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Abstract This study aimed at comparing the storage stability, fatty acids profile, volatile flavor compounds and eating quality of Hanwoo beef *longissimus thoracis* (LT) muscles between two marbling morphological groups (fineness and coarseness). The 1^+ grade Hanwoo LT muscles were measured for marbling fleck morphology at the 13^{th} thoracic vertebrae location by using computerized image analysis. Results reveal that both marbling groups had similar proximate composition (protein, fat, moisture and collagen), color and technological quality traits ($p>0.05$). However, the coarse marbling group presented greater C18:2n6 and polyunsaturated fatty acids contents compared to the fine marbling group ($p<0.05$). Furthermore, the fine marbling group presented lower volatile basic nitrogen (VBN) and 2-thiobarbituric acid reactive substance (TBARS) contents compared to the coarse marbling group after 14 d storage ($p<0.05$). Also, higher amounts of C18:2n6 oxidation-derived volatile compounds such as hexanal and 3-ethyl-2-methyl-1,3-hexadiene, were found in the coarse marbling group. In term of sensory aspects, the finely-marbled group received higher scores of flavor and taste compared to the coarse marbling group ($p<0.05$). Overall, the marbling flecks morphology partially showed its effects on the storage stability, fatty acids profile and eating quality of the 1^+ grade Hanwoo beef LT muscle.

Keywords: Marbling morphology, Hanwoo beef, flavor compound, eating quality

Introduction

In many markets, marbling (a term describing the white flecks of intramuscular fat) is considered as the most important factor determining the market price as well as the purchasing decision by consumers for beef (Lucherk et al., 2016; O'Quinn et al., 2012). Therefore, to satisfy the consumer's demand, beef producers have paid much attentions to the production of highly marbled beef (Morales et al., 2013; O'Quinn et al., 2012). This is because the degree of marbling is correlated positively to eating quality especially tenderness, juiciness, flavor and overall acceptability of beef (Ba et al., 2017; Corbin et al., 2015). However, recent studies have revealed that morphological characteristics of marbling flecks (e.g., coarse and fine flecks) showed some effects on the palatability and consumer's acceptability for beef (Konarska et al., 2017; Lee et al., 2018). According to studies on consumer's feedbacks, within a same marbling degree, the consumers do not prefer beef cuts with high amount of coarse marbling flecks (Lee et al., 2019; Motoyama et al., 2016). The characteristics of marbling flecks are characterized by several parameters: number and size of marbling flecks (coarseness and finess) (Kuchida et al., 2006). The size of marbling flecks can be determined by using a computerized image analysis system (Lee et al., 2018; Lee et al., 2019).

Because the Hanwoo beef industry sector contributes an enormous revenue to the total agricultural production, and is considered as the premium meat type in the country (Chung et al., 2018). Additionally, the marbling degree is considered as a primary factor determining its market price in which the Hanwoo beef with high marbling degree such as 1^{+} and 1^{++} grades usually has the highest value. Therefore, providing further scientific information regarding the effects of marbling on the meat quality in general and eating quality in particular is necessary for the producers to produce more palatable Hanwoo beef. Thus, the main objective of this study was to compare the meat quality traits, storage stability, volatile flavor compounds and eating quality between the coarsely- and finely-marbled grade 1^+ Hanwoo loins.

Materials and Methods

Sample preparation

Beef loin samples used in the present investigation were collected from Hanwoo steers. The cattle were reared under identical conditions, slaughtered at 28-30 months-old with average body weight of around 720 kg. After approximately 12 h fasting from food, the cattle were slaughtered following the commercial slaughtering procedure at a commercial slaughterhouse (Jeonju, Korea). Following 24 h chilling, the carcasses were evaluated by an official grader for quality grade using the Korean Carcass Grading System of Korea Institute of Animal Products Quality Evaluation (KAPE, 2017). The quality grade evaluation was carried out on the surface of *longissimus thoracis* (LT) muscles between the 13^{th} thoracic and 1^{st} lumbar vertebra (the standard location for beef quality grade evaluation). After classifying the quality grades (as shown in Table 1), the carcasses with quality grade 1^+ (beef marbling scores of 6-7) were collected and the LT muscles obtained from these carcasses were used for the marbling flecks characteristics analysis. After photographing the cut surface of the muscles (at 13^{th} thoracic vertebrae location), the images were analyzed for morphological characteristics of marbling flecks (e.g., size) by using a Beef Analyzer developed by Kuchida et al. (2006). Firstly, the images were binarized into muscle (black) and fat flecks (white) using Otsu's methods as described by Lee (2019). Based on the sizes of white marbling flecks, the LT muscles with their flecks size of > 0.5 cm^2 were grouped into the coarse marbling whereas, the ones with flecks size of 0.01 to 0.5 cm^2 were grouped into the fine marbling. For each the marbling group, LT muscle samples (9^{th} to 13^{th} thoracic vertebrae, $n = 10$) were used for analysis of meat quality characteristics and eating quality. The muscles were then cut into sub-samples depending on the analyses. In order to determine the storage stability, a 3-cm thick steak (approximately 300 g) was aseptically taken from each the muscle (10 steaks per marbling group). The steaks were immediately vacuum- packaged in plastic bags and stored at 0, 7 and 14 days at 2 ^{o}C. The analyses of chemical composition, fatty acids, free amino acids, volatile compounds, and sensory properties were carried out using the 0-day stored samples (sampling day) while, the storage stability of the samples was evaluated during storage (0, 7 and 14 d).

Proximate composition

The protein, moisture, fat and collagen contents were determined using a Food Scan™ Lab 78810 (Foss Tecator Co., Ltd., DK) according to the instruction by manufacturer. Each sample (approximately 200 g ground sample) was distributed onto a round sample dish and then was loaded into the instrument’s sample chamber. Each sample was determined in duplicate.

pH measurement

The pH values of samples during storage were measured using a pH*K 21 (NWK-Technology GmbH, Kaufering, Germany) equipped with a stainless steel and solid-state probe. After calibration with standard solutions (pH 4.0 and 7.0), the probe was inserted deeply into the muscle tissue. Three readings were taken for each sample.

Meat color measurement

The meat color was determined on the freshly cut surface of each sample (3 different locations per sample) after 30 min blooming at 2 ^{o}C using a Minolta Chroma Meter CR-400 (Minolta Camera Co., Ltd., Osaka, Japan). Prior to use, the device was standardized with a white plate (Y = 86.5, X = 0.3166 and Y = 0.3242). According to the Commission International de l'Eclairage (CIE) system the color traits were expressed as CIE L* (lightness), CIE a* (redness), CIE b* (yellowness), chroma and hue angle (h°). In which the chroma and hue angle were calculated as $(a^{*2}+b^{*2})^{0.5}$ and $\tan^{-1}(b^*/a^*)$, respectively.

Cooking loss and Warner-Bratzler shear force (WBSF) measurement

The cooking loss and WBSF were measured on the same steak (3.0-cm in thickness) of each muscle sample, as described in our previous work (Cho et al., 2017). After the initial weight was recorded, the steaks were placed into plastic bags, sealed with double clips and put in a pre-heated water bath until the core temperature reached 80°C. Thereafter, the cooked samples were immediately cooled under running water (18-20 °C) for 30 min, removed from the plastic bags and absorbed with wiping papers to remove the surface water. The weights of the cooked samples were recorded to determine the cooking loss. The cooking loss was determined as the weight loss percentage as follows:

Cooking loss (%) = [(raw meat weight − cooked meat weight) ÷ raw meat weight] x 100

After the cooking loss measurement was completed, the cooked samples (each) were made into eight cores with an average diameter of 1.27 cm. The cores were carefully removed parallel to the muscle fiber direction using a 0.5-inch metal corer. The WBSF values were obtained by completely cutting the cores using a V-shaped shear blade of Instron Universal Testing Machine (Model 4465, Instron Corp, High Wycombe, UK) using a crosshead speed of 400 mm/min and a 40 N load cell and expressed as kilograms of force (kgf)

Water holding capacity (WHC)

The WHC of the samples during storage was determined using centrifugation-based method as described in our previous study (Cho et al., 2017). Briefly, each the ground muscle (approximately 0.51 g) was taken and placed in a 2 mL ultra-centrifugal filter unit, inserted into an ultra-centrifugal filter device (Millipore Corp., Bedford, MA, USA) and then heated in an 80 ℃ pre-heated water bath for 20 min. Thereafter, the centrifugation tubes containing samples were cooled at room temperature for 10 min and centrifuged at 2000 × *g* for 10 min at 4℃. The weight of ultra-centrifugal filter unit containing the heated sample was recorded to determine the water loss. The WHC percentage was calculated as a ratio of moisture to the water loss. Each sample was analyzed in duplicates.

Volatile basic nitrogen (VBN)

The VBN content produced in the samples during storage was determined using the Conway method as described by Seong et al. (2017). The VBN content was calculated and expressed as mg% VBN/100g meat. Each sample was analyzed in duplicate.

Lipid oxidation

To elucidate whether the marbling type affects the lipid oxidation, the content of 2-thiobarbituric acid reactive substance (TBARS) was determined on the samples stored at 0, 7 and 14 d, using the method as described in our previous study (Cho et al., 2017). The TBARS content was calculated and expressed as mg malonaldehyde (MA)/kg meat. Each sample was analyzed in duplicate.

Fatty acids

The lipid content in each sample was extracted using a solvent mixture of chloroform: methanol (2:1, v/v) as described by Folch et al. (1957). Briefly, each sample (10 g) and 150 mL of the solvent mixture was homogenized at 2,500 × rpm for 3 min using a homogenizer (Polytron, PT-MRC. 2100, Switzerland). The homogenate was then filtered through a Whatman filter paper. After adding with approximately 20g of Na_2SO_4, the filtrate was thoroughly mixed for 1 min, and then the upper lipid layer was separated and transferred into Erlenmeyer flask. Thereafter, the lipids layer was dried at 55 °C using a rotary evaporator. After dissolving in 1 mL tricosanic and 1 mL of 0.5N NaOH, the lipid was converted to fatty acid methyl esters following the procedure of Morrison and Smith (1964). The fatty acids analyzed by a Gas Chromatography (GC, Model Star 3600, Varian Technologies, Palo Alto, CA, USA) connected with a capillary column (30 m x 2 mm x 0.25 μm film thickness) using nitrogen as a carrier gas at flow rate of 1mL/min. The sample (1 μL) was injected into the injection port at 250°C, while the oven temperature was held for 1 min at 50 °C, and then raised to 200 °C at a rate of 25°C/min, and further increased to 260 °C at a rate of 5°C/min. The temperature of detector was fixed at 300 °C. The fatty acids in samples were identified by comparing their retention times with those obtained from standard fatty acids. The results were expressed as relative percent (%) of total fatty acids based on total peak area.

Sensory evaluation

The sensorial characteristics of the samples in both marbling groups were evaluated using the procedure as described by Ha et al. (2019) with minor modifications. The procedure used for sensory evaluation was approved by the Institutional Review Board of National Institute of Animal Science (No.11-1390744-000007-01). The panel consisted of 7 trained members (at 24 to 40 years old) who were the institution's staffs. For samples preparation, each LT muscle sample was prepared into 4-mm thick slices using a meat slicer, and 7 representative slices (50 × 75 × 4 mm) were finally chosen for the sensory evaluation. Each session had 7 panelists;

each panelist evaluated 7 samples, and two sessions per day were carried out. The slices were cooked on an open tin-coated grill for approximately 2 min and turned at the start of shrinkage (30 s intervals). The cooking temperature was monitored using an infrared thermometer and was maintained at around 160-170ºC. Immediately after cooking, the slices were placed on individual paper dishes and served to the panelists. The panelists tasted and then evaluated for the following attributes: beefy flavor (intensity of beef flavor after chewing), umami taste intensity of umami taste after chewing), juiciness, tenderness and overall acceptability using a 6-point scale as described by Meilgaard et al. (1999).

Free amino acids (FAA) analysis

The FFA content in the samples was analyzed following the procedure as described by Dashdorj et al. (2013) with suitable modifications. Briefly, 2.5 g of each sample was homogenized with 5 mL DDW at 13,000 × rpm for 1 min. After filtering through Whatman filter paper, 100 μL filtrate taken and mixed with 900 μL methanol. After centrifuging at 13,000 × *g* for 10 min at 4 ^{o}C, the supernatant was collected and then filtered through a 0.45-μm membrane filter. The filtrate was derivatized using AccQ-Tag™ (Waters Co., Milford, MA, USA) according to the manufacturer's instrument. Finally, each 5 μL of each derivatized sample was injected into a Waters ACQUITY UPLC (model: Xevo TQ-S, Waters Co. Milford, MA, USA) connected to a IMTAKA Intrada Amino Acid column (2×50 mm, 3μm). The separation was carried out with two buffers: A [acetonitrile: 100 mM amonium formate; 20:80 v/v] and B [acetonitrile: trifluoroacetic acid: 25 mM amonium formate: formic acid: 9:75:16:03 v/v/v]. The separation conditions such as temperature and mobile phase ingredient were same as those used in previous study (Dashdorj et al., 2013). The amino acids standard was used for identifying and quantifying the FAA, and the detected FAA were expressed as milligram per

100 g meat (mg/100 g meat).

Volatile flavor compounds analysis

The analysis of volatile flavor compounds was performed using the method developed by Ba et al. (2010). Briefly, the volatile flavor compounds were extracted using solid-phase micro-extraction (SPME). Before extracting, the samples were cooked under the conditions same as those used for the sensory samples preparation as mentioned above. In order to minimize the loss of volatile compounds due to evaporation, after cooking the cooked samples (1 g each) were immediately placed into 20-mL headspace vials (Agilent, USA) and tightly capped with PTFE-faced silicone septum. The vials containing samples were then kept at 4^{o}C for use. For extraction of volatile flavor compounds, a SPME device containing carboxen–polydimethylsiloxane (75 µm) fiber (Supelco) was inserted into the vials and the extraction was carried out at 65^{o}C and agitated at 250 × rpm for 60 min. All steps such as; extraction, absorption, desorption of the flavor compounds were done using a fully automated SPME sample preparation instrument (Model: AOC-5000 Plus) connected to Gas Chromatography (Model: 7890B GC, Agilent Technologies) with Mass Spectrophotometry (Model: 5977B MSD, Agilent Technologies). The fiber was desorbed at GC injection port for 5 min at 250^{o}C. The GC and MS conditions set were same as those mentioned in the above cited literature. Identifications of volatile compounds were performed by comparing their mass spectra with those already present in the Wiley registry library (Agilent Technologies) and/or by comparing their retention times with those of external standards. Concentrations of the identified volatile compounds were determined by comparison of their peak areas with that of the internal standard (1.0 µL of 2-methyl-3-heptanone, 816mg /mL in methanol was added together with the sample) obtained from the total ion chromatogram using a response factor of 1.

Statistical analysis

Obtained data were analyzed using the Statistic Analysis System (SAS) package (SAS Institute, Cary, NC, USA, 2015). The data were analyzed by using the General Linear Model procedure considering marbling fleck group as the main effect. Means were compared using Duncan's Multiple Range Test. Significance was set at $p<0.05$.

Results and Discussion

The summary statistics on the traits of carcasses used for sampling in the present investigation is presented in Table 1. The beef marbling scores were almost similar in both marbling groups (6.30 and 6.80 for the fineness and coarseness, respectively) ($p>0.05$). Also, the back-fat thickness, loin area, carcass weight and meat yield etc. of both marbling groups were not different from each other ($p>0.05$).

Effects of marbling type on chemical composition, meat color and quality traits

The results on the chemical composition, color and technological quality traits of the LT muscles from both the marbling groups are summarized in Table 2. The contents of protein, moisture, fat and collagen were 18.79 vs 18.23%, 59.34 vs 58.96%, 19.88 vs 20.65%, and 20.7 vs 20.5% for the fine and coarse marbling groups, respectively. And no differences occurred between the two marbling groups for all of these contents ($p>0.05$). The Intramuscular fat (IMF) is well recognized as the most important factor determining the eating quality of beef (Gajaweera et al., 2018; Jung et al., 2015; O'Quinn et al., 2012). Generally, the fat content in both groups were proportional to its standard beef marbling scores (KAPE, 2017) and was similar to the levels (18-20%) reported for 1^{+} or 2^{++} grade Hanwoo loin in literature (Gajaweera et al., 2020; Lee et al., 2019).

In term of instrumental colors, results show that the marbling group did not influence any traits (p>0.05). This could be due to the similar IMF and protein contents in both the marbling groups as mentioned above because the meat color is fundamentally related to proteins in muscle tissues. The mean values of lightness, redness and yellowness obtained in the present work were slightly lower than values reported for same beef muscle of 1^{+} grade Hanwoo steers by Gajaweera et al. (2020).

There were no differences in the technological quality traits such as; cooking loss and water holding capacity as well as WBSF values between the two marbling groups studied (p>0.05). Our results are in accordance with those of Lee et al. (2018) and Lee et al. (2019): marbling morphology did not affect cooking loss level of Hanwoo loin muscle regardless of marbling scores. In contrast to the current finding, Lee et al. (2019) reported that finely-marbled beef samples had a higher shear force value compared to coarsely-marbled samples, however, these researchers used higher marbling scores (7-9) beef muscles.

Effects of marbling type on fatty acids composition

Fatty acids not only reflect the nutritional value (Doyle, 2004) but also remarkably affect the development of cooked meat flavor (Mottram, 1998). This study for first time, the fatty acid profiles as affected by marbling group was investigated. The relative percent (%) of fatty acids in the LT muscles from both marbling groups are summarized in Table 3. The outcome of our analysis depicts that the marbling type significantly affected the C18:2n-6, C20:5n-3 and total polyunsaturated fatty acids (PUFA) contents, with significantly (p<0.05) greater levels for the coarse marbling group. However, the precise reason underlying this phenomenon still remains unknown. Whereas, the marbling group showed no effects on the other remaining fatty acids, total saturated fatty acids (SFA) as well as the PUFA/SFA ratio (p>0.05). Three the most predominant fatty acids we found in the both marbling groups were C16:0 (30-31%), C18:0 (11-12%) and C18:1n-9 (45-47%). Similar to the present results, Lee et al. (2017) and Gajaweera et al. (2020) also found that C16:0, C18:0 and C18:1n-9 were the most predominant fatty acids present in the 1^+ grade Hanwoo beef.

Effects of marbling type on storage stability

In order to elucidate whether the marbling group affects the storage stability of beef, the TBARS and VBN contents were determined during storage up to 14 days. Lipid oxidation is well recognized as a major process responsible for the perishability in quality of meat and meat products during storage (Amaral et al., 2018). The results (Table 4) show that at the initial measurement (0 d), no differences were found between the two marbling groups for the level of TBARS ($p>0.05$). However, after 7 and 14 d storage, the coarse marbling group showed a significantly ($p<0.05$) greater TBARS content compared to the fine marbling group. It means that the degree of lipid oxidation occurred at a faster rate in the coarsely-marbled samples, this may be attributed to its higher linoleic acid (C18:2n-6) as well as the total PUFA contents (Table 3). Because researchers have found that the lipid oxidation occurs mainly in fatty acids especially the PUFAs such as C18:2n-6 (Amaral et al., 2018; Laguerre et al., 2007).

VBN content is generally considered as an important index reflecting the freshness of meats; an increase in the VBN content may indicate the putrefaction process and decomposition of amino acids in meat (Min et al., 2007). Our results depict that the VBN content differed significantly between the two marbling groups for instance; at initial measurement (0 d) the coarse marbling group presented higher content (7.83 mg%/100g) compared to the fine marbling group, and a similar trend was also observed on this marbling group after 14 d storage

($p<0.05$). It is known that the VBN content is produced as a result of protein degradation, mainly caused by spoilage bacteria or endogenous enzymes (Cai et al., 2011; Huang et al., 2014). Thus, the result indicating the VBN contents difference could be attributed to the differences in initial bacteria loads and/or the endogenous enzymes between the two marbling groups. Unfortunately, these factors were not determined in the present work. Compared to the present results, Sujiwo et al. (2019) reported a higher VBN level (29.31 mg%/100g) for the same Hanwoo beef muscle after 15 d storage. According the Korea Ministry of Food and Drug Safety (2014), beef is considered as spoilage (inedible) when its VBN content exceeds 20 mg%/100 g. Thus, according to this standard the beef samples in the both marbling groups were considered fresh and edible after 14 d storage.

Effects of marbling type on free amino acids composition

Free amino acids are known as the important compounds responsible for the sensory quality of cooked meat, especially umami (Dashdorj et al., 2013; Jayasena et al., 2013). The outcome of our analysis depicts that nine-teen FAAs were detected in the samples (Table 5). Out of these FAAs, alanine and glutamine were the most predominant FAAs we found in the both marbling groups, followed by glutamate, arginine, leucine, glycine and serine etc. The statistical analysis revealed no significant differences in the concentrations of all the FAAs occurred between two marbling groups ($p>0.05$). However, it was observed that the samples of the fine marbling groups tended to possess higher amounts of glycine, serine, threonine and glutamate. The synergistic effect of these amino acids has been demonstrated to contributes to the umami taste of cooked meat (Cho et al., 2007).

Effects of marbling type on the volatile flavor compounds

Regarding the volatile flavor compounds (Table 6), we observed that both the marbling groups were generally predominant in the fatty acids-derived flavor compounds such as aldehydes, alcohols, ketones, hydrocarbons and furans. Supporting the present results, Ba et al. (2017) and Ha et al. (2019) have reported a similar trend for the fatty acids-derived flavor compounds in highly-marbled Hanwoo beef. The statistical analysis showed that out of fifty-two compounds only two were significantly ($p<0.05$) different between the two marbling groups (Table 6). Particularly, hexanal and 3-ethyl-2-methyl-1,3-hexadiene are known as the products derived from the oxidation/degradation of C18:2n6 during cooking (Ba et al., 2013). The amount of hexanal (1.32 μg/g) was significantly ($p<0.05$) higher in the coarse marbling group compared to the fine marbling group (0.77 μg/g). This obtained result could be related to the higher level of C18:2n6 in the coarse marbling group (Table 3). Hexanal has been found to contributed positively to cooked beef flavor, but may produce undesirable flavor when its amount is produced at a high concentration (Calkins and Hodgen, 2007). Likewise, Hanwoo beef is characterized by its high IMF (marbling) especially the C18:1n9 content (Gajaweera et al., 2018), which are known the major precursors for production of volatile flavor compounds associated with fatty aroma in cooked meat during cooking (Motram, 1998). In the present study, the similar IMF and C18:1n9 contents (Table 3) may be the main reason why both of the marbling groups did not differ in the other remaining monounsaturated fatty acids-derived flavor compounds (e.g., octanal, nonanal and decanal etc.).

Effects of marbling type on sensory properties

In term of sensorial quality aspects, significant differences in beefy flavor and umami taste scores occurred between the two marbling groups (Table 7). Noticeably, the panelists gave higher scores for these two traits for the samples of the fine marbling group ($p<0.05$). Currently, we cannot yet offer a satisfactory explanation for the flavor difference; however, it may be related to the variations in the PUFA contents (Table 3) which affected the flavor compounds (Table 6) as well as the flavor quality of the cooked beef samples between the marbling groups. Likewise, the results indicating a greater umami taste score for the fine marbling group could be attributed to its slightly higher amounts of some amino acids (e.g., glycine, serine, threonine and glutamate) which have been reported to contribute to the umami taste (Cho et al., 2007). In contrast to the current finding, Lee et al. (2019) reported no differences in flavor scores for 1^{++} grade Hanwoo beef samples between coarse and fine marbling groups. Conversely, a study by Wieck et al. (2018) showed that coarsely-marbled steaks exhibited higher scores for flavor and juiciness compared to finely-marbled steaks in the USDA choice beef grade. Regarding the tenderness and juiciness, the panelists reported no differences in their scores between the two marbling groups ($p>0.05$). This is, in part because both the marbling groups had a similar IMF content (Table 2), since this content is considered as a primary factor affecting the beef juiciness and tenderness (O'Quinn et al., 2012). In the present study, the result of sensorial tenderness was in line with that of the instrumental shear force measurement (Table 2). Although no statistical differences, the finely-marbled samples were rated slightly higher juiciness, tenderness and overall acceptability scores. Partially supporting the present results, Lee et al. (2019) also reported a higher overall acceptability score for finely-marbled Hanwoo beef samples than for the coarsely-marbled beef samples.

Conclusion

This study for the first time, compared the storage stability, fatty acids profile and volatile flavor compounds between two morphological marbling groups (coarse and finesse flecks). Our results reveal that the coarse marbling group presented greater C18:2n6 and PUFA contents as well as higher amounts of fatty acids-derived flavor compounds (hexanal and 3-ethyl-2-methyl-1,3-hexadiene). Noticeably, the finely-marbled group showed a better storage stability (indicated by lower VBN and TBARS contents) compared to the coarse marbling group after 14 d storage. Likewise, the finely-marbled group exhibited greater flavor and taste scores compared to that of the coarse marbling group. The present study thus indicate that the marbling morphology partially shows its particular effects on the storage stability and eating quality of 1^+ grade Hanwoo beef loin. Further study on the effect of fat (marbling) type on the quality characteristics of Hanwoo beef according to the grades (e.g., 1 and 1^{++} grade) is necessary.

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Table 1. **Carcass traits*) of Hanwoo steers used for sampling in the present study**

Item	Marbling type	
	Fineness	Coarseness
Beef marbling score	6.30±0.15	6.80±0.13
Back-fat thickness (mm)	15.30±1.67	15.20±0.79
Loin area (cm^2)	101.90±3.41	101.10±2.82
Carcass weight (kg)	469.00±14.55	467.60±14.55
Yield index	63.84±1.13	63.83±0.59
Meat color	4.90±0.10	5.00±0.00
Fat color	2.90±0.10	3.00±0.00
Texture	12.10±0.10	12.00±0.00
Maturity	2.00±0.00	2.10±0.10

*): The carcass traits were determined according to the Korean Carcass Grading System (KAPE, 2017).

Table 2. Proximate composition, color and quality traits of coarsely-and finely-marbled 1+ grade Hanwoo loins

Item	Marbling type	
	Fineness	Coarseness
Proximate composition		
Protein (%)	18.79±0.18	18.23±0.35
Moisture (%)	59.34±0.24	58.96±1.04
Crude fat (%)	19.88±0.46	20.65±1.28
Collagen (%)	2.07±0.04	2.05±0.07
Color traits		
L* (Lightness)	37.89±0.76	36.41±0.50
a* (Redness)	20.85±0.70	21.98±0.59
b* (Yellowness)	10.87±0.64	11.55±0.46
Chroma	23.54±0.89	24.84±0.73
Hue angle	27.35±0.87	27.65±0.47
Technological quality traits		
Cooking loss (%)	22.74±0.77	23.16±0.61
Shear force (kgf)	2.80±0.15	2.74±0.10
Water holding capacity (%)	54.64±1.29	56.62±1.37

Table 3. **Relative percent (%) fatty acids in coarsely-and finely-marbled 1^{+} grade Hanwoo loins**

Item	Marbling type	
	Fineness	Coarseness
C14:0	3.46±0.30	3.19±0.02
C16:0	31.35±1.61	30.13±0.55
C16:1n7	4.48±0.47	4.41±0.34
C18:0	12.79±0.92	11.36±0.73
C18:1n7	0.37±0.06	0.38±0.03
C18:1n9	45.91±2.86	47.65±0.97
C18:2n6	2.21±0.17^{b}	2.54±0.17^{a}
C18:3n3	0.10±0.01	0.09±0.01
C18:3n6	0.04±0.00	0.05±0.00
C20:1n9	0.15±0.03	0.14±0.04
C20:4n6	0.10±0.05	0.06±0.01
C20:5n3	0.00±0.00^{b}	0.01±0.00^{a}
C22:4n6	0.03±0.00	0.05±0.00
SFA	47.60±2.40	44.70±1.14
UFA	53.70±2.40	55.30±1.14
MUFA	50.92±2.53	52.58±1.13
PUFA	2.49±0.12^{b}	2.81±0.14^{a}
MUFA/SFA	1.07±0.12	1.18±0.05
PUFA/SFA	0.05±0.00	0.06±0.00
n3	0.10±0.01	0.10±0.01
n6	2.28±0.14	2.62±0.17
n6/n3	25.40±1.66	30.66±3.67

Means within a same row with different superscripts (a,b) differ significantly $p<0.05$; SFA: saturated fatty acids; UFA: unsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Table 4. Storage stability coarsely-and finely-marbled 1⁺ grade Hanwoo loins

Item	storage days	Marbling type	
		Fineness	Coarseness
pH	0	5.50±0.03	5.55±0.02
	7	5.60±0.04	5.60±0.03
	14	5.57±0.02	5.60±0.01
TBARS (mg MA/kg meat)	0	0.25±0.03	0.26±0.04
	7	0.41±0.02^{b}	0.56±0.04^{a}
	14	0.49±0.04^{b}	0.91±0.14^{a}
VBN (mg%)	0	6.82±0.20^{b}	7.83±0.36^{a}
	7	7.33±0.25	8.04±0.37
	14	7.65±0.15^{b}	8.96±0.47^{a}

Means within a same row with different superscripts (a,b) differ significantly $p<0.05$

VBN: Volatile basic nitrogen; TBARS: 2-thiobarbituric acid reactive substance.

Table 5. Concentration (mg/100 g) of free amino acids in coarsely-and finely-marbled 1+ grade Hanwoo loins

Items	Marbling type	
	Fineness	Coarseness
Glycine	6.01±0.41	5.19±0.24
Alanine	25.27±0.49	23.27±0.22
Serine	6.01±0.72	4.28±0.50
Proline	2.89±0.29	3.17±0.44
Valine	6.46±0.53	5.43±0.27
Threonine	5.15±0.52	4.48±0.78
Leucine	9.34±1.03	7.81±0.63
Isoleucine	3.98±0.34	3.30±0.56
Asparagine	2.53±0.15	2.32±0.11
Aspartate	0.26±0.05	ND
Lysine	5.49±0.60	4.85±0.37
Glutamine	22.74±1.05	26.42±0.89
Glutamate	10.59±0.12	10.36±0.64
Methionine	3.16±0.47	2.09±0.39
Histidine	4.97±0.56	4.78±0.57
Phenylalanine	5.55±0.64	4.49±0.89
Arginine	7.25±0.90	6.34±0.73
Tyrosine	6.30±0.57	4.71±0.21
Tryptophan	1.71±0.01	1.47±0.02

ND; not detectable.

Table 6. Amount (µg/g) of volatile flavor compounds in coarsely-and finely-marbled 1^{+} grade Hanwoo loins

Volatile compounds	RT (min)	Marbling type		Identification method[1)]
		Coarseness	Fineness	
Aldehydes				
Propanal	1.701	0.01±0.00	0.01±0.00	MS+STD
Butanal	2.139	0.01±0.01	0.01±0.00	MS+STD
3-methyl butanal	2.699	0.01±0.00	0.01±0.01	MS+STD
2-methyl butanal	2.812	0.01±0.00	0.02±0.01	MS+STD
Pentanal	3.286	0.11±0.05	0.10±0.02	MS+STD
Hexanal	6.101	1.32±0.24[a]	0.77±0.01[b]	MS+STD
E,2-hexenal	7.808	ND	0.01±0.00	MS
Heptanal	9.262	0.62±0.29	0.55±0.23	MS+STD
E,2-heptenal	10.754	0.03±0.02	0.02±0.01	MS
Benzaldehyde	10.866	0.05±0.02	0.05±0.01	MS+STD
Octanal	11.921	0.47±0.02	0.53±0.03	MS+STD
Benzenacetaldehyde	12.873	0.01±0.00	0.01±0.01	MS
E,2-octenal	13.185	0.05±0.05	0.03±0.01	MS+STD
Nonanal	14.198	0.76±0.04	0.73±0.31	MS+STD
E,2-nonenal	15.334	0.14±0.07	0.19±0.06	MS+STD
E,4-decenal	15.938	0.03±0.01	0.04±0.00	MS
Decanal	16.227	0.07±0.03	0.05±0.02	MS+STD
E,2-decenal	17.267	0.21±0.12	0.14±0.07	MS+STD
Undecanal	18.088	0.03±0.01	0.03±0.00	MS
E,E,2,4-decadienal	18.296	0.01±0.01	0.01±0.00	MS+STD
2-undecenal	19.076	0.09±0.05	0.07±0.04	MS
Tridecanal	19.823	0.01±0.01	0.01±0.00	MS
2-methyl-undecenal	20.651	0.01±0.00	0.01±0.00	MS
Alcohols				
1-Pentanol	5.013	0.05±0.03	0.03±0.01	MS+STD
1-hexenol	8.344	0.03±0.02	0.04±0.02	MS+STD
Ketones				

3-heptanone	8.775	0.01±0.00	0.01±0.00	MS
2-Heptanone	8.889	0.03±0.02	ND	MS+STD
2-methyl-4-heptanone	10.458	0.03±0.00	0.03±0.00	MS
4-octanone	11.134	0.05±0.05	0.04±0.02	MS
2,5-octanedione	11.474	0.09±0.09	0.05±0.03	MS
2-nonanone	13.887	0.04±0.02	0.03±0.00	MS
Hydrocarbons				
Toluene	4.942	0.01±0.00	0.01±0.00	MS+STD
2-octene	5.751	0.01±0.00	0.01±0.00	MS
1,3-dimethylbenzene	8.253	0.01±0.00	0.01±0.01	MS
1-nonene	8.934	ND	0.03±0.02	MS
Nonane	9.193	0.01±0.00	0.01±0.00	MS
2-methyl hexane	11.091	0.005±0.00	0.01±0.00	MS
Hexanoic acid	11.34	0.02±0.01	0.03±0.02	MS
Decane	11.832	0.02±0.01	0.03±0.02	MS
2-methyl decane	12.346	ND	0.01±0.00	MS
3-ethyl-2-methyl-1,3-hexadiene	12.565	0.007±0.01^{b}	0.01±0.00^{a}	MS+STD
2,2-dimethyl octane	13.289	ND	0.01±0.00	MS
Undecane	14.088	0.01±0.01	0.02±0.01	MS
Z-3-dodecene	14.758	0.01±0.01	0.02±0.00	MS
Benzoic acid	15.48	0.23±0.13	0.09±0.03	MS
Furans				
2-pentyl furan	11.586	0.10±0.08	0.06±0.03	MS+STD
2-heptyl furan	16.097	0.02±0.01	0.02±0.00	MS+STD
2-n-octyl furan	17.887	0.02±0.01	0.01±0.01	MS+STD
Sulfur and nitrogen compounds				
Carbon disulfide	1.867	0.003±0.00	0.004±0.00	MS
2,5-dimethyl pyrazine	9.534	0.02±0.01	0.03±0.02	MS+STD
2-ethyl-3,5-dimethyl pyrazine	13.568	0.01±0.00	0.02±0.00	MS+STD
5-butylhydro-2(3H)-furazone	17.161	0.01±0.01	ND	MS

Means within a same row with different superscripts (a,b) differ significantly $p<0.05$.
ND: Not detectable;
[1)]The flavor compounds were identified by mass spectra (MS) from library or external standard (STD).

Table 7. Sensory evaluation of coarsely-and finely-marbled 1$^+$ grade Hanwoo loins

Item	Marbling type	
	Fineness	Coarseness
Beefy flavor	4.01±0.07[a]	3.74±0.11[b]
Umami taste	4.11±0.09[a]	3.85±0.06[b]
Juiciness	4.50±0.11	4.24±0.13
Tenderness	4.19±0.13	4.07±0.17
Overall acceptability	4.30±0.09	4.24±0.09

Means within a same row with different superscripts (a,b) differ significantly p<0.05. Score distribution: Beef flavor: intensity of beefy flavor, (1) very weak to very strong (6); juiciness: (1) very dry to extremely juicy (6); umami taste: intensity of umami taste, (1) very weak to very strong (6); tenderness: (1) very tough to very tender (6); overall acceptability: (1) extremely dislike to extremely like (6).