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Correlation of Marbling Characteristics with Meat Quality and Histochemical Characteristics in *Longissimus Thoracis* Muscle from Hanwoo Steers

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Abstract The objective of this study was to investigate the correlations of marbling characteristics, including marbling score, intramuscular fat (IMF) content, and fleck traits, with meat quality traits and histochemical characteristics of the *longissimus thoracis* muscle from Hanwoo steers. Marbling fleck characteristics, especially area, number, and fineness (F) index, measured by computerized image analysis were strongly correlated with marbling score and IMF content ($p < 0.05$). However, coarseness (C) index and F/C ratio were somewhat limited relationships with marbling score. In contrast, the IMF content and the number of smaller white flecks increased with increasing lightness value ($p < 0.05$). Moreover, beef with higher marbling scores showed lower cooking loss and Warner-Bratzler shear force value compared to beef with lower marbling scores ($p < 0.05$). Regarding the muscle bundle traits, as number of bundle increased, number of marbling flecks increased ($p < 0.05$), although most marbling characteristics did not have significant correlation with muscle fiber or bundle characteristics.

Keywords marbling fleck, meat quality, muscle fiber, muscle bundle, Hanwoo

Introduction

The degree of marbling is generally considered a contributing factor to the sensory quality, especially tenderness, of cooked meat (Lee et al., 2018a; Nishimura, 2010). Consumers tend to believe that heavily-marbled beef is more palatable than lightly-marbled beef (Hunt et al., 2014; Lee et al., 2018b; Motoyama et al., 2016). In addition, various characteristics of marbling fleck, as measured by computer image analysis, are also associated with the palatability of cooked beef (Konarska et al., 2017). A previous study reported that highly-marbled steaks with coarser marbling flecks exhibited a lower score of overall acceptability, although were more tender compared to highly-marbled steaks with evenly-distributed marbling flecks. In contrast, quality traits of fresh meat, including water holding capacity (WHC) and meat color, can vary depending on intramuscular fat (IMF) content (Lee et al., 2018a; Nam et al., 2009). For instance,

Nam et al. (2009) reported that, as IMF content increased, drip and cooking losses decreased in the porcine *longissimus dorsi* muscle. Bovine muscle with a higher marbling score has been associated with higher lightness compared to muscle with a lower marbling score in Hanwoo steer (Lee et al., 2018a). However, there is limited information about the relationships between marbling fleck characteristics and meat quality traits in Hanwoo steer.

Muscle fibers are classified into various fiber types according to their diverse characteristics, including morphological, structural, contractile, and metabolic properties (Choi and Kim, 2009). These characteristics of muscle fiber have an impact on postmortem conversion of muscle to meat and therefore can contribute to variation in meat quality characteristics and palatability in poultry (Choi et al., 2016), pork (Choi et al., 2013; Ryu and Kim, 2005), and beef (Klont et al., 1998). In addition, muscle bundle, which is group of muscle fibers surrounded by the perimysium, can influence the organoleptic characteristics of cooked beef (Purslow, 1985). Lee et al. (2018a) reported that muscle bundle size and fiber number per each bundle could influence the surface texture features, including texture and firmness, of bovine *longissimus thoracis* muscle and thus affects the sensory quality traits determined by trained panelists. Moreover, muscle bundle characteristics are associated with IMF content and marbling fleck characteristics, as marbling flecks are located between muscle bundle (Fortin et al., 2005). Thus, Fortin et al. (2005) suggested that a smaller marbling fleck (frost-like) could be associated with a smaller bundle size. However, the correlation between muscle bundle and marbling fleck characteristics have not been extensively elucidated. Therefore, the aim of this study was to use the spearman correlation coefficients in order to investigate the correlation of marbling characteristics with meat quality traits and histochemical characteristics, especially bundle characteristics, of the *longissimus thoracis* muscle from Hanwoo steers.

Materials and Methods

Animals and muscle samples

A total of 65 loin cuts from Hanwoo steers (aged 27–32 months, mean carcass weight of 458.4±68.3 kg) were used in this study. Hanwoo steers were transported and then slaughtered at the same slaughterhouse in three batches (20, 20, and 25 cattle per day). At 45 min postmortem, samples were taken from the *longissimus thoracis* muscle at the 13th thoracic vertebrae (carcass grading site) for histochemical analysis, and muscle pH was directly measured at the same position. After 24 h of chilling at 4°C, carcasses were classified according to the carcass grading standard of the Korea Institute of Animal Products Quality Evaluation (KAPE, 2017). The KAPE provided grades for beef marbling standard (BMS) score. Muscle surface images for marbling fleck analysis were taken with a mirror-type digital camera (D3200; Nikon Co., Japan) at the carcass grading site. A strobe (Nikon Co.) was used to prevent irregular reflection on the muscle surface. After images were captured, muscle samples were dissected between the 13th to 9th thoracic vertebrae for the measurements of IMF contents and meat quality characteristics. The Soxhlet method was performed in order to determine the percentage of IMF (AOAC, 2012).

Image analysis of marbling fleck characteristics

Digital images of beef loins obtained from 65 Hanwoo steers were used for image analysis of marbling fleck characteristics. Image analysis was performed using the Beef Analyzer G software (Hayasaka Ricoh Co. Ltd., Japan) according to previously reported protocol (Kuchida et al., 2006). To measure marbling fleck characteristics, digital color images were first converted into binary images. After binarization, marbling fleck distribution, including the number and area of fleck, was clearly visible. The number of marbling flecks on the loin-eye area was counted. However, the smallest particles

(<0.01 cm²) were not included in the calculation of marbling fleck number. The total marbling area, consisting of all the marbling flecks, was measured and the marbling area percentage was calculated as the total marbling area divided by the muscle surface area at the 13th rib cross-section. In order to calculate the marbling fleck index, marbling particles were then separated into two groups with bigger (>0.5 cm²) and smaller (0.01 to 0.5 cm²) marbling flecks (Konarska et al., 2017; Kuchida et al., 2006). Coarseness index (C) was calculated as the total area of the bigger marbling flecks divided by the total marbling area. Fineness index (F) was calculated as the number of small particles (0.01 to 0.5 cm²) per loin-eye area (cm²) and the F/C ratio was then calculated (Konarska et al., 2017; Kuchida et al., 2006; Lee et al., 2018b).

Meat quality measurements

Muscle pH at 45 min (pH_{45 min}) and 24 h (pH_{24 h}) postmortem was measured directly on the *longissimus thoracis* muscle using spear-type pH meter (IQ-150 pH meter with PH77-SS probe, IQ Scientific Instrument, USA). Meat lightness was measured at 24 h postmortem using a Minolta chromameter (CR-400; Minolta Camera Co., Japan). Lightness value (L*) was expressed according to the recommendations of the Commission Internationale de l'Eclairage (CIE, 1978). To determine WHC, drip loss, filter-paper fluid uptake (FFU), and cooking loss were measured according to previously published procedures (Honikel, 1998; Kauffman et al., 1986). Sample preparation for Warner-Bratzler shear force (WBS) analysis was similar to preparation of the cooking loss method. Beef samples were cut in a 4°C cold room (approximately 80 g of initial weight), and then placed in a thin polyethylene bag and placed in a continuously heated water-bath at 80°C until the internal temperature of the beef sample reached 71°C. After cooking, meat samples were immediately transferred to an ice-slurry until equilibration. More than six cores (1.27 cm diameter) were used for WBS analysis. WBS was measured using an Instron Universal Testing Machine (Model 1011; Instron Corp., USA) with a WBS device (cross-head speed 200 mm/min; AMSA, 1995).

Histochemical characteristics

Serial muscle cross-sections were obtained using a cryostat (CM1860, Leica, Germany) at -20°C. The myofibrillar ATPase method with acid-preincubation (pH 4.3) was used to assess muscle fiber characteristics (Fig. 1A), and muscle fibers were classified as type I, IIA, or IIX according to their contractile and metabolic characteristics (Brooke and Kaiser, 1970; Lind and Kernell, 1991). The area and number percentages of each fiber type were calculated. To evaluate the characteristics of

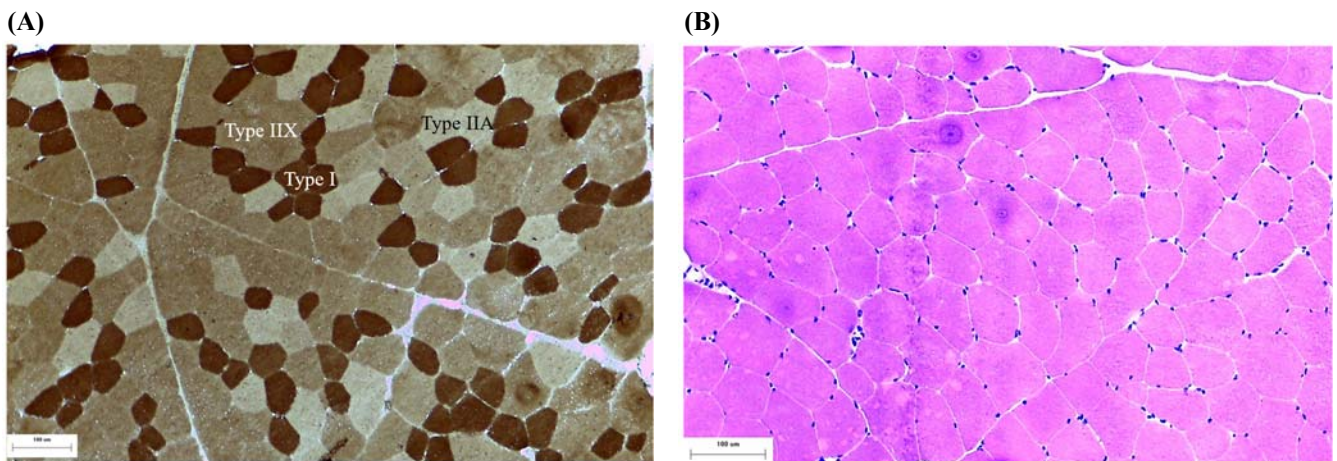


Fig. 1. Schematic view of muscle fiber and bundle in Hanwoo *longissimus thoracis* muscle. A, myosin ATPase staining (pH 4.3); B, hematoxylin and eosin staining. Scale bar=100 µm.

muscle bundle, each muscle section was stained with hematoxylin and eosin staining (Fig. 1B) (Cardiff et al., 2014). Muscle bundle area and fiber number per bundle were calculated at 40× magnification (Albrecht et al., 2006), and at least 30 bundles per each sample were used for the determination of these characteristics. The total number of bundle was calculated by dividing the loin-eye area without marbling area by the mean bundle area of each sample.

Statistical analysis

Marbling characteristics, including IMF content, marbling score, and fleck characteristics data were analyzed using SAS software (2014) to calculate means, standard deviation, minimum, and maximum. Spearman correlation coefficients were calculated using the PROC CORR procedure in SAS software (2014) to determine the correlation of marbling characteristics with meat quality and histochemical characteristics. Principal component analysis (PCA) was performed based on the correlation matrix using the PRINCOMP procedure in SAS software (2014).

Results

Relationship between marbling and fleck characteristics

Table 1 shows the means, standard deviations, and overall ranges for marbling characteristics including marbling score, IMF content, and marbling fleck characteristics of *longissimus thoracis* muscle from Hanwoo steers. Broad ranges were observed for IMF content, as BMS grades of muscle samples were from 2 to 9. IMF content of Hanwoo steers from BMS 2 to 9 grades ranged from 5.69% to 29.3%. Due to these reasons, large variations were also detected in the characteristics of marbling fleck, especially fleck number and area.

The correlations between marbling characteristics are presented in Table 2. Marbling score and IMF content tended to be related to marbling fleck characteristics. Both marbling score and IMF content were strongly correlated with marbling area ($r=0.80$ and 0.88 , respectively), marbling area ratio ($r=0.87$ and 0.67 , respectively), and fineness ($r=0.76$ and 0.60 , respectively). Marbling area and marbling area ratio were positively correlated with coarseness ($r=0.31$ and 0.26 , respectively) and fineness ($r=0.43$ and 0.60 , respectively), and were negatively correlated with marbling fleck number ($r=-0.27$ and -0.44 , respectively). However, no correlation was observed between F/C ratio and IMF content.

Table 1. Marbling characteristics, including marbling score, intramuscular fat (IMF) content, and marbling fleck characteristics, of Hanwoo *longissimus thoracis* muscle

	Mean	Standard deviation	Minimum	Maximum
Marbling score	5.28	1.81	2.00	9.00
IMF content (%)	14.8	5.03	5.69	29.3
Marbling fleck characteristics				
Fleck number	2,762	740.7	474.0	4,209
Marbling area (cm ²)	24.4	6.40	13.7	48.1
Marbling area ratio (%)	24.5	5.13	16.4	40.8
Coarseness (C)	0.13	0.05	0.03	0.25
Fineness (F)	2.26	0.60	1.10	4.08
F/C ratio	20.2	12.0	5.62	60.7

Table 2. Correlation coefficients (r) of marbling characteristics of the *longissimus thoracis* muscle from Hanwoo steers

Variables	IMF content	Marbling fleck characteristics					
		Fleck number	Marbling area	Marbling area ratio	Coarseness (C)	Fineness (F)	F/C ratio
Marbling score	0.87***	-0.30*	0.80***	0.87***	0.23	0.76***	0.11
IMF content		-0.44***	0.88***	0.68***	0.26*	0.60***	0.04
Marbling fleck characteristics							
Fleck number			-0.27*	-0.44***	-0.49***	-0.04	0.31*
Marbling area				0.88***	0.31*	0.43***	-0.08
Marbling area ratio					0.26*	0.60***	0.04
Coarseness (C)						-0.21	-0.78***
Fineness (F)							0.56***

* p<0.05; *** p<0.001.
IMF, intramuscular fat.

Marbling characteristics and meat quality traits

Table 3 presents the correlations between marbling characteristics and meat quality traits of Hanwoo steer. No significant correlations were observed between age at slaughter and marbling fleck characteristics. There was significantly positive relationship between marbling score and lightness ($r=0.36$, $p<0.01$), and marbling score was negatively correlated with cooking loss ($r=-0.29$, $p<0.05$) and WBS value ($r=-0.34$, $p<0.01$). IMF content showed a positive relationship with lightness ($r=0.38$, $p<0.01$) and negative relationships with cooking loss ($r=-0.31$, $p<0.05$) and WBS value ($r=-0.28$, $p<0.05$). For marbling fleck characteristics, marbling fleck number was negatively correlated with muscle pH at 24 h postmortem ($r=-0.27$, $p<0.05$). As marbling area increased, the WBS value decreased ($r=-0.27$, $p<0.05$). The percentage of marbling area had positive correlation with lightness ($r=0.38$, $p<0.01$) and negative correlation with cooking loss ($r=-0.31$, $p<0.05$). Fineness index exhibited a positive correlation with lightness ($r=0.36$, $p<0.01$), but a negative correlation with cooking loss ($r=-0.30$, $p<0.05$).

Table 3. Correlation coefficients (r) of marbling characteristics and meat quality traits of the *longissimus thoracis* muscle from Hanwoo steers

Variables	Marbling score	IMF content	Marbling fleck characteristics					
			Fleck number	Marbling area	Marbling area ratio	Coarseness (C)	Fineness (F)	F/C ratio
Age at slaughter	-0.09	0.08	-0.09	0.06	0.08	0.02	-0.10	-0.06
pH _{45 min}	-0.15	0.05	-0.04	0.09	0.05	0.15	-0.13	-0.15
pH _{24 h}	0.04	0.15	-0.27*	0.11	0.15	0.08	-0.02	-0.02
Lightness	0.36**	0.38**	-0.03	0.22	0.38**	0.05	0.36**	0.10
Drip loss	-0.08	0.11	0.12	0.01	0.11	0.13	0.03	-0.11
FFU	-0.02	-0.14	-0.02	-0.04	-0.14	0.09	-0.12	-0.02
Cooking loss	-0.29*	-0.31*	0.09	-0.22	-0.31*	-0.03	-0.30*	-0.01
WBS	-0.34**	-0.28*	-0.03	-0.27*	-0.23	0.01	-0.22	-0.01

* p<0.05; ** p<0.01.
IMF, intramuscular fat; FFU, filter-paper fluid uptake; WBS, Warner-Bratzler shear force.

Marbling characteristics and histochemical characteristics

The correlation coefficients between marbling characteristics and histochemical characteristics are presented in Table 4. Marbling score and IMF content did not have a significant correlation with muscle fiber and bundle characteristics. Additionally, marbling fleck number did not correlate with muscle fiber characteristics, whereas positively correlated with total bundle number ($r=0.26$, $p<0.05$). Coarseness was positively correlated with the number percentage of type I fiber ($r=0.26$, $p<0.05$) and negatively correlated with the number percentage of type IIA fiber ($r=-0.28$, $p<0.05$). Fineness and F/C ratio were negatively correlated with the area ($r=-0.33$ and -0.34 , respectively) and number ($r=-0.26$ and -0.37 , respectively) percentage of type I fiber.

Principal component analysis

PCA was performed to reduce the number of variables and to clearly understand the correlation between the variables, including marbling characteristics, meat quality traits, and histochemical characteristics (Fig. 2). The first three PCs accounted for 60.4% of the variance observed in the 16 variables analyzed (PC1=28.9%, PC2=18.0%, and PC3=13.5%). Marbling score, IMF content, marbling area, and marbling area ratio were located close to one another indicating that they were positively correlated with each variable (Fig. 2A). Fineness and lightness were distributed in the area with positive PC1 and PC2 loading. In Fig. 2B, marbling fleck number and coarseness are placed on opposite quadrants, indicating that these were negatively correlated. Fineness, cooking loss, and WBS were distributed in the area with negative PC1 loading; however, fineness and marbling fleck number were distributed in the area with positive PC3 loading.

Discussion

Beef marbling score is evaluated in terms of area and distribution of visible marbling flecks on the muscle surface by trained

Table 4. Correlation coefficients (r) of marbling characteristics and histochemical characteristics of the *longissimus thoracis* muscle from Hanwoo steers

Variables	Marbling score	IMF content	Marbling fleck characteristics					
			Fleck number	Marbling area	Marbling area ratio	Coarseness (C)	Fineness (F)	F/C ratio
Muscle fiber area percentage								
Type I	0.14	-0.07	0.09	0.09	-0.07	0.22	-0.33**	-0.34**
Type IIA	0.07	-0.06	0.13	0.05	-0.06	-0.22	0.10	0.17
Type IIX	0.02	0.10	-0.18	-0.10	0.10	0.06	0.12	0.06
Muscle fiber number percentage								
Type I	-0.12	-0.03	-0.06	0.05	-0.03	0.26*	-0.26*	-0.37**
Type IIA	0.13	-0.03	0.12	0.05	-0.03	-0.28*	0.19	0.26*
Type IIX	-0.01	0.05	-0.06	-0.09	0.05	0.01	0.08	0.11
Muscle bundle characteristics								
Bundle area	-0.04	-0.16	-0.06	-0.14	-0.16	-0.09	0.07	0.08
Fiber number per bundle	0.07	0.01	-0.03	-0.07	0.01	0.01	0.20	0.07
Total bundle number	0.07	-0.02	0.26*	0.13	-0.02	0.13	-0.09	-0.15

* $p<0.05$; ** $p<0.01$.

IMF, intramuscular fat.

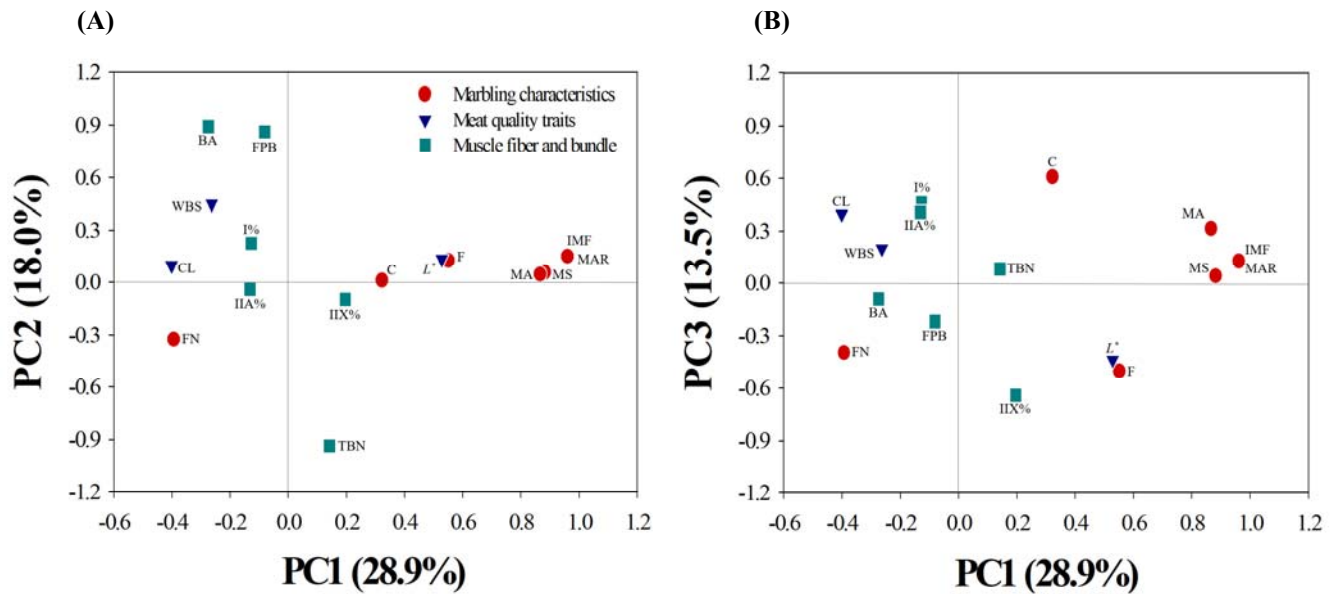


Fig. 2. Principal component analysis (PCA) plots for PC1 versus PC2 (A) and PC1 versus PC3 (B). The percentage variance explained by the three PCs was 60.4%. MS, marbling score; IMF, intramuscular fat content; FN, fleck number; MA, marbling area; MAR, marbling area ratio; C, coarseness; F, fineness; L*, lightness; CL, cooking loss; WBS, Warner-Bratzler shear force; I%, area percentage of type I fiber; IIA%, area percentage of type IIA fiber; IIX%, area percentage of type IIX fiber; BA, bundle area; FPB, fiber number per bundle; TBN, total bundle number.

graders according to the BMS (Gerrard et al., 1996). Marbling grades can also be determined using computerized image analysis to improve the scoring accuracy (Cheng et al., 2015; Lee et al., 2018b), as a strong association was observed between marbling score and marbling fleck characteristics by image analysis, especially marbling area (Kuchida et al., 2000). In the current study, total marbling fleck area and area percentage were also strongly associated with marbling score in Hanwoo steers. Additionally, in PCA results, IMF content was associated with total fleck area and fineness index of marbling fleck. In contrast, marbling score based on the USDA grading system was positively correlated with fineness and coarseness indices ($r=0.69$ and 0.14 , respectively), although the correlation with coarseness index was not strong (Konarska et al., 2017). Additionally, Lee et al. (2018b) reported that the fineness index gradually increased with increasing BMS score from 2 to 9, whereas the area of coarser marbling flecks was similar between different grades in high-marbled Hanwoo steers (BMS grades 7 to 9). These results support the relationship observed between marbling score and marbling fleck characteristics in the current study. In Hanwoo steers, somewhat limited relationships were observed between marbling score and marbling fleck indices including coarseness and F/C ratio, and strong association was observed between marbling score and fineness index.

Generally, glycolytic rate during the postmortem period measured by metabolite contents and muscle pH influence the characteristics of meat quality (Choi et al., 2007; Choi and Oh, 2016). In the current study, muscle pH measured at 45 min and 24 h postmortem were not associated with marbling characteristics ($p>0.05$) with the exception of fleck number and pH_{24h} ($p<0.05$), even though muscle pH_{24h} was negatively correlated with meat lightness ($p<0.05$, data not shown). Pflanzler and de Felicio (2011) reported that beef with higher marbling scores exhibited higher lightness values compared to beef with lower marbling grades. Our result was similar to previous studies, and beef loin harboring higher IMF content showed lighter meat surface compared to beef loin harboring lower IMF content. Additionally, the number of smaller white flecks increased with increasing lightness value ($p<0.05$). On the other hand, beef with higher marbling score or IMF content exhibited lower cooking loss and WBS value compared to beef with lower marbling score ($p<0.01$). Furthermore, strong association was also

observed between marbling area and WBS value in PCA results. These relationships can be explained by IMF characteristics. In beef with a higher amount of fat, heat is not easily transferred to the meat compared to beef with a lower amount of fat, as the fat acts as a heat insulator (Wheeler et al., 1994). Thus, highly-marbled beef may contain a lower quantity of heat-denatured proteins compared to lightly-marbled beef, which resulted in higher WHC and more tender of cooked (Miller, 2014). Meanwhile, heavily-marbled beef (BMS grades 7 to 9) with a higher value of coarseness required a lower initial force to penetrate meat compared to beef loin with a lower value of coarseness (Lee et al., 2018b). In study of low- and medium-marbled cattle determined by the USDA grading system, the extent of coarser marbling fleck was not associated with tenderness attributes evaluated by trained panelists (Vierck et al., 2018). In the current study, Hanwoo steers were used from all BMS grades except BMS 1 grade. Therefore, extent of coarser marbling flecks on the muscle cut surface tended to have limited effects on the WBS value.

IMF content or marbling degree is influenced by both genetic and environmental factors, such as age, gender, muscle fiber, feeding system, and etc. (Jeong et al., 2010; Purslow, 2005). A higher amount of lipid is present within type I fiber compared to type IIB fiber, since lipids are the predominant fuel source for oxidative metabolism in type I fiber (Choi et al., 2013). Therefore, IMF content was positively correlated with the percentage of type I fiber (Choi and Kim, 2009), and the percentage of type IIB fiber was negatively correlated with beef marbling score (Calkins et al., 1981). However, the higher IMF content in heavily marbled cattle, such as Hanwoo and Japanese Black cattle, is mainly achieved by longer fattening periods and a feedlot diet (Hocquette et al., 2010). Thus, in the current study, there were no effects of fiber type composition on IMF content and marbling degree ($p>0.05$) and somewhat limited relationships were observed between marbling fleck characteristics and muscle fiber composition.

IMF deposits form as fat cells accumulate, mainly between the bundles of muscle fiber during the fattening period. If more than 10 cells are present, they will appear as a white marbling fleck (approximately 0.01 cm^2 ; Aberle et al., 2012). Thus, the morphological characteristics of muscle bundle, especially bundle size, are associated with the marbling fleck distribution. Highly-marbled beef loin with a smaller bundle area tended to exhibit evenly distributed marbling flecks compared to highly-marbled beef loin with a large bundle area (Lee et al., 2018b). In the current study, as the number of muscle bundle increased, the number of marbling flecks increased ($p<0.05$), although most of the marbling characteristics and muscle bundle characteristics were not associated in PCA results.

Conclusion

Taken together, marbling fleck characteristics, especially marbling area, number, and fineness index, measured via computerized image analysis were strongly associated with IMF content and marbling score. However, marbling score did not have significant correlation with coarseness and F/C ratio in Hanwoo steer. Marbling fleck characteristics exhibited a somewhat limited correlation with meat quality and histochemical characteristics including muscle bundle traits. However, marbling fleck indices, including fineness, coarseness, and F/C ratio, were developed for Japanese Black steer by Kuchida et al. (2006). Therefore, it is necessary to develop new marbling fleck indices suitable for Hanwoo cattle, since the marbling fleck characteristics differ between Hanwoo and Japanese Black cattle.

Conflicts of Interest

The authors declare no potential conflict of interest.

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Author's Contributions

Conceptualization: Choi YM. Data curation: Choi YM, Lee B. Formal analysis: Lee B. Methodology: Choi YM, Lee B. Software: Lee B. Validation: Choi YM. Investigation: Lee B. Writing - original draft: Choi YM, Lee B. Writing - review & editing: Choi YM, Lee B.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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