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Effects of Marbling on Meat Quality Characteristics and Intramuscular Connective Tissue of Beef *Longissimus* Muscle

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ABSTRACT : This study was designed to explore the effects of marbling on meat quality characteristics and intramuscular connective tissue of beef *longissimus* muscle. Chemical determinations, histological and mechanical measurements were performed on the raw and cooked meat at d 4 postmortem. The results showed that crude fat, collagen, fiber diameter and maximum transition temperature of intramuscular connective tissue increased ($p<0.05$) with the increase of marbling score. The cooking losses, collagen solubility, WBSF and perimysial thickness decreased ($p<0.05$) with the increasing marbling. WBSF correlated ($p<0.05$) with moisture, crude fat, collagen, cooking losses, sarcomere length and perimysial thickness. The development of marbling results in the decline in cooking losses, the avoidance of sarcomere shortening, and the disorganization of the perimysia, which accounts for the improvement of beef tenderness. (**Key Words :** Marbling, Tenderness, Connective Tissue, *Longissimus*)

INTRODUCTION

Tenderness is one of the most important eating quality traits and it is affected by numerous factors. Marbling, i.e. intramuscular fat, is an importantly intrinsic factor contributing to beef palatability and thus used as an indicator for beef quality grading (USDA, 1997; CMA, 2003).

Numerous studies have been done on the relationship between marbling score or quality grade and beef palatability attributes in the past four decades (Savell et al., 1987; Brooks et al., 2000; Kim and Lee, 2003). It seems acceptable that beef muscle with a higher marbling level should have a higher rating in sensory attributes (juiciness, tenderness, flavor intensity). However, it is still controversial whether marbling has some influence on the Warner-Bratzler shear force (WBSF) of beef. Some studies showed a significant decrease in WBSF for beef muscle with advancing marbling score (Kim and Lee, 2003), whereas others noted no differences in WBSF of beef among marbling groups (Brooks et al., 2000).

In addition to intramuscular fat, intramuscular connective tissue (IMCT) and myofibrils are important components of meat that influence meat tenderness. According to Nishimura, Hattori and Takahashi (1999), the development of intramuscular fat contributes to tenderization of highly marbled beef by disorganizing the structure of IMCT. However, it is uncertain whether there were effects of animal age and myofibrillar components on beef tenderness in their study.

Therefore, this study aimed to: (1) compare the traits of meat quality and intramuscular connective tissue of beef at three levels of marbling score; (2) determine whether, and if so, how marbling has an impact on beef tenderness.

MATERIALS AND METHODS

Sampling

Twenty-one USDA Slight-to-Small, Modest-to-Moderate, and Abundant marbling carcasses from pure Luxi steers (n = 7 each marbling group, carcass weight: 245 to 270 kg) were selected on a cutting line and two 2.54-cm-thick *longissimus* (LM) steaks were obtained at d 4 postmortem from the right carcass side, which were hung at

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4°C in a commercial meat processing company (Hebei Hua'an Meat Ltd.). At slaughter day, over one hundred pure Luxi steers (36±2 mo) were slaughtered humanely according to the *Requirements of Islamic Slaughtering*. Prior to slaughter, animals had been fattened on a similar feeding regime for approximately 90 days in a commercial feedlot, which is affiliated to the above meat company. Therefore, differences in marbling levels were considered to result from the individual animals themselves.

Instrumental measurement

Of the two steaks from each carcass, one was directly used for the following analyses. The other was cooked in an 80°C water bath until an internal temperature of 70°C was reached. All visible subcutaneous fat and connective tissue were removed before the steaks were individually placed inside polyethylene bags. During cooking, the internal temperature of steaks was tracked by a digital needle-tipped thermometer (HI145, HANNA, Italy). The steaks were immediately taken out of the water bath when the designed internal temperature was reached and then cooled with running water for about half an hour. After cooling, five 1.27-cm-diameter cylindrical cores were removed from each steak parallel to the muscle fiber orientation. A single, peak shear force measurement was obtained for each core using a Warner-Bratzler meat shear machine (Salter 235, G-R, USA) and an average shear force (WBSF) was calculated and recorded for each steak. After shearing, each core was used for determination of chemical composition and histological observation.

Chemical composition

Determinations of moisture, crude fat, total and insoluble collagen in raw meat were based on the whole steak, whereas those of cooked meat were determined on the basis of cores. Moisture was determined using the freeze drying method. About 5.0 g of meat sample from raw or cooked samples was dried in a freeze drying machine (Alpha 2, Christ, Germany) for 24 h until the sample weight was constant. Crude fat content in dried samples was extracted in ether solution for about 15 h. Collagen content and its solubility were determined according to the procedures of Hill (1966) and Bergman and Loxley (1963).

Preparations of the perimysia and the endomysia, and differential scanning calorimetry

The perimysia and the endomysia were prepared according to the method of Light and Champion (1984). Before DSC, the purified perimysial and endomysial portions were concentrated by freeze drying. The endothermal transition of perimysial and endomysial collagen was measured using a calorimeter (DSC 7, Perkin Elmer, USA). Temperature calibration was run using the Indium thermogram. The samples (20 mg) were accurately weighed into aluminum pans and sealed. The samples were scanned at 10°C min^{-1} over the range of 20 to 90°C using liquid nitrogen as the cooling medium. An empty pan was used as the reference. The maximum transition temperature (T_{max}) was estimated from the thermogram using the software Pyris Manager Series (DSC 7, Perkin Elmer, USA).

Sarcomere length

The sarcomeres of both raw and cooked muscle were measured on a whole steak basis. The sarcomere length was measured according to the method of Cross, West and Dutson (1981) with some modifications. Briefly, 5 g of meat sample was cut into 0.5×0.5×0.5 cm cubes and homogenized in 30 ml of cold 0.25 M sucrose for 60s at a low speed (5.000 rpm). A drop of homogenate was observed with the oil objective using a phase-contrast microscopy (BX41, Olympus, Japan), and 25 single myofibrils were photographed by an Olympus camera (Olympus, Japan). All the images were analyzed by the software Image-Pro Plus (5.1, Media Cybernetics, USA). Five measurements of sarcomere length were performed at different points on each image. Finally, the average of 125 measurements (25×5) was designated as the sarcomere length of one steak.

Histological observations

A 0.5×0.5×0.5 cm cube was removed from each raw and cooked steak. The cubes were rapidly frozen in nitrogen for 3 to 4 h, and cut into 10 μm sections, perpendicular to the orientation of muscle fibers, in a cryostat (1850, Leica, Germany). The sections were stained according to Flint and Pickering (1984). Slides were examined under bright-field illumination with a 10× objective using a microscope (BX41, Olympus, Japan), and 15 photographic images were taken from each slide, using a digital camera (Olympus, Japan) affixed to the microscope, for measurements of perimysial thickness and fiber diameter.

The perimysial thickness and the fiber diameter were measured by the software Image-Pro Plus (5.1, Media Cybernetics, USA). Fifteen points were randomly selected from each image. The perimysial thickness was designated as the shortest distance between the two edges of the membrane, and the fiber diameter was designated as the shortest diameter of a single muscle fiber. Finally, the perimysial thickness and the fiber diameter for each sample were recorded as the averages of 225 measurements (15×15).

Mechanical strength of IMCT for raw meat

The IMCT preparation was prepared according to the procedures of Nishimura et al. (1999). The shear force

Table 1. Chemical composition, mechanical strength, fiber diameter, and sarcomere length of raw beef steaks with different marbling scores (n = 7 each group)

	Slight-to-small		Modest-to-moderate		Abundant	
	Means	SE	Means	SE	Means	SE
Moisture (g kg^{-1})	726.1^{a}	10.8	683.1^{b}	39.6	618.4^{c}	14.8
Crude fat (g kg^{-1})						
on a dry basis	220.3^{a}	31.9	371.7^{b}	30.9	484.8^{c}	22.7
on a wet basis	62.2^{a}	11.4	119.5^{b}	14.3	186.5^{c}	13.8
Collagen (g kg^{-1})						
Total collagen						
on a non-fat, dry basis	17.3^{a}	1.4	21.7^{b}	3.1	22.6^{b}	3.8
on a dry basis	13.3	0.9	13.7	2.1	12.0	2.7
on a wet basis	3.6	0.3	4.3	0.6	4.4	0.7
Insoluble collagen						
on a non-fat, dry basis	13.6^{a}	0.7	16.8^{ab}	1.9	19.8^{b}	3.0
on a dry basis	10.6	0.5	10.6	1.3	10.5	2.2
on a wet basis	2.9	0.2	3.3	0.4	3.9	0.6
Solubility (%)	19.2^{a}	5.2	19.0^{a}	6.1	11.3^{b}	3.2
IMCT mechanical strength (Newton)	6.4^{a}	0.7	4.2^{b}	0.8	4.9^{b}	0.7
Fiber diameter (μm)	41.3^{a}	1.1	45.0^{b}	0.7	44.9^{b}	0.9
Sarcomere length (μm)	2.4^{a}	0.1	2.7^{b}	0.1	2.5^{a}	0.1
Transition temperature (°C)						
Endomysium	58.7^{a}	0.8	60.8^{b}	0.7	62.3^{c}	0.8
Perimysium	62.5	1.1	67.3	0.9	69.5	1.3

^{a,b,c} Means with different superscript letters differ ($p<0.05$).

values of IMCT preparations embedded in acrylamide gels were measured using a texture analyzer (TA-XT2i, Godalming, England) with a V-type blade (HDP/BSW).

Scanning electron microscopy (SEM)

A 0.5×0.5×0.5 cm cube was cut from raw muscles. The cubes were macerated and treated as described by Nishimura et al. (1999) for SEM. Finally, the samples were observed under a scanning electron microscope (SX-40, Topcon Technologies Inc., Paramus, Japan) with an accelerating voltage of 20 kV.

Statistical analyses

The effects of marbling on WBSF, cooking losses, moisture content, crude fat content, collagen content and its solubility, the perimysial thickness, sarcomere length, mechanical strength of IMCT and fiber diameter were evaluated by one-way analysis of variance where these measurements were dependent variables and marbling group was the independent variable. Means of the measurements of different marbling groups were compared using the Duncan's multiple-range test at the significance level of 0.05. Correlation coefficients between the variables were evaluated by descriptive analysis of correlation. To investigate the differences in WBSF, moisture content, crude fat content, collagen content, and perimysial thickness between individual steaks within the same marbling group and between individual cores within the same steak, ANOVAs were performed on the measurements of each core. A further analysis of correlation was performed on the basis of the measurements of each core. Furthermore, factorial regression analysis was performed to select the factors that could be used to predict the WBSF. All statistical analyses were performed by SAS8.12 (SAS Inst. Inc. Cary, NC. 2001).

RESULTS AND DISCUSSION

Raw meat

Crude fat increased with the increase in marbling score, i.e., the LM muscles in the Slight to Small group had the lowest quantities of crude fat, whereas those in the Abundant group had the highest ($p<0.05$). Inversely, moisture content declined ($p<0.05$) as the marbling score increased (Table 1). This is in agreement with previous studies (Hedrick et al., 1981; Von Seggern et al., 2005). However, the quantities of ether extractable fat corresponding to USDA marbling levels in the present study were higher than those reported by Savell, Cross and Smith (1986), which probably results from differences in visual evaluation.

The quantity of total collagen, expressed on a nonfat basis, increased ($p<0.05$) as the marbling score increased. When expressed on a dry or wet basis, the percentages of total collagen of beef from the three marbling groups were not significantly different ($p>0.05$), which was also the case for the quantity of insoluble collagen. The means of insoluble collagen, on a nonfat basis, had a similar tendency to those of total collagen, but no differences ($p>0.05$) existed between the three marbling groups (Table 1). Kim

Table 2. Cooking losses, WBSF, fiber diameter, perimysial thickness and sarcomere length of cooked beef steaks with different marbling scores (n = 7 each group)

	Slight-to-small		Modest-to-moderate		Abundant	
	Means	SE	Means	SE	Means	SE
Cooking losses (%)	21.0[a]	1.1	14.0[b]	0.8	11.4[c]	1.1
WBSF (Newton)	72.1[a]	5.3	45.3[b]	4.0	47.3[b]	3.0
Moisture (g kg^{-1})	642.5[a]	2.9	607.9[b]	8.9	595.8[b]	5.7
Crude fat (g kg^{-1})						
on a dry basis	206.2[a]	10.1	356.4[b]	23.6	409.3[c]	12.9
on a wet basis	74.4[a]	4.3	146.3[b]	13.2	167.3[c]	7.2
Collagen (g kg^{-1})						
Total collagen						
on a non-fat (dry basis)	15.7[a]	0.8	18.1[b]	0.8	18.8[b]	0.8
on a dry basis	12.3	0.4	11.3	0.4	11.0	0.5
on a wet basis	4.4	0.2	4.4	0.2	4.4	0.2
Insoluble collagen						
on a non-fat (dry basis)	14.0[a]	0.5	16.0[b]	0.7	15.7[b]	0.5
on a dry basis	11.0	0.3	9.9	0.3	9.2	0.4
on a wet basis	3.9	0.1	3.9	0.1	3.7	0.1
Fiber diameter (μm)	29.48	0.35	29.98	0.79	30.74	0.43
Sarcomere length (μm)	1.81[a]	0.04	2.05[b]	0.03	1.96[b]	0.06
Perimysial thickness (μm)	50.94[a]	3.90	25.61[b]	1.09	25.54[b]	1.19

[a, b, c] Means with different superscript letters differ ($p<0.05$).

and Lee (2003) also reported that the amount of total collagen in the beef LM muscles was not influenced by quality grade/marbling score. It was notable that both the quantities of total and insoluble collagen differed between marbling groups when adjusted to a nonfat and dry basis. This indicates that intramuscular fat and moisture content minimize the differences in collagen content between marbling groups. Collagen solubility had a tendency ($p>0.05$) to decrease with advancing marbling score (Table 1), which may be ascribed to the formation of mature crosslinks during intramuscular fat deposition. Similarly, Nishimura et al. (1999) also reported a decline in collagen solubility for beef *longissimus* and *semitendinosus* muscles from Japanese Black cattle with the increase of marbling score. However, in their study animal age could have been the major contributor to the decline in collagen solubility. Mechanical strength of IMCT was higher ($p<0.05$) for the low-marbled muscle than for moderately- and highly-marbled muscles (Table 1). This could be due to the development of intramuscular fat disorganizing the structure of the perimysia (Figure 3). In contrast to this study, Nishimura et al. (1999) demonstrated an increase in mechanical strength values with marbling scores, which could still be attributed to the effect of animal age. Corresponding to the decrease in collagen solubility, the maximum transition temperatures of the endomysia and the perimysia increased with advancing marbling score (Table 1), which could be due to formation of more mature crosslinks during the deposition of intramuscular fat.

The Slight- to Small- Marbled muscles had a smaller ($p<0.05$) fiber diameter than the other two marbling groups (Table 1). This is consistent with the study of Romans, Tuma and Tucker (1965) who reported that muscle fibers from the Moderate marbling level were significantly larger in diameter than those from the Slight level of marbling. But the fiber diameters of muscles from modestly-to-moderately and abundantly marbled carcasses did not differ ($p>0.05$). The contributor to a small fiber diameter for the low marbled muscle may be the occurrence of pre-rigor cold shortening due to thinner fat cover over the carcasses (Savell, Mueller and Baird, 2005). This is also proven by the increase ($p<0.05$) in sarcomere length with advancing marbling score (Table 1). But Kim and Lee (2003) showed no change in sarcomere length for LM muscles with quality grade/marbling. Cooper et al. (1968) reported that fiber diameter was not influenced by marbling score. The differences in sarcomere length and fiber diameter among the mentioned studies should be attributed to the method of sample treatment.

Cooked meat

The quantities of cooking losses for LM muscles tended to decrease ($p<0.05$) with increasing marbling scores (Table 2), which supports previous studies (Parrish et al., 1973; Jeremiah, 1996; Ozawa et al., 2000). The cooked muscles had a similar trend to raw meat in moisture content, crude fat content, total and insoluble collagen, i.e., highly marbled cooked LM had higher quantities of crude fat and collagen but lower quantities of moisture than low marbled muscle ($p<0.05$) (Table 2).

Cooking led to a great decrease ($p<0.05$, Tables 1 and 2) in the fiber diameter and the sarcomere length of all LM

Table 3. WBSF, fiber diameter, perimysial thickness and chemical composition for beef steaks within USDA Slight-to-small marbling group (means±SE, n = 5 each)

Characteristics	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7
WBSF (Newton)	61.8±5.9^{a}	94.0±3.7^{b}	55.1±4.4^{c}	74.6±5.5^{d}	87.5±5.5^{e}	64.0±6.3^{a}	67.9±4.8^{f}
Moisture (g kg^{-1})	651.8±5.3^{a}	654.5±3.1^{a}	637.9±5.5^{b}	624.7±10.9^{c}	648.7±9.0^{ab}	642.3±5.7^{ab}	637.6±7.3^{b}
Crude fat (g kg^{-1})							
on a dry basis	187.5±19.1^{a}	161.1±12.3^{b}	200.9±13.4^{c}	256.7±51.0^{d}	241.0±22.3^{d}	224.2±7.9^{cd}	172.0±15.4^{ab}
on a wet basis	65.3±6.9^{a}	55.7±4.7^{b}	72.8±5.3^{a}	98.5±22.2^{c}	85.4±10.5^{c}	80.3±3.7^{c}	62.5±6.3^{ab}
Collagen (g kg^{-1})							
Total collagen							
on a non-fat (dry basis)	14.8±0.7^{a}	15.0±1.1^{a}	16.7±0.9^{b}	20.9±4.1^{c}	14.1±1.4^{a}	16.2±1.0^{b}	12.4±0.7^{d}
on a dry basis	12.0±0.4^{a}	12.5±0.8^{a}	13.3±0.6^{b}	14.7±2.0^{b}	10.6±0.9^{c}	12.6±0.6^{a}	10.3±0.5^{c}
on a wet basis	4.2±0.2^{a}	4.3±0.3^{a}	4.8±0.3^{ab}	5.6±0.9^{b}	3.7±0.4^{c}	4.5±0.3^{a}	3.7±0.2^{c}
Insoluble collagen							
on a non-fat (dry basis)	13.7±0.6^{a}	13.4±0.3^{a}	14.6±0.8^{ab}	15.6±3.3^{b}	13.9±1.0^{a}	14.4±0.5^{ab}	12.5±0.5^{c}
on a dry basis	11.1±0.6	11.2±0.4	11.7±0.8	11.0±1.5	10.6±0.7	11.1±0.4	10.3±0.3
on a wet basis	3.9±0.2^{ab}	3.9±0.1^{ab}	4.2±0.2^{a}	4.2±0.7^{a}	3.7±0.3^{b}	4.0±0.1^{ab}	3.7±0.2^{b}
Fiber diameter (μm)	30.8±1.3^{a}	28.6±0.7^{b}	29.0±0.8^{b}	31.4±0.6^{a}	30.5±0.6^{a}	28.8±0.8^{b}	27.6±0.5^{c}
Perimysial thickness (μm)	53.8±12.6^{ab}	40.4±8.9^{a}	41.3±8.2^{a}	64.7±15.0^{b}	50.0±8.1^{ab}	48.9±9.8^{a}	57.3±8.8^{b}

$^{a, b, c, d, e, f}$ Means with different superscript letters differ ($p<0.05$).

Table 4. WBSF, fiber diameter, perimysial thickness and chemical composition for beef steaks within USDA Modest-to-moderate marbling group (means±SE, n = 5 each)

Characteristics	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7
WBSF (Newton)	27.7±0.9^{a}	56.9±8.3^{b}	33.5±4.1^{c}	25.4±4.8^{a}	53.9±8.1^{b}	42.5±3.3^{d}	43.0±3.3^{d}
Moisture (g kg^{-1})	663.8±5.7^{a}	594.6±9.7^{b}	533.7±30.4^{c}	621.0±16.9^{d}	616.3±19.5^{d}	562.6±23.8^{bc}	643.0±6.6^{e}
Crude fat (g kg^{-1})							
on a dry basis	187.5±19.1^{a}	412.1±41.8^{b}	515.8±58.1^{c}	378.8±50.9^{b}	308.4±30.1^{d}	439.2±67.9^{b}	254.7±14.0^{e}
on a wet basis	62.6±7.6^{a}	167.8±19.2^{b}	237.0±41.9^{c}	146.9±25.9^{b}	120.4±17.9^{c}	198.4±39.8^{c}	91.0±5.6^{d}
Collagen (g kg^{-1})							
Total collagen							
on a non-fat (dry basis)	16.7±2.1^{a}	15.9±0.5^{a}	22.0±2.9^{b}	16.9±1.6^{a}	17.0±1.5^{a}	20.9±3.2^{b}	17.4±0.4^{a}
on a dry basis	13.5±1.5^{a}	9.4±0.7^{b}	10.0±0.7^{b}	10.3±1.0^{b}	11.6±0.6^{ab}	11.6±1.9^{ab}	13.0±0.3^{a}
on a wet basis	4.6±0.6^{a}	3.8±0.3^{b}	4.5±0.4^{a}	3.9±0.3^{b}	4.5±0.4^{a}	5.0±0.8^{c}	4.6±0.1^{a}
Insoluble collagen							
on a non-fat (dry basis)	13.7±0.4^{a}	14.0±0.7^{a}	20.8±2.9^{b}	14.8±1.3^{a}	15.2±2.0^{a}	17.6±2.0^{c}	15.5±0.9^{a}
on a dry basis	11.1±0.2a	8.2±0.7b	9.5±0.7c	9.0±0.7bc	10.3±0.9^{cd}	9.8±1.5c	11.5±0.6d
on a wet basis	3.7±0.1^{ab}	3.3±0.2^{a}	4.2±0.4^{b}	3.4±0.2^{a}	4.0±0.6^{b}	4.2±0.6^{b}	4.1±0.2^{b}
Fiber diameter (μm)	25.9±0.6^{a}	29.3±0.9^{b}	28.9±1.7^{b}	36.2±3.4^{c}	28.9±1.1^{b}	33.0±1.5^{d}	27.8±1.0^{e}
Perimysial thickness (μm)	31.5±1.4^{a}	23.8±1.3^{b}	19.2±3.0^{c}	22.3±1.1^{b}	27.4±4.2^{de}	29.6±1.2^{d}	26.1±2.5^{e}

$^{a, b, c, d, e}$ Means with different superscript letters differ ($p<0.05$).

muscles. But a larger decline in fiber diameter occurred for the Modest-to-Moderate and Abundant marbled muscles than for muscles with Slight-to-Small level of marbling (33.31%, 31.58% vs. 28.61%). Nevertheless, muscles in the Slight-to-Small and the Modest-to-Moderate groups had a larger decrease in sarcomere length than those in the Abundant group (24.27%, 23.79% vs. 20.00%). This is attributed to the protection of intramusular fat against the denaturation of myofibrillar protein. The differences in the fiber diameter and sarcomere length among raw muscles from the three marbling groups were eliminated by cooking.

The mean perimysial thickness of cooked muscles from the Slight-to-Small group was double to those from the Modest-to-Moderate and Abundant groups, which seems not to be derived from the quantities of intramuscular fat but from other sources such as cooking.

No panel testing data were obtained in the present study to support the viewpoint, accepted by many researchers (Tatum, Smith and Carpenter, 1982; Obuz et al., 2004), that marbling is an important contributor to the improvement of beef palatability traits (myofibrillar tenderness, connective tissue amount, flavor intensity and overall tenderness). However, the objective measurements showed that marbling substantially improved ($p<0.05$) the tenderness of beef LM muscles, evaluated by the WBSF method. This agrees with previous studies (McBee and Wiles, 1967; Luchak et al., 1998; Fiems et al., 2000; McKenna et al., 2004) and it is seen more clearly from the study of Wheeler, Cundiff and

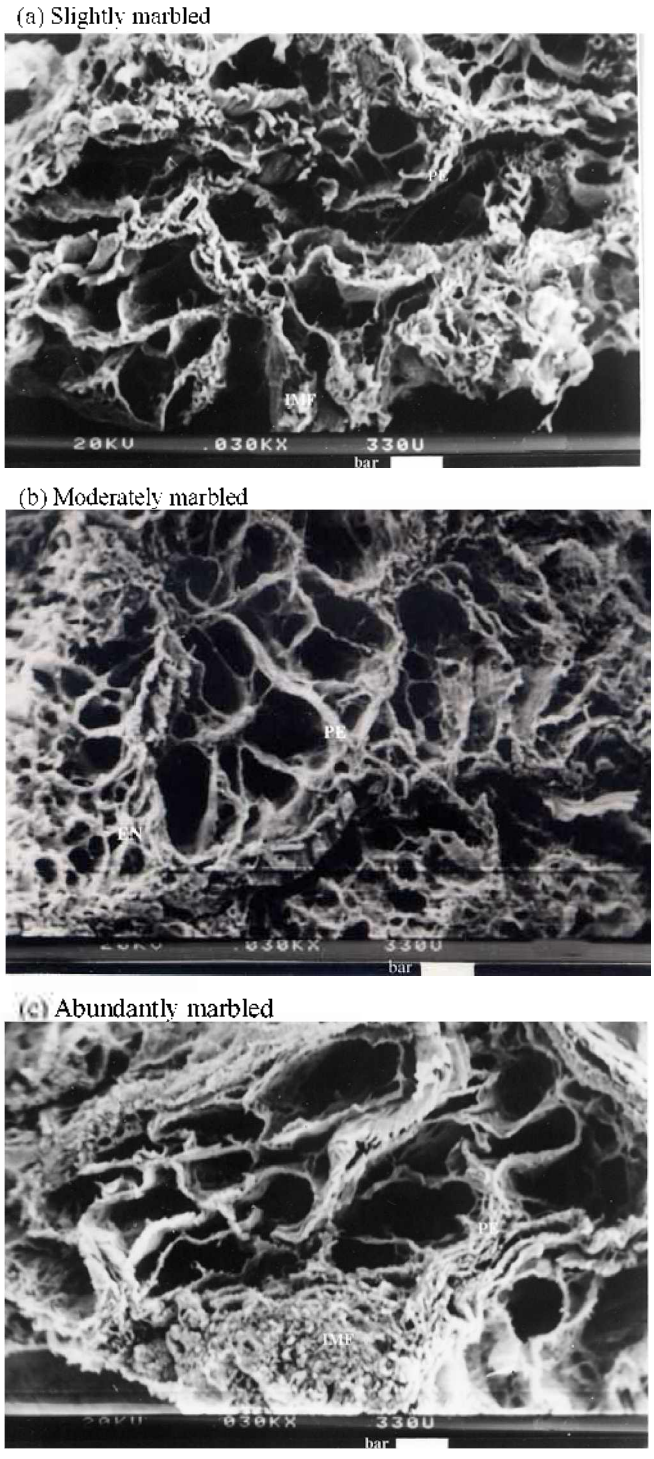


Figure 1. Microscopic structures of intramuscular connective tissue for slightly, moderately, and abundantly marbled beef *longissimus* muscle at a low magnification (30×).

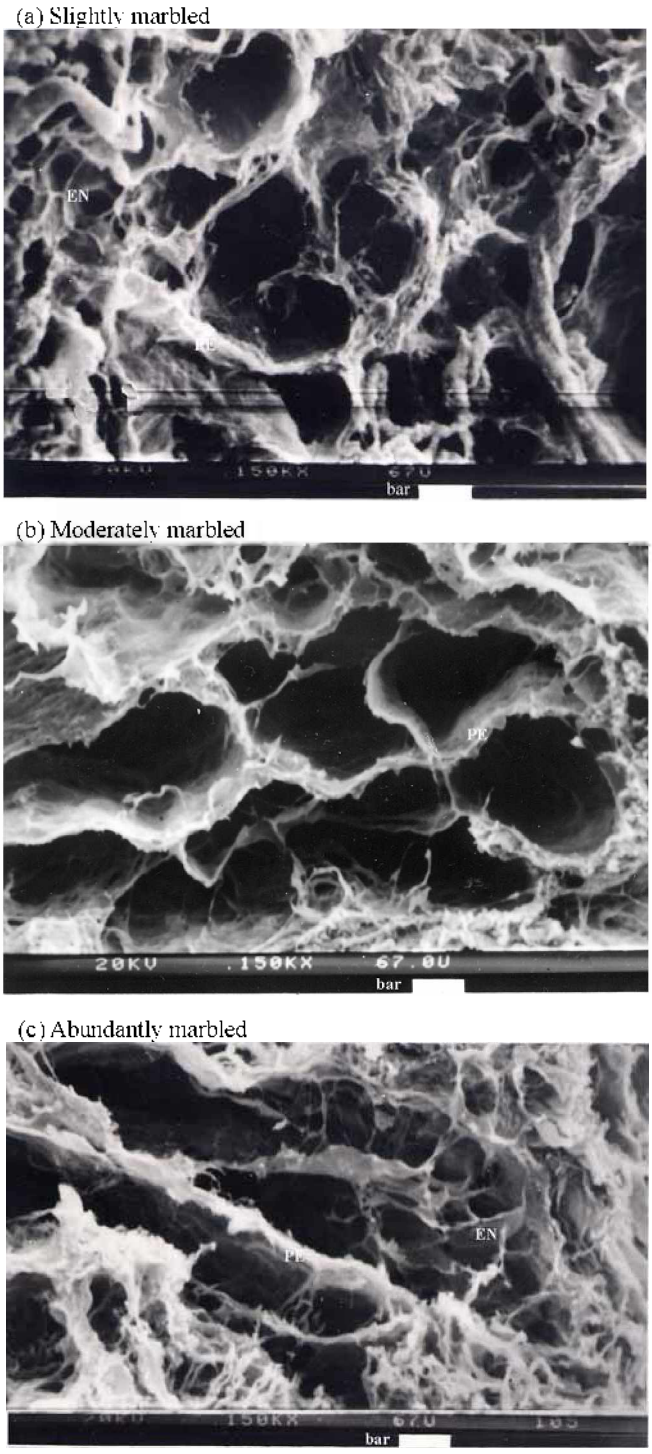
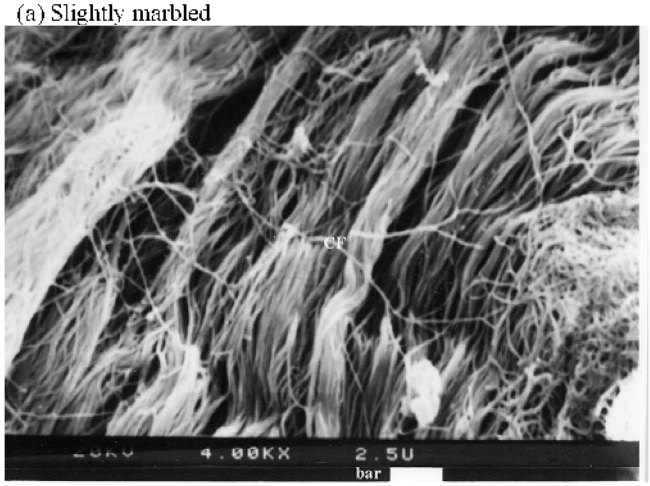


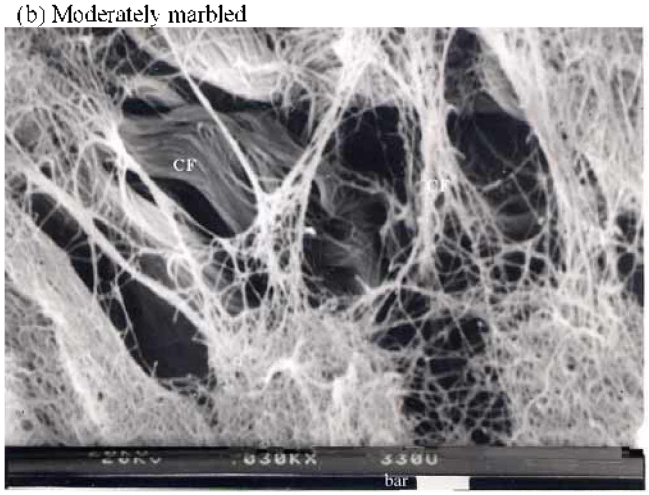
Figure 2. Microscopic structures of intramuscular connective tissue for slightly, moderately, and abundantly marbled beef *longissimus* muscle at a moderate magnification (150×)

Koch (1994) who reported that shear force value was not different among marbling scores ranging from U.S. Small through Moderate, but U.S. Slight marbling was higher in shear force value than Small through Modest marbling scores, and Traces marbling had a higher shear force than Slight marbling. However, the effect of marbling on WBSF was negligible in many studies (Romans et al., 1965; Kim and Lee, 2003; Riley et al., 2005). The lack of difference in WBSF values between muscles of different marbling levels in these studies could be due to tenderization of myofibrillar components with long-time aging (at least 14 d), which is further proven by Bratcher et al. (2005).

(a) Slightly marbled



(b) Moderately marbled



(c) Abundantly marbled

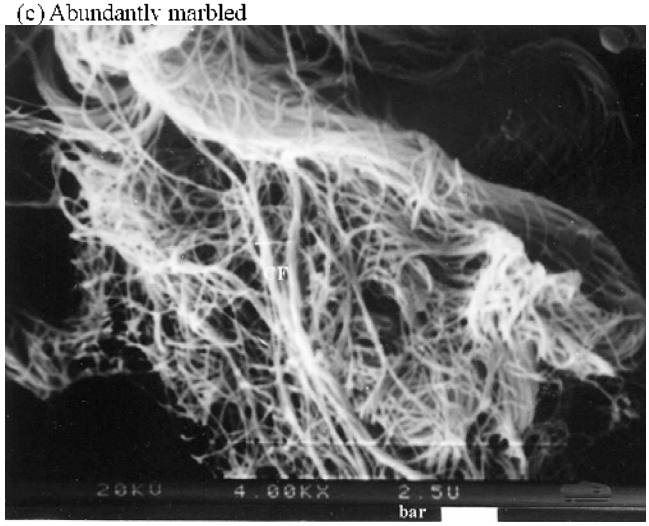


Figure 3. Microscopic structures of the perimysia for slightly, moderately, and abundantly marbled beef *longissimus* muscle at a high magnification (4,000×)

The variances among cores from the same steaks in WBSF, crude fat, total and insoluble collagen, and in the perimysial thickness were relatively high, but those of moisture content and fiber diameter were lower (Tables 3, 4 and 5). The means of WBSF, moisture, crude fat, perimysial thickness differed greatly ($p<0.05$) for individual steaks within the same marbling group (Tables 3, 4 and 5). This is supported by other studies (Swanson, Kline and Goll, 1965; Shackelford, Wheeler and Koohmaraie, 1997; Reuter, Wulf and Maddock, 2002; Denoyelle and Lebiham, 2004; Bratcher et al., 2005). Janz et al. (2005) further presented the differences in WBSF through the cross-section of *longissimus thoracis et lumborum* and concluded that the medial to lateral gradient was more pronounced than that observed in the superficial to deep cross-section.

Microscopic observations

At low and moderate magnifications (×30, ×150), all the endomysia and part of perimysia were destroyed during the NaOH maceration, but there seemed no significant microstructural differences for the intramuscular connective tissue between the three marbling levels (Figures 1 and 2). However, high magnification observations showed that the structures of the perimysia were seriously disorganized with increasing marbling level (Figure 3), which probably led to the decline of the mechanical strength of IMCT.

Correlation analyses

Table 6 presents correlation coefficients between WBSF, chemical composition, fiber diameter, and perimysial thickness of cooked meat according to the measurements of steaks.

The cooking losses correlated highly ($p<0.01$) with the amounts of moisture and crude fat, and the fiber diameter of raw meat, which indicates that the cooking losses are influenced by marbling levels, as well as the size of muscle fiber, accounting for the change in WBSF (Table 6). Collagen solubility correlated with total collagen content, but not with insoluble collagen.

WBSF correlated ($p<0.05$) with cooking losses, moisture content, crude fat content, and sarcomere length of raw muscle, and also with sarcomere length ($p<0.01$) of cooked meat. This is in accordance with some earlier studies (Wheeler et al., 1994; Fiems et al., 2000; Fortin et al., 2005) where a low but significant correlation was observed between marbling score and WBSF. Jones and Tatum (1994) accounted for 9% and 5.1% of the variation in WBSF and muscle fiber tenderness (after 10 d of aging), respectively, with marbling score. However, other studies (Jeremiah, 1996; Renand et al., 2001) did not found any relationship between marbling score and WBSF. As described above, the effect of marbling on WBSF of LM muscle was overcome by the tenderizing effect of postmortem aging. Although the traits of IMCT (collagen content and its solubility, mechanical strength, maximum transition temperature and microstructure) changed with

Table 5. WBSF, fiber diameter, perimysial thickness and chemical composition for beef steaks within USDA Abundant marbling group (means±SE, n = 5 each)

Characteristics	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7
WBSF (Newton)	37.66±6.03^{ab}	41.70±10.52^{ac}	31.04±5.72^{b}	32.40±3.73^{b}	39.76±2.37^{a}	39.32±2.59^{a}	45.74±3.86^{c}
Moisture (g kg^{-1})	639.6±10.9^{a}	592.2±8.2^{b}	595.3±5.3^{b}	566.0±11.9^{c}	594.5±21.7^{b}	609.4±11.1^{b}	573.7±10.3^{c}
Crude fat (g kg^{-1})							
on a dry basis	365.9±25.5^{a}	412.0±23.5^{b}	413.4±10.6^{b}	424.3±26.4^{bc}	421.5±53.9^{bc}	375.7±51.9^{a}	452.1±31.9^{c}
on a wet basis	132.4±11.8^{a}	168.4±11.4^{b}	167.5±6.3^{b}	185.3±15.9^{bc}	175.3±31.1^{bc}	148.4±23.2^{a}	193.9±18.4^{c}
Collagen (g kg^{-1})							
Total collagen							
on a non-fat (dry basis)	16.3±2.9^{ac}	18.4±1.6^{ab}	21.2±3.0^{b}	15.6±1.2^{c}	20.7±2.2^{b}	21.0±1.6^{b}	18.2±1.5^{ab}
on a dry basis	10.1±1.4^{ac}	10.8±0.9^{a}	12.5±2.0^{bc}	8.9±0.4^{c}	11.6±0.7^{b}	13.2±1.8^{c}	9.8±0.5^{a}
on a wet basis	3.7±0.6^{a}	4.4±0.3^{ab}	5.0±0.7^{b}	3.9±0.3^{a}	4.7±0.4^{ab}	5.1±0.7^{b}	4.2±0.2^{ab}
Insoluble collagen							
on a non-fat (dry basis)	11.8±0.6^{a}	16.5±0.8^{b}	16.6±1.3^{b}	14.7±0.9^{c}	17.2±0.8^{b}	18.3±1.9^{b}	15.2±1.1^{c}
on a dry basis	7.5±0.4^{a}	9.7±0.6^{b}	9.7±1.1^{b}	8.4±0.4^{ab}	9.8±0.7^{b}	11.5±1.7^{c}	8.2±0.3^{ab}
on a wet basis	2.7±0.1^{a}	3.9±0.2^{b}	3.9±0.4^{b}	3.6±0.2^{b}	3.9±0.2^{b}	4.5±0.7^{c}	3.5±0.2^{b}
Fiber diameter (μm)	33.3±0.4^{a}	32.8±1.1^{a}	29.5±0.4^{b}	32.6±1.4^{a}	28.2±0.5^{c}	29.0±0.3^{c}	30.1±0.5^{b}
Perimysial thickness (μm)	25.4±3.2^{a}	19.6±3.1^{b}	25.6±1.7^{b}	31.7±3.2^{c}	31.3±1.9^{c}	22.0±3.3^{b}	24.3±2.9^{a}

^{a,b,c} Means with different superscript letters differ ($p<0.05$).

Table 6. Correlation coefficients between cooking losses, WBSF, chemical composition, fiber diameter, and sarcomere length of whole beef *longissimus* steaks (n = 28) [1, 2, 3]

	WBSF	Moisture	CFD	CFW	TCNF	TCD	TCW	ICNF	ICD	ICW	CS	FD	SLR	SLC
CL	0.75**	0.73**	-0.79**	-0.76**	-0.10	0.24	-0.03	-0.17	0.23	-0.09	0.17	-0.69**	-0.21	-0.42*
WBSF		-0.52*	-0.54*	-0.53*	-0.13	0.13	-0.07	-0.20	0.08	-0.15	0.26	-0.34	-0.41*	-0.63**
Moisture			-0.92**	-0.98**	0.01	0.42*	0.05	-0.15	0.36	-0.09	0.22	-0.38	0.07	-0.24
CFD				0.98**	0.16	-0.28	0.06	0.25	-0.28	0.14	-0.09	0.49*	0.02	0.36
CFW					0.07	-0.37	-0.01	0.20	-0.33	0.10	-0.16	0.42*	-0.01	0.31
TCNF						0.90**	0.99**	0.86**	0.78**	0.83**	0.42*	0.28	-0.15	0.03
TCD							0.92**	0.70**	0.86**	0.72**	0.49*	0.08	-0.17	-0.16
TCW								0.84**	0.81**	0.85**	0.43*	0.22	-0.21	-0.06
ICNF									0.86**	0.98**	-0.08	0.40*	0.06	0.19
ICD										0.90**	-0.01	0.17	0.05	-0.01
ICW											-0.10	0.34	0.02	0.11
SC												-0.18	-0.48*	-0.36
FD													0.23	0.23
SLR														0.68**

[1] Except CL, WBSF and SLC (cooked steak measurement), all the other variables were based on raw steak measurement. Maximum transition of intramuscular connective tissue was not included because it did not correlate with other variables.

[2] CL: cooking losses, WBSF: Warner-Bratzler shear force; CFD/CFW: crude fat content on a dry/wet weight basis; TCNF/TCD/TCW: total collagen content on a nonfat/dry/wet weight basis; ICNF/ICD/ICW: insoluble collagen on a nonfat/dry/wet weight basis; CS: collagen solubility; FD: fiber diameter; SLR: sarcomere length of raw meat; SLC: sarcomere length of cooked meat.

[3] * $p<0.05$; ** $p<0.01$.

marbling level, there were no relationships between these changes and that of WBSF.

Table 7 lists correlation coefficients between WBSF, chemical composition, diameter of muscle fiber, and perimysial thickness of cooked meat according to the measurements of cores.

Significant correlations existed ($p<0.05$) between moisture, crude fat, total and insoluble collagen, fiber diameter, and perimysial thickness. WBSF of cores correlated ($p<0.05$) with the quantities of moisture, collagen and crude fat, and perimysial thickness of cores.

When WBSF was fitted against cooking losses, moisture, crude fat, traits of IMCT (total and insoluble collagen content, collagen solubility, and mechanical strength), fiber diameter and sarcomere length of raw and/or cooked steaks using factorial regression analysis, the stepwise independent variates selection procedure finally picked cooking losses and sarcomere length of cooked meat which accounted for 75% of total variation (Equation 1, Table 8, model 1).

$$\text{WBSF} = 9.16+0.26\times\text{cooking loss} -4.22\times\text{sarcomere length of cooked meat} \quad (R^2 = 0.75) \qquad (1)$$

Table 7. Correlation coefficients between WBSF, chemical composition, fiber diameter, and perimysial thickness of cores for beef steaks (n = 140) [1, 2]

	Moisture	CFD	CFW	TCNF	TCD	TCW	ICNF	ICD	ICW	FD	PT
WBSF	0.38*	-0.51**	-0.46**	0.25*	0.09	-0.06	0.29*	0.17	-0.03	-0.01	0.58**
Moisture		-0.87**	-0.93**	-0.54**	0.18	-0.24*	-0.55**	0.33*	-0.18	-0.21*	0.31**
CFD			0.98**	0.52**	-0.28*	0.10	0.51**	-0.48**	-0.03	0.29*	-0.48**
CFW				0.54**	-0.27*	0.13	0.54**	-0.45**	0.02	0.26*	-0.43**
TCNF					0.65**	0.88**	0.67**	0.16	0.46**	0.17	-0.14
TCD						0.91**	0.27*	0.59**	0.52**	-0.05	0.24*
TCW							0.51**	0.44**	0.60**	0.05	0.09
ICNF								0.49**	0.81**	0.08	-0.23*
ICD									0.86**	-0.20*	0.19
ICW										-0.08	0.02
FD											0.06

[1] CFD/W: Crude fat content on a dry/wet weight basis; TCNF/D/W: total collagen content on a nonfat/dry/wet weight basis; ICNF/D/W: insoluble collagen content on a nonfat/dry/wet weight basis; FD: fiber diameter; PT: perimysial thickness.
[2] * $p<0.05$; ** $p<0.01$.

Table 8. Stepwise selection regression analyses for WBSF

Step	Variable entered	Partial R-square	Model R-square	F value	Pr>F
Model 1: based on whole steak measurement					
1	Cooking loss	0.66	0. 66	34.69	<0.01
2	Sarcomere length of cooked meat	0.09	0.75	6.06	0.02
Model 2: based on core measurement					
1	Perimysial thickness	0.30	0.30	40.11	<0.01
2	Fat content on a dry basis	0.11	0.41	12.10	<0.01

However, when WBSF was fitted against the quantities of moisture, crude fat, total and insoluble collagen, solubility of collagen, fiber diameter and perimysial thickness of cores using factorial regression analysis, the stepwise independent variates selection procedure finally picked percent crude fat and the perimysial thickness, which accounted for 41% of total variation (Equation 2, Table 8, model 2).

$$\text{WBSF} = 6.64 - 0.22 \times \text{crude fat on a dry basis} + 0.04 \times \text{perimysial thickness} \quad (R^2 = 0.41) \qquad (2)$$

According to the models (1) and (2), both the natures of myofibrillar components and intramuscular connective tissue are important contributors to beef marbling-related tenderness. Meanwhile, beef tenderness is only related to the measurements of cooked meat, but not to the nature of raw meat, which agrees with Denoyelle and Lebihan (2004) who concluded that compression measurements on raw meat are not suitable parameters for meat tenderness as perceived by consumers.

IMPLICATIONS

In this study, all of WBSF, moisture content, crude fat content, collagen content and its solubility, mechanical strength of intramuscular connective tissue, perimysial thickness, fiber diameter, sarcomere length and T_{max} of LM muscle were observed to change with advancing marbling score. The development of marbling results in a decline in cooking losses, the avoidance of sarcomere shortening, and the disorganization of the perimysia, which accounts for the improvement of beef tenderness.

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REFERENCES

Bergman, I. and R. Loxley. 1963. Two improved and simplified methods for spectrophotometric determination of hydroxyproline. Analyt. Chem. 35:1961-1965.

Bratcher, C. L., D. D. Johnson, R. C. Littell and B. L. Gwartney. 2005. The effects of quality grade, aging, and location within muscle on Warner-Bratzler shear force in beef muscles of locomotion. Meat Sci. 70:279-284.

Breidenstein, B. B., C. C. Cooper, R. G. Cassens, G. Evans and R. W. Bray. 1968. Influence of marbling and maturity on the palatability of beef muscle. I. Chemical and organoleptic considerations. J. Anim. Sci. 27:1532-1541.

Brooks, J. C., J. B. Belew, D. B. Griffin, B. L. Gwartney, D. S. Hale, W. R Henning, D. D. Johnson, J. B. Morgan, F. C.

Parrish, J. O Reagan and J. W. Savell. 2000. National beef tenderness survey-1998. J. Anim. Sci. 78:1852-1860.

China's Ministry of Agriculture (CMA). 2003. Beef quality grading (NY/T676-2003). China's Standard Publishing, Beijing, China.

Cooper, C. C., B. B. Breidenstein, R. G. Cassens, G. Evans and R. W. Bray. 1968. Influence of marbling and maturity muscle. II. Histological considerations. J. Anim. Sci. 27:1542-1546.

Cross, H. R., R. L. West and T. R. Dutson. 1981. Comparison of methods for measuring sarcomere length in beef *semitendinosus* muscle. Meat Sci. 5:261-266.

Denoyelle, C. and E. Lebihan. 2004. Intramuscular variation in beef tenderness. Meat Sci. 66:241-247.

Fiems, L. O., E. De Campeneere, S. De Smet, G. Van de Voorde, J. M. Vanacker and C. V. Boucque. 2000. Relationship between fat depots in carcasses of beef bulls and effect on meat color and tenderness. Meat Sci. 56:41-47.

Flint, F. O. and K. Pickering. 1984. Demonstration of collagen in meat products by an improved picro-sirius red polarization method. Analyst. 109:1505-1508.

Fortin, A., W. M. Robertson and A. K. W. Tong. 2005. The eating quality of Canadian pork and its relationship with intramuscular fat. Meat Sci. 69:297-305.

Hedrick, H. B., G. F. Krause, M. R. Ellersieck, R. J. Epley, J. C. Riley and G. B. Thompson. 1981. Beef carcass composition as influenced by yield and quality grade. J. Anim. Sci. 53:102-106.

Hill, F. 1966. The solubility of intramuscular collagen in meat animals of various ages. J. Food Sci. 31:161-166.

Janz, J. A. M., J. L. Aalhus, M. E. R. Dugan and M. A. Price. 2006. A mapping method for the description of Warner-Bratzler shear force gradients in beef *longissimus thoracis et lumborum* and *semitendinosus*. Meat Sci. 72:79-90.

Jeremiah, L. E. 1996. The influence of subcutaneous fat thickness and marbling on beef. Palatability and consumer acceptability. Food Res. Int. 29:513-520.

Jones, B. K. and J. D. Tatum. 1994. Predictors of beef tenderness among carcasses produced under commercial conditions. J. Anim. Sci. 72:1492-1501.

Kim, C. J. and E. S. Lee. 2003. Effects of quality grade on the chemical, physical and sensory characteristics of Hanwoo (Korean native cattle) beef. Meat Sci. 63:397-405.

Light, N. and A. E. Champion. 1984. Characterization of muscle epimysium perimysium and endomysium collagens. Biochem. J. 219:1017-1026.

Luchak, G. L., R. K. Miller, K. E. Belk, D. S. Hale, S. A. Michaelsen, D. D. Johnson, R. L. West, F. W. Leak, H. R. Cross and J. W. Savell. 1998. Determination of sensory, chemical and cooking characteristics of retail beef cuts differing in intramuscular and external fat. Meat Sci. 50:55-72.

McBee, J. L. and J. A. Wiles. 1967. Influence of marbling and carcass grade on the physical and chemical characteristics of beef. J. Anim. Sci. 26:701-704.

McKenna, D. R., C. L. Lorenzen, K. D. Pollok, W. W. Morgan, W. L. Mies, J. J. Harris, R. Murphy, M. McAdams, D. S. Hale and J. W. Savell. 2004. Interrelationships of breed type, USDA quality grade, cooking method, and degree of doneness on consumer evaluations of beef in Dallas and San Antonio, Texas, USA. Meat Sci. 66:399-406.

Nishimura, T., A. Hattori and K. Takahashi. 1999. Structural changes in intramuscular connective tissue during the fattening of Japanese Black Cattle, effect of marbling on beef tenderization. J. Anim. Sci. 77:93-104.

Obuz, E., M. E. Dikeman, J. P Grobbel, J. W. Stephens and T. M. Loughin. 2004. Beef *longissimus lumborum*, *biceps femoris*, and *deep pectoralis* Warner-Bratzler shear force is affected differently by endpoint temperature, cooking method, and USDA quality grade. Meat Sci. 68:243-248.

Ozawa, S., T. Mitsuhashi, M. Mitsumoto, S. Matsumoto, N. Itoh, K. Itagaki, Y. Kohno and T. Dohgo. 2000. The characteristics of muscle fiber types of *longissimus thoracis* muscle and their influences on the quantity and quality of meat from Japanese Black steers. Meat Sci. 54:65-70.

Parrish, F. C., D. G. Olson, B. E. Miner and R. E. Rust. 1973. Effect of degree of marbling and internal temperature of doneness on beef rib steaks. J. Anim. Sci. 37:430-434.

Renand, G., B. Picard, C. Touraille, P. Berge and J. Lepetit. 2001. Relationship between muscle characteristics and meat quality traits of young Charolais bulls. Meat Sci. 59:49-60.

Reuter, B. J., D. M. Wulf and R. J. Maddock. 2002. Mapping intramuscular tenderness variation in four major muscles of the beef round. J. Anim. Sci. 80:2594-2599.

Riley, D. G., D. D. Johnson, C. C. Chase, R. L. West, S. W. Coleman, T. A. Olson and A. C. Hammond. 2005. Factors influencing tenderness in steaks from Brahman cattle. Meat Sci. 70:347-356

Romans, J. R., H. J. Tuma and W. L. Tucker. 1965. Influence of carcass maturity and marbling on the physical and chemical characteristics of beef. I. Palatability, fiber diameter and proximate analysis. J. Anim. Sci. 24:681-685.

Savell, J. W., H. R. Cross and G. C. Smith. 1986. Percentage ether extractable fat and moisture content of beef *longissimus* muscle as related to USDA marbling score. J. Food Sci. 51:838, 840.

Savell, J. W., R. E. Branson, H. R. Cross, D. M. Stiffler, J. W. Wise, D. B. Griffin and G. C. Smith. 1987. National consumer retail beef study: palatability evaluations of beef loin steaks that differed in marbling. J. Food Sci. 52:517-519.

Savell, J. W., S. L. Mueller and B. E. Baird. 2005. The chilling of carcasses. Meat Sci. 70:449-459.

Shackelford, S. D., T. L. Wheeler and M. Koohmaraie. 1997. Repeatability of tenderness measurements in beef round muscles. J. Anim. Sci. 75:2411-2416.

Swanson, L. A., E. A. Kline and D. E. Goll. 1965. Variability of muscle fiber size in bovine *longissimus dorsi*. J. Anim. Sci. 24:97-101.

Tatum, J. D., G. C. Smith and Z. L. Carpenter. 1982. Interrelationships between marbling, subcutaneous fat thickness and cooked beef palatability. J. Anim. Sci. 54:777-784.

USDA. 1997. United States standards for grades of carcass beef. Agriculture Marketing Service, USDA, Washington, DC.

Von Seggern, D. D., C. R. Calkins, D. D. Johnson, J. E. Brickler, and B. L. Gwartney. 2005. Muscle profiling: characterizing the muscles of the beef chuck and round. Meat Sci. 71:39-51.

Wheeler, T. L., L. V. Cundiff and R. M. Koch. 1994. Effects of marbling degree on beef palatability in *Bos taurus* and *Bos indicus* cattle. J. Anim. Sci. 72:3145-3515.