Color Determination of Beef Rib Eye Using Near Infrared Spectroscopy

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ABSTRACT : Beef samples of loin eye area from New Zealand, USA and three quality grades of Hanwoo were analyzed using near infrared spectrophotometer with reference values from laboratory optical Chromameter to determine effective spectrum range and mathematical treatment for determination of color values. R^2s of prediction models were not improved much by calibrating with whole light range (400~2500 nm) compared to using visible range (400~1100 nm). Standard errors of calibration and prediction were influenced by possible bias due to sampling non-homogeneous sample sources. However, partial differentiation in the first order was more stable against sampling biases than second derivatives of the spectra. Lightness value was little different among the five sample sources of beef. Beef samples from USA were brighter and more reddish than beefs of Hanwoo or from New Zealand ($p<0.05$). Yellowness of USA beef was the highest followed by beef from New Zealand, which was also higher than Hanwoo beefs of three quality grades ($p<0.05$). (*Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 2 : 263-267*)

Key Words : Loin Eye, NIR, Color, Spectrum Range, Mathematical Treatment

INTRODUCTION

Use of Near Infrared (NIR) spectroscopy has advantages over traditional qualitative or quantitative analytical procedures (Kim, 1997; Park, 1999). It saves time for analysis, labor, and preparation of samples before analysis. Therefore, the NIR technique can be applied to concurrent monitoring of product qualities and compositional changes such as during on-line processing. It also can be applied to a variety of physical or chemical characteristics of materials, liquid, ground or natural phase solid. Recently, NIR has become widely applied in agricultural product analyses (Cho, 1998) and quality control. With the developments in statistical (chemometric) applications and delicate calibration approaches, NIR techniques will become more customized and feasible in many agri-businesses.

There has been considerable research in the field of meat science and processing. However, as pointed out by Shenk and Westerhaus (1991a, b), a wider range of samples should be used for calibration to improve quality or precision and accuracy of NIR analysis with fresh meat or processed meat products. There are many factors affecting the results of NIR analyses, both mechanical effects and sampling effects. Mechanical variations arise from noise, light sources, linearity of signals, wavelengths chosen, mathematical treatment, temperature control, power management, sample cells, or electro-magnetic environment. Sampling variations arise from chemical composition, physico-chemical properties, moisture, density, ambient temperature, shape and inner temperature of the sample, and particle size and distribution.

The purpose of this study was to determine coloring of beef samples of domestic and imported samples. We also examined the effect of mathematical treatment (the first and the second partial derivatives with respect to selected wavelengths) for non-homogeneous samples of beef loins. Another inspection was made to see if the pattern of spectrum and the results would be different by using two sets of wavelength ranges.

MATERIALS AND METHODS

Sample preparation

Cooled beef rib eye samples of Hanwoo (Korean Native Cattle) were taken from Korea Refrigerators Inc. and Karakdong slaughterhouse of NLCF (National Livestock Co-operatives Federation), Korea. Three different beef quality levels (1, 2, 3; for information about Korean beef quality scoring, see Lee, 1997) of Hanwoo beef products were taken.

Beef rib eye samples of foreign breeds were purchased from Dongbang Mart, Chonan and Shinsegae department store, Seoul, Korea. Those beef products were imported as frozen from New Zealand and the United States.

Rib eye samples were kept refrigerated at 4℃ and held for 30 minutes at room temperature before NIR analyses. All samples were sliced to 10 mm thickness and cut to fit the coarse sample cup provided with NIR equipment.

Spectrophotometry

A near infrared spectroscope manufactured by Foss NIRSystems, Inc., Maryland, USA (Model No. 6500) was provided for use by the National Livestock Research Institute, Korea. A total set of 152 samples

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was used for calibrations by reflectance mode with modified partial least squares (MPLS) regression model. Before fitting calibration equations, a total of 103 samples out of 152 samples were selected. Outliers were determined arbitrarily by the global (center) and neighborhood (select) distance algorithms suggested by Shenk and Westerhaus (1991a).

Colorimetry

Beef samples after NIR analyses were immediately put into a Tristimulus colorimeter (Chromameter Model CR-200b, Minolta Inc., Japan) to determine color values (L*, a* and b*) using standard illuminant C (daylight). L*, a* and b* scales (also referred to as CIELAB) represent lightness, redness and yellowness, respectively, according to the standards developed by Commission Internationale de l'Eclariage (CIE) in 1976. Hunter scale of optical reflectance was set to L=96.7, a=0.0 and b=2.3.

Statistical Procedure

Mean differences of Chromameter readings between sources of beef loins were made for prediction samples by Duncan's Multiple Range Test at 95% confidence level. Analyses of variance with source effect in the linear model were performed using SAS ANOVA procedure (SAS Institute Inc., 1989).

Statistical procedures for instrumental calibration and output data summarization were performed using WinISI II (version 1.02A) program developed by Infrasoft International, LLC (FOSS NIRSystems/ TECATOR, 1999).

Modified multiple partial regression models were fitted to estimate color parameters (L*, a*, b*) combining reflectance values of different wavelengths. The first and second derivatives of reflectance energy (log 1/R) were taken mathematically to reduce baseline offsets from variations in particle size and composition.

Multiple partial correlations between NIR measures and colorimetric measures were estimated as R^2 values from the above models taken separately for each parameter. Standard errors for calibration (SEC) and for prediction (SEP) were calculated by the methods described by Park (1999):

$$SEC=\{\sum(x_i-y_i)^2 / (N-k)\}^{1/2}$$
$$SEP=\{\sum(x_i-y_i-d)^2 / (N-1)\}^{1/2}$$

Where, x_i : predicted value of ith sample from NIR measures from regression of parameter on reflectance at each wavelength,

y_i : parameter estimator of ith sample from colorimetric measure,

d : bias as average deviation of predicted values from colorimetric measures ($=\sum(x_i-y_i) / N$),

N : number of samples,

k : number of parameters in the regression models.

RESULTS AND DISCUSSIONS

Colorimetry

Mean values and variations in the color parameters (L*, a*, b*) from Chromameter are presented in table 1. Corresponding measures from NIR reflectance are

Table 1. L*, a*, and b* values of calibration set (N=103) by Chromameter

Parameter[1]	Mode	Mean	SD	Range	CV (%)
L*	35.8	37.0	3.49	30.0~49.4	9.45
a*	23.0	21.1	4.34	13.7~31.8	20.57
b*	11.8	9.2	2.86	3.8~14.1	31.10

[1] L*=lightness, a*=redness, b*=yellowness.

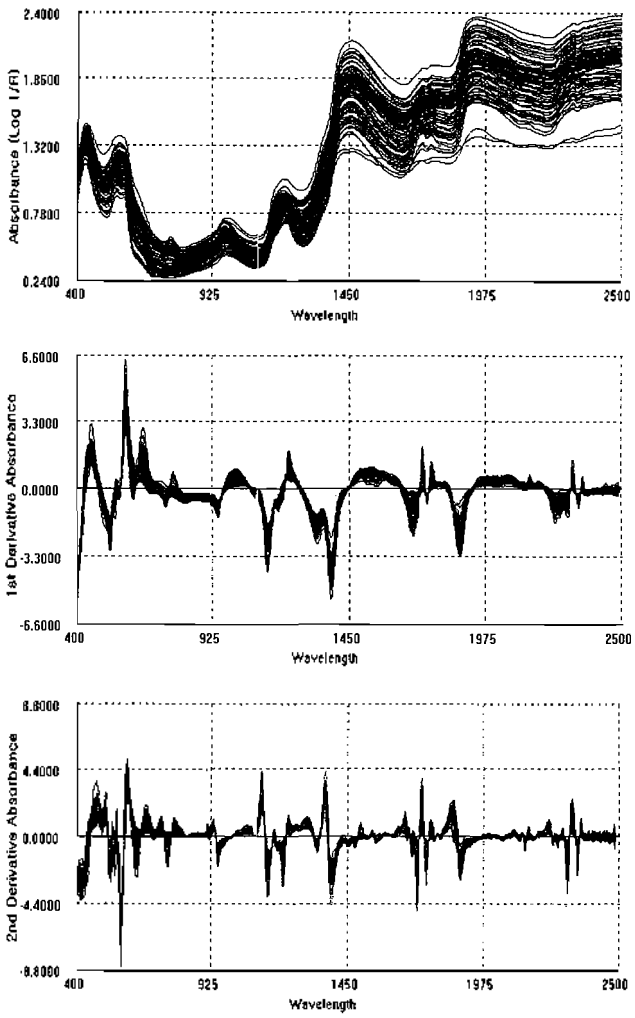

Figure 1. Spectra of beef using reflectance mode by NIR (Top: Original Scale, Middle: 1st derivative, Bottom: 2nd derivative)

illustrated in figure 1. Data on a total of 103 beef samples out of 152 samples were fitted to regression models as a calibration set for NIR spectrophotometry, as summarized in table 1.

Lightness (L*) values were quite small in coefficient of variation. However, yellowness (b*) showed larger variation than lightness. Redness (a*) showed the largest variation of all three parameters. Mode of b* was about one standard deviation apart from its mean. This wider range in yellowness might be due to different degrees of marbling in Hanwoo beef samples and also due to different fat coloring between Hanwoo and imported beef samples. Therefore, there can be two different major source of variation, physical structure of meat and source of meat, which varies in myoglobin and fat constituents. It was proposed (FOSS NIRSystems/TECATOR, 1999) that a current heterogeneous calibration set would be a better estimate when absorbed energy measures of NIR analyses were differentiated in the second order rather than in the first order.

Color values determined by Chromameter are summarized in table 2. The effects of source of beef loins were all significant ($p<0.01$) for all three color variables. These samples, 10 samples for each beef source, were set for prediction in NIR analyses. Coefficients of variations (CV) were smaller than for the calibration set. Mean values of each parameter in prediction samples were within range of one standard deviation from the means of the calibration samples (table 1).

Lightness value (L*) was the greatest for beef samples from the USA, and the lowest for Hanwoo beef samples of quality grade 3. R^2 of the model for L* was 0.47. Redness value (a*) was the greatest for beef from USA and the lowest for beef from New Zealand. The mean of b* values of American beef samples was the greatest of all five sources and that of New Zealand samples was the second highest. Yellowness values of imported beef, which were significantly higher than three Korean beef samples of three quality gradings, partly support our assumptions about different coloring of intramuscular fat particles. Higher a* values in imported beef samples can be explained by their higher myoglobin contents than Korean beef samples (Kang and Kim, 1999) which might be affected by age and exercise from grazing practice or partly by over-dosage of vitamin E during the fattening procedure (personal communication with Dr. Kang). Higher b* values in imported beef samples than those in domestic beef samples might be due to higher β-carotene consumption by American and New Zealand beef cattle by grazing fresh pasture which is much less in Korean roughage nutrition. Statistically non-significant differences in color values between three quality grades of Korean beef samples may be due to application of the colorimetric device only on the red (muscle) portion of the sample beef slices.

Table 2. Color determination (L*, a* and b*) of loin eye prediction set by Chromameter by sources

Variable	Source[1]	N	Mean[2]	Min.	Max.	SD	CV (%)
L*	KOR1	10	35.28^{a}	33.6	36.8	33.6	3.80
	KOR2	10	36.08^{a}	33.2	40.1	33.2	7.49
	KOR3	10	33.09^{b}	29.8	35.9	29.8	5.91
	NZ	10	35.44^{a}	33.8	37.9	33.8	3.62
	USA	10	38.99^{c}	35.5	44.5	35.5	7.11
Average		50	35.78				5.90
a*	KOR1	10	17.25^{a}	14.8	19.1	1.41	8.16
	KOR2	10	16.57^{ab}	15.0	18.8	1.18	7.12
	KOR3	10	17.73^{a}	15.6	20.0	1.45	8.19
	NZ	10	15.48^{b}	12.4	18.6	2.06	3.33
	USA	10	26.18^{c}	23.5	31.5	2.76	10.53
Average		50	18.64				9.99
b*	KOR1	10	6.04^{a}	4.2	7.9	1.23	
	KOR2	10	5.27^{a}	2.9	6.5	1.17	
	KOR3	10	5.80^{a}	4.4	7.4	0.95	
	NZ	10	8.35^{b}	6.9	9.8	0.92	
	USA	10	12.31^{c}	10.1	14.7	1.38	
Average		50	7.55				

[1] Sources: KOR1=Hanwoo beef, Korean quality grade 1, KOR2 =Hanwoo beef, Korean quality grade 2, KOR3=Hanwoo beef, Korean quality grade 3, NZ=Beef from New Zealand, USA=Beef from the United States of America.

[2] Means with different superscripts in the same column of each parameter differ significantly ($p<0.05$).

Table 3. Prediction of color values (L*, a* and b*) of beef loin eye area by partial differentiation

Wavelengths		400~2500 nm				400~1100 nm			
		Calibration		Prediction		Calibration		Prediction	
		R^2	SEC#	R^2	SEP#	R^2	SEC	R^2	SEP
L*	1st Derivative	0.78	1.50	0.78	1.45	0.76	1.58	0.74	1.50
	2nd Derivative	0.89	0.89	0.89	1.40	0.86	1.09	0.85	1.43
a*	1st Derivative	0.84	1.65	0.83	1.60	0.84	1.66	0.78	1.94
	2nd Derivative	0.96	0.79	0.82	1.89	0.94	1.00	0.53	2.79
b*	1st Derivative	0.88	1.02	0.90	0.97	0.92	0.83	0.91	0.87
	2nd Derivative	0.97	0.50	0.90	0.87	0.91	0.82	0.89	1.04

SEC=standard error of calibration; SEP=standard error of prediction.

Therefore, fat particles were not the major part estimated for coloring.

NIR spectrophotometry

Figure 1 shows the NIR spectra using reflectance mode in original and the first and second derivative scales. In all three scales, there were characteristic peaks and the bottom lines were clear between 400 and 1400 nm region. However, in its original scale, spectra over 1400 nm region showed high overlapping to make it difficult to separate characteristic peaks. Therefore, the first or the second derivative scales of the spectra were recommended (Mitsumoto et al., 1991; FOSS NIRSystems/TECATOR, 1999). Cho (1998) also pointed that mathematical treatment of the spectrum was necessary to make a stable calibration equation and to separate spectra as in chromatographic techniques. However, choice of the order of partial differentiation must be considered with respect to possible creation of 'noise', clarity of characteristic peaks or prediction power through the NIR procedure.

Table 3 shows model fitting of the samples used for calibration and for prediction in NIR analyses using the first and the second derivatives of absorbed energy by reflectance mode at wavelengths from 400 to 2500 nm. R^2 represents the proportion of variation explained by the regression models fitted relative to the total variation adjusted for the means. The regression models vary depending on the properties of samples and the variables to be predicted.

There was not any great difference in R^2 using spectra of whole visible and NIR range (400~2500 nm) or a shorter range close to the visible (400~1100 nm).

Multiple coefficients of determination (R^2) of the NIR spectra were all high enough to explain most of the variation in dependent parameters (L*, a* and b*) at calibration or at prediction. Standard errors of prediction for all parameters were not different greatly from standard errors of calibration for the regression equations with the first derivatives in the models. However, the SEP of a* (2.79) using the second derivatives of the spectral values of 400~1100 nm range was unexpectedly greater than SEC of the parameter (1.00) with much lower R^2 (0.53). This might be the case of overfitting a calibration model (Chung and Kim, 2000), or the sampling bias due to culling with global and neighborhood H values before getting calibration. Hildrum et al. (1994) proposed that, after scatter correction, useful information from the data might have been removed as well. Because the samples used were not homogeneous by the nature of samples or by the source of getting samples, those used for prediction might have been taken mostly from different sources from a major portion of calibration sample sets after culling outlying values. Therefore, the source of bias involved in SEP would be mainly from sampling error because the R^2 decreased greatly while SEP increased.

We also found a minor deviation of prediction set for b* using second derivative spectra of 400~2500 nm range. Sampling errors mentioned above would also be applied to b*. As seen in table 2, the proportion of significantly higher a* and b* value of USA beef among sampling units might have contributed to this discrepancies.

In general, differentiation of spectra improved separation of characteristic peaks. The first derivatives were more stable and unaffected by bias due to sampling as opposed to the recommendation of FOSS NIRSystems/TECATOR (1999), which preferred second derivatives for non-homogeneous materials.

Figure 2 compares results from colorimetric analyses and NIR analyses. Lightness values were not different much among different sources of beef samples. However, the average redness value of USA beef samples was higher than any other beef samples as shown by the colorimetric observations. Yellowness value of New Zealand beef samples was higher than Hanwoo samples of all three quality grades, and that of USA beef was the highest of all five sources of beef samples. This coincides with the results from colorimetry.

We could not find any detectable differences in L*

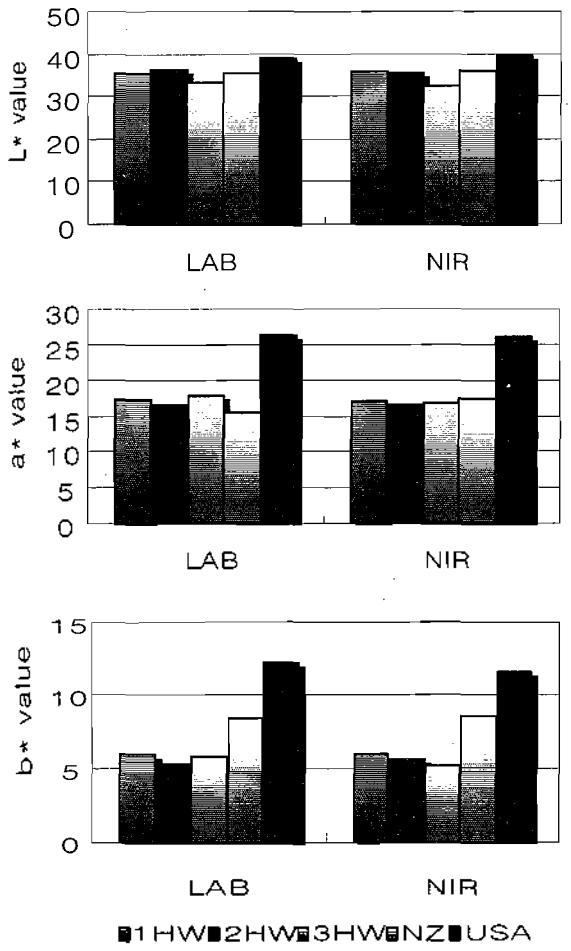

Figure 2. Histograms comparing colorimetric analyses (LAB) and NIR analyses of L*, a* and b*. 1HW=Hanwoo beef of quality grade 1, 2HW=Hanwoo beef of quality grade 2, 3HW=Hanwoo beef of quality grade 3, NZ=New Zealand beef, USA=USA Beef.

or a* values among samples of three quality grades of Hanwoo and of New Zealand in both LAB and NIR analyses.

CONCLUSION

This study indicates advantages in using NIR analyses to determine color parameters of meat. Heterogeneity from different sources of beef adds another source of variation to determination of beef colors. NIR analyses using the first derivatives of the spectral energy were more stable to sampling errors associated with different sources of material. There were differences in yellowness values between different sources of beef. This difference was well detected by the NIR analyses and by conventional colorimetric analyses. The correlation between the two analytical procedures was high enough to prove the advantage of replacing conventional colorimetric analysis with NIR because the latter can provide the valuable information in addition to color parameters of beef.

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