

Multivariate Calibration of Fourier Transform Infrared Spectra for Determining Thiobarbituric Acid-Reactive Substance Content in Palm Oil

M.E.S. Mirghani^a, Y.B. Che Man^{a,*}, S. Jinap^b, B.S. Baharin^a, and J. Bakar^a

Departments of ^aFood Technology and ^bFood Science, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

ABSTRACT: Fourier transform infrared (FTIR) spectra of palm oil samples between 2900 and 2800 cm^{-1} and 1800 and 1600 cm^{-1} were used to compare different multivariate calibration techniques for quantitative determination of their thiobarbituric acid-reactive substance (TBARS) content. Fifty spectra (in duplicate) of palm oil with TBARS values between 0 and 0.25 were used to calibrate models based on partial least squares (PLS) and principal components regression (PCR) analyses with different baselines. The methods were compared for the number of factors, coefficients of determination (R^2), and accuracy of estimation. The standard errors of prediction (SEP) were calculated to compare their predictive ability. The calibrated models generated three to eight factors, R^2 of 0.9414 to 0.9803, standard error of estimation (SEE) of 0.0063 to 0.0680, and SEP of 1.20 to 6.67.

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KEY WORDS: FTIR spectroscopy, palm oil, partial least squares (PLS), principal components regression (PCR), TBARS.

Fourier transform infrared (FTIR) spectroscopy has been used for food analysis (1). Che Man and co-workers also used FTIR spectroscopy to measure several quality parameters of palm oil and palm oil products, such as iodine value (2), anisidine value (3), moisture content (4), peroxide value (5) and free fatty acids (6). In the past, data acquisition and analysis were often time-consuming and tedious activities in analytical laboratories. High-speed digital computers have provided analytical chemists with opportunities to use many measurements for calibrating an instrument (e.g., absorbances at multiple wavelengths). The multivariate models used were usually calibrated using the classical least squares, multiple linear regression, partial least squares (PLS) or principal components regression (PCR) analyses (7). Multivariate calibration methods have also been used successfully for quantification by near-infrared, mid-infrared, and Raman spectrometry (8). Che Man and Setiowaty (9) applied both the PLS and PCR multivariate statistical methods on the FTIR spectra from a set of palm oil products to determine their iodine value. Multivariate statistical methods also have been used for food analyses (10).

*To whom correspondence should be addressed at the Department of Food Technology, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia, 434 UPM, Serdang, Selangor, Malaysia.
E-mail: yaakub@fsb.upm.edu.my

The primary products of lipid oxidation are hydroperoxides, and those from polyunsaturated fatty acids are readily decomposed to produce various thiobarbituric acid-reactive substances (TBARS). The thiobarbituric acid (TBA) values, as well as the anisidine values, are representative parameters of secondary oxidation (11). The TBA test is based on the development of a complex colored compound (chromogen) that develops from the reaction of malonaldehyde (MDA) with TBA followed by colorimetric determination of the pigment at 530 nm. The TBA test (11), modified TBA test (12,13), and the AOCS method (14) are used to determine MDA, other aldehydes, and some oxidation products, particularly carbonyl compounds, as TBARS. These methods are unable to avoid interference from other compounds that also react with TBA (12).

Multivariate calibration methods are more precise, more informative, and faster than previous data analysis methods. In the process of calibration, they also estimate the precision of analysis and yield important qualitative and diagnostic information. In this study, both the PLS and PCR statistical methods were applied to FTIR spectra using different baseline types from a set of palm oil samples to determine their TBARS content without any spectral correction. The methods were then compared by their standard errors of prediction (SEP), coefficients of determination (R^2), standard errors of estimation (SEE), and F -ratios.

The objective of this study was to use FTIR spectroscopy to develop a suitable method for the measurement of free MDA in palm oil as one of the essential products of the second step in the oil oxidative process.

MATERIALS AND METHODS

Materials. Palm oil was obtained from a local refinery. All reagents were of analytical grade. MDA for the chemical analysis was derived from 1,1,3,3-tetramethoxypropane (Sigma Chemical Company, St. Louis, MO) as a TBARS (13). Fifty freshly refined palm oil samples were spiked with MDA at 0–60 $\mu\text{mol/kg}$ oil and used as standards for calibration and cross-validation.

Chemical analysis. American Oil Chemists' Society (AOCS) method Cd 19-90 (14) was used to estimate the MDA content for palm oil as TBARS in the spiked samples.

Instrumental analysis. The instrument used was a Perkin-Elmer FTIR Spectrometer, Series 1600 (PerkinElmer Corporation, Norwalk, CT), with a room temperature deuterated triglycine sulfate detector controlled by a PerkinElmer Professional Computer, Model 7300. The software used for FTIR data collection was the infrared data management system. The instrument was maintained with two automatic dehumidifiers to minimize interference from water vapor.

Melted drops of each standard were placed between the sodium chloride (NaCl) windows, and the transmission path was fixed at 100 μm by adjusting the polytetrafluoroethylene spacer. The cell was then placed in the cell holder and the sample scanned. After each sample scanning, the NaCl windows of the transmission cell were rinsed three times with acetone and then dried with soft tissue for the next sample. For the calibration, spectra were obtained from 64 scans at 4 cm^{-1} resolution and a gain of 2.0 with strong apodization through the frequency region 4000–600 cm^{-1} . The spectra were ratioed against the background air spectrum. All the scans were done in duplicate with the spectra recorded as absorbance and stored in a disk as Joint Committee on Atomic and Molecular Physical Data—Data Exchange (JCAMP-DX) files for subsequent chemometric analysis (15).

Statistical multivariate analysis. All the experiments and measurements were done in duplicate. The relationships between each of the FTIR spectrum parameters and data from the standard chemical method were determined using the software program Nicolet Turbo Quant IR-Calibration and Prediction Package, Version 1.1 (Nicolet Instrument Co., Madison, WI).

PLS and PCR regressions were used to analyze the concentration data from the multicomponent mixtures, based on inverse modeling. Only the concentrations of the model components needed to be known, although the levels of interference had to be distinct.

In the PLS, the chemical and spectral data were correlated and the correlation coefficients (r) were taken as estimates of the factor scores, which were then used as regressors to model both the spectral and chemical data. In the PCR, the absorbance spectral variables were first screened by “data compression” for the most important causes of variation. The selected factors were used in a multiple linear regression to predict the spectral values, which were then used in a Microsoft Excel 97 spreadsheet to derive regressions between the FTIR predictions and chemical data. The similarity between the chemical data and FTIR predictions of TBARS values in the 50 calibration samples indicated the adequacy of the FTIR calibration. Accuracy was assessed on the smallest standard error and the highest R^2 (16).

Validation. The “leave-one-out” cross-validation technique was used to verify the calibration model. The predicted residual error sum of squares (PRESS) was computed from the error in prediction from the standards by cross-validation and plotted as a function of the number of factors employed in the calibration. PRESS values are indications of how closely a model fits the calibration data. The best model in-

cludes the fewest number of factors so that the PRESS for that model is not significantly higher than the minimum PRESS value (17). The PRESS value was calculated as follows:

$$\text{PRESS}(k) = \sum_{i=1}^{\text{Samples}} [C_{i\text{true}} - C_{i\text{calc}}(k)]^2 \quad [1]$$

where $C_{i\text{true}}$ is the real concentration of analyte in sample i and $C_{i\text{calc}}$ is the concentration predicted by multivariate calibration methods; k refers to the number of variables used in the model. The accuracy was assessed by the SEP and R^2 . Further verification was by the mean difference (MD) and standard deviation of difference (SDD) for repeatability and closeness of fit (accuracy) between the chemical data and FTIR predicted TBARS values.

RESULTS AND DISCUSSION

Chemical method. Table 1 shows the FTIR predicted values as means and standard deviations (SD) of the chemically analyzed data (AOCS direct method) (14) for TBARS content (as MDA) using the PLS and PCR statistical methods. With PLS, they were 0.0878 and 0.00264, and 0.0839 and 0.00364 for calibration and cross-validation, respectively. With PCR, they were 0.0794 and 0.0030, and 0.0752 and 0.00339, respectively.

Spectra. Forty samples were used for calibration and 10 for validation for both the PLS and PCR quantitative methods. Figure 1 shows the spectra for MDA-spiked palm oil and pure palm oil, the difference between them representing the spectrum of MDA in the frequency 4000–600 cm^{-1} . The palm oil spectrum showed the characteristic absorption bands of common vegetable oils (18). The MDA spectrum showed bands at 2832, 1683, 1391, 1118, and 907 cm^{-1} , the frequency regions assigned to C–H stretching of the aldehydic group, vibration mode of C=O from carbonyl of the aldehydes and C–O linkage (19,20).

The carbonyl functional group of the MDA-spiked oil in the FTIR spectra appeared in the frequency region 1750–1600 cm^{-1} . The aldehydic C=O exhibited a stretched band in 1730–1680 cm^{-1} (21), which is close to and may overlap with the absorption band of the ester carbonyl functional group of palm oil in 1748–1746 cm^{-1} , especially because under oxidative conditions, the frequency of this band can decrease to 1743 cm^{-1} depending on the carbonyl absorbencies of the saturated aldehydes (22). Hamilton (23) also reported the gener-

TABLE 1
Calibration and Cross-Validation Statistics for Malonaldehyde (TBARS) Content in Palm Oil Obtained by the PLS and PCR Methods with Zero (None) Baseline^a

Data set	PLS		PCR	
	Mean	SD	Mean	SD
Calibration	0.08787	0.00246	0.0794	0.00301
Validation	0.08391	0.00364	0.0752	0.00339

^aTBARS, thiobarbituric acid-reactive substance; PLS, partial least squares; PCR, principal components regression; SD, standard deviation.

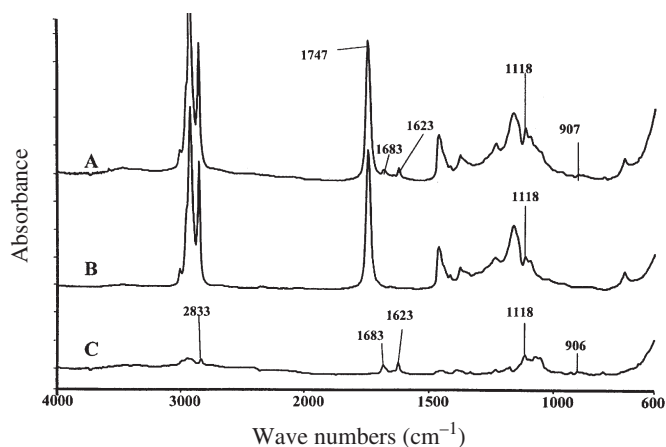


FIG. 1. Spectra of (A) malonaldehyde-spiked palm oil, (B) palm oil, and (C) the difference between A and B.

ation of aldehydes during the auto-oxidation of oils, which reduces the band frequency from 1746 cm^{-1} to below 1744 cm^{-1} . The band at $1730\text{--}1728\text{ cm}^{-1}$ could only be detected as the difference spectrum between the oxidized and unoxidized oils (21). Aldehydes also showed two moderate absorption bands in the frequency region $2699\text{--}2695\text{ cm}^{-1}$.

Selection of the optimal frequency region for MDA prediction. The correlation spectra between 4000 and 600 cm^{-1} (Fig. 2) showed a mathematical correlation between spectral changes within the regions of $2900\text{--}2800\text{ cm}^{-1}$ and $1800\text{--}1600\text{ cm}^{-1}$ in the MDA content of the palm oil samples as described by Fuller *et al.* (24).

Fuller *et al.* (25) used the variance spectrum (Fig. 3) to distinguish between the active and inactive spectral regions. High variances were seen at $3000\text{--}2800\text{ cm}^{-1}$, $1800\text{--}1600\text{ cm}^{-1}$, and $1250\text{--}1100\text{ cm}^{-1}$ for C–H, C=O, and C–O–, respectively (19). Although the frequencies of $1440\text{--}1325\text{ cm}^{-1}$, $1320\text{--}1260\text{ cm}^{-1}$, and $1230\text{--}1160\text{ cm}^{-1}$ have been quoted for aldehydes, Bellamy (20) has argued that it is unwise to use $1440\text{--}1160\text{ cm}^{-1}$ for the identification of aldehydes because of the multiplicity of factors involved in determination within the frequency of this band.

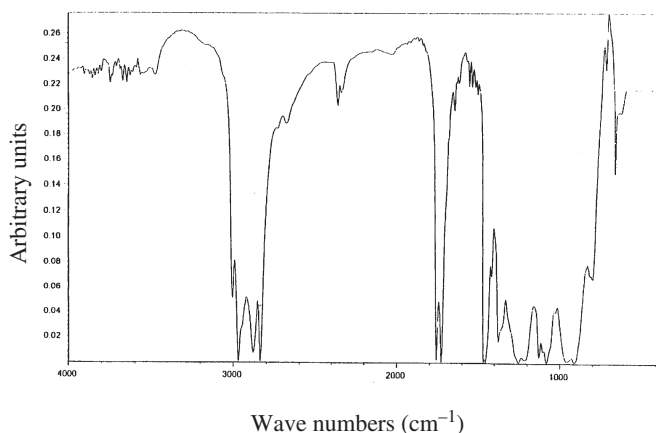


FIG. 2. Correlation spectrum showing good correlation of malonaldehyde absorption at frequency regions $2900\text{--}2800\text{ cm}^{-1}$ and $1800\text{--}1600\text{ cm}^{-1}$.

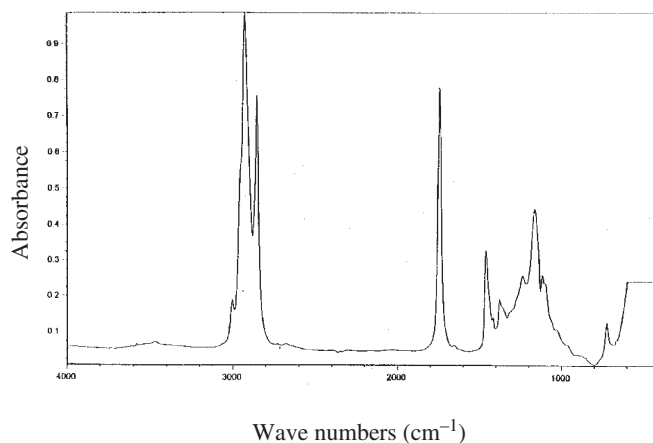


FIG. 3. Variance spectrum obtained from the calibration set.

The correlation and variance spectra were used to select the best regions ($2900\text{--}2800\text{ cm}^{-1}$ and $1800\text{--}1600\text{ cm}^{-1}$) for prediction using both the PLS and PCR statistical techniques calibrated from the FTIR spectra and chemical data (as shown in Table 2).

Statistical analysis. Figure 4 plots the data from the AOCS method against the PLS FTIR-predicted values for MDA content in the palm oil samples using the zero (none) baseline type. This was the best correlation obtained as judged by its R^2 (the highest at 0.9803) and SEE (the lowest at 0.0063) in Table 2 (Method 2 in Table 2: $R^2 = 0.9093$ and $SEE = 0.0205$; Method 3 in Table 2: $R^2 = 0.9745$ and $SEE = 0.0070$). Although all three PLS methods gave good predictions, Method 2 was the worst with the lowest R^2 and highest SEE.

Figure 5 shows the estimations of the TBARS content in the palm oil samples using Methods 4–6, derived using the PCR technique at different baseline types. As in Table 2, the R^2 were high at 0.9506, 0.9414, and 0.9443, and the SEE low at 0.0065, 0.0680, and 0.0150, respectively. Although all three methods were again good, Method 5 was the least accurate, with the lowest R^2 and highest SEE despite having eight factors.

TABLE 2
Accuracy of Prediction of Results by the PLS and PCR Calibration Models of the Reference Method Assessed Against the FTIR Spectroscopic Method at the Frequency Regions $2900\text{--}2800\text{ cm}^{-1}$ and $1800\text{--}1600\text{ cm}^{-1}$ with Different Baseline Types for Malonaldehyde in Palm Oil^a

No.	Method	Baseline type	Factor	R^2	SEP	SEE
1	PLS	Zero (none)	5	0.9803	0.0075	0.0063
2	PLS	Linear removed	4	0.9093	0.0410	0.0205
3	PLS	One point (1500 cm^{-1})	5	0.9745	0.0090	0.0070
4	PCR	Zero (none)	6	0.9506	0.0078	0.0065
5	PCR	Linear removed	8	0.9414	0.0520	0.0680
6	PCR	One point (1500 cm^{-1})	7	0.9443	0.0330	0.0150

^aFTIR, Fourier transform infrared; R^2 , coefficient of determination; SEP, standard error of prediction; SEE, standard error of estimation. See Table 1 for other abbreviations.

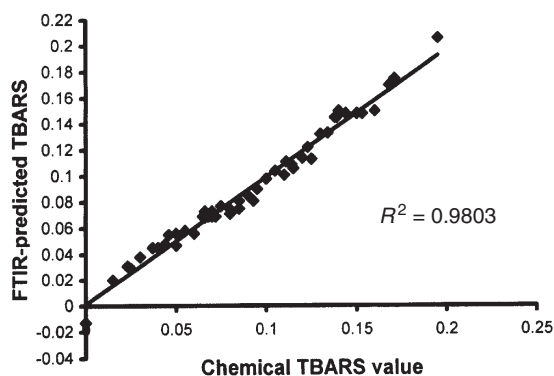


FIG. 4. Data from the AOCS direct method vs. Fourier transform infrared (FTIR)-predicted thiobarbituric acid-reactive substance (TBARS) values by the partial least squares (PLS) calibration method.

The results of statistical analysis (Table 3) show the mean difference of repeatability (MD_r) to be close to zero for both the FTIR and AOCS methods, implying that there was little difference between them in the two series of analyses. The standard deviation of difference of repeatability (SDD_r), which measures variability around the MD, was also very low but showed the FTIR method to have better repeatability.

Although there was good agreement between the FTIR predictions and chemically analyzed data for TBARS content as MDA in the MDA-spiked palm oil samples, the FTIR-predicted values were generally higher than the chemically derived data.

Table 3 shows the assessment of accuracy (in the forms of MD_a and SDD_a) for the FTIR method of determining TBARS content in palm oil. The standard error of calibration relative to the mean value for the reference chemical method is expressed as the coefficient of variation (CV). It has been argued that a CV of <20% suffices for most analytical purposes (26). The CV obtained from using the two statistical methods (PLS and PCR) to predict FTIR results from the AOCS method data were 7.39–19.85% and 8.18–23.6%, respectively. The optimal number of factors employed in the calibrated models was indicated by the PRESS values of 4–5 for the PLS methods and 6–8 for the PCR ones. An example of the PRESS plot is shown in Figure 6.

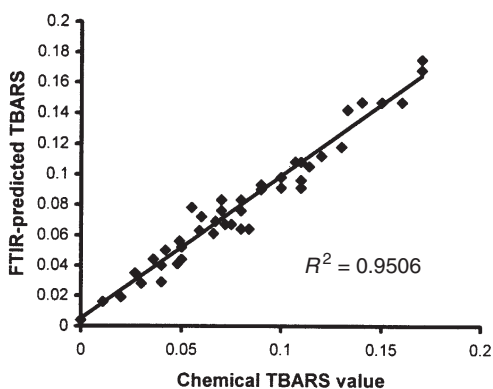


FIG. 5. Data from the AOCS direct method versus FTIR-predicted TBARS values by the principal components regression (PCR) calibration method. See Figure 4 for abbreviations.

TABLE 3
Calibration Statistics for Malonaldehyde (TBARS) Content in Palm Oil Obtained by Reference and FTIR Methods using the PLS and PCR Techniques^a

Statistics	PLS FTIR	AOCS method	PCR FTIR
MD_r	0.0016	0.0071	0.0029
SDD_r	0.00196	0.0077	0.0034
Max. value	0.2060	0.1710	0.2250
Min. value	-0.0130	0.000	-0.0160
MD_a		-0.0067	-0.0080
SDD_a		0.0021	0.0031

^a MD , mean difference; SDD , standard deviation of difference; subscript a , accuracy; subscript r , repeatability. See Tables 1 and 2 for other abbreviations.

The critical F -values and SEP ratios were used to compare the predictive capabilities of the methods objectively (27). Table 4 shows the SEP ratios between Methods 1 and 2, and between Methods 2 and 3 to be greater than the F -value (3.06). The PLS Methods 1 and 3 obviously gave better estimates than Method 2. Among the PCR statistical methods, Method 4 gave more accurate TBARS values than Methods 5 and 6. The SEP comparison among Methods 1, 3, and 4 showed that the prediction error variances were not significantly different.

Thus, FTIR spectroscopy with the PLS and PCR multivariate methods can be an important analytical tool for determination of the TBARS content as MDA in palm oil, as it gives a better repeatability than the chemical method presently in use. Predictions by the PLS FTIR spectroscopy with zero (none) and one-point baseline types, and the PCR method with a zero baseline type gave similar results for the TBARS content as MDA in palm oil samples. Finally, multivariate calibration methods can provide better accuracy, precision, reliability, and significantly more information in considerably less time than previous data analysis methods.

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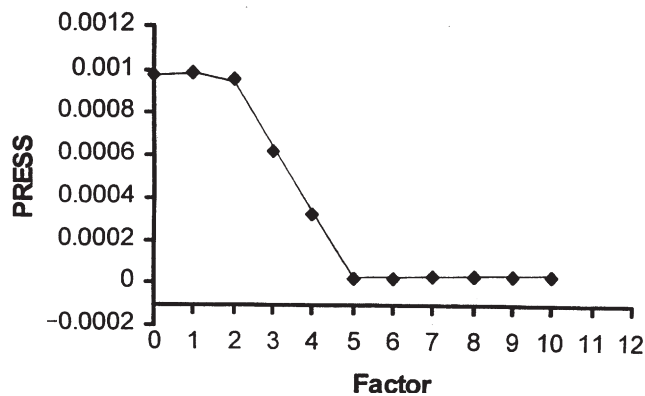


FIG. 6. Predicted residual error sum of squares (PRESS) plot from cross-validation of PLS calibration for the AOCS method with five factors. See Figure 4 for abbreviations.

TABLE 4
SEP Ratios of the Different FTIR Methods and Their F-Value (critical)
at a 95% Confidence Level^a

Method	Baseline type	SEP ratio	F-value (critical)
			3.06
PLS	Linear removed and none (2 and 1)	5.47	
PLS	Linear removed and one point (2 and 3)	4.55	
PLS	One point and none (3 and 1)	1.20	
PCR	Linear removed and none (5 and 4)	6.67	
PCR	Linear removed and one point (5 and 6)	1.58	
PCR	One point and none (6 and 4)	4.23	

^aF, F-statistic (27); see Table 2 for methods and Tables 1 and 2 for other abbreviations.

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