Determination of Free Fatty Acids in Palm Oil by Near-Infrared Reflectance Spectroscopy

Y.B. Che Man* **and M.H. Moh**

Department of Food Technology, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia,
43400 UPM Serdang, Selangor, Malaysia

ABSTRACT: A near-infrared (NIR) spectroscopy calibration was developed for the determination of free fatty acids (FFA) in crude palm oil and its fractions based on the NIR reflectance approach. A range of FFA concentrations was prepared by hydrolyzing oil with 0.15% (w/w) lipase in an incubator at 60°C (200 rpm). Sample preparation was performed in Dutch cup, and the spectra were measured in duplicate for each sample. The optimized calibration models were constructed with multiple linear regression analysis based on C=O overtone regions from 1850–2050 nm. The best wavelength combinations were 1882, 2010, and 2040 nm. Multiple correlation coefficients squared (R^2) were: 0.994 for crude palm oil, 0.961 for refined-bleached-deodorized (RBD) palm olein, and 0.971 for RBD palm oil. Calibrations were validated with an independent set of 8–10 samples. R^2 of validation were 0.997, 0.943, and 0.945, respectively. The developed method was rapid, with a total analysis time of 5 min, and environmentally friendly, and its accuracy was generally good for raw-material quality control.
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KEY WORDS: Free fatty acids, MLR, NIR, palm oil, spectroscopy.

The use of analytical instrumentation in fats and oils analyses is no longer uncommon. High-performance liquid chromatography (HPLC) has been used in separating and identifying triglycerides (1,2) and determining minor components in palm oil (3,4). Fourier transform infrared (FTIR) spectroscopic methods were used by van de Voort *et al.* (5–7) to determine total unsaturation and saponification value, monitor oxidation, and determine the peroxide value in edible oils. Ismail *et al.* (8) and Teo and Goh (9) also used FTIR to determine the free fatty acid (FFA) contents in fats and oils.

Near-infrared (NIR) spectroscopy is a common technique in agriculture (10,11) and the food industry (12). Recently, it has gained popularity in protein, moisture, and fats and oils analyses (13,14). NIR general absorption bands around 1600–1800 and 2100–2200 nm contain useful information regarding fatty acid compositions in various fats and oils (15). It particularly gives strong and sharp absorption bands in the 2300–2400 nm NIR region (16). Holman and Edmondson (17) showed that the absorptions at 1680, 2450, and 2190 nm might be assigned to vibration of C–H bonds bound to *cis*-unsaturation. Bewig *et al.* (18) used NIR as an alternative method to differentiate vegetable oil types and to classify unknown oil samples. The utility of NIR transmission spectroscopy for prediction of oil content in single maize kernels was explored by Orman and Schumann (19).

Based on the number of studies conducted on different edible oils using NIR spectroscopy, we postulated that it could also be applicable to measure some quality parameters of palm oil and its products. Therefore, the objective of this study was to develop an NIR calibration that is capable of replacing the common laborious, wet-chemical determination of FFA in palm oil and thus minimizing the environmental concern associated with the use of organic solvents and hazardous reagents.

MATERIALS AND METHODS

Sample preparation. Crude palm oil (CPO), refined, bleached and deodorized (RBD) palm olein, and RBD palm oil were purchased from Ngo Chew Hong Oils and Fats (M) Sdn. Bhd. (Selangor, Malaysia) and Lypozyme IM (Novo Nordisk A/S, Denmark) was purchased from Science Technik Sdn. Bhd. (Petaling Jaya, Malaysia). A range of FFA was prepared by spiking a 400-g sample with 0.15% w/w enzyme. The sample was inoculated in an incubator (CERTOMAT, B. Braun; Melsungen AG, Germany) at 60°C and 200 rpm. A sample (*ca.* 70 g) was collected at each 15-min interval and filtered through No. 2 Whatman (Whatman International Ltd., Maidstone, England) filter paper in an oven (90°C) to remove enzyme. All chemicals used were of either analytical or HPLC grade.

Chemical analysis. Acid values of enzyme free samples were determined by AOCS titration method (20). The results were expressed as the number of mg KOH necessary to neutralize the FFA (referenced to palmitic acid) in 1 g of sample.

Instrumentation. An NIR spectroscopic instrument (InfraAlyser 500; Bran and Luebbe, Norderstedt, Germany) was used to measure the NIR reflectance. The system was run by InfraAlyser Data Analysis Software (IDAS) (Bran and Luebbe). Sample presentation was performed in a Dutch cup (Bran and Luebbe), and the spectra were measured in duplicate for each sample. The wavelengths measured ranged from

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*To whom correspondence should be addressed.
E-mail: yaakub@fsb.upm.edu.my.

TABLE 1
Samples Used in Calibration and Validation Analyzed by Standard Chemical Methods[a]

			FFA (%)	
Samples	No. of samples for calibration	No. of sample for validation	Mean of duplicates	Range
CPO	25	10	4.89	3.46–6.20
RBD palm olein	25	10	0.31	0.07–0.52
RBD palm oil	20	8	0.18	0.07–0.44

[a]FFA, free fatty acids; CPO, crude palm oil; RBD, refined-bleached-deodorized.

TABLE 2
Major Analytical Bands and Their Relative Peak Position of NIR Spectroscopy from 1850–2050 nm

Functional groups	Approximate band position (nm)
RCO_2H	1880–1900[a]
H_2O	1940
RCO_2R'	1900–1950[a]
CO	2020–2050

[a]Wheeler (Ref. 24). NIR, near infrared.

1100 to 2500 nm in 2-nm intervals (701 data points) to obtain the entire spectrum.

Calibration and validation procedure. Samples (20–25) of each oil type were used as calibration sets for NIR analysis (Table 1). Data from chemical analysis were entered for each sample scan. Wavelengths for determination of FFA were selected by taking measurements of the energy log $1/R$ values, where R is reflectance energy, directly from 1850–2050 nm without any mathematical transformation, followed by stepwise multiple linear regression (MLR) analysis of the reflected energy at each wavelength point against the concentration. Separate sets of 8–10 samples were used for validation to verify the reliability of the calibration constant.

RESULTS AND DISCUSSION

General concepts. FFA develop naturally in CPO and can be increased by the action of enzymes in the palm fruit and by microbial lipases. During storage, FFA were produced by reaction of oil with water. According to the Palm Oil Refiners Association of Malaysia (PORAM), standard specifications for the FFA content (as palmitic acid) are: CPO, max. 5.0%; RBD palm olein, max. 0.1%; and RBD palm oil, max. 0.1%. For trading purposes, such standards have to be accomplished.

The routine procedure to determine the FFA content in palm oil is through the conventional wet-chemical method. For preparation of the standard reagent, analysis, and expression of the result, substantial personnel time and glassware are required. Therefore, an alternative to reduce the problem is needed. Based on previous work with FTIR (21), FFA are characterized by their carboxylic acid, C=O, absorption in the mid IR region (1711 cm^{-1}). Thus, the determination of FFA in palm oil by NIR is proposed based on the C=O stretching bands that lie in the overlap region of the first overtone and the combination region (1850–2050 nm), as presented in Table 2.

Development of calibration equations. The original FFA contents used in this study were 3.45, 0.07, and 0.07% for CPO, RBD palm olein, and RBD palm oil, respectively. To develop a calibration set with a wider range of concentration, the oils were subjected to hydrolysis. In this experiment, the lipase broke down the fatty acid chains of the triglyceride randomly. The hydrolysis was accelerated in an incubator at 60°C. At 15-min intervals, samples were collected and filtered, and the FFA contents were determined by conventional method. The samples were kept frozen pending NIR analysis. Because NIR spectroscopy determined the total C=O functional groups, it was not important which fatty acid was favored in hydrolysis.

Figure 1 represents an overlaid spectrum of palm oil from 1100 to 2500 nm. The absorption bands at 2140 and 2190 nm are due to *cis*-unsaturation in fatty acids (17), whereas 1700–1800 nm and 2200–2400 nm are due to CH stretching. The OH absorption band is located at approximately 1940 (22).

For a multiple component system such as palm oil, the relationship between the absorbance and concentration is not linear for a single wavelength by the NIR spectroscopic method. The deviations are due to chemical interactions, such as association, dissociation or interaction of the absorbing species or molecules, or to the difference in scattering and reflecting of radiant power. To overcome the problem, the equation is expanded to include more than one wavelength in the MLR analysis. The MLR analysis showed that a three-wavelength (λ) combination (λ_1, λ_2, λ_3) was adequate to produce good calibration (Table 3). Addition of one other wavelength (e.g., 1902 nm) did not improve the calibration results. The determination was based on the highest multiple correlation coefficient (R^2) with smallest standard error of estimate (SEE) of calibration. The regression models to predict FFA contents by NIR spectroscopy can be written in equation form as below:

$$\text{FFA} = -4.26 + 15.47\lambda_1 - 831.71\lambda_2 + 844.87\lambda_3 \qquad [1]$$

$$\text{FFA} = -5.53 - 98.61\lambda_1 - 474.30\lambda_2 + 591.38\lambda_3 \qquad [2]$$

$$\text{FFA} = -8.47 - 57.61\lambda_1 - 522.62\lambda_2 + 608.65\lambda_3 \qquad [3]$$

TABLE 3
Effect of Different Wavelengths with 2040 nm as Measurement Wavelength in Constructing the Calibrations in Palm Oil Samples

Reference wavelengths (nm)	CPO		RBD palm olein		RBD palm oil	
	SEE[a]	R^2[a]	SEE	R^2	SEE	R^2
None	0.52	0.81	0.11	0.59	0.08	0.16
2010	0.09	0.99	0.06	0.92	0.04	0.85
2010, 1882	0.09	0.99	0.04	0.96	0.03	0.97

[a]SEE = standard error of estimate. R^2 = multiple correlation coefficient. For other abbreviations see Table 1.

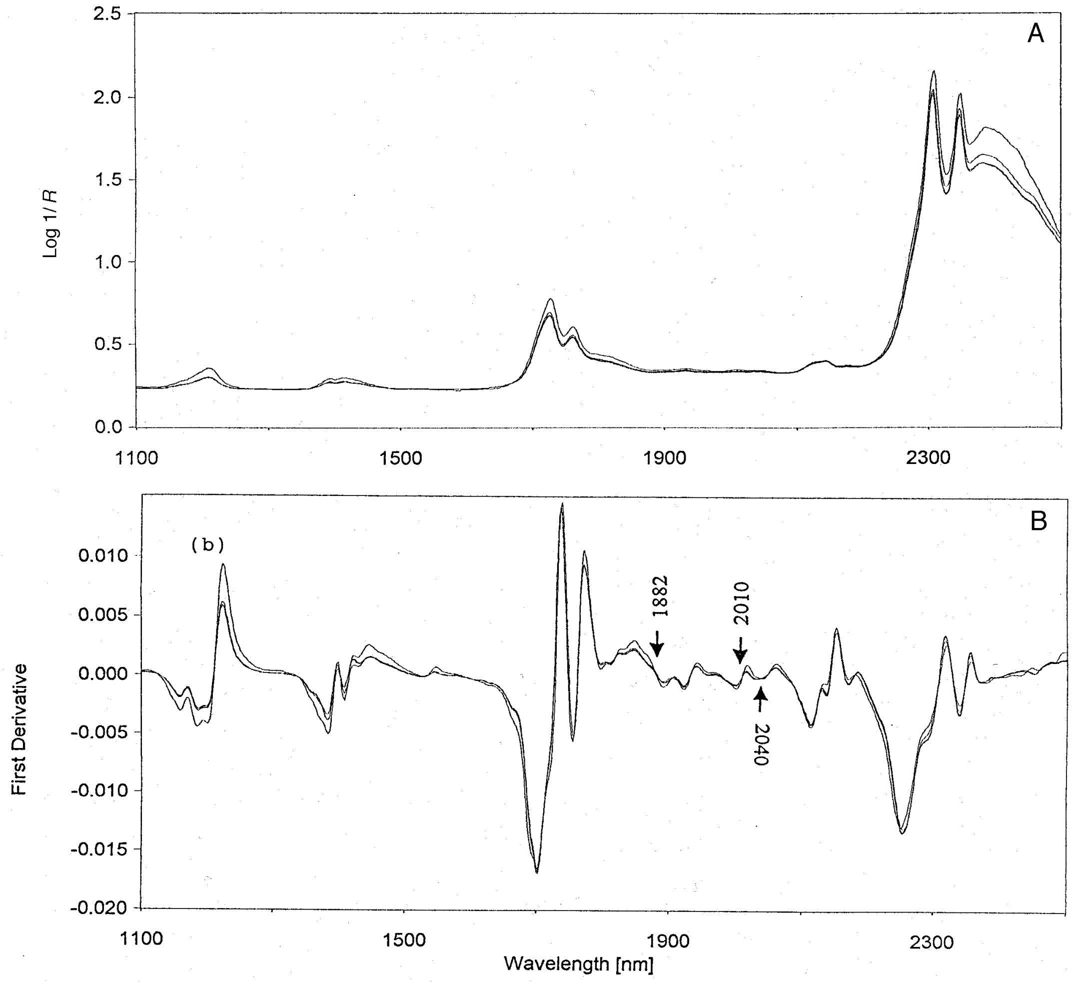

FIG. 1. Original (A) and first-derivative (B) near-infrared spectra of palm oil samples.

where Equation 1 is for determining FFA content in CPO, Equation 2 for RBD palm olein, and Equation 3 for RBD palm oil, respectively.

The standard deviations (SD) for all samples at the selected wavelengths are given in Table 4. The multiple correlation coefficient of calibration, R^2, for CPO is 0.994, for RBD palm olein 0.961, and for RBD palm oil 0.971. The high coefficients were the result of repeated calibration processes (two times) that excluded large outliers. For the calibration set, the stepwise regression process excluded two sets of values as outliers from CPO, one set from RBD palm olein, and one set from RBD palm oil. The values of SEE for calibration decrease as the number of the wavelengths increases (Table 3). The final SEE values from three wavelengths for

TABLE 4
Standard Deviation Values at Selected Wavelengths Obtained for Both Calibration and Validation Sets by Multiple Linear Regression[a]

	Wavelengths (nm)					
	1882	2010	2040	R^2	Bias	RMSEP
Calibration sets						
CPO	0.003	0.003	0.004	0.994	—	0.008
RBD palm olein	0.002	0.002	0.003	0.961	—	0.041
RBD palm oil	0.002	0.002	0.002	0.971	—	0.026
Validation sets						
CPO	0.004	0.004	0.004	0.997	0.002	0.077
RBD palm olein	0.002	0.002	0.002	0.943	−0.003	0.041
RBD palm oil	0.004	0.004	0.004	0.945	−0.003	0.059

[a] R^2, multiple correlation coefficient; RMSEP, root mean square error of prediction. For other abbreviations see Table 1.

CPO were 0.09, for RBD palm olein 0.04, and for RBD palm oil 0.03.

Validation of calibration equations. Once the calibration equations were developed, the applicability of the equations was confirmed through validation of the calibration with 8–10 external sets of samples, which were not included in constructing the calibration. The multiple corrrelation coefficients of validation, R^2, obtained were: 0.997, 0.943, and 0.945, respectively. These R^2 for validation samples were close to those of calibration samples, though no outliers were included. The equations for the validation sets were: $FFA = 3.13 + 103.38\lambda_1 - 1059.65\lambda_2 + 965.43\lambda_3$ for CPO, $FFA = -2.40 - 102.35\lambda_1 - 586.39\lambda_2 + 698.39\lambda_3$ for RBD palm olein, and $FFA = -3.52 - 117.00\lambda_1 - 418.42\lambda_2 + 548.15\lambda_3$ for RBD palm oil.

Figures 2, 3, and 4 illustrate the distribution of the predicted data in reference to the calibration regressions for the CPO, RBD palm olein, and RBD palm oil, respectively. The data indicate that prediction by NIR spectroscopy with MLR analysis is linear (23) throughout the ranges tested. The quality of the predictive ability of calibration can be further justified from the minor squared differences of the root mean square error of prediction between the chemical and NIR values (Table 3). The close-to-zero bias values (≤ ±0.03) indicate that there were no systematic error in the predicted values, and the slopes of the regression lines or the skew values were close to one (i.e., CPO 0.993, RBD palm olein 1.005, and RBD palm oil 0.956 and also showed that there was significant systematic variation in the predicted values.

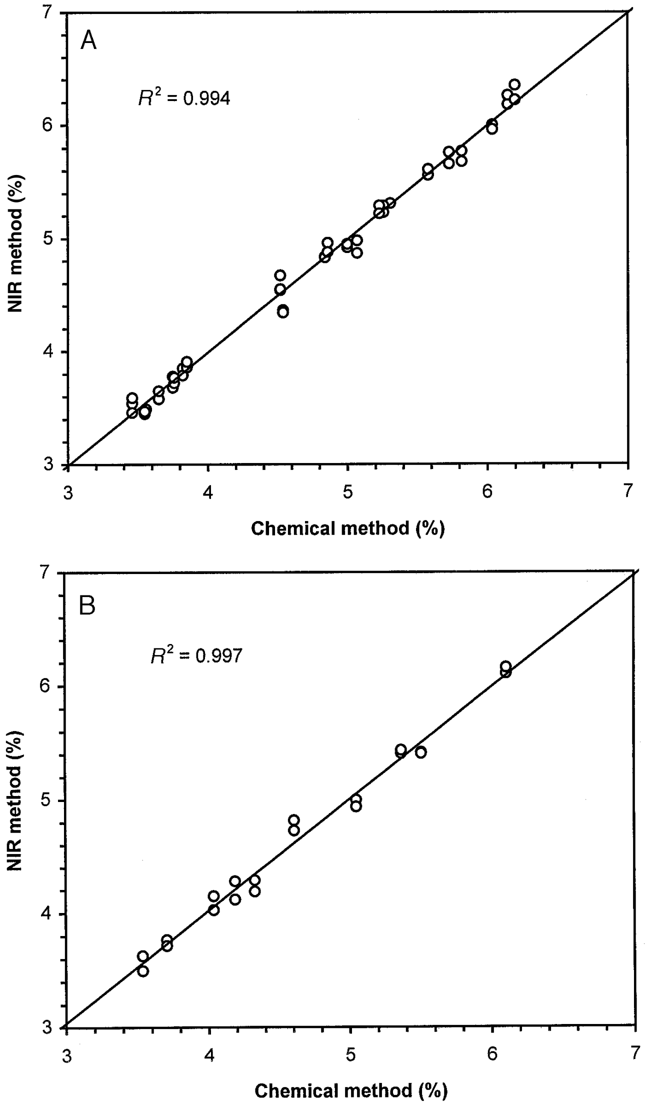

FIG. 2. Scatter diagrams of calibration (A) and validation (B) data of free fatty acid content in percentage for crude palm oil. R^2, multiple correlation coefficient; NIR, near infrared.

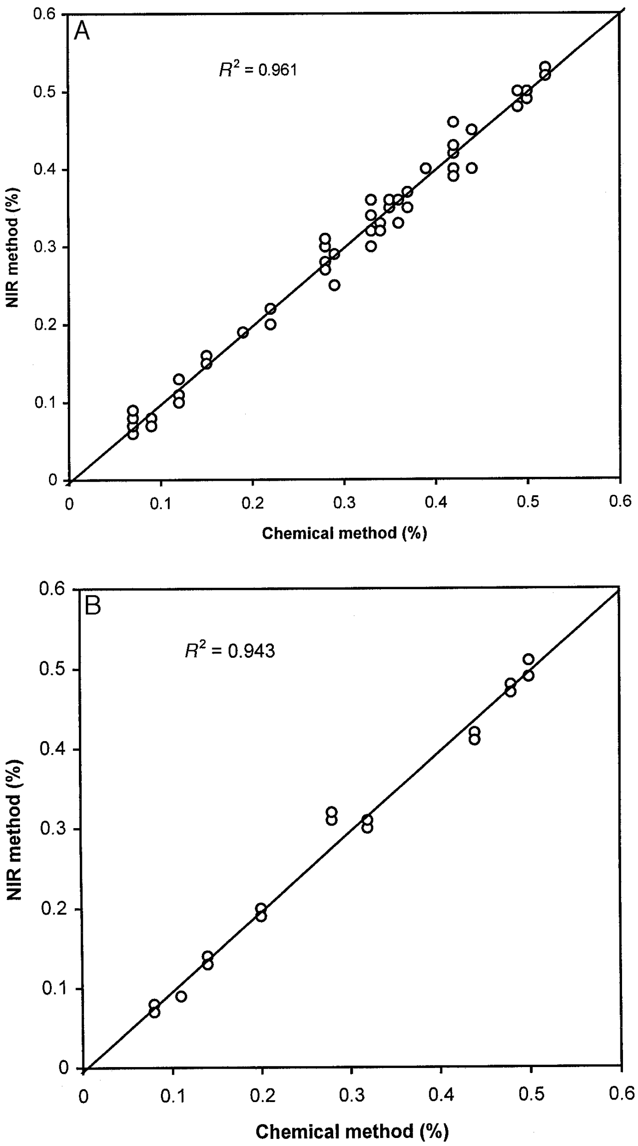

FIG. 3. Scatter diagrams of calibration (A) and validation (B) data of free fatty acid content in percentage for refined, bleached, deodorized palm olein. For other abbreviations see Figure 2.

Results from this study indicate that NIR spectroscopy is a useful technique for measuring palm oil quality parameters such as FFA. The total analysis takes less than 5 min once the spectrometer is precalibrated. Compared to the conventional

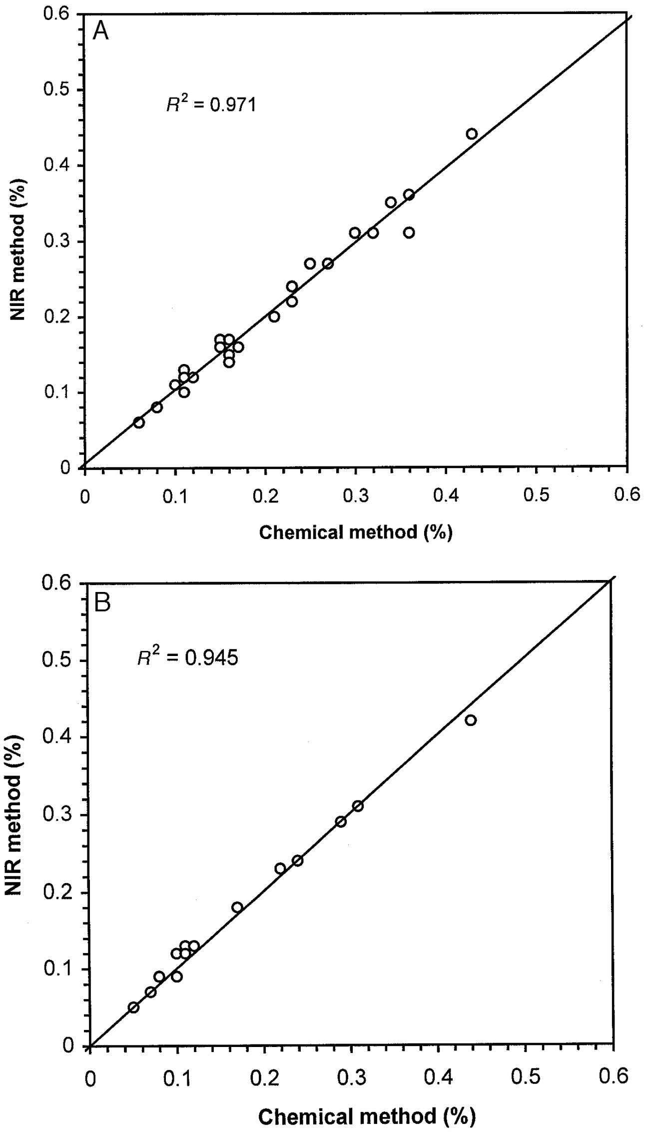

FIG. 4. Scatter diagrams of calibration (A) and validation (B) data of free fatty acid content in percentage for refined, bleached, deodorized palm oil. For other abbreviations see Figure 2.

wet chemical analysis, NIR spectroscopy is capable of measuring a hundred samples in a day and yet still meets the trading specification. In addition, by applying this technique, the amount of hazardous solvents can be reduced dramatically as well as the cost of labor.

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