Near-Infrared Spectroscopic Technique for Detection of Beef Hamburger Adulteration

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A near-infrared spectroscopic technique was developed to detect beef hamburgers adulterated with 5−25% mutton, pork, skim milk powder, or wheat flour with an accuracy up to 92.7%. The accuracy of detection increased with the increase of adulteration level. When an adulterant was detected, the adulteration level was further predicted by calibration equations. The established calibration equations for predicting adulteration levels with mutton, pork, skim milk powder, and wheat flour had standard errors of cross-validation of 3.33, 2.99, 0.92, and 0.57% and coefficients of variance of 0.87, 0.89, 0.99, and 1.00, respectively. The results of this study indicate that near-infrared spectroscopy is potentially useful in detection of beef hamburger adulteration.

Keywords: *NIR; beef hamburger; adulteration; authenticity*

INTRODUCTION

Adulteration of beef hamburgers with cheaper meat or nonmeat materials has been reported in the literature (Barai et al., 1992; Patterson, 1985). Such adulteration is not only a commercial malpractice but also a health risk as certain consumers may be allergic to the adulterant material. To prevent such adulteration, a screening method is required. Several adulterant detection methods, such as gel electrophoresis (Santin and Centrich, 1997), enzyme-linked immunosorbent assay (Whittaker et al., 1983), DNA probe (Meyer and Candrian, 1996), and composition analysis (Cantoni et al., 1973; Peric et al., 1984; Abe and Okuma, 1995) have been reported. However, all these methods are laborious and technically demanding.

Near-infrared spectroscopy is a rapid analytical method that uses spectra in the near-infrared region. The technique has been used not only for food composition determination but also for food authentication or classification (Davis and Grant, 1987; Downey, 1996). The technique has been employed successfully to discriminate normal and abnormal chicken carcasses (Chen and Massie, 1993), fresh and frozen-then-thawed beef (Thyholt and Isaksson, 1997), beef and kangaroo meat (Ding and Xu, 1999), and broiler and local chicken carcasses (Ding et al., 1999). It has also been used to detect adulteration in fruit and oil products (Evans et al., 1993; Wesley et al., 1998). The present study examined the potential of using near-infrared spectroscopy to detect adulteration of beef hamburgers with mutton, pork, skim milk powder, or wheat flour. A spectroscopic technique was also developed to determine the adulteration level when an adulterant was detected.

EXPERIMENTAL PROCEDURES

Hamburger Preparation. Frozen beef, pork, and mutton were purchased at local meat shops. Creamy butter, table salt, white pepper powder, plain wheat flour, and skim milk powder were purchased at local supermarkets. The main components in wheat flour were protein and starch, and the compositions of skim milk powder were protein (37.5%), sugar (51%), and fat (less than 10%).

Authentic beef hamburgers were made according to the following procedure. For each hamburger, 70 g of minced beef was mixed with 10 g of butter, 1.5 g of salt, 0.5 g of white pepper powder, and 10 g of water. The mixture was then wrapped in food film and pressed into a thin round patty of about 0.6 cm in thickness. The patty was cooked in a microwave oven with the power output set at 900 W for 2 min. After cooking, the hamburger was cooled to room temperature and wrapped in food film. For adulterated hamburgers, beef was partially substituted with minced pork, mutton, paste of skim milk, or paste of wheat flour at a 5, 15, or 25% level. The paste of skim milk or wheat flour consisted of 30% skim milk powder or wheat flour and 70% water. In total, 50 authentic beef hamburgers and 144 adulterated hamburgers (12 for each adulterant at each adulteration level) were prepared. Spectroscopic analysis was performed on the same day of hamburger preparation.

Spectroscopic Analysis. Reflectance spectra of raw hamburger mixtures, cooked hamburgers, and minced hamburgers were obtained using a visible/near-infrared spectrophotometer (NIRSystem 6500, Perstorp Analytical Inc., Silver Spring, MD). Raw hamburger mixtures were packed in polyethylene bags before being presented to the spectrophotometer with a high fat/moisture cell (205 mm in height, 40 mm in width and 5 mm in depth). Cooked hamburgers were trimmed into a disk shape of approximately 38 mm in diameter and 8 mm in thickness before being presented to the spectrophotometer in a ring cup cell (38 mm in diameter and 8 mm in depth). Samples of minced hamburgers were prepared by mincing the cooked hamburgers with an electric food chopper and were presented to the spectrophotometer in the same way as for raw hamburger mixtures. All samples were scanned three times at room temperature, and the average reflectance spectrum of each sample was used for further analysis. The spectra between 400 and 2500 nm in 2-nm steps were recorded as $\log(1/R)$, where R represented reflected energy. Spectra within each treatment group were centered to detect any outliers using 3.0 as the cutoff H value (Rodrigues-Otero et al., 1994). In the present study, two outlying spectra, one from a raw mixture sample with 15% mutton adulteration and the other from a sample of minced beef hamburger, were found and deleted. Spectrum recording and all data handling were

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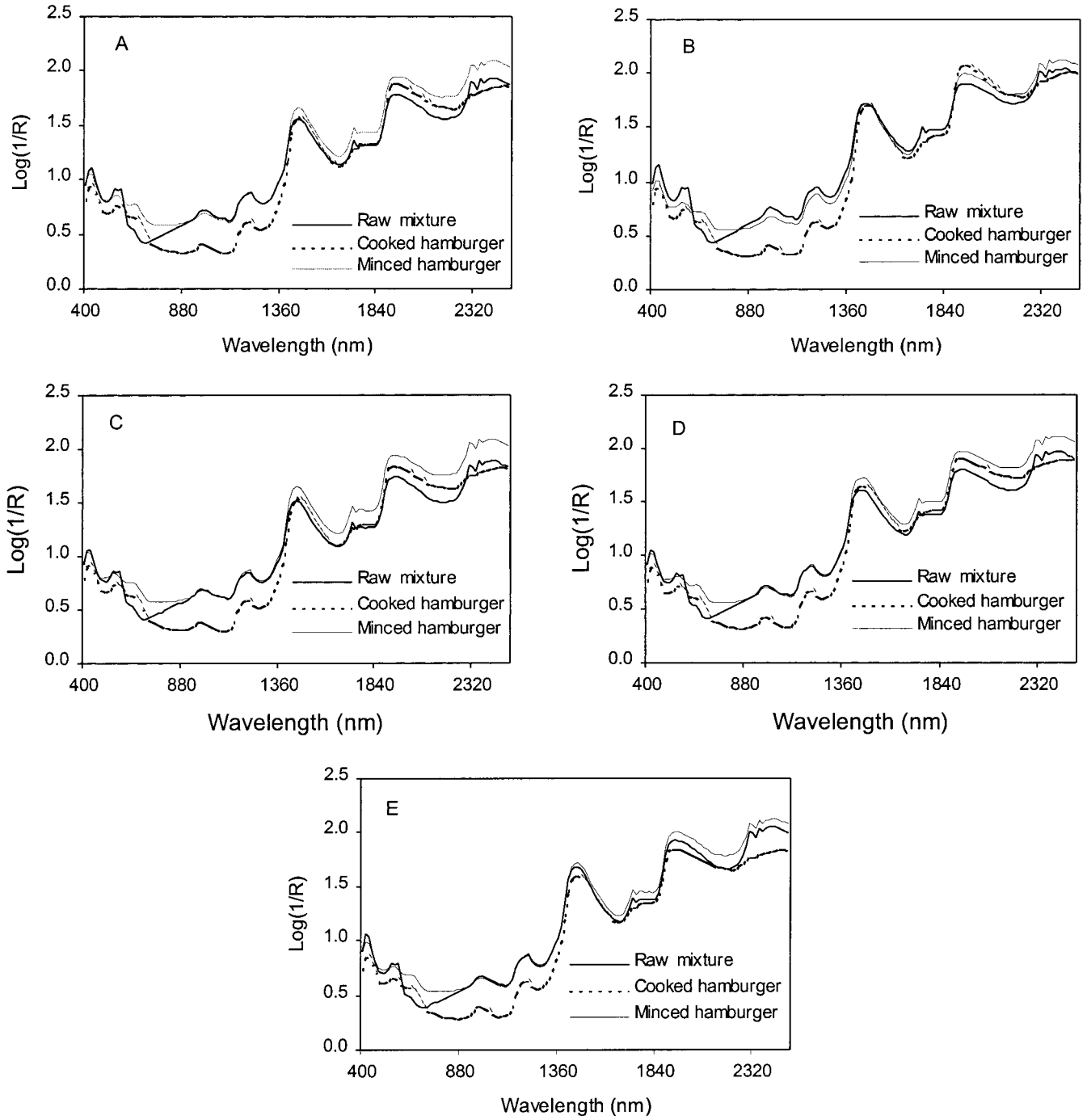

Figure 1. Average reflectance spectra of raw, cooked, and minced beef hamburgers (A) and hamburgers adulterated with 25% mutton (B), pork (C), skim milk powder (D), or wheat flour (E).

carried out using computer software (ISI, version 3.00, Infrasoft International, Port Matida, PA).

Discriminant Analysis. Discriminant analysis was performed using canonical discriminant analysis (CDA) and *K*-nearest-neighbor (KNN) method. Prior to CDA and KNN analyses, spectral data of 194 samples were compressed into principal components (PC) by principal component analysis (PCA). CDA and KNN were performed with the first 20 PCs (less than one-third of the sample number), which comprised 100% variations of the original spectral information. CDA, a parametric method, was based on a normal distribution within each class, and linear discriminant function was developed from pooled covariance matrices (SAS, 1990). KNN was a nonparametric method, and pooled covariance matrices were chosen for calculating the distance. During KNN analysis, *K* was chosen as an odd number between 1 and 15. Cross-validation was performed in both CDA and KNN analyses, and each observation was classified by the discriminant function developed from the data set excluding that observation. In this way, samples would not affect their own classification, and hence a realistic estimation of performance would be obtained. PCA, CDA, and KNN were all performed using a statistical program SAS (version 6, SAS Institute Inc., Cary, NC). A total of 194 samples was grouped into beef hamburgers and hamburgers adulterated with mutton, pork, skim milk powder, or wheat flour regardless of adulteration level. Classification accuracy was calculated as the percentage of samples correctly allocated to their original groups by the discriminant functions.

Quantitative Prediction. To predict the adulteration level, calibration equations were developed for each adulterant. In the calibration process, beef hamburgers were assigned an artificial value of 0, and hamburgers with 5, 15, and 25% adulteration were assigned values of 5, 15, and 25, respectively. Modified partial least squares (mPLS) was used as

regression method. Sample outliers were deleted during calibration using a cutoff T value of 2.5 and a cutoff H value of 4.0 as described by Murray (1990). The number of outlying samples detected in the present study was between 0 and 5, and the number of samples used to establish each calibration equation was between 81 and 85. The performance of established calibration equations was evaluated by cross-validation. The results were expressed as standard error of cross-validation (SECV) and coefficients of variance (R^2). Effects of sample presentation and scatter correction by standard normal variance and de-trend (SNVD) and second derivative treatment of spectral data on calibration performance were examined. The calibration was performed using the ISI computer software (version 3.00, Infrasoft International, Port Matida, PA).

RESULTS AND DISCUSSION

Spectral Characteristics. The average reflectance spectra of beef hamburgers and hamburgers adulterated with pork, mutton, skim milk powder, or wheat flour at 25% level are shown in Figure 1. The spectrum of beef hamburgers showed absorption bands at 428, 546, and 574 nm, possibly related to meat pigments; at 978 and 1448 nm related to O−H second and first overtones; at 1212 nm associated with C−H stretch second overtone; at 1936 nm due to water absorption; at 1732 and 1768 nm related to C−H stretch first overtones; and at 2312 nm associated with C−H combination tone (Osborne et al., 1993; Cozzolino et al., 1996). The spectra of adulterated hamburgers were similar to that of beef hamburgers, but position shifts of band peaks were observed after adulteration. The shifts increased with the increase of adulteration level (data not shown). Cooked hamburgers reflected less energy than raw mixtures (Figure 1). The positions of spectral peaks of absorption bands also shifted slightly after cooking. For example, absorption bands at 428, 574, 1448, 1732, and 1936 nm in the spectrum of raw hamburger mixtures shifted to 426, 642, 1452, 1730, and 1938 nm after cooking. The spectral shifts incurred by cooking may be due to alterations of chemical surroundings of the relevant chemical bonds.

Detection of Adulteration. Discriminant functions were developed with both CDA and KNN methods. During CDA analysis, canonical variates were first constructed from the first 20 principal components of the spectral data, and then the discriminant function was developed. Scatter plot of the first two canonical variate, which comprised over 70% intergroup variations, showed that beef hamburgers were separated clearly from hamburgers adulterated with skim milk powder or wheat flour (Figure 2). The separation among the beef hamburgers and hamburgers adulterated with pork or mutton was not very clear on the plot, but a separation trend was evident.

Discriminant functions developed with CDA classified all samples into beef hamburgers or hamburgers adulterated with pork, mutton, skim milk powder, or wheat flour regardless of adulteration level with an accuracy up to 90% (Table 1). Sample presentation had a marked impact on classification accuracy, and the sample presented as minced hamburgers gave the best discriminant performance (Table 1).

The performance of the discriminant function developed with KNN method was influenced by the preset K values. The performance was the best when $K = 1$. Therefore, the discriminant function used for classification of hamburger samples was developed with the K value set at 1. As shown in Table 1, the discriminant function thus developed classified samples into beef hamburgers and hamburgers adulterated with pork, mutton, skim milk powder, or wheat flour regardless of adulteration level with an accuracy up to 92.7%. The highest classification accuracy was achieved when samples were presented to the spectrophotometer as minced hamburgers (Table 1).

In general, the classification accuracy increased with the increase of adulteration level. For both CDA and KNN methods, most misclassified samples were those hamburgers adulterated at the 5% level (Table 2). For samples adulterated at 15% or above, misclassification was less than 6% when samples were presented to the spectrophotometer as minced hamburgers (Table 2). For

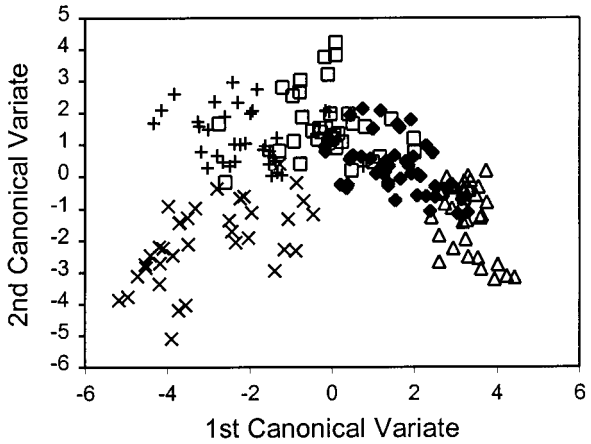

Figure 2. Score plots of first versus second canonical variates of authentic and adulterated beef hamburgers; regardless of adulteration level when minced hamburger samples were presented to the spectrophotometer. Filled squares, beef hamburgers; empty squares, hamburgers adulterated with mutton; triangles, hamburgers adulterated with pork; +, hamburgers adulterated with skim milk powder; ×, hamburgers adulterated with wheat flour.

Table 1. Classification Accuracy of Authentic and Adulterated Beef Hamburgers by Canonical Discriminant Analysis (CDA) and *K*-Nearest-Neighbor Method (KNN) with Cross-Validation Based on NIR Reflectance Spectra

	classification accuracy[a] *(%)*	
sample presentation	CDA	KNN
raw mixture	76.6	83.5
cooked hamburger	68.8	81.6
minced hamburger	90.0	92.7

[a] Classification accuracy was calculated as the percentage of samples allocated correctly into their original groups by the discriminant functions.

Table 2. Percentage of Misclassified Samples at Different Adulteration Levels

		adulteration level[b] (%)			
sample presentation	method[a]	0	5	15	25
raw mixture	CDA	28.0	38.9	36.1	13.9
	KNN	24.0	22.2	19.4	16.7
cooked hamburger	CDA	20.0	66.7	47.2	22.2
	KNN	20.0	27.8	30.6	11.1
minced hamburger	CDA	8.2	33.3	2.8	5.6
	KNN	6.1	22.2	5.6	2.8

[a] Discriminant function was developed with either canonical discriminant analysis (CDA) or K-nearest-neighbor method (KNN). [b] Samples with 0% adulteration were the authentic beef hamburgers, and the remaining samples were those adulterated with pork, mutton, skim milk powder, or wheat flour at 5, 15, and 25% respectively.

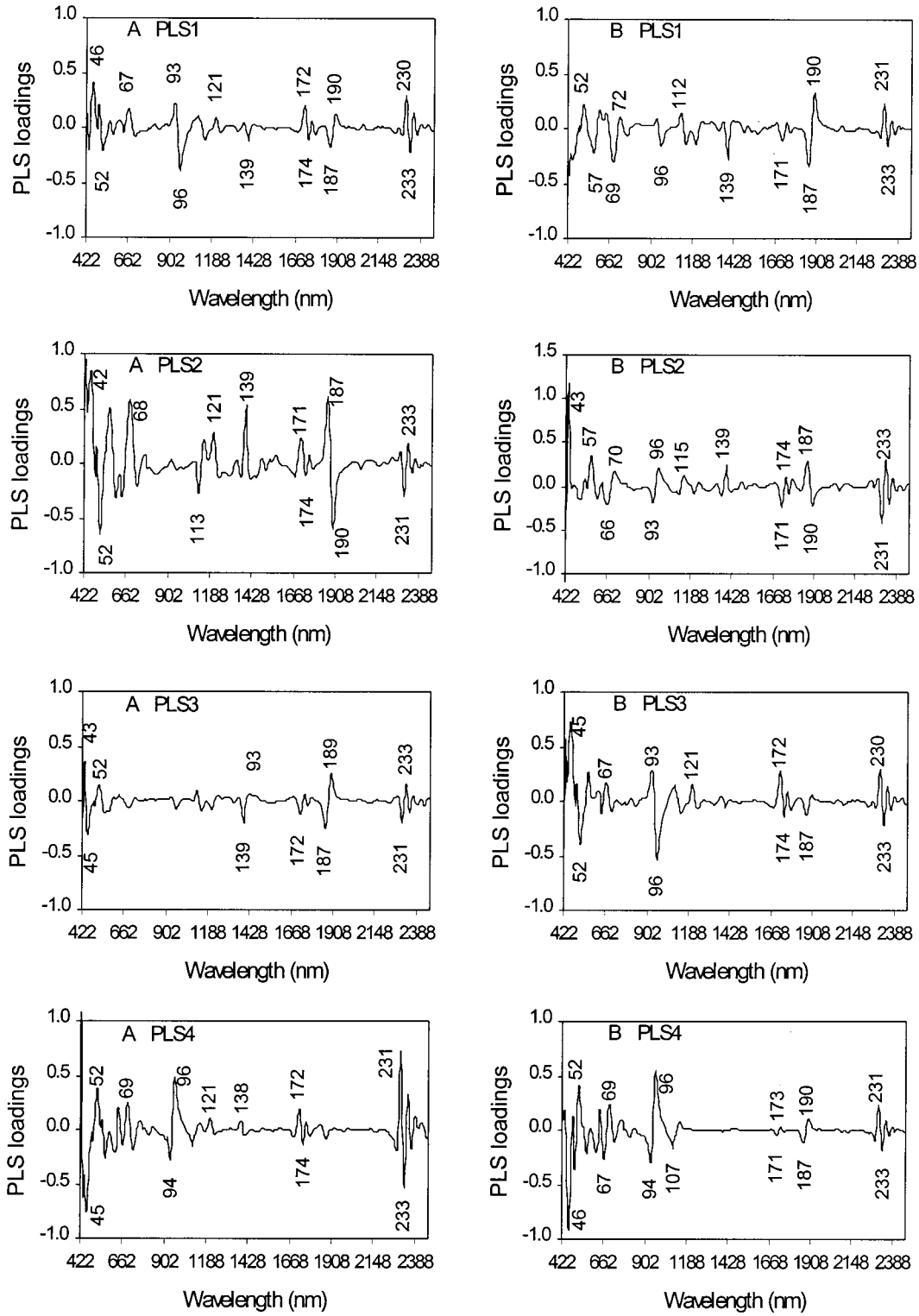

Figure 3. PLS loadings of the first four factors used in the regression calibrations to determine the level of adulteration with mutton (A) or pork (B). The spectra of minced hamburgers were used.

authentic beef hamburgers, less than 9% were misclassified when using the discriminant functions developed with the spectra of minced hamburgers.

Determination of Adulteration Level. When an adulterant has been detected, a further determination of adulteration level is often required. In this study, calibration equations were developed to predict the adulteration level for each adulterant. Spectra data with scatter correction and second derivative operation produced the optimal calibration equations. Statistics of the established calibration equations are presented in Table 3. The spectra of minced hamburgers produced the best calibration equations for all adulterants with SECV between 0.57 and 3.40% and R^2 between 0.86 and 1.00 (Table 3). These results indicate that the adulteration level can be accurately predicted when a hamburger adulterated with pork, mutton, skim milk powder, or wheat flour has been detected.

The performance of the calibration equations was affected by spectral scatter correction and derivative

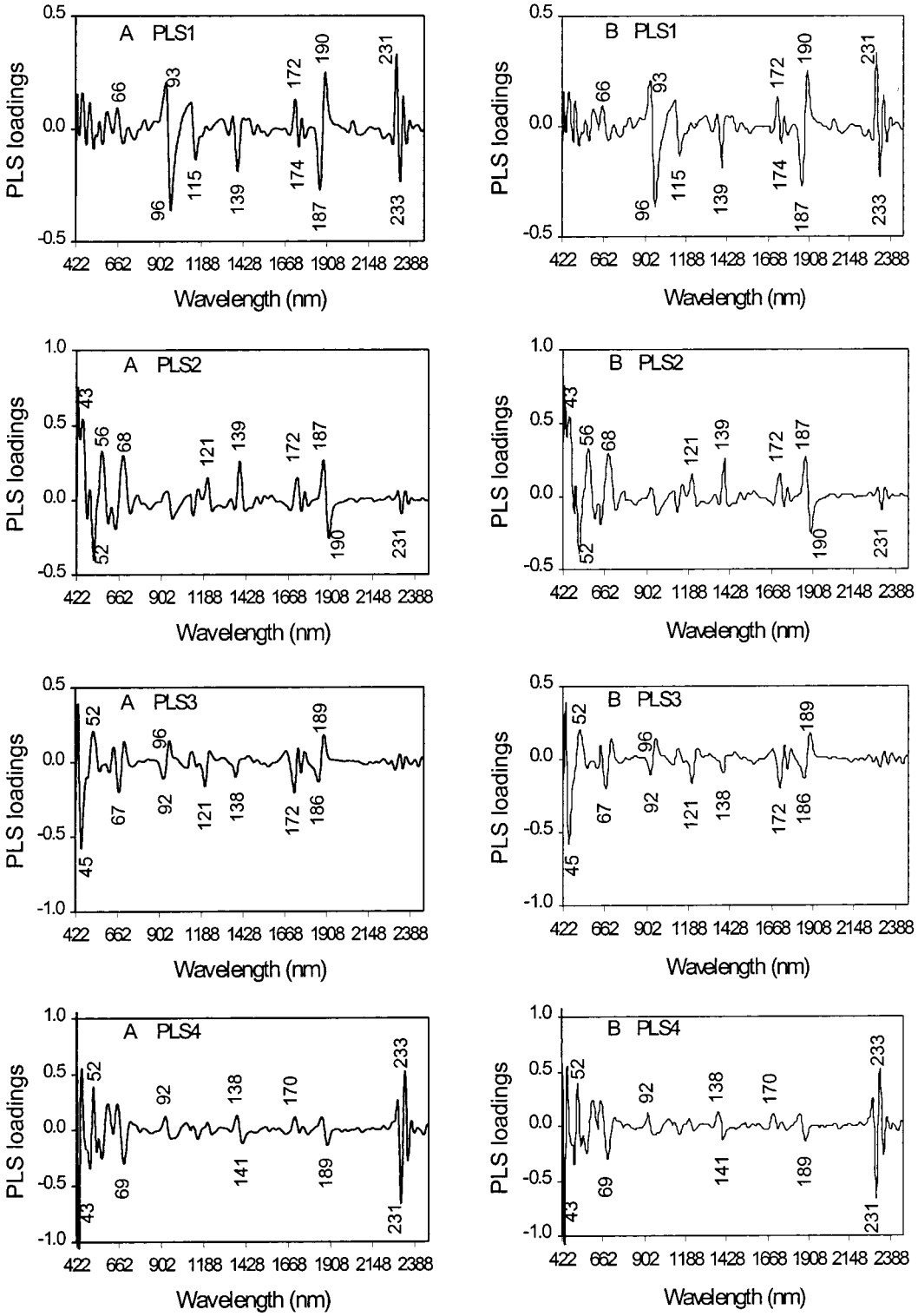

Figure 4. PLS loadings of the first four factors used in the regression calibrations to determine the level of adulteration with skim milk powder (A) or wheat flour (B). The spectra of minced hamburgers were used.

treatment. The effects of scatter correction and derivative treatment may be due to improvement in spectral resolution and reduced scatter effect (Stuart, 1996). The performance of the calibration equations was also affected by sample presentation methods. The lowest SECV and the highest R^2 were achieved with minced hamburgers followed by raw mixture samples and cooked hamburger samples (Table 3).

The PLS loadings of the major factors used in the construction of the calibration equations are illustrated in Figures 3 and 4. It was shown that the PLS loadings for each adulterant had peaks in the visible region and also shared common NIR peaks at around 966, 1212, 1396, 1732, 1748, 1870, 1900, 2310, and 2330 nm. The absorption bands in the visible region are associated with pigments. The bands at 966, 1900, and 1870 nm may be related to the second and first stretch overtones and the combination of O−H bonds associated with water. The absorption bands at 1212, 1732, 1748, 1396, 2310, and 2330 nm may be related to the second and first stretch overtones and the combinations of C−H bonds associated with fat (Osborne et al., 1993). The

Table 3. Statistics of Calibration Equations for Prediction of Adulteration Level of Beef Hamburgers Adulterated with Mutton, Pork, Skim Milk Powder, or Wheat Flour[a]

adulterant	sample presentation	term	SECV	R^2	mean	N
mutton	raw	11	3.33	0.87	6.33	83
	cooked	10	4.12	0.79	6.14	84
	minced	11	3.40	0.86	6.36	85
pork	raw	11	3.59	0.84	6.24	81
	cooked	10	4.59	0.74	5.96	84
	minced	11	2.98	0.89	6.51	83
skim milk powder	raw	11	1.14	0.98	6.36	85
	cooked	10	1.69	0.97	6.51	83
	minced	11	0.92	0.99	6.26	84
wheat flour	raw	11	0.88	0.99	6.51	83
	cooked	10	1.45	0.97	6.14	84
	minced	11	0.57	1.00	6.59	82

[a] Calibration equations were developed from the spectra of raw, cooked, or minced hamburgers after scatter correction and second derivative operation with both gap and smoothing set at 8 data points. Term: number of PLS factors employed in calibration equations; SECV: standard error of cross-validation; R^2: coefficient of variance; N: number of samples used in calibration.

results suggest that the spectral information related to color, moisture, and fat are important for determination of adulteration level. The differences in color and contents of moisture and fat between the beef hamburgers and the hamburgers adulterated with mutton, pork, skim milk powder, or wheat flour might be originated from the differences between beef and adulterants in compositions, water-holding capacity, and emulsion ability.

In conclusion, the present study demonstrates that visible/near-infrared spectroscopy can be used to detect beef hamburgers adulterated with 5−25% mutton, pork, skim milk powder, or wheat flour with a detection accuracy up to 92.7%. When an adulterated beef hamburger is detected, the adulteration level can be further predicted by the spectroscopic technique with a predication error between 0.6 and 4.6% as estimated by SECV (Table 3).

ABBREVIATIONS USED

CDA, canonical discriminant analysis; ISI, Infrasoft International; KNN, *K*-nearest-neighbor method; mPLS, modified partial least squares; NIR, near-infrared; NIRS, near-infrared spectroscopy; PC, principal component; PCA, principal component analysis; PLS, partial least squares; R^2, coefficient of variance; SECV, standard error of cross validation; SNVD, standard normal variance and de-trend.

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