST. There were no significant differences in total collagen content between LT and GM for the combined quality grade. The results agree with the findings of Torrescano et al. (2003), who found that the PM out of 14 muscles had the lowest total collagen content, and the total collagen content of ST was higher than those of SM, GM, LT or PM.

In the combined quality grade, WBSF values ranged from 3.4 kg (PM) to 7.2 kg (SM). ST and SM had the highest (p<0.05) WBSF values of six muscles, whereas PM was lowest (p<0.05). No significant difference in WBSF value was found between LT and GM. The results are in agreement with the findings of Torrescano et al. (2003), who reported that Warner-Bratzler shear force of PM was lower compared with those of SV, GM, SM, ST or LT, and that of ST was higher than those of PM, SV, GM, SM or LT. The results are also in agreement with the findings of Belew et al. (2003), who reported that Warner-Bratzler shear force of muscles were numerically in the order of PM<SV<LT<GM<SM<ST. They reported that muscles considered “very tender” were PM and SV, “tender” were LT and GM and “intermediate” were ST and SM. Shackelford et al. (1995), however, reported that Warner-Bratzler shear forces were not different between LD, ST, GM and SM muscles aged until 14 d postmortem, whereas overall tenderness ratings were different between muscles.

The PM with the lowest WBSF value in general had a significantly lower (p<0.05) total collagen content and IMCT shear force value, whereas ST and SM with higher (p<0.05) WBSF value had significantly higher (p<0.05) total collagen contents and IMCT shear force values. Soluble collagen contents of muscles, however, showed a different trend from the results of WBSF values of muscles. Soluble collagen contents showed in most cases LT and SV to be higher (p<0.05) than other muscles, being SM and PM to be lower (p<0.05) than other muscles. Those of ST and GM were not significantly different. These results are in agreement with the findings of Young and Braggins (1993), who reported that sensory tenderness and shear force were closely related to total collagen content. However, soluble collagen result was inconsistent with findings of Hall and Hunt (1982), who reported that more soluble collagen contents were associated with higher tenderness. They also found that soluble collagen content in muscle seamed out from cow carcass was generally increased with age. In present study, muscles were removed from carcasses, which had the same overall maturity (Table 1). Dutson (1974) found that the total collagen content was not closely related to the changes in tenderness associated with advancing age, whereas percent soluble collagen content was related to tenderness changes associated with advancing age.

Effect of quality grade on total and soluble collagen contents of muscles

For LT, total collagen content was significantly higher (p<0.05) for quality grade 3 than those for quality grades 1

Table 3. Effect of quality grade on total collagen contents (mg/g) of each muscle

Muscles	Grade 1	Grade 2	Grade 3
Gluteus medius	5.24±0.30^{y}	5.33±0.22^{y}	6.29±0.27^{x}
Longissimus thoracis	5.14±0.18^{yz}	4.97±0.30^{z}	6.35±0.44^{x}
Semimembranosus	5.55±0.31^{y}	6.99±0.21^{x}	6.97±0.22^{x}
Semitendinosus	7.48±0.25^{y}	8.15±0.22^{x}	8.10±0.25^{x}
Psoas major	3.46±0.23^{y}	3.80±0.22^{x}	4.05±0.23^{x}
Serratus ventralis	5.95±0.21^{y}	6.52±0.31^{x}	5.80±0.27^{y}

$^{x-z}$ Means in the same row with different letters are significantly different ($p<0.05$).

Table 4. Effect of quality grade on soluble collagen contents (mg/g) of each muscle

Muscles	Grade 1	Grade 2	Grade 3
Gluteus medius	1.34±0.12	1.35±0.09	1.35±0.12
Longissimus thoracis	1.58±0.15	1.54±0.10	1.61±0.18
Semimembranosus	0.63±0.09	0.65±0.11	0.68±0.14
Semitendinosus	1.25±0.21	1.26±0.13	1.26±0.17
Psoas major	0.79±0.15	0.79±0.13	0.78±0.12
Serratus ventralis	1.44±0.13	1.47±0.13	1.46±0.17

and 2 (Table 3). For ST, SM, PM and SV in quality grade 1, total collagen contents were lower ($p<0.05$) than those for quality grades 2 and 3, but there were no significant differences between quality grades 2 and 3, with the exception of SV. The results correspond with the findings of Nishimura et al. (1999), who reported that highly marbled beef had a lower total collagen content and weaker intra-connective tissue strength. There was no significant difference in total collagen content of GM between all quality grades. No significant differences in soluble collagen contents were found between the three quality grades.

Effect of quality grade on IMCT and myofibrillar protein shear force of muscles

IMCT shear force of muscles decreased ($p<0.05$) with an increase in quality grade, (i.e. better quality grade), and was higher ($p<0.05$) for quality grade 3 than for quality grades 1 and 2 (Table 5). These results are in agreement with those of Nishimura (1999), who found that highly marbled beef weakened structures of the intramuscular connective tissue and contributed to tenderisation of muscles. For IMCT shear force of SV, however, there was little significant difference between quality grades 1 and 2. This might be due to the higher fat content of SV within all quality grades. The results indicate that higher intramuscular fat content could improve the IMCT tenderness. Within each quality grade, IMCT shear force of ST was highest ($p<0.05$) of five muscles, while PM was lowest ($p<0.05$).

WBSF values for GM and LT decreased ($p<0.05$) with an increase, i.e. better quality grade, in quality grade, and

Table 5. Effect of quality grade on IMCT shear force (g/cm^2) of each muscle

Muscles	Grade 1	Grade 2	Grade 3
Gluteus medius	22.5±9.2^{z}	59.7±9.5^{y}	76.8±3.3^{x}
Longissimus thoracis	46.2±3.5^{z}	50.1±12.0^{yz}	80.6±3.8^{x}
Semimembranosus	50.0±4.8^{z}	57.0±4.2^{yz}	82.3±9.0^{x}
Semitendinosus	81.2±5.4^{z}	84.0±4.2^{yz}	125.6±8.2^{x}
Psoas major	22.4±6.0^{y}	29.6±3.4^{y}	56.3±1.8^{x}
Serratus ventralis	34.8±7.5^{z}	41.0±3.5^{yz}	57.8±7.0^{x}

$^{x-z}$ Means in the same row with different letters are significantly different ($p<0.05$).

Table 6. Effect of quality grade on Warner-Bratzler shear force (g/cm^2) of each muscle

Muscles	Grade 1	Grade 2	Grade 3
Gluteus medius	4.56±0.45^{y}	5.22±0.67^{xy}	5.88±0.57^{x}
Longissimus thoracis	4.79±0.65^{y}	5.35±0.91^{xy}	6.18±0.30^{x}
Semimembranosus	5.77±0.70^{z}	6.92±0.85^{xy}	7.29±0.36^{x}
Semitendinosus	3.08±0.34^{z}	3.27±0.44^{xy}	3.75±0.68^{x}
Psoas major	4.07±0.45^{z}	4.77±0.85^{yz}	5.05±0.35^{x}
Serratus ventralis	5.74±0.66^{z}	7.76±0.84^{xy}	8.40±0.67^{x}

$^{x-z}$ Means in the same row with different letters are significantly different ($p<0.05$).

Table 7. Simple correlation coefficients for Warner-Bratzler shear force of six muscles of cooked Hanwoo beef

	SM	GM	SV	PM	ST
LT	0.73**	0.77**	0.78**	0.46*	0.55*
SM		0.66**	0.59**	0.50*	0.73**
GM			0.67**	0.57**	0.71**
SV				0.62*	0.58**
PM					0.53*

** $p<0.01$, * $p<0.05$.

LT = *longissimus thoracis*; SM = *semimembranosus*; GM = *gluteus medius*; SV = *serratus ventralis*; PM = *psoas major*; ST = *semitendinosus*.

that for PM was higher ($p<0.05$) for quality grade 3 than for quality grades 1 and 2 (Table 6). SM, ST and SV in quality grade 1 had a lower ($p<0.05$) WBSF value than those in quality grades 2 and 3, but WBSF values of SM, ST and SV were not significantly different between quality grades 2 and 3. Nishimura et al. (1999) reported that the tenderisation effect of marbling fat could be applied only to some breeds of cattle that are capable of depositing large amounts of intramuscular fat. In the present study, however, fat content of SM, ST and PM was lower ($p<0.05$) than that of GM and LT and also SV had very high fat content in all quality grades.

The simple correlation coefficient for WBSF of muscles

The simple correlation coefficients for WBSF among five major muscles were significant, and ranged from r = 0.46 to r = 0.78 (Table 7). Also, the simple correlation coefficient for WBSF between LT and other major muscles ranged from r = 0.46 (PM) to r = 0.78 (SV). For simple correlation among muscles, LT had the strongest correlation with SV (r = 0.78) and GM (r = 0.77), and SM had the strongest correlation with ST (r = 0.73) and LT (r = 0.73). Also, PM had the strongest correlation with SV (r = 0.62). The results showed a higher correlation among muscles, when compared with previous research. Joseph and Connolly (1979) reported that correlation among these muscles for trained sensory tenderness rating were 0.20 to 0.32 in beef, and they concluded that using one muscle as an index of carcass toughness would not be feasible. Shackelford et al. (1995) reported that correlation between LD and major muscles in beef for Warner-Bratzler shear force were 0.12 to 0.40. Wheeler et al. (2000) reported that LD was related to GM (r = 0.68) and SM (r = 0.58), and GM was related to SM (r = 0.62). The differences of correlation among muscles might be due to the postmortem period when tenderness was estimated. The results indicate that tenderness of SM, GM and SV was related more closely with LT, compared to the other muscles measured at 24 h postmortem.

IMPLICATIONS

A better quality grade with a higher marbling score and a better texture score tended to have a lower total collagen content, IMCT and Warner-Bratzler shear force. Tender muscle (PM) had a lower total collagen content and IMCT shear force value, whereas tough muscles had a higher total collagen content and IMCT shear force value. The results of the simple correlation indicate that tenderness of SM, GM and SV was related more closely with LT, compared to the other muscles measured at 24 h postmortem. Further studies might be required to understand the relationship of collagen properties with meat tenderness between muscles.

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