Determination of Peroxide Value in Thermally Oxidized Crude Palm Oil by Near Infrared Spectroscopy

M.H. Moh[a], Y.B. Che Man[a,]*, F.R. van de Voort[b], and W.J.W. Abdullah[c]

Departments of [a]Food Technology, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia, Selangor, Malaysia, [b]Food Science and Agricultural Chemistry, Macdonald Campus of McGill University, St. Anne de Bellevue, Québec H9X 3V9, Canada, and [c]Mathematics, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ABSTRACT: A near infrared (NIR) spectroscopic method was developed to measure peroxide value (PV) in crude palm oil (CPO). Calibration standards were prepared by oxidizing CPO in a fermentor at 90°C. A partial least squares (PLS) calibration model for predicting PV was developed based on the NIR spectral region from 1350 to 1480 nm with reference to single-point baseline at 1514 nm. The optimization of calibration factors was guided by the predicted residual error sum of squares test. The standard error of calibration obtained was 0.156 over the analytical range of 2.17–10.28 PV and the correlation coefficient (R^2) was 0.994. The method was validated with an independent set of samples which was prepared in the same manner on a different day. A linear relationship between the American Oil Chemists' Society and the NIR methods was obtained with R^2 of 0.996 and standard error of performance of 0.17. This study has demonstrated that the prediction of PV in the NIR region is possible. The method developed is rapid, with total analysis time less than 2 min, is environmentally friendly, and its accuracy is generally good for quality control of CPO.

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The autoxidation of palm oil is an important deteriorative reaction that has significant commercial implication in terms of product value. It is generally accepted that autoxidation of unsaturated fatty acids proceeds as a free-radical mechanism. Hydroperoxides are the major primary products during the initial reaction of unsaturated fatty acids with oxygen, and they subsequently break down to form secondary oxidation products (alcohols, ketones and aldehydes) leading to off-flavors (1). Consumers normally judge oils based on the taste or smell of the oil. The primary oxidation products of oils are normally measured using the American Oil Chemists' Society (AOCS Method Cd 8b-90) iodometric procedure for the determination of peroxide value (PV) (2). This method is based on the stoichiometric conversion of potassium iodide to molecular iodine by hydroperoxides. The liberated iodine in the acidic environment is titrated with standardized sodium thiosulfate. The analytical procedure is rather cumbersome and involves large amounts of glassware and solvents.

Recently, various instrumental techniques have been developed to replace the chemical method. Lovaas (3) used spectrophotometry to measure hydroperoxides based on the strongly absorbing triiodine ion at 360 nm in the visible region. Infrared analysis of hydroperoxides has also been studied. Van de Voort *et al.* (4) used Fourier transform infrared (FTIR) spectroscopy to measure the hydroperoxides related functional groups to quantify the PV in various edible oils. On the other hand, Dong *et al.* (5) quantified the PV in the near infrared (NIR) using Fourier transform near infrared (FT-NIR) spectroscopy. The calibration standards of the latter group were stoichiometrically prepared by reacting triphenylphosphine (TPP) with ROOH to produce triphenylphosphine oxide (TPPO). The calibration matrix was developed by spiking the TPP and TPPO of various concentrations in high-erucic-acid rapeseed oil and PV were predicted using the NIR spectral region from 4710 to 4540 cm^{-1}.

Our research group has been working on the development of rapid NIR spectroscopy methods for the quantitative analysis of palm oil such as free fatty acid (FFA) contents (6). The purpose of the present study was to optimize a calibration model to predict the PV in palm oil using an NIR spectroscopic method.

MATERIALS AND METHODS

Sample preparation. Freshly extracted crude palm oil (CPO) with PV of 2.17 was purchased from a local refinery (Ngo Chew Hong Oils and Fats Sdn. Bhd., Selangor, Malaysia). The oil was thermally oxidized in the laboratory. A kilogram of CPO was loaded into a 1-L fermentor (B. Braun, Melsungen AG, Germany). The fermentor was placed in a waterbath with temperature set at 90°C. A gas line from a normal laboratory compressor was inserted through the inlet hole of the fermentor, and air was continuously purged into the oil throughout the oxidation process. At each 15-min interval, a 50-g sample was withdrawn and the hydroperoxide level was determined in duplicate using the AOCS (2) PV procedure. The PV is expressed in terms of milliequivalents of active O_2 per kilogram which

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*To whom correspondence should be addressed at the Faculty of Food Science and Biotechnology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
E-mail: yaakub@fsb.upm.edu.my

oxidize potassium iodide (KI) under the conditions of the test. A total of 30 samples analyzed ranging from 2.2 to 10.28 PV were used to develop the calibration matrix which was adequate to cover the average range of 2–5 PV in commercial CPO. An additional 10 samples prepared on a different day using the same procedure were also analyzed to determine their PV as a validation set. All solvents and reagents used were of analytical grade.

Instrumentation and sample handling. A scanning NIR spectrometer (model 6500 monochromator; NIRSystems, Inc., Silver Spring, MD) was interfaced to a 486/33 MHz PC and run by NSAS software. For the purpose of oil analysis, a liquid module (NIRSystems) was used to measure the NIR transmittance to meet all the specifications guided by the manual. The instrument and detector were warmed for 30 min before the instrument was aligned and noise level was tested (1100–2500 nm). The oil sample was handled in a 10-mm path length quartz transmission cuvette (NIRSystems). A background spectrum of the clean and emptied cuvette was taken by co-adding 64 scans and stored in the memory. The spectrum of a sample preheated at 65°C in an oven was also recorded by co-adding 64 scans. The actual spectrum of the sample was obtained by ratioing to the background spectrum after every scan. Both the calibration and validation spectral information were saved in separate files for further data manipulation.

Calibration/validation procedures. All the spectral data were converted and saved to diskettes under JCAMP-DX format. This file format is a pure ASCII text format and is used as a standard for exchange of infrared spectral data between spectrometer and computer system. These spectral data were then downloaded to Nicolet Turbo Quant-IR Calibration and Prediction Package (Nicolet Instrument Corp., Madison, WI) for subsequent partial least squares (PLS) calibration development. The selection of spectral regions for calibration development was assisted by means of correlation and variance spectra. Each calibration was assessed by using the leave-one-out cross-validation procedure. The optimization of calibration factors was guided by the predicted residual error sum of squares (PRESS) test where the minimal number of factors was obtained. The calibration was further refined by using the mean difference (MD) and standard deviation of the difference (SDD) between the predicted and actual PV as a measure of performance of the calibration. The correlation coefficient (R^2) was used to measure the strength of the linear relationship between the predicted and the actual values, and the standard error (SE) generated during analysis. Prediction was carried out using an independent set of samples to test the applicability of the developed calibration model.

RESULTS AND DISCUSSION

Calibration matrix. Quantitative analysis by NIR spectroscopy is a secondary method. Before conducting a NIR spectroscopy analysis, calibration is required (7). The calibration set must be selected randomly and must contain the complete concentration range of analyte(s) of interest. Collecting samples can be quite simple for some analyses. However, in a daily-operating palm oil plant, consistency of quality is always guided. As a result, it is difficult to find samples which represent a wide range of PV. To create a broader range for the purpose of calibration development, a synthetic approach is necessary. Many calibration matrices have been developed by the spiking of different concentrations of standards into an original sample. In developing the calibration standards for PV analysis using FTIR spectroscopy, van de Voort and colleagues (4) spiked *t*-butyl hydroperoxide (TBHP) standards into peroxide-free oil. In developing the FTIR method for FFA analysis, Ismail *et al.* (8) used oleic acid at different concentrations. In our previous study with NIR spectroscopy for the determination of FFA in CPO, refined-bleached-deodorized (RBD) palm oil and RBD palm olein, the calibration standards with the broader range of FFA were developed by hydrolyzing the oils with lipase in a controlled manner. The calibration results obtained were satisfactory with the slopes close to unity (6).

Unlike chromatographic methods that separate the sample into its components, NIR analyzes the complete sample matrix. Therefore, great care must be taken when transferring a calibration consisting of synthetic samples to the production line. In order to develop a calibration matrix that represents the real matrix for determination of PV in CPO in this study, the oil with minimum PV of 2.17 units was oxidized with normal air supplied from a laboratory compressor. To accelerate the oxidation process, the temperature of the oil was set at 90°C. Stirring the CPO in the fermentor established homogeneity. The range of PV generated in this manner was from 2.17 to 10.28.

General NIR statistical spectra. Figure 1 shows the general NIR mean spectrum of CPO from 1100 to 2500 nm. NIR spectra mainly consist of overtones and combinations of the fundamental vibrations involving the hydrogenic (X-H) stretching modes (9), where X can be oxygen, carbon, or metal halides. The combination bands of -CH stretching motions and deformations are at 2200–2400 nm. The 1500–1800 nm region consists of -CH and -OH stretch first overtones, and the 1100–1450 nm region includes -CH stretch second overtones, along with some -CH stretch and deformation combination bands, and -OH first overtones. By visually studying the raw spectrum, it is impossible to assign an exact band position for an organic molecule in the NIR spectral region. However, the prediction (7) can be done based on the position of the fundamental bands in the mid-IR region by using a formula:

$$\nu = n\nu_o(1 - nx) \qquad [1]$$

where ν is the wavenumber of the overtone band; ν_o is the corresponding fundamental wavenumber in the mid-IR region; n is an integer, i.e., 2 for first overtone, 3 for second overtone in the NIR region; and x is the anharmonicity constant 0.01. The overtone bands are reported in wavenumbers. To convert the units to nm, the following formula can be used:

$$\text{nanometer} = 10{,}000{,}000 \div \text{wavenumber} \qquad [2]$$

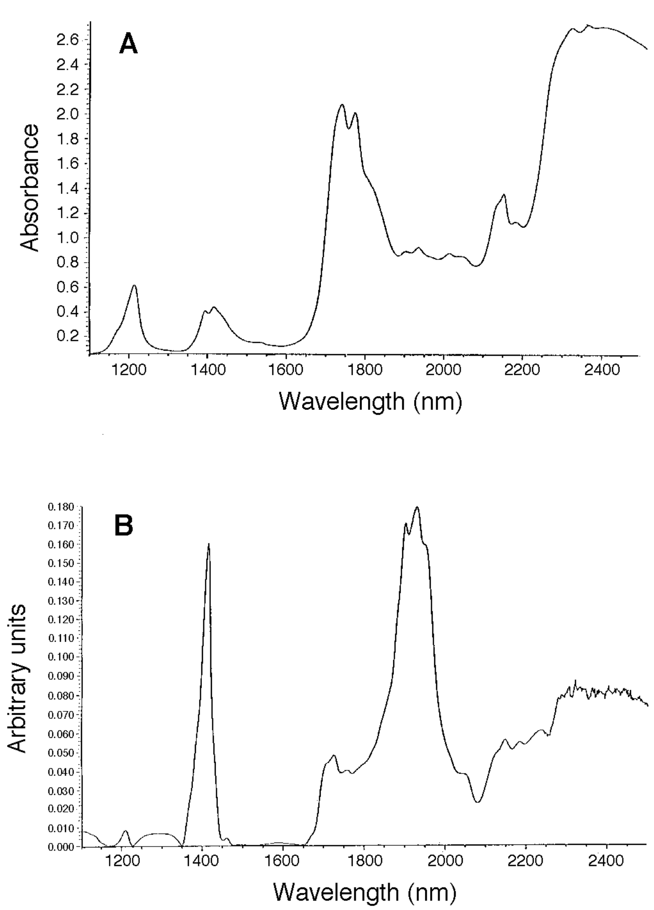

FIG. 1. (A) Mean spectrum of crude palm oil obtained by near infrared spectroscopy from 26 calibration standards; (B) correlation spectrum obtained from calibration standards, illustrating the spectral correlation occurring to the chemical peroxide value.

In the FTIR determination of PV, van de Voort *et al.* (4) showed some characteristic absorption bands that were related to hydroperoxide moieties between 3600 and 3400 cm^{-1} which were due to an -OO-H stretching vibration. By using the formula above, the overtones of these bands in the NIR spectral regions were estimated at 1417–1500 nm for the first overtone of -OO-H stretching band to be in the second overtone of the NIR spectral region, whereas the second overtone of -OO-H stretching band was found at the third overtone region (954–1011 nm). This was in agreement with Osborne and Fearn (7) who assigned the first overtone of -OH stretching to be from 1400 to 1560 nm and the second overtone from 950 to 1040 nm, respectively, for both free and intermolecular H-bonded peroxide. Trott *et al.* (10) also observed the intermolecular hydrogen-bonded OH group to be at 1589 nm in carbohydrates. Further confirmation of this band was done by studying the correlation spectrum (Fig. 1B). The correlation showed a sharp peak from 1350 to 1450 nm indicating that PV can be well correlated here, hence, the assignment of this band in the NIR spectra of oil to the -OO-H stretching overtone of hydroperoxides.

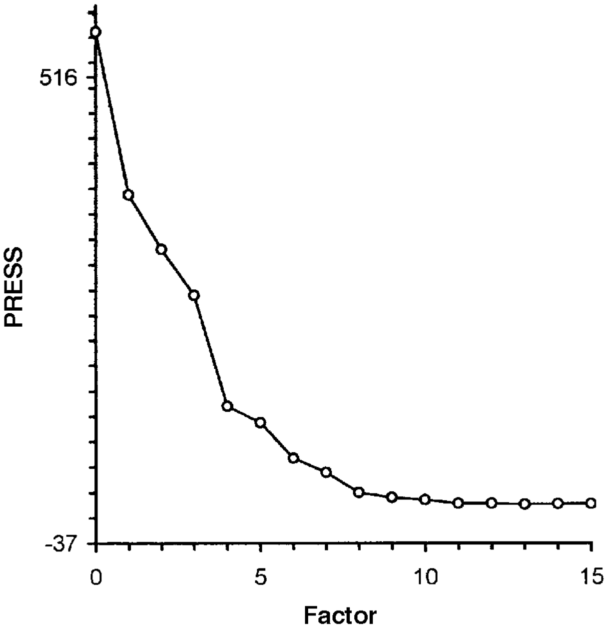

FIG. 2. Plot of predicted residual error sum of squares (PRESS) vs. number of partial least squares (PLS) factors used in calibration model.

PLS calibration. Another problem encountered in the NIR spectral region was during the process of calibration development. Assignment of specific wavelengths using simple Beer's Law was difficult mainly owing to the extensive overlapping of bands in the combination and overtone regions. Therefore, the power of chemometric software based on PLS regression was an alternative approach (11) as a basic tool for calibration in the NIR region which allows the whole spectrum in the selected region to be investigated simultaneously to establish correlations between spectral and compositional data.

In this study, the selections of wavelength region, baseline reference, and number of factors used in the calibration model were guided by the PRESS test (Fig. 2). Then the calibration model was further optimized using the leave-one-out cross-validation test. It was demonstrated that the NIR spectral region from 1350 to 1480 nm, with single-point baseline at

TABLE 1
Calibration Results Generated from Different Spectral Regions in Terms of Standard Error (SE) and Correlation Coefficient (R^2)[a]

Region (nm)					
1350–1480	1850–2100	2100–2200	SE	R^2	Factors
+[b]	+	+	0.343	0.997	10
+	+	—[c]	0.349	0.973	11
+	—	—	0.156	0.994	10
—	+	—	0.454	0.962	10
—	—	+	0.568	0.759	10

[a] Number of factors used was selected from a PRESS (predicted residual error sum of squares) test.
[b] +, Used for calibration development.
[c] —, Not used for calibration development.

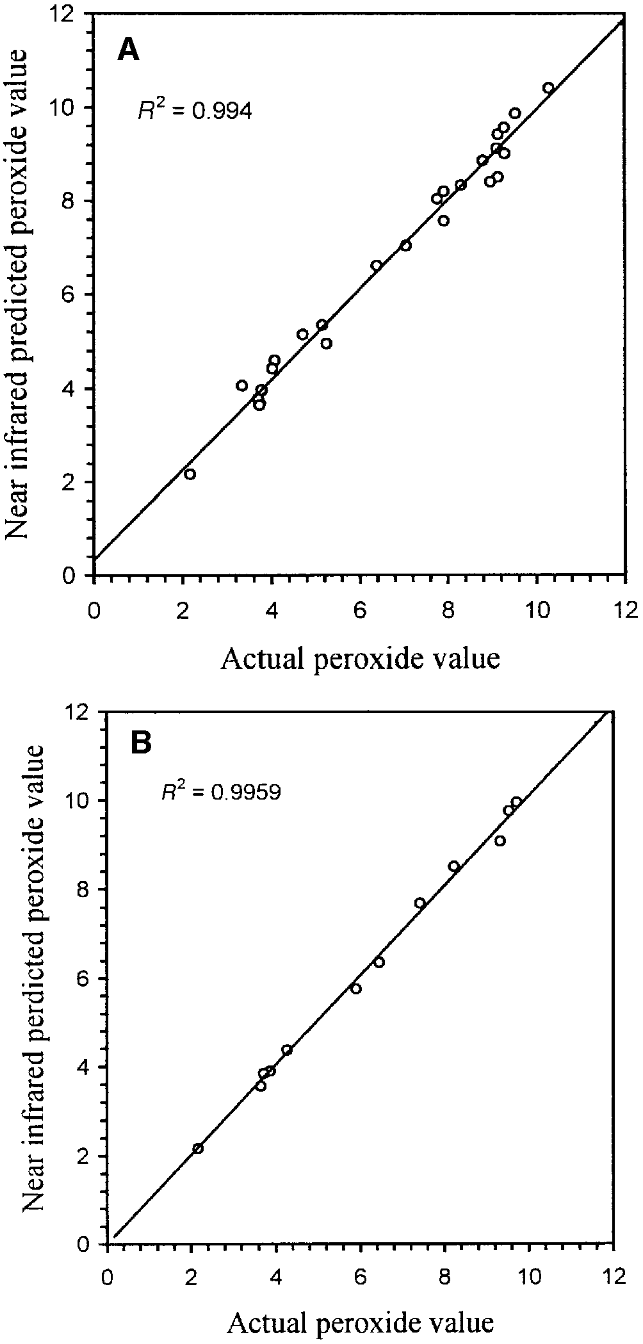

FIG. 3. (A) Calibration and (B) validation plots showing the near infrared predicted vs. the actual peroxide value based on 10 PLS factors. R^2, correlation coefficient. See Figure 2 for abbreviation.

1514 nm, was the best region for predicting PV as given in Table 1. Figure 3A illustrates the calibration plot obtained from 26 calibration standards (Table 2) based on 10 factors in terms of predicted against actual PV. The plot showed strong linearity with R^2 of 0.994 and SE of 0.156.

Validation of equation. Figure 3B presents a plot of the means of duplicate PV determinations using the chemical method (CHEM-PV) plotted against the corresponding means of the NIR prediction (NIR-PV) for the validation set. The regression equation for the line was:

$$\text{NIR-PV} = 1.018\ \text{CHEM-PV} - 0.061 \qquad [3]$$

The plot was linear and had a slope close to unity, with R^2 of 0.996 and standard error of performance (SEP) of ±0.17 PV. The statistical results between the two methods are summarized in Table 3. From the reproducibility standpoint, both

TABLE 2
Mean of Actual[a] Peroxide Value (PV) vs. Mean of Near Infrared (NIR) Predicted PV Generated Using Partial Least Squares Analysis Based on 1350–1480 nm Region

PV[b]	
Actual method	NIR method
2.17	2.17
3.35	4.06
3.7	3.68
3.73	3.64
3.74	3.77
3.78	3.97
4.03	4.42
4.08	4.59
4.71	5.14
5.16	5.34
5.25	4.95
6.93	6.61
7.05	7.03
7.76	8.04
7.91	7.57
7.91	8.21
8.3	8.34
8.97	8.41
8.79	8.86
9.11	9.13
9.14	9.42
9.14	8.51
9.28	9.56
9.29	9.02
9.53	9.81
10.28	10.4

[a] Using American Oil Chemists' Society procedure (2).
[b] Mean of duplicate analyses.

TABLE 3
Statistical Comparison of PV Obtained by American Oil Chemists' Society (AOCS) Reference and NIR Method

	PV	
Statistic	AOCS reference method	NIR method
Max	10.28	10.4
Min	2.17	2.17
Mean	6.531	6.617
MD_r	0.032	−0.073
SDD_r	0.049	0.009
MD_a	−0.058	
SDD_a	0.051	

Abbreviations: MD, mean difference reproducibility; MD_a, mean difference accuracy; SDD_r, standard deviation of the difference reproducibility; SDD_a, standard deviation of the difference accuracy. For other abbreviations see Table 2.

chemical and instrumental results have comparable means, with the chemical method having a somewhat lower standard deviation between duplicates. A paired comparison test showed that the two data were not significantly ($P > 0.001$) different. In terms of accuracy, the NIR predictions were ~0.06 PV higher than the chemical method. The SSD_a value indicated NIR can accurately determine PV to within 0.05 PV.

Results obtained from this study indicate that NIR spectroscopy is a useful technique for measuring PV in crude palm oil. Once the instrument is pre-calibrated, the total analysis takes less than 2 min. Compared to the conventional wet chemical method, NIR spectroscopy is capable of measuring hundreds of samples in a day. In addition, by applying this technique, the amount of hazardous solvents as well as the cost of labor can be dramatically reduced.

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