BEEF MARBLING IN THREE DIMENSIONS – BREED COMPARISON BETWEEN ANGUS AND HANWOO CATTLE

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***Abstract*—A new approach is presented for analysing beef marbling from a 3 dimensional (3D) perspective. This paper describes a method which included sample segmentation into 4 mm thick sections, photography of each section, application of specifically designed software to assess 2D marbling parameters, and utilisation of the individual sections for input into another computer software program to reconstruct the 3D pattern of marbling. Ten 12 month-old steers in Australia and 9 Hanwoo steers and bulls (3 and 6, respectively) 12-14 months old in Korea were used to test the application of the method. The results demonstrated a significant difference in 3D structures (voxflecks) between the 2 breeds of cattle, with Hanwoo having more and smaller voxflecks than the Angus steers. The results may also suggest that the superiority of Hanwoo beef eating quality may be associated with the quantity and 3D distribution of fat voxflecks within the muscles.**

***Index Terms* — 3D structure, Angus, Hanwoo, Marbling.**

I. INTRODUCTION

Marbling of meat is defined as the appearance of white flecks or streaks of fatty tissue that spread within the muscle, located between the individual muscle fibers (Harper and Pethick, 2001). Marbling is an important parameter in assessing beef quality as its abundance increases the commercial value of carcasses in certain Asian beef markets (Hart, 2001). Although it has been demonstrated that genetics and nutritional factors affect marbling quantity, the aetiology of this phenomenon is still not fully understood. Therefore, the aim of this study was to analyse beef marbling by visualising it from a 3D perspective, and then compare the 3D parameters between the two breeds of cattle known to be diverse in marbling appearance.

II. MATERIALS AND METHODS

A. Animals and management

Ten 12 month-old Angus steers in Australia that had been fed on pasture were placed in a feedlot where they were fed a standard ration containing 75% grain supplemented with a medium level of vitamin A (60,000 IU retinyl palmitate/100 kg/day) for 308 days. In Korea, 3 steers and 6 bulls (12-14 months of age) that were also fed on pasture were placed in a feedlot where they were fed hay and a 75% concentrate ration. The steers in Australia were slaughtered at 22 months of age and the animals in Korea at 24 months of age. Approximately 12 cm long striploin samples were collected from the left side of the carcass quartered at 12th/13th ribs, vacuum packed and frozen until required for measurements.

B. Marbling parameters and ultrasound scanning

Marbling parameters (including various marbling fleck characteristics), were measured in *M. longissimus dorsi* using image analysis technology as described by Kruk et al., (2006). Intramuscular fat percentage (IMF%) in Australia was determined by a solvent extraction method as described by Christie (1989) with modifications described by Siebert et al., (2006). Chemical fat extraction in Korea was performed by solvent extraction method using a FAS 9001 system with meat samples trimmed from visible fat and homogenised in a food processor.

Ultrasound scanning for marbling was performed on striploins which were heated to 38°C in a water bath. The scans were taken by accredited graders in Australia and Korea. The Esaote-Pie Medical 200 SLC Scanner (Netherlands) with a 2 x 10 cm scanner head was used to take ultrasound measurements of the *M. longissimus dorsi* muscle samples in both countries.

C. 3D marbling reconstruction

Three-dimensional reconstruction of marbling was based on two-dimensional images which were taken from consecutively sliced 10 cm long frozen striploin samples. Before the slicing, in order to recognise the orientation of each slice, 2 landmark points (3 mm in diameter) were drilled perpendicular to the slices through each striploin. Each slice (4 mm thick) was photographed and various 2D marbling parameters were measured (Kruk et al., 2006). In order to reconstruct the 3D structure Amira 3.1 computer software was used. Colour slice images (25 from each striploin) were converted to binary (black and white) images. Additional processing steps and programs were written to obtain accurate alignment of meat slices using the landmark points. They included the identification of centers of the circular landmarks, and alignment of all slice images from individual *M. longissimus dorsi* samples according to the initial slice centres of the two landmarks.

The next step included a selection of the connectivity method between the 25 slices using a *ConnectComponent* module (Amira 3.1 User guide). A connected region is a set of adjacent voxels (the smallest box-shaped part of a three-dimensional image or scan derived from volume pixel) with intensity values based on a range defined empirically. Hence, an intensity range of 192-255 was selected, as histogram information of images demonstrated that the fat grey-scale region lies within this peak.

In addition to the connectivity method, the type of connectivity was defined under *ConnectedComponent* (Amira 3.1 User guide). Among many types of connectivity options such as six-neighbours connectivity (voxels are viewed as connected if they share a common face), 18-neighbours connectivity (voxels are viewed as connected if they share a common face or edge) and 26-neighbours connectivity (voxels are viewed as connected if they share a common face, edge or vertex), the 26-neighbours connectivity was selected. This type of connectivity was the most efficient as the slice thickness was not in the nano-magnitude but in the mm-magnitude. Fat flecks change and shift more significantly between the thicker than thinner slices. Thus, as observed with 6-neighbours connectivity, it would produce a large number of flecks in 3D image. Nevertheless, the connectivity of 18-neighbours and 26-neighours was more similar, supporting the decision to select the 26-neighbours connectivity. Furthermore, the type of connectivity selected did not affect the visualization of marbling structure. Computation of *ConnectedComponent* displayed a number of detected regions, the volume of the smallest and largest region, and the average volume. Voxels outside intensity range were considered to be background. The units in volume were the same as the specified voxel size in the descriptive file. Thus, the voxel size was in mm and, the volume was in mm^3.

The regions could then be visualized in 3D by using the *Isosurface* module, with threshold of 0.5 calculated by Amira 3.1 if the identified region was more than 255. The *Isosurface* module had a threshold range varying according to the number of connected regions. This enabled a specified number of regions of flecks to be viewed. This was not ideal since the observation of a marbling pattern of all fleck regions was desirable. The threshold 0 displayed all fleck regions, however, and this caused a highly congested the 3D image. Therefore, a number of filters available in Amira 3.1 software, such as minimum (erosion), maximum (dilation), Unsharpen Masking, median, Gasus, Sobel, Histogram, etc., were investigated. The 3D visualisation filtered data were correlated with ultrasound scanning results and the filter with the best correlation was chosen. The *minimum* filter appeared to be the most effective, which showed marbling structure without any change in threshold (T=0), and was used for 3D data acquisition. A 26-neighbours connectivity parameter was selected for all samples. The developed method had the potential to measure the total volume and the number of regions connected (fat flecks) in 3D reconstructed samples.

E. Statistical analysis

The two-dimensional data obtained from the binary images included 19 animal samples (10 Angus and 9 Hanwoo) with 25 slices per each animal, each slice containing more than 100 flecks, which were described by 10 parameters. Thus, approximately 19 animals x 25 slices x 100 flecks x 10 parameters, gave at least 475,000 data points to evaluate. The data was analyzed using Genstat, and the linear mixed-model included breed as a fixed factor, slice as a covariate, and animal as a random effect.

III. RESULTS AND DISCUSSION

Intramuscular fat content in the rib-eye region did not differ between the two breeds. Both Angus and Hanwoo cattle had 9.60% and 9.47% of IMF, respectively, and the difference was not statistically significant (Table 1). While the intramuscular fat levels were similar, the Hanwoo cattle had significantly more (and hence smaller) flecks than Angus steers. Hanwoo flecks were also shorter and narrower, as described by the fleck major and minor axes lengths, than the flecks of Angus steers (Table 1). Consequently, the Hanwoo flecks are more desirable from the consumer's perspective since it has been reported (Albrecht et al. 1996; Thompson 2004) that consumers appreciate small evenly distributed marbling flecks as this enhances eating quality.

There was a large difference between the breeds in the 3D parameters. The Angus steers had significantly less marbling voxflecks within a 10 cm length of striploin than Hanwoo cattle. The difference between the breeds was over 153-fold higher in favour of Hanwoo cattle and was highly statistically significant. These data demonstrate that Hanwoo cattle, which are known to be superior in marbling, have a more even distribution of marbling when assessed from a 3D perspective. Moreover, voxflecks of the Hanwoo cattle had an approximately 40-fold smaller volume when compared with Angus voxflecks. Such a highly dispersed distribution of a large number of voxflecks, which are smaller in volume, has the potential to contribute to a superior eating experience with Hanwoo beef. The more regularly distributed the intramuscular fat in the muscle, the more the connective tissue is dispersed, leading to increased tenderness (Albrecht et al. 1996). Until now, Hanwoo beef has been highly appreciated, especially by Korean consumers, due to its perceived superior quality. However, there was no strong scientific evidence explaining the superior eating quality of this breed. The 3D preliminary results from the herein study suggest that the size, number and distribution of fat voxflecks could explain why meat from Hanwoo cattle is highly valued for eating quality.

Table 1. Marbling fleck characteristics of Angus and Hanwoo steers and bulls fed commercial diets

Variable	Angus	Hanwoo
IMF%	9.60 ± 0.15^{a}	$9.47.16\pm0.23^{a}$
No. flecks per slice	105.5 ± 8.8^{a}	185.5 ± 15.1^{b}
Ave. fleck area	383.2 ± 13.9^{a}	153.3 ± 22.5^{b}
Major length	12.99 ± 0.08^{a}	7.91 ± 0.16^{b}
Minor length	5.20 ± 0.03^{a}	3.42 ± 0.06^{b}
Voxfleck #	197 ± 0.1355^{a}	30252 ± 0.2474^{b}
Voxfleck volume	2.34 ± 0.49^{a}	0.06 ± 0.89^{b}

Note: IMF% = intramuscular fat extracted with organic solvents, fleck number = the mean total of all the flecks calculated for each slice (unit), fleck area = area of marbling flecks (number of pixels), major and minor length = length in pixels of the major and minor axis of the best fitting ellipse, voxflecks# = number of flecks in 3D parameters, voxfleck volume = average volume of voxflecks. Within each row, values that are not significantly different ($p>0.05$) share a common superscript.

IV. CONCLUSION

This study demonstrated a novel method of viewing beef marbling and described a technique which was developed to reconstruct the 3-dimentional (3D) structure of marbling. The 3D image reconstruction technique demonstrated that the application of sequential slicing, photographing and computer software reconstruction is very precise and provides high-resolution images and reconstructs.

When the newly developed technique was applied to assess the marbling of Angus and Hanwoo meat, the results showed a significant difference between the breeds with Hanwoo cattle having a smaller size, and larger number and distribution of the fat voxflecks within the muscles. In this preliminary study, we conclude that Hanwoo cattle possess a predisposition to produce more even and therefore, desirable marbling characteristics. The 3D marbling parameters provide more concrete evidence of why Hanwoo beef is regarded as superior in eating quality. However, more research is needed to further investigate the breed and nutritional effects on 3D marbling distribution and its association with beef eating quality.

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