Prediction and Visualization of Fat and Fatty Acid Content of Beef Using Near-Infrared Multispectral Imaging

Ken-ichi KOBAYASHI[1)], Yasunori MATSUI[2)], Yosuke MAEBUCHI[1)], Ken NISHINO[1)], Toshihiro TOYOTA[1)], and Shigeki NAKAUCHI[1)]
[1)] Department of Computer Sciences, Toyohashi University of Technology; Toyohashi, JAPAN
[2)] Mie Prefecture Livestock Research Institute; Matsusaka, JAPAN

Abstract

The beef quality grade is greatly affected by visible fat content. Especially, in Japanese black (Wagyu) cattle, high fat content is typically valued highly. In this paper, we describe the feasibility of beef evaluation by visualizing fat characteristics using near-infrared (NIR) multispectral imaging. An intact raw beef cut from Wagyu cattle was used as an evaluation target. The content of fat and fatty acid, such as the total saturated fatty acid (SFA) content, the total unsaturated fatty acid (UFA) content, myristic acid (C14:0) , palmitic acid (C16:0), stearic acid (C18:0), myristoleic acid (C14:1), palmitoleic acid (C16:1), oleic acid (C18:1), and linoleic acid (C18:2) were estimated and visualized. The total SFA content was calculated as the sum of myristic acid, palmitic acid, and stearic acid. Also, the total UFA content was calculated as the sum of myristoleic acid, palmitoleic acid, oleic acid, and linoleic acid. Reference values for the fat content and fatty acid composition were determined by conventional physical and chemical methods. The fatty acid composition was determined from the extracted lipids by Folch's method, by gas chromatography (GC) using its methyl ester. The fat content was determined by using the Gerhardt SOXTHERM. The NIR multispectral images of the sample were acquired by using the SPECIM Spectral Camera SWIR. It works in the wavelength range of 970-2500 nm with 6.3 nm of bandwidth at 320 pixels resolution in spatial domain. The absorbance spectra of each pixel calculated from pixel intensity of subject and reference white standard was used for constructing the prediction model. In total, 33 samples from various parts of the 2 head of Wagyu cattle were measured. Calibrations were performed by a partial least squares (PLS) regression using mean extracted spectra from each individual sample, limited wavelength range from 1000 to 2300 nm. The coefficients of determination (R^2) were between 0.68 and 0.87. The ranks by evaluation index (EI) were “B (high accuracy)” and “C (slightly high)”. The ratios of the standard error of prediction to the standard deviation (RPD) were between 1.74 and 2.74. These results indicate a sufficient feasibility of the prediction except for myristoleic acid content. The visualizations, which show the spatial distribution of fatty acid content, were performed by applying the model to predict the content of each pixel.

Introduction

The quality of beef carcasses is currently evaluated based on the standard by the “Japan Meat Grading Association (JMGA)” in Japan. Generally, the quality of beef carcass graded on a scale of one to five as a result of the evaluating from the four standpoints. The four standpoints are “fat marbling”, “meat color and brightness”, “firmness and texture of meat”, and “fat color, luster and quality”. The determination of these evaluations is carried out conventionally by visual observation. The visual observation is performed on the only single standard area (longissimus dorsi). Nevertheless, whole body of a head of cattle is represented by a graded value. For example, “fat marbling” is one of the most important indicators of meat quality, which was evaluated by comparing with Beef Marbling Standard (BMS) by JMGA. The very high level of marbling is preferred especially in Japan.

Yoshikawa et al. [1] were proposed an automatic grading system for beef marbling using monochromatic image. Okamoto et al. (2003), Hori et al. (2005), Takahashi et al. (2006) were developed an objective beef carcass evaluation system by using an image of digital still camera [2-4]. These evaluations were based on fat marbling, and aim to estimate BMS. To estimate the BMS, the evaluations were performed based on “a pixel is fat or not”. Therefore, the fat characteristics were not considered.

On the other hand, fat characteristics of beef have been analyzed by physical and chemical methods. However, these methods are destructive, messy, and time-consuming. As a nondestructively method, some studies based on NIR spectroscopy were reported [5-7]. In these studies, NIR spectra were obtained by point measurement.

The overall objective of this study is to develop a objective, nondestructive, and rapid quality evaluation method applicable to intact raw beef (not minced) based on the composition. We use NIR multispectral imaging technique to predict the beef composition. By visualizing the special distribution of the content, we aim to indicate characteristics rather than grade.

Materials and Methods

Meat Sample Preparation

A total of 33 meat samples from 2 heads of Japanese Black (Wagyu) cattle were collected. The beef carcasses were kept at −25 degrees Celsius after about 60 days of aging at 0-5 degrees Celsius. A sample was made from a cut of many parts of cattle: shoulder clod, sirloin, clod, knuckle, rump, spencer roll, short plate, silver side, chuck, rib, foreshank, brisket, and boneless short rib. Samples from one to five, each approximately 8 cm long, 12 cm wide, and 0.5 cm thick, were made from different positions of the same region of each carcass. For two different analyses, by the conventional method and multispectral imaging, two identical samples were needed. We used two adjacent cuts for both the samples. Just before measurement by multispectral imaging, the samples were thawed at room temperature until the temperature increased to about 0-5 degrees Celsius.

Physical and Chemical Analyses (Reference)

The fat content and fatty acid compositions used for reference values were analyzed by physical and chemical methods. The reference fat content was determined using the Gerhardt SOXTHERM for the percentage in the muscle. The fatty acid compositions were determined by gas

chromatography of fatty acid methyl esters of lipids. The lipids were extracted from about 10 grams of minced muscle by Folch's method. This minced muscle was made from a limited area of the sample. The area was selected manually to avoid intermuscular fat areas, whose characteristics were of interest to us, as much as possible. The seven main fatty acids—myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), myristoleic acid (C14:1), palmitoleic acid (C16:1), oleic acid (C18:1), and linoleic acid (C18:2)—were analyzed by their percentage in the lipid.

The total saturated fatty acid (SFA) composition was calculated as the sum of myristic acid, palmitic acid, and stearic acid; and, the total unsaturated fatty acid (UFA) composition was calculated as the sum of myristoleic acid, palmitoleic acid, oleic acid, and linoleic acid.

Target of Prediction and Visualization

The reference value of fatty acid composition by physical and chemical methods is the proportion of the fatty acid in the lipids. Generally, the absorption spectrum from an object is proportional to the concentration of its content, in accordance with the Lambert-Beer law. To predict fatty acid quantities using the absorbance spectra from intact raw beef, the fatty acid content was defined as:

$$C_i = F \times K_i \tag{1}$$

where C_i is the fatty acid content as the proportion in the whole beef, F is the fat content as the proportion in the whole beef, K_i is the fatty acid composition as the proportion in the lipids. In this paper, we use C_i as the target of prediction and visualization.

Near-infrared (NIR) Multispectral Imaging

We developed the spatial image acquisition system with multispectral bands shown in Figure 1. This system consists of a linear image sensor (Spectral Camera SWIR, SPECIM Spectral Imaging Ltd., Finland), a linear slide table, and 3 halogen light sources. (Figure 2.) The resulting 2-dimensional image represents the light intensities along the observation line (X-axis in the spatial domain) and the wavelength axis. The spectral camera works in the wavelength range of 970-2500 nm with a 6.3 nm of bandwidth at a resolution of 320 pixels (X-axis). To acquire a spatially 2-dimensional image, the linear slide table below the camera was moved at a constant speed along the Y-axis. To achieve sufficient light intensity for reflection measurements in the NIR, the samples were illuminated with 3 halogen lamps surrounding the observation line. Three multispectral images—the dark current image ($I_{Dark}(\lambda)$), white diffuse reflectance standard image ($I_{Reference}(\lambda)$), and sample image of intact raw beef ($I_{Subject}(\lambda)$)—were acquired for camera calibration and dark current correction.

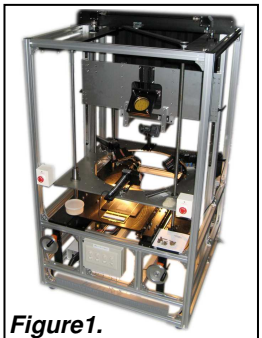


Figure1. *The imaging system.*

Mathematical Pre-treatments

A software package "The MathWorks MATLAB 7.5 (R2007b)" was used for analyzing the multispectral image data. The absorbance was calculated for each pixel in each

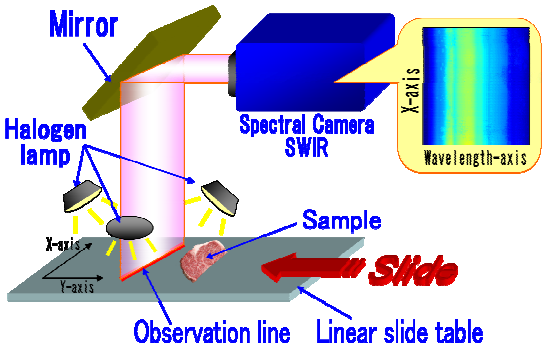


Figure2. *A schematic of the multispectral imaging system.*

wavelength frame from the pixel intensity of the subject and reference. The pixel intensity was corrected by dark measurement $I_{Dark}(\lambda)$ to reduce the dark current noise. The reflectance $R(\lambda)$ was calculated as the ratio of the subject intensity to reference intensity. Thus $R(\lambda)$ is given by

$$R(\lambda) = \frac{I_{Subject}(\lambda) - I_{Dark}(\lambda)}{I_{\mathrm{Re}\,ference}(\lambda) - I_{Dark}(\lambda)} \tag{2}$$

Then the absorbance ($A(\lambda)$) was given by the negative logarithm of the reflectance ($R(\lambda)$) as:

$$A(\lambda) = \log_{10}(1/R(\lambda)) \tag{3}$$

Multiplicative Scatter Correction (MSC)

Multiplicative scatter correction (MSC) was applied to the absorbance $A(\lambda)$ to correct spectra intensity differences [8].

$$A(\lambda) = a(\lambda)A_0 + b(\lambda) + e(\lambda) \tag{4}$$

$$A_{MSC}(\lambda) = \frac{A(\lambda) - b(\lambda)}{a(\lambda)} \tag{5}$$

where λ is the wavelength, $A(\lambda)$ is the raw data of the spectrum value at wavelength λ, $A_0(\lambda)$ is the mean value of all $A(\lambda)$ spectra of the data set at wavelength λ, $A_{MSC}(\lambda)$ is the MSC corrected spectra, $a(\lambda)$ is a multiplicative correction factor, $b(\lambda)$ is an additive correction factor, and $e(\lambda)$ is the residual error. Correction was performed by estimating $a(\lambda)$ and $b(\lambda)$ from the data set by the least-squares method.

Extract Spectra

To create a prediction model, spectra were extracted from a selected part of the multispectral image. These parts were selected manually by comparison with corresponding photos of the areas used for physical and chemical analysis. The extracted spectra were averaged by each sample, because the reference values from the physical and chemical method produced only one value for each sample, which was the average of the whole sample. Therefore, each sample has corresponding single spectra.

Development of Prediction Model

The prediction models for estimating the fatty acid content of beef from NIR absorbance spectra were obtained using PLS (partial least squares regression)[9] as a regression method for each content. Calibrations were developed using a number of samples which was analyzed by physical and chemical methods. Factor number f, which is a key parameter for PLS modeling, was varied from 1 to 20 using leave-one-out cross-

validation method. The f were evaluated using prediction residual error sum of squares (PRESS), shown by

$$PRESS(f) = \sum_{n} (C_{\text{Re}\,ference} - C_{\text{Pr}\,ediction}(f))^2 \quad (6)$$

where $C_{Reference}$ is a reference value of fatty acid content analyzed by physical and chemical methods, $C_{Prediction}(f)$ is a prediction from the model using f factors from NIR spectrum, f is factor number of PLS (from 1 to 20), and n is the sample number of validation. The value of f minimizing PRESS was selected.

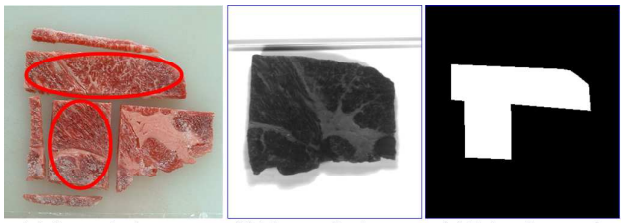
Results

Ranges of the Samples

Table 1 shows the results of analyses by physical and chemical methods for calibration and validation samples. These values are the percentage of content in lipids, except for the value of fat content. The fat content is the percentage of lipids in the entire sample. The content of each fatty acid as a proportion of the entire sample is calculated as shown in Table 2.

Result of Measurements

An area analyzed by physical and chemical methods is indicated by the photo shown in Figure 3 as an example of a mask used to extract spectra.



(a) An analyzing area by physical and chemical methods. (b) Mean reflectance image (c) Extracting mask

Figure3. *A mask for extracting spectra.*

Figure 4 indicates the spectra of pixel intensity of the white diffuse reflectance standard and dark current. This white standard plot is a multiplication result of the camera sensitivity and intensity of the light source. We used the wavelength range of 1000-2300 nm, which could achieve sufficient pixel intensity. The collected raw absorbance spectra from 33 samples, presented in Fig. 5 and Fig. 6, show the spectra corrected by applying the MSC to the raw absorbance.

Prediction Results

Calibrations were developed using 33 samples, by each content: Fat content(%), total SFA content(%), total UFA content(%), myristic acid content(%), palmitic acid content(%), stearic acid content(%), myristoleic acid content(%), palmitoleic acid content(%), oleic acid content(%), and linoleic acid content(%). The PLS factor number f was also determined for each content. The ranges of predicted values are shown in Table 3.

SDP was calculated as follows

$$SDP = \sqrt{\frac{\sum_{n}(C_{\text{Re}\,ference} - C_{\text{Prediction}})^2 - \{\sum_{n}(C_{\text{Re}\,ference} - C_{\text{Prediction}})\}^2 / n}{n-1}} \quad (7)$$

RPD and EI were calculated as follows

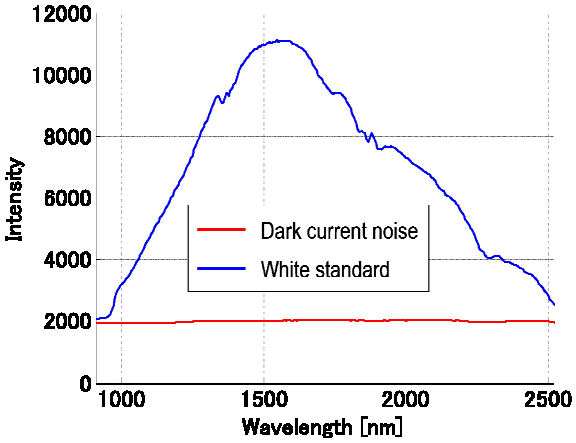


Figure 4. *An example of the pixel intensity of a white diffuse reflectance standard and dark current.*

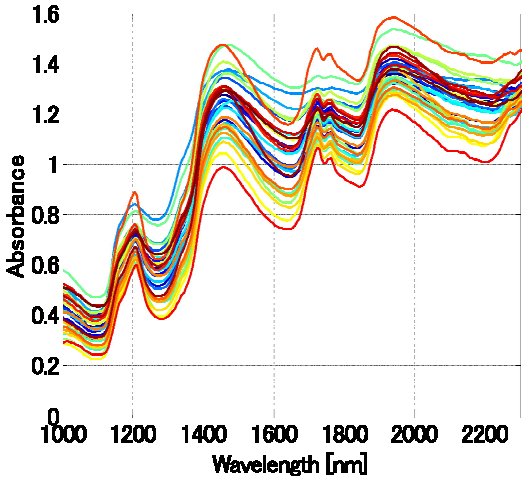


Figure 5. *The collected raw absorbance spectra.*

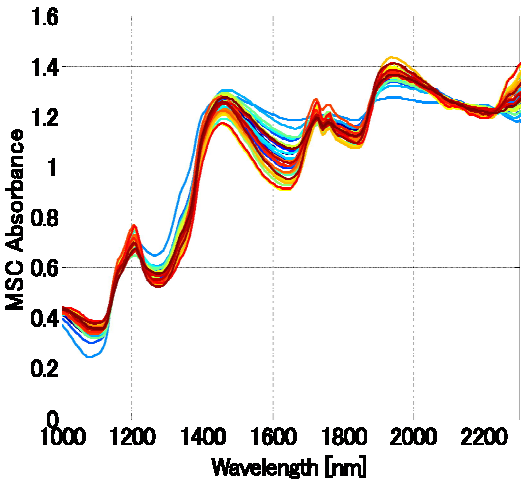


Figure 6. *The absorbance spectra after correction by MSC*

$$EI = \frac{2 \times SDP}{Range} \times 100 \quad (8)$$

$$RPD = SD / SEP \quad (9)$$

Calibrations were evaluated by the value of EI and RPD. The criterion based on EI shown in Table 4 [10]. In the

evaluations using EI, the calibrations of Fat content(%), total SFA content(%), total UFA content(%), myristic acid content(%), palmitic acid content(%), palmitoleic acid content(%), oleic acid content(%), and linoleic acid content(%) were ranked "B", the accuracy rated as "high", and the practical usability was "good". The calibrations of stearic acid content (%), and myristoleic acid content(%) ranked "C", the accuracy was "slightly high", and the practical usability was "fair". The RPD values were classified into four levels of prediction accuracy: an RPD < 1.5 indicates very bad model/predictions; an RPD between 1.5 and 2.0 is poor model/predictions; an RPD between 2.0 and 2.5 is good model/predictions; and an RPD > 2.5 indicates very good/excellent model/predictions [11]. In the evaluations using RPD, the calibrations of fat content(%), total UFA content(%), and oleic acid content(%) were "excellent model/predictions"; total SFA content(%), myristic acid content(%), palmitic acid content(%), palmitoleic acid content(%), and linoleic acid content(%) were "good model/predictions"; stearic acid content(%) and myristoleic acid content(%) were "poor model/predictions". Although these rankings do not reflect an absolute standard, they indicate sufficient feasibility of the prediction.

Figure 7 shows correlation plots for each content of prediction and measurements. They clearly confirm the goodness of the prediction. The coefficients of correlation R were ranged from 0.824 to 0.933.

Table 1. Composition analyzed by physical and chemical *method*

	Fat(%)	Total SFA(%)	Total UFA(%)	Myristic acid(%)	Palmitic acid(%)	Stearic acid(%)	Myristoleic acid(%)	Palmitoleic acid(%)	Oleic acid(%)	Linoleic acid(%)
Min	5.25	31.7	57.8	2.1	22.8	5.3	0.9	3.5	50.1	1.9
Max	55.4	42.2	68.3	2.9	28.7	12.5	2.6	8.9	58.3	4.7
Mean	27.42	35.95	64.05	2.40	25.52	8.05	1.65	5.87	53.96	2.59
SD	12.67	2.48	2.48	0.21	1.47	1.53	0.45	1.37	1.81	0.52

Table 2. Content (percentage of whole sample.)

	Fat(%)	Total SFA(%)	Total UFA(%)	Myristic acid(%)	Palmitic acid(%)	Stearic acid(%)	Myristoleic acid(%)	Palmitoleic acid(%)	Oleic acid(%)	Linoleic acid(%)
Min	5.25	1.79	3.46	0.12	1.33	0.35	0.09	0.36	2.76	0.25
Max	55.4	19.5	35.93	1.33	13.3	4.88	1.17	3.56	31.78	1.27
Mean	27.42	9.81	17.61	0.66	6.91	2.24	0.45	1.57	14.92	0.67
SD	12.67	4.38	8.38	0.31	2.97	1.15	0.25	0.75	7.27	0.26

Table 3. Statistical parameters of prediction (n=33)

	Fat(%)	Total SFA(%)	Total UFA(%)	Myristic acid(%)	Palmitic acid(%)	Stearic acid(%)	Myristoleic acid(%)	Palmitoleic acid(%)	Oleic acid(%)	Linoleic acid(%)
R	0.926	0.886	0.933	0.918	0.890	0.824	0.840	0.888	0.931	0.900
R^2	0.857	0.785	0.870	0.842	0.792	0.678	0.705	0.788	0.867	0.811
Factor(f)	6	3	6	6	3	2	5	6	6	6
SEP	4.87	2.07	3.06	0.13	1.38	0.67	0.14	0.35	2.69	0.12
SDP	4.80	2.03	3.02	0.12	1.36	0.65	0.13	0.35	2.65	0.11
RPD	2.60	2.12	2.74	2.48	2.16	1.74	1.81	2.14	2.70	2.26
	(>2.5)	(>2.0)	(>2.5)	(> 2.0)	(>2.0)	(>1.5)	(>1.5)	(>2.0)	(>2.5)	(>2.0)
	Excellent	Good	Excellent	Good	Good	Poor	Poor	Good	Excellent	Good
EI	19.14	22.97	18.57	20.56	22.65	28.91	25.05	21.68	18.25	22.17
(Rank)	B	B	B	B	B	C	C	B	B	B

Table 4. Criterion based on evaluation index (EI)

EI	Rank	Accuracy	Practical usability
– 12.4	A	Very good	Very good
12.5 – 24.9	B	High	Good
25.0 – 37.4	C	Slightly high	Fair
37.5 – 49.9	D	Low	Poor
50.0 –	E	Very low	Poor

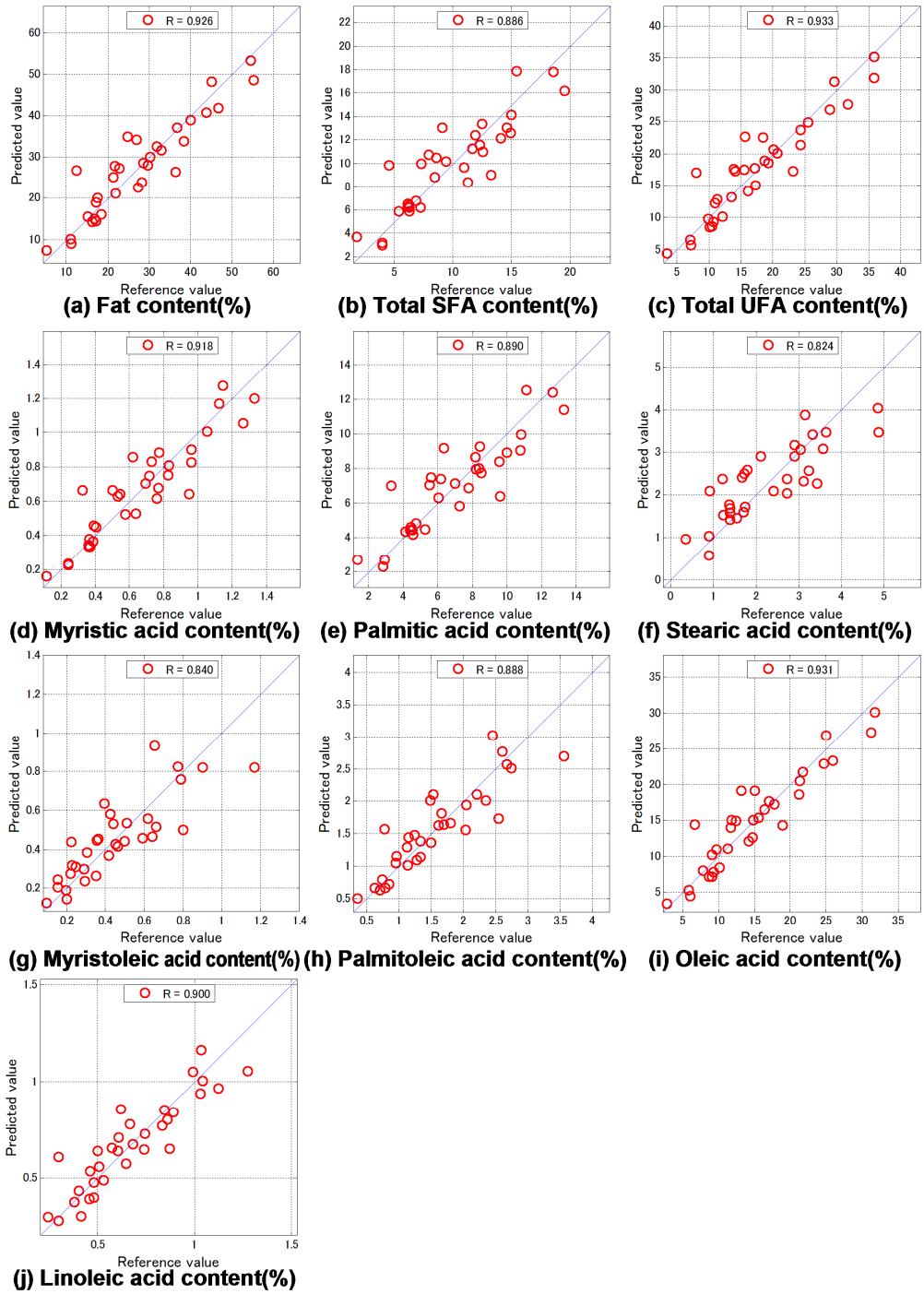


Figure 7. *Correlation plots*

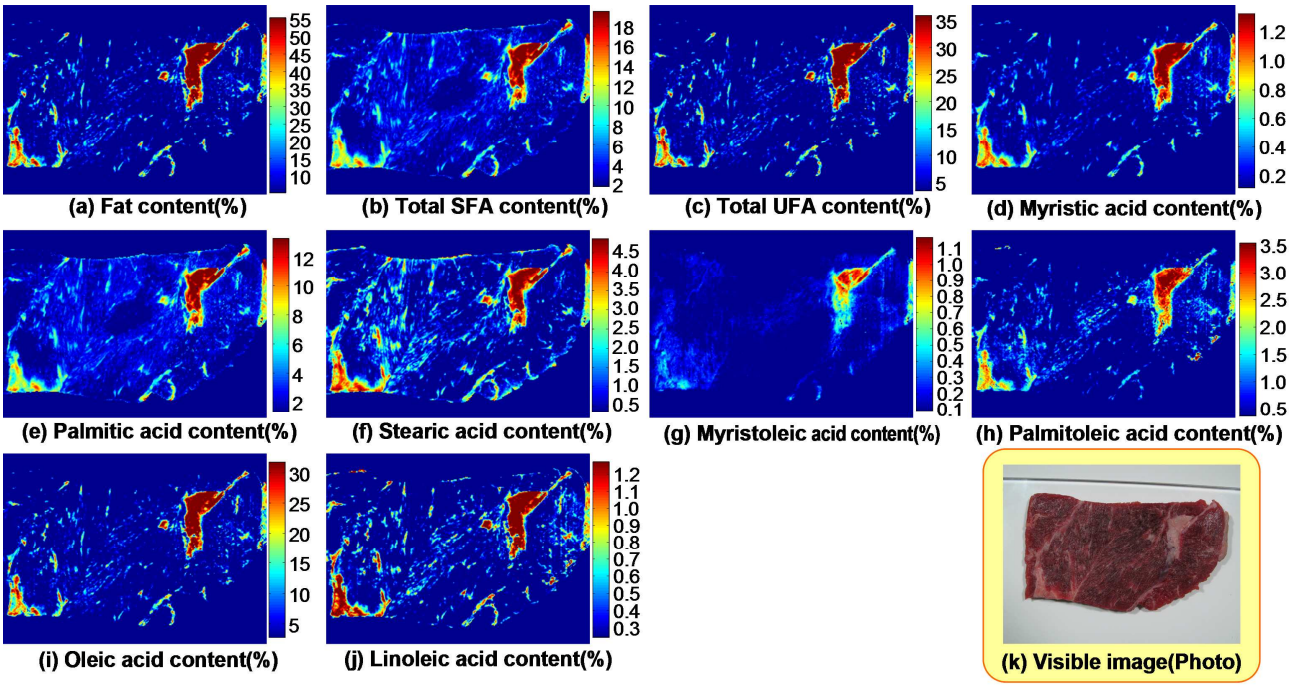


Figure 8. *Examples of visualization results.(Silver-Side region)*

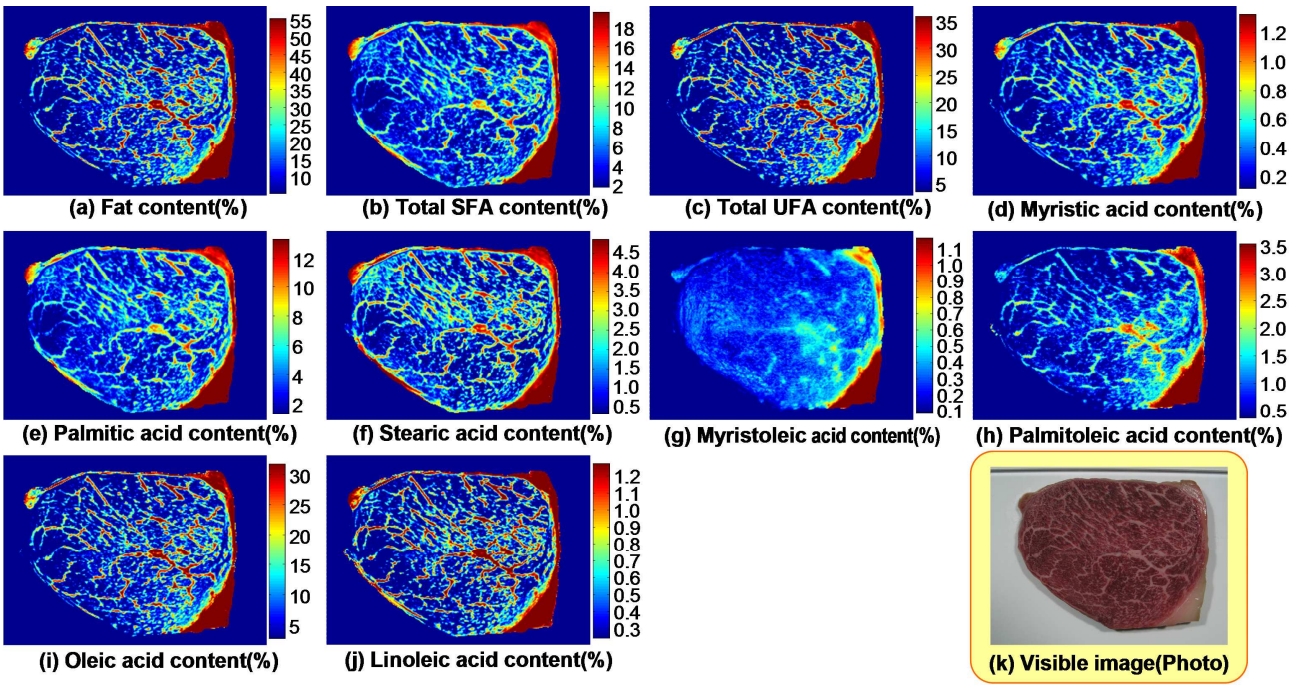


Figure 9. *Another examples of visualization results.(Clod region)*

Visualization of Fatty Acid Content

By applying the model to each pixel of the multispectral image, visualization was performed to show the spatial distribution of fatty acid content. Figure 8 shows examples of the visualization for a single sample (Silver-Side region). Because the physical and chemical analysis needs several grams of samples, it is difficult to confirm each pixel of the visualized result. These results indicate that the intermuscular fat area (that was judged by visual observation) was visualized as a high pixel value. Also, every pixel indicates a value that indicates not only whether it is fat, but a certain range. Additionally, there are some gristle (not fat) areas. It is difficult to distinguish fat area from other white area by appearance in visible image.

Figure 9 shows the results of another sample (Clod region). Especially in lean area, the contents were indicated as higher pixel value compared with Figure 8.

Conclusions

In this paper, we developed prediction model predicting various fatty acid content based on NIR spectra. Calibrations were evaluated according to the EI and RPD value. Evaluation results of the most calibrations showed good accuracy of prediction. By applying the model to visualize the spatial distribution of content, the feasibility of evaluating beef based on its fat characteristics, using NIR multispectral imaging, was indicated.

Acknowledgments

This study was supported in part by the Knowledge Cluster Initiative (2nd Stage) "Hamamatsu Optronics Cluster" from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), and Global COE (Centers of Excellence) Program of MEXT.

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Author Biography

Ken-ichi Kobayashi received his Master of Engineering from the Toyohashi University of Technology (2007). Since then he worked in the development department at JUKI DENSHI KOGYO Corporation in Akita, Japan. Currently (2009-), he is a Ph.D. student in the department of computer sciences at the Toyohashi University of Technology.