Relationships between monounsaturated fatty acids of marbling flecks and image analysis traits in longissimus muscle for Japanese Black steers[1]

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ABSTRACT: The percentage of MUFA to total fatty acids of beef differs among intermuscular, intramuscular, and subcutaneous fat even within an individual cow. Our objective was to investigate the variation of the percentage of MUFA by geometric and sectional change of marbling flecks in rib eye. Longissimus muscles of 8 Japanese Black steers from a common sire and a common maternal grand sire were used. Three slices (1 from rib roast and 2 from sirloin) from each animal were selected for analysis. Five marbling flecks from each slice were randomly taken to obtain the percentage of MUFA using gas chromatography. High-quality digital images of all slices were taken with a mirror-type camera. The area and location of each marbling fleck were calculated by image analysis. The marbling flecks were categorized by area [small <0.4 cm^2, medium 0.4 to 2.0 cm^2, large >2.0 cm^2], by location (dorsal and ventral), and by slice section through the LM (front, middle, and back). The effects of classification according to the area, location, and slice section were statistically significant ($P < 0.05$) for the percentage of MUFA. Least squares means of the percentage of MUFA for marbling flecks of sizes small, medium, and large were 56.8, 58.4, and 60.2%, respectively, indicating that larger marbling flecks had greater MUFA ($P < 0.05$). Those of dorsal, ventral, front, middle, and back were 59.1, 57.8, 55.4, 59.9, and 60.1%, respectively. The percentages of MUFA of the marbling flecks located in the dorsal part were greater than those in the ventral part ($P < 0.05$). The percentages of MUFA from middle and back were greater than those from front ($P < 0.01$). We suggest that the area, location, and slice section of marbling would be the determining factors for the percentage of MUFA of marbling.

Key words: image analysis, Japanese Black, monounsaturated fatty acid

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INTRODUCTION

In Japan, beef carcasses are ribbed on the left side between the sixth and seventh ribs. The yield and meat quality scores are evaluated by certified graders of the Japan Meat Grading Association (JMGA, 1988). In general, marbling in rib eye (musculus longissimus thoracis) is an important factor in determining the meat quality and carcass value. Additionally, physicochemical traits such as AA of muscle and fatty acid composition of fat have attracted attention recently (Oka et al., 2002). Monounsaturated fatty acids have a lower melting point compared with SFA, and, in particular, oleic acid (C18:1) positively correlates with the flavor of beef (Westerling and Hedrick, 1979). Therefore, some approaches measuring fatty acids have been investigated to evaluate meat palatability.

The percentage of MUFA to total fatty acids is affected by factors such as sex, breed, sire, and deposit site (Zembayashi et al., 1995; Oka et al., 2002). He et al. (1997) reported that the fatty acid composition of adipose tissue differed among the sizes of fat cells, even for an individual cow and within fat tissue from 1 animal. These results suggest that fatty acid composition differs by size and location of the marbling fleck in the rib eye, or by the cross-section through LM even for 1 cow. Although marbling in rib eye was treated as a main determining factor for beef carcass quality in Japan, there have been no studies investigating the variation of MUFA due to the various sizes of marbling flecks, because it was difficult to evaluate the

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shape or size of marbling flecks in detail. However, in recent years, some investigations for the coarseness and fineness of marbling flecks were performed using high-quality digital cameras (Kuchida et al., 2006; Okamoto et al., 2007). Our objective was to investigate the relationship between the percentage of MUFA and geometric characteristics of marbling flecks calculated using image analysis at different cross-sections.

MATERIALS AND METHODS

All animals were cared for and killed according to Japanese rules and regulations for animal care.

Image Analysis

Eight Japanese Black steers from a common sire and a common maternal grand sire were used in this study. The age of each animal ranged from 25 to 29 mo. Steers were fed concentrate feed 7.8 kg/d from 9 to 26 mo of age. The concentrate feed consisted of corn, barley, wheat bran, and roughage. After slaughtering and chilling in a day, the meat quality scores of the carcasses were evaluated between the sixth and seventh ribs by an official Japanese grader. Marbling score was graded from 1 (poor) to 12 (very abundant) according to the Beef Marbling Standard (JMGA, 1988).

Longissimus muscle sections were taken from each carcass and sliced at 2-cm intervals from the sixth and seventh rib cross-section to the last lumbar vertebrae. Three slices from each animal were randomly selected (1 from rib roast and 2 from sirloin) and categorized as follows: front (from rib roast), middle (from cranial sirloin), and back (from caudal sirloin).

High-quality digital images were taken with a mirror-type camera (HK-333, Hayasaka Rikoh Co. Ltd., Sapporo, Japan). Image analysis was performed using the Beef Analyzer II software (Hayasaka Rikoh Co. Ltd., Sapporo, Japan), which minutely analyzes marbling of rib eye.

To specify the area of muscle to be analyzed, a border line (line width is 1 pixel) of the rib eye was semiautomatically drawn and manually corrected using an image analysis program developed by Kuchida et al. (1997). According to the procedure reported by Kuchida et al. (2006), the fat area ratio (**FAR**), overall coarseness of marbling particles (**OCM**), coarseness of maximum marbling particle (**CMM**), and number of small flecks (**NSF**) were calculated as image analysis traits at the sixth and seventh rib cross-section and each of the slices. The calculation methods for the image analysis traits used in this study were as follows:

- FAR: Images were binarized into lean and fat using the image analysis program (Figure 1b). Fat area ratio was calculated by dividing all pixels of the fat image by the rib eye area.
- OCM: Because Japanese Black cattle meat has so much marbling, marbling flecks tend to be connected. The thinning process is the technique for separating the marbling flecks individually. The binarized image was thinned, and the hairlines were removed to pick up the rough marbling. The OCM was calculated from all pixels of the fat after thinning and removing the hairline image (Figure 1d) by the fat image used in FAR calculation. A high OCM indicates a muscle containing a large number of rough marbling particles. The thinning process was performed 1 time for the rib roast and 5 times for the sirloin. The difference of thinning times between rib roast and sirloin was derived from the difference in amount of marbling flecks.
- CMM: The CMM was calculated by dividing pixels of the largest marbling particle (after thinning and removing hairlines) by all pixels of the fat image used in FAR calculation. A high CMM indicates the existence of extremely large marbling particles in the muscle.
- NSF: We counted the number of marbling flecks, which ranged from 0.01 to 0.5 cm^2.

The area and locations of marbling flecks that were separated by the thinning process were measured using Photoshop (Adobe Systems Inc., San Jose, CA) after image analysis. Subsequently, marbling flecks were categorized by size into small (**S**, <0.4 cm^2), medium (**M**, 0.4 to 2.0 cm^2), and large (**L**, >2.0 cm^2). Marbling flecks located in the external part from the first inertial axis of rib eye, which is the longest line passing through the center of gravity, and those located in the internal part from the axis were categorized as dorsal and ventral, respectively (Figure 2).

Fatty Acid Analysis

Five samples of marbling flecks were collected randomly from each slice for chemical analysis. Numbers of marbling flecks categorized as size S, M, and L were 44, 34, and 42, respectively. Adipose samples from whole rib eye and subcutaneous fat were also collected. Seven samples were extracted in total to calculate MUFA for each slice. An example of sampling points is shown in Figure 2.

The fatty acid profile was measured according to the method of He et al. (1997) as follows: the target marbling (approximately 10 mg) was scraped off using a metal spatula and methylated in 5 mL of methanol containing 5% hydrochloric acid for 3 h at 100°C. Adipose tissues of whole rib eye and subcutaneous fat were sampled by wiping the surface of the rib eye or fat with polyester plates 2.5 cm in width. The fatty acid methyl esters were extracted by hexane (5 mL) and analyzed using a flame ionization detector on a gas chromatograph (GC2010, Shimadzu Corporation, Kyoto, Japan). Nitrogen was the carrier gas, with a flow rate of 1.2 mL/min. A capillary column (CP-Sil88, 0.25 mm × 50 m, GL Science, Tokyo, Japan) was programmed to heat up from 140 to 180°C at 10°C/min and then from 180

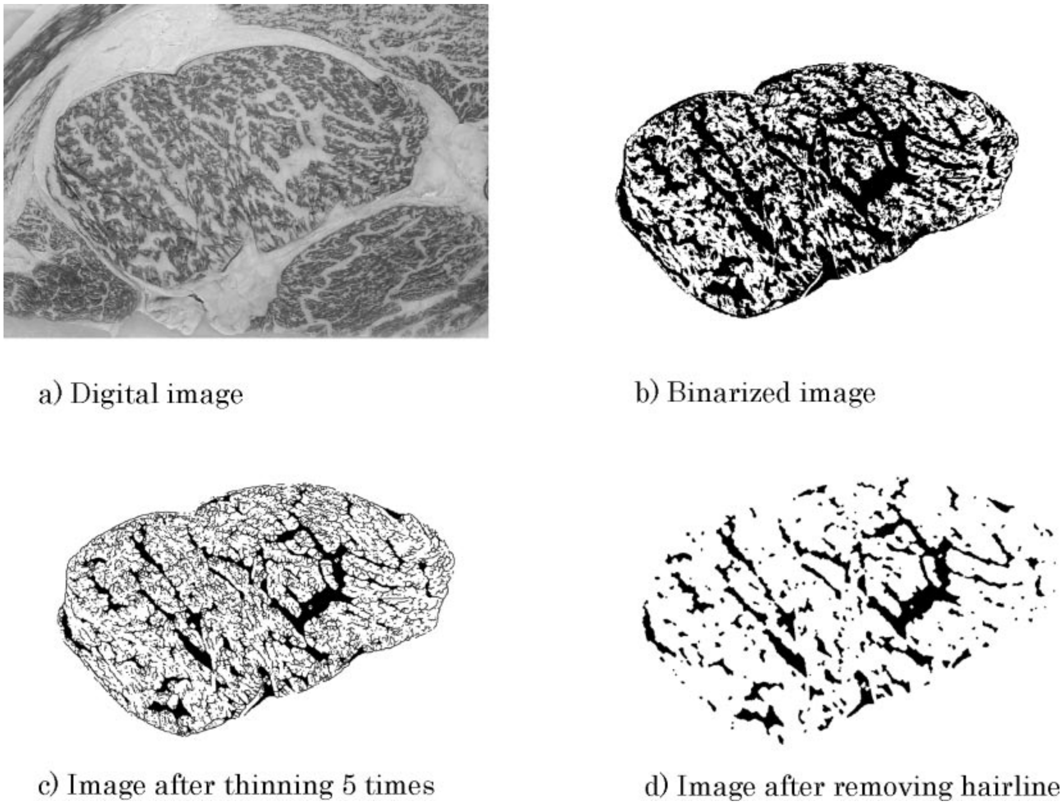

Figure 1. Detail of image analysis to calculate coarseness of marbling particles.

to 220°C at 2°C/min, followed by 5 min at 220°C. The injector and detector temperatures were 220 and 250°C, respectively. A standard fatty acid sample (GL Science) of known composition was analyzed to compare with the fatty acids in the samples, and the peak areas of fatty acids were quantified. The identified fatty acids

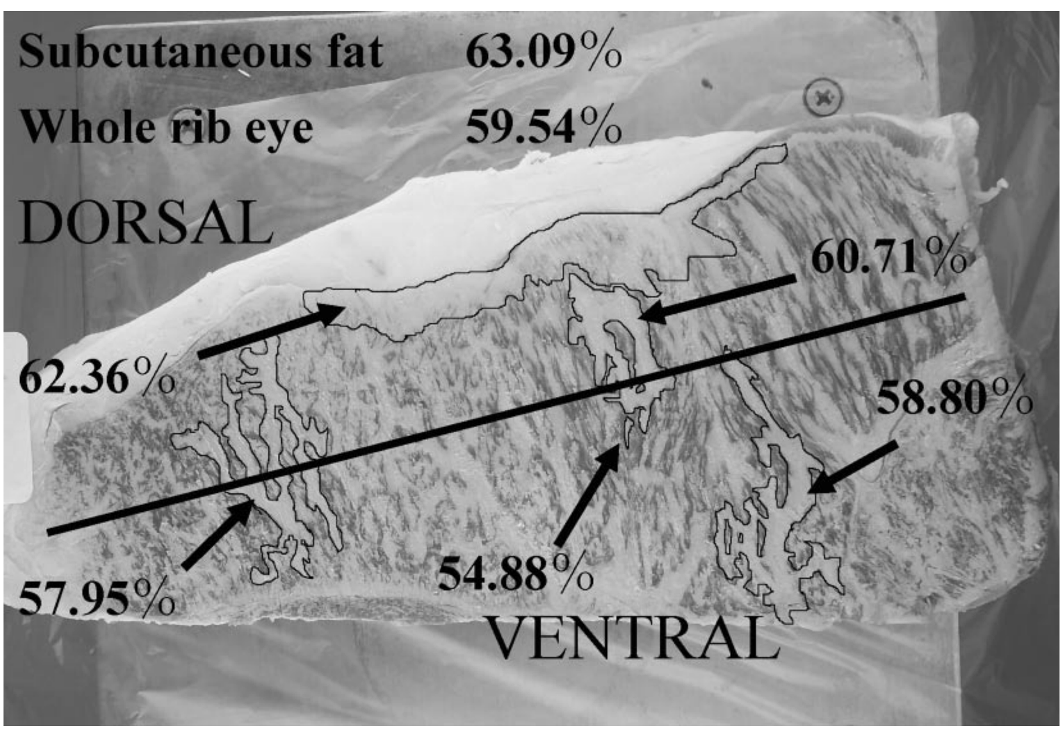

Figure 2. Example of an image of randomly sampled marbling flecks with MUFA. Sections separated by the first inertial axis (black line).

Table 1. Summary statistics of carcass traits and image analysis traits evaluated at the sixth and seventh rib cross-section of Japanese Black steers (n = 8)

Traits	Mean	SD	Minimum	Maximum
Carcass trait				
Carcass weight, kg	434.5	26.3	402.9	475.6
Rib thickness, cm	7.6	0.3	7.3	8.0
Subcutaneous fat, cm	2.7	0.7	1.8	3.8
Beef marbling standard	6.1	1.2	5.0	8.0
Image analysis trait[1]				
Rib eye area, cm^2	58.8	5.1	51.7	65.9
FAR, %	55.3	6.6	45.4	64.9
OCM, %	35.5	4.7	29.6	40.8
CMM, %	8.4	3.0	3.6	12.0
NSF	230.6	27.4	176.0	267.0

[1]FAR = fat area ratio; OCM = overall coarseness of marbling particles; CMM = coarseness of maximum marbling particle; NSF = number of small flecks.

were myristic acid (C14:0), myristoleic acid (C14:1), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3). The percentage of the sum of myristoleic acid (C14:1), palmitoleic acid (C16:1), and oleic acid (C18:1) to the total identified fatty acids was calculated as percentage of MUFA.

Statistical Analysis

Simple correlations of MUFA between the average value of the 5 marbling flecks and the value of the whole rib eye were determined to verify the validity of sampled points. In addition, simple correlations and Spearman's rank correlation coefficients among slice sections were obtained. An ANOVA was performed to investigate the effect of the image analysis traits on MUFA. Percentage of MUFA was treated as a dependent variable, whereas the categories of area, locations, and slice sections were treated as fixed effects. Multiple comparisons by least squares means were performed, and they verified the interactions. Statistical analysis was performed with SAS software (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Summary statistics are shown in Table 1. The average carcass weight was 434.5 kg with approximately a 70-kg range. Okamoto et al. (2007) reported similar carcass weights (437.4 kg) for the Japanese Black cattle in the same carcass market. Averages of rib thickness, subcutaneous fat, and Beef Marbling Standard number were 7.6 cm, 2.7 cm, and 6.1, respectively. Okamoto et al. (2007) reported similar value (8.0 cm, 2.5 cm, and 6.3, respectively) with this study.

There was a strong correlation (r = 0.93) between the average MUFA for the 5 marbling flecks in rib eye and the percentage of whole rib eye. This suggests that marbling fleck samples represented the whole rib eye well.

Subcutaneous fat had a significantly greater percentage of MUFA than whole rib eye (63.96 and 58.97%, respectively, $P < 0.01$). This result was consistent with that of Elias-Calles et al. (2000) and Oka et al. (2002), who investigated the difference between subcutaneous and intramuscular fats of Japanese Black cattle. The percentage of MUFA of subcutaneous fat in this study was greater than that reported by Taniguchi et al. (2004), who found 57.3% in Japanese Black cattle of almost the same age. Oka et al. (2002) measured a decreased percentage of MUFA of whole rib eye (51.73%) using Japanese Black cattle at 21 mo of age.

Each fixed effect significantly affected MUFA according to the ANOVA ($P < 0.05$). This result suggests that the percentage of MUFA differed in size and locations of marbling flecks and slice section even for a single cow. Figure 2 shows an example of MUFA for the marbling flecks.

The results of multiple comparisons by least squares means for each fixed effect are shown in Table 2. Middle and back slice sections had a greater MUFA than the front ($P < 0.05$), indicating that the percentage of MUFA tends to be greater toward the caudal end. The back section had a slightly greater percentage of MUFA than those of the middle section ($P > 0.05$).

The percentage of MUFA of marbling flecks categorized as L was significantly greater than M and S ($P < 0.05$), suggesting that larger marbling flecks tend to have a greater MUFA. Lending support to our findings, He et al. (1997) reported that large adipocytes had greater MUFA, and Yang et al. (2006) reported that the diameters of adipocytes in large marbling flecks were larger than those of small flecks on average.

Marbling flecks in the dorsal region had a significantly greater MUFA than those in the ventral region ($P < 0.05$). Subcutaneous fat had greater values than intramuscular fat, and the dorsal region had greater values than the ventral region in rib eye. These results indicate that external sections had a greater MUFA than internal sections even for identical muscle. Although the difference was small, this result corresponded to

Table 2. Multiple comparisons for least squares means of the percentage of MUFA for the effects of slice section, fleck size, and fleck location

Class	MUFA ± SE
Slice section[1]	
Front	$55.41^{x} \pm 0.48$
Middle	$59.89^{y} \pm 0.49$
Back	$60.13^{y} \pm 0.48$
Fleck size[2]	
Large	$60.22^{x} \pm 0.47$
Medium	$58.41^{y} \pm 0.53$
Small	$56.80^{y} \pm 0.46$
Fleck location[3]	
Dorsal	$59.15^{x} \pm 0.38$
Ventral	$57.81^{y} \pm 0.40$

x,yValues with different superscripts within the same class and column differ significantly ($P < 0.05$).

[1]Front = rib roast; middle = cranial sirloin; back = caudal sirloin.

[2]Large = >2.0 cm^2; medium = 0.4 to 2.0 cm^2; small = <0.4 cm^2.

[3]Dorsal = external part of rib eye from axis; ventral = internal part of rib eye from axis.

Table 3. Comparison for least squares means of image analysis traits of the rib eye images separated by the first inertial axis

Image analysis trait[2]	Slice section[1] Front	Middle	Back
FAR, %			
Dorsal[3]	47.7	65.3^{x}	67.7
Ventral[3]	52.6	56.0^{y}	64.8
OCM, %			
Dorsal	9.8^{x}	26.7^{x}	24.6
Ventral	19.3^{y}	18.8^{y}	19.7
CMM, %			
Dorsal	2.0^{x}	9.0	12.2^{x}
Ventral	7.9^{y}	4.0	4.7^{y}
NSF			
Dorsal	193	193	143
Ventral	168	225	166

x,yValues with different superscripts within the same class and column differ significantly ($P < 0.05$),

[1]Front = rib roast; middle = cranial sirloin; back = caudal sirloin.

[2]FAR = fat area ratio; OCM = overall coarseness of marbling particles; CMM = coarseness of maximum marbling particle; NSF = number of small flecks.

[3]Dorsal = external part of rib eye from axis; ventral = internal part of rib eye from axis.

the results of Oka et al. (2002), who reported that the percentages of MUFA at subcutaneous, intermuscular, intramuscular, and perinephric adipose tissues were 59.66, 55.12, 51.73, and 44.42%, respectively.

The interaction between location and slice section was significant ($P < 0.05$). In the front region, ventral marbling flecks had greater MUFA than the dorsal region. However, they were lesser than dorsal in middle and back.

To further investigate the interaction between locations and slice sections, we separated images of each slice by the first inertial axis into dorsal and ventral parts and performed image analysis again for these 2 images. The results for FAR, OCM, CMM, and NSF showed different trends between dorsal and ventral parts (Table 3). Although FAR, OCM, and CMM in the dorsal part were greater than those in the ventral part in the middle and back sections, these traits in the dorsal part were less than in the ventral part in the front section. The NSF of dorsal was greater than ventral in the front section but was lesser in the middle and back sections. Because the coarser marbling had a greater MUFA, image analysis traits such as OCM, CMM, and NSF may explain the difference. A significant interaction was not recognized among the area and slice section or the location.

Simple correlations and Spearman's rank correlations between the percentage of MUFA of subcutaneous fat and whole rib eye were calculated for each slice section (front, middle, and back) to investigate the variation by slice section. The percentage of MUFA of whole rib eye showed high values of simple and Spearman's rank correlation coefficients for all section combinations. Simple correlation coefficients for these combinations were 0.85 (front and middle), 0.92 (front and back), and 0.83 (middle and back), and rank correlation coefficients were 0.81, 0.93, and 0.91, respectively ($n = 8$, $P < 0.05$). These results suggested that LM with great percentage of MUFA in the front section tended to contain high MUFA throughout the muscle. Although MUFA of subcutaneous fat among section combinations showed moderate to high values of simple and rank correlation coefficients (0.52 to 0.70 and 0.57 to 0.83, respectively), most of them were not statistically significant. Only rank correlation between the middle and back sections was significant ($P < 0.05$).

Moreover, using ANOVA, we found that the effect of slice section was significant ($P < 0.05$) for whole rib eye, whereas it was not for subcutaneous fat. Although the percentage of MUFA of whole rib eye tended to be greater toward the caudal side, the values for subcutaneous fat were constant in every section.

There were some new findings in our study. Because the percentage of MUFA was affected by the activity of Δ^9-desaturase enzyme (Taniguchi et al., 2004), perhaps the enzyme activities were greater at the larger marbling flecks, located in the dorsal section, and caudal cross-section. For further investigation, especially in studies using the image analysis method, these findings would help to consider the differences between breeds, lines, animals, and so on. The image analysis traits showing the coarseness of the marbling flecks in rib eye (such as OCM, CMM, and NSF), which affect the percentage of MUFA, may become new factors in determining the quality of fat.

In conclusion, the differences in the percentage of MUFA between subcutaneous fat and intramuscular fat have been reported by some researchers, and this study presents a need for more investigations on the effects of sizes and locations of marbling flecks to facilitate the understanding of the variation of fatty acids. The area and location of marbling flecks were considered as a new approach to investigate the determining factors of the percentage of MUFA in this study. We feel these were important points and suggest that there are reasons to sample adipose tissue evenly from the rib eye. However, further analysis with more records and other breed line needs to be conducted.

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