Determination of Free Fatty Acids in Crude Palm Oil and Refined-Bleached-Deodorized Palm Olein Using Fourier Transform Infrared Spectroscopy

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ABSTRACT: A rapid direct Fourier transform infrared (FTIR) spectroscopic method using a 100 μ BaF_2 transmission cell was developed for the determination of free fatty acid (FFA) in crude palm oil (CPO) and refined-bleached-deodorized (RBD) palm olein, covering an analytical range of 3.0–6.5% and 0.07–0.6% FFA, respectively. The samples were prepared by hydrolyzing oil with enzyme in an incubator. The optimal calibration models were constructed based on partial least squares (PLS) analysis using the FTIR carboxyl region (C=O) from 1722 to 1690 cm^{-1}. The resulting PLS calibrations were linear over the range tested. The standard errors of calibration (SEC) obtained were 0.08% FFA for CPO with correlation coefficient (R^2) of 0.992 and 0.01% FFA for RBD palm olein with R^2 of 0.994. The standard errors of performance (SEP) were 0.04% FFA for CPO with R^2 of 0.998 and 0.006% FFA for RBD palm olein with R^2 of 0.998, respectively. In terms of reproducibility (*r*) and accuracy (*a*), both FTIR and chemical methods showed comparable results. Because of its simpler and more rapid analysis, which is less than 2 min per sample, as well as the minimum use of solvents and labor, FTIR has an advantage over the wet chemical method.

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KEY WORDS: FTIR, free fatty acids, palm oil, palm olein, partial least squares analysis, spectroscopy.

Free fatty acids (FFA) are released naturally in crude palm oil (CPO) and can be increased by the action of enzymes in the palm fruit and by microbial lipases. During storage, FFA were produced by the reaction of oil with water. The Palm Oil Refiners Association of Malaysia standard specifications for the FFA content (as palmitic acid) is 5% maximum in CPO and 0.1% maximum in refined-bleached-deodorized (RBD) oils, respectively. For trading purposes, such standards have to be met. The routine procedure to determine the FFA content in palm oil is through the conventional wet chemical method adopted from the American Oil Chemists' Society (AOCS; Ca 5a-40) titration method (1). For preparation of the standard reagent, analysis, and expressing the result, substantial personnel time and glassware are required.

Fourier transform infrared (FTIR) spectroscopy has received great attention in quantitative analysis of fats and oils over the years (2). It has a major advantage over the conventional grating instruments, having more energy throughput (the Jacquinot advantage), excellent reproducibility, and accuracy from the laser source (3). With increasing use of the microcomputer, FTIR is capable of manipulating spectral information (subtraction, ratioing, derivative spectra, and deconvolution) and advanced chemometric software to handle calibration development. Coupling with an attenuated total reflectance (ATR) accessory or a flow-through transmission cell has simplified many problems associated with liquid sample handling in infrared analysis (4).

The only widespread application of infrared in fats and oils is the determination of *trans* isomers, which is an official method of the AOCS (Cd 14-95) (1). However, with some minor modification, FTIR spectrometry shows potential to meet the present need for rapid, accurate *trans* analysis (5). Other analyses based on FTIR, such as degree of unsaturation, saponification number (6) and peroxide value (7), have been developed. A method for measuring the FFA content in edible oils has also been developed by Ismail *et al.* (8). Though a variety of types of oils were used in the studies, palm oil was not included.

Malaysia is the largest palm oil producer in the world. However, the use of FTIR spectroscopy as a quality control tool is yet to be implemented by the industry. Because of the number of samples needing to be analyzed daily, chemical analysis is too time consuming. Therefore, the objective of this study was to develop an FTIR spectroscopic technique for rapid determination of FFA in CPO and RBD palm oil.

MATERIALS AND METHODS

Sample preparation. CPO (2.0% FFA) and RBD palm olein (0.07% FFA) were purchased from a local refinery. Lypozyme IM (Novo Nordisk A/S, Denmark) used in this study was purchased from Science Technik Sdn. Bhd. (Petaling Jaya,

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Malaysia). A range of FFA was achieved by spiking 400 g sample with 0.15% w/w enzyme. The sample was then incubated (Certomat, B. Braun, Melsungen AG, Germany) at 60°C and 200 rpm. Samples (70 g) were collected at each 15-min interval and filtered through No. 2 Whatman filter paper in an oven (90°C) to remove enzyme. Acid values of enzyme-free samples were determined by the AOCS (1) titration method. The results were calculated from the number of milligrams of KOH necessary to neutralize the FFA in 1 g of sample and expressed as percent palmitic acid.

Instrumentation. A Perkin-Elmer model 1000 Paragon FTIR spectrometer capable of covering the spectral range of 4000–400 cm^{-1} was used in this study. The instrument was controlled by a Digital-Pentium personal computer run under Windows-based Perkin-Elmer Spectrum Light Software (version 1.5) and equipped with a specially designed heated sample-handling accessory for palm oil analysis. The temperature of the flow cell was set at 80°C (±2°C) by a temperature controller constructed by attaching strips of heating tapes on the cell window and the stainless steel tubing so that all components of the accessory were heated to prevent crystallization of oil in the lines or cell. Prior to the analysis, the sample compartment was purged with pure N_2 gas at slow rate for 30 min to minimize water vapor and CO_2 interferences. An outlet line from the flow-through transmission cell (100 μ BaF_2) was emptied into a collection vessel which was attached to a vacuum pump unit to automate sample handling.

Calibration and validation procedures. All spectra were collected from eight scans at 4 cm^{-1} resolution and gain of 2. After every scan, a new reference air background spectrum was taken and the flow cell was cleaned by flushing through with *n*-hexane. The spectral data of calibration standards were stored to disk under JCAMP file and transferred to the Nicolet Turbo Quant-IR Calibration and Prediction Package (Nicolet Instrument Co., Madison, WI) for subsequent partial least square (PLS) calibration development. Spectral regions for calibration development were explored by means of correlation and variance spectra. Each calibration was also assessed by using the leave-one-out cross-validation procedure and optimized in terms of the minimal number of factors by using the predicted residual error sum of squares (PRESS) test. The calibration was further refined by using the mean difference (MD) and standard deviation of the difference (SDD) between the predicted and actual FFA as a measure of improved performance of the calibration. The correlation coefficient (R^2) was used to measure the strength of the linear relationship between the predicted and actual values, and the standard errors were generated during analysis. Prediction was carried out using independent sets of samples to test the applicability of the calibration model developed.

RESULTS AND DISCUSSION

General FTIR statistical spectra. Figure 1 shows a typical mean spectra of 29 CPO samples obtained using a BaF_2 transmittance technique that allows the full spectrum to be examined to ~800 cm^{-1}. The region of intense absorption, i.e., the triglyceride ester linkage at 1744 cm^{-1}, major CH bands from 3050 to 2800 cm^{-1}, and the fingerprint region of oil (1500–1000 cm^{-1}), where the absorbance values are >2.0 absorbance units, is off-scale (10). This is because the signal reaching the detector is too small to be sampled properly. This problem can be avoided by reducing the effective path length of the cell so that such bands are not as intense. However,

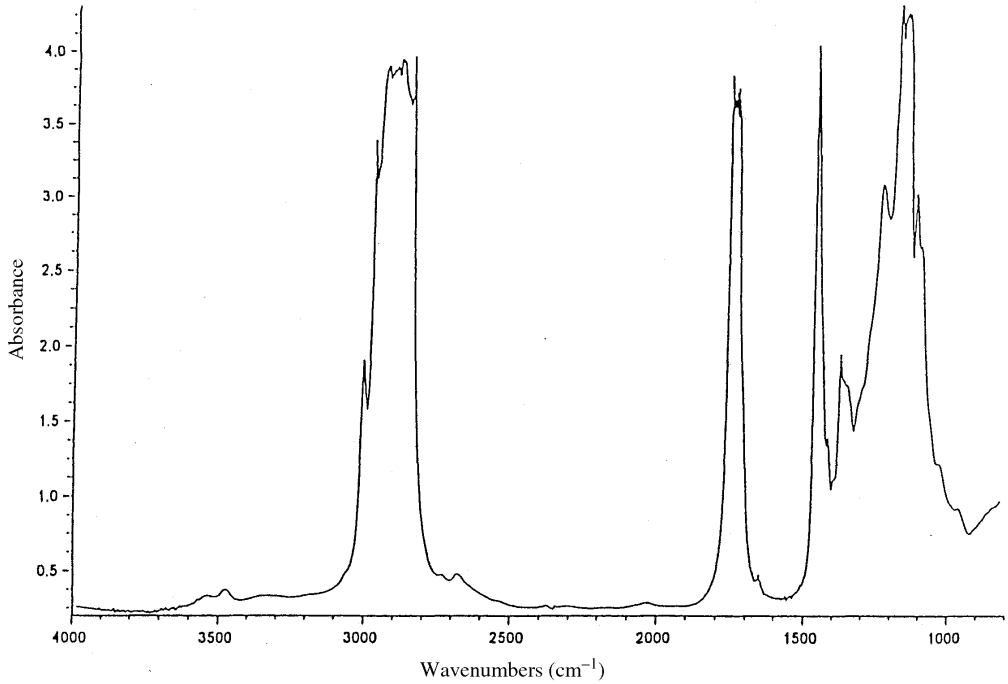

FIG. 1. A typical mean Fourier transform infrared (FTIR) spectrum of palm oil.

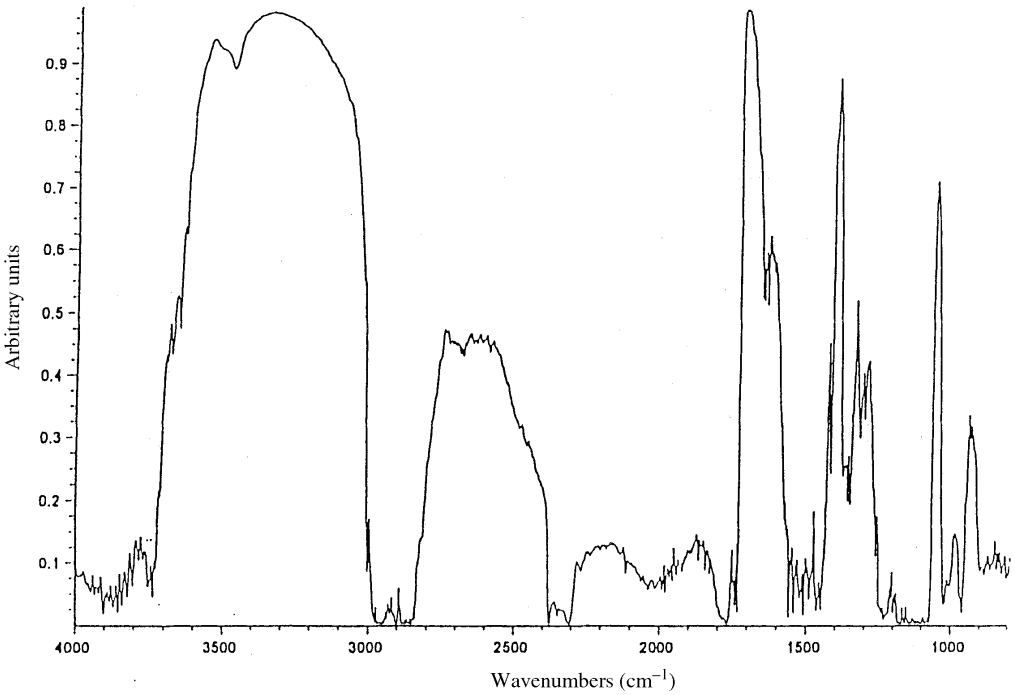

FIG. 2. Correlation spectrum obtained from the crude palm oil (CPO) calibration standards.

components at other regions that have low concentrations are at the expense of sensitivity, i.e., <1% might be too weak to be detected. As a result, no signal will be picked up by the detector. Thus, path length selection for a proper analysis is very crucial.

Figures 2 and 3 illustrate the correlation spectra of the CPO and RBD palm olein from the calibration standards, respectively. FFA property is related to a large part of the spectrum, such as the bands due to bonded O–H, C=O stretching in ester, and C–H olefinic stretch region at around 3350, 1745, and 2900 cm^{-1}, respectively. From the correlation spectra, the highest correlation region exhibits at 1711 cm^{-1} due to the carboxyl acid functional group of FFA followed by the OH region. However, by studying the variance spectra, the only significant region is at 1711 cm^{-1}. There is zero variance at the OH region. Other absorption bands are from the digitiza-

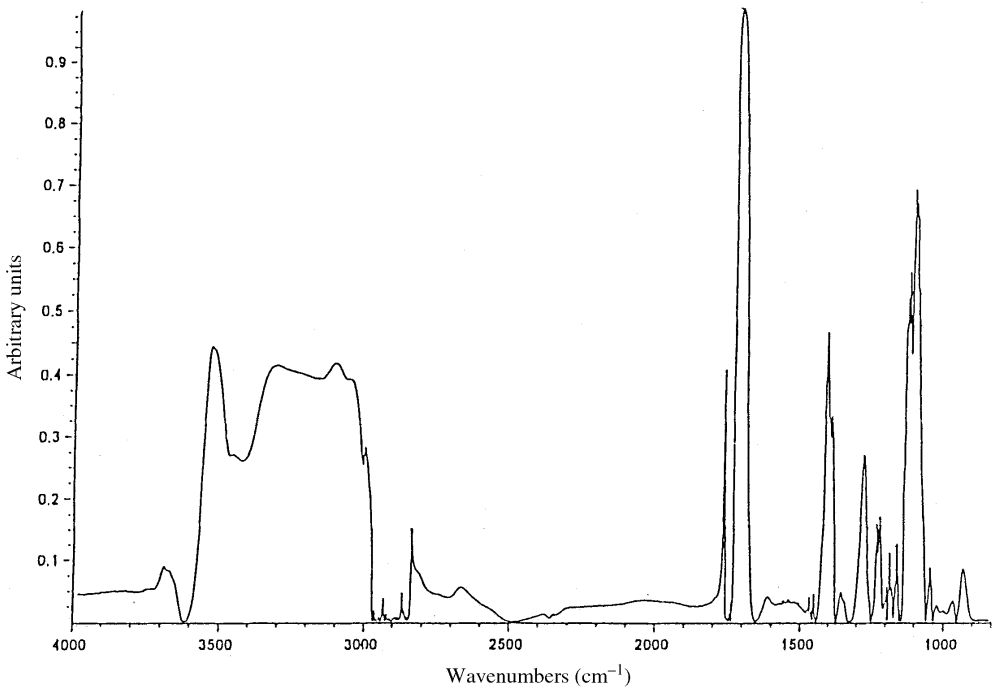

FIG. 3. Correlation spectrum obtained from the refined-bleached-deodorized (RBD) palm olein calibration standards.

tion noise due to the off-scale absorbance units. Therefore, the carboxyl absorption band proves once again to be the best region to quantify FFA in palm oil samples.

PLS calibration. Chemometric software based on PLS was used in this study. PLS is a powerful tool in quantitative IR analysis (11) as a basis for calibration, allowing the whole spectrum to be investigated correlationally (6). The regions that correlate with the measure of interest are readily determined by studying the correlation spectra. Instead of using a single absorbance point, PLS analysis uses regions of the spectrum that exhibit variations with changes in the concentration. In a PLS calibration model, the spectral data for a calibration set are compressed into the loading spectra or factors. Each loading spectrum represents an independent source of variation in the data. Generally, the first loading spectrum describes most of the variation in the calibration standards, whereas the additional loading spectra describe the remaining variations, which are more specific, representing small variations in the data. Thus, the last loading spectra generated will mostly represent noise in the spectra of the calibration standards, and their inclusion in the PLS calibration model will degrade its performance in prediction of unknowns.

To develop a robust PLS calibration model, it is important that the concentration range of the component(s) of interest in the samples to be analyzed must be adequately spanned with the presence of interfering components which have no correlation with the components of interest. However, in a real production plant, a wide range of FFA concentration is difficult to obtain. Thus, in developing a PLS calibration model to predict the FFA content in palm oil, a hydrolysis process was employed. The hydrolysis was set at 60°C to prevent the formation of oxidative products to avoid building in any correlation with FFA. Furthermore, higher temperature may denature the lipase, as a result easing hydrolysis. Table 1 summarizes the calibration standard matrix for both CPO and RBD palm olein used to develop the PLS calibration. An optimized PLS calibration developed for predicting FFA content in CPO and RBD palm olein calibration standards was based on the 1722–1690 cm^{-1} spectral region referenced to a single-point baseline at 1590 cm^{-1}. The PRESS test was used in selecting the optimal wavelength region, baseline, and number of factors for the calibration. The optimal number of factors was determined when the PRESS plot reached a minimum or began to level off. Figures 4 and 5 illustrate the calibration plot in terms of predicted vs. actual FFA for CPO and RBD palm olein obtained from the PLS calibration model. A plot of the means of duplicate readings, derived by averaging duplicate samples, was linear, with correlation coefficient (R^2) of 0.992 and standard error of calibration (SEC) of 0.08 for CPO calibration standard and R^2 of 0.994 with SEC of 0.01 for RBD palm olein using factor 3.

Validation of calibration for predicting CPO samples. A plot of the means of duplicate readings of FTIR-predicted vs. FFA concentration determined by the chemical method of the validation set obtained from the PLS is represented in Figure 6. The standard curve obtained for FFA in CPO (3.46–6.11%) with the 100 μ transmission cell produced the following calibration equation:

$$\text{CPO} - \text{FTIRFFA} = 0.9792\ \text{CPO} - \text{CHEMFFA} + 0.0975 \quad [1]$$

where FTIRFFA is FFA predicted by FTIR method and CHEMFFA is FFA determined by AOCS method with R^2 = 0.998 and standard error of performance (SEP) = ±0.03% FFA. This plot illustrates that the slope is close to 1 and an excellent linear relationship exists between FTIR-determined FFA and chemically-determined values within the range tested. The comparisons between duplicate of chemical and instrumental results in terms of the mean difference (MD) and standard deviation of the difference (SDD) for reproducibility (r) and the MD and SDD for overall accuracy (a) between the chemical and the instrumental results is shown in Table 2.

Both chemical and instrumental results have comparable mean differences in terms of reproducibility. Comparison of all the data by a two-way analysis indicated that there were no significant differences between any of the data sets ($P <$

TABLE 1
Determination of FFA as Percentage of CPO and RBD Palm Olein Obtained from AOCS Reference and the FTIR Methods[a]

	CPO		RBD palm olein	
Sample number	AOCS reference method	FTIR method	AOCS reference method	FTIR method
1	3.46	3.64	0.07	0.07
2	3.54	3.61	0.07	0.08
3	3.65	3.65	0.08	0.1
4	3.71	3.6	0.11	0.13
5	3.74	3.81	0.12	0.13
6	3.76	3.75	0.14	0.15
7	3.82	3.81	0.15	0.15
8	3.84	3.72	0.2	0.2
9	4.19	4.18	0.27	0.27
10	4.33	4.23	0.3	0.3
11	4.52	4.53	0.32	0.33
12	4.61	4.58	0.33	0.32
13	4.84	4.84	0.34	0.33
14	4.86	4.77	0.37	0.36
15	5.05	4.88	0.39	0.39
16	5.26	5.35	0.42	0.41
17	5.31	5.4	0.44	0.44
18	5.58	5.56	0.49	0.49
19	5.73	5.74	0.49	0.49
20	6.15	6.17	0.5	0.52
21	6.2	6.21	0.52	0.54
22	3.54	3.58	0.09	0.09
23	4.04	4.05	0.12	0.12
24	4.52	4.57	0.19	0.19
25	5	4.96	0.23	0.24
26	5.23	5.18	0.27	0.27
27	5.37	5.37	0.33	0.32
28	6.11	6.13	0.42	0.42

[a]Mean of duplicate readings. Abbreviations: FFA, free fatty acids; CPO, crude palm olein; RBD, refined-bleached-deodorized; AOCS, American Oil Chemists' Society; FTIR, Fourier transform infrared.

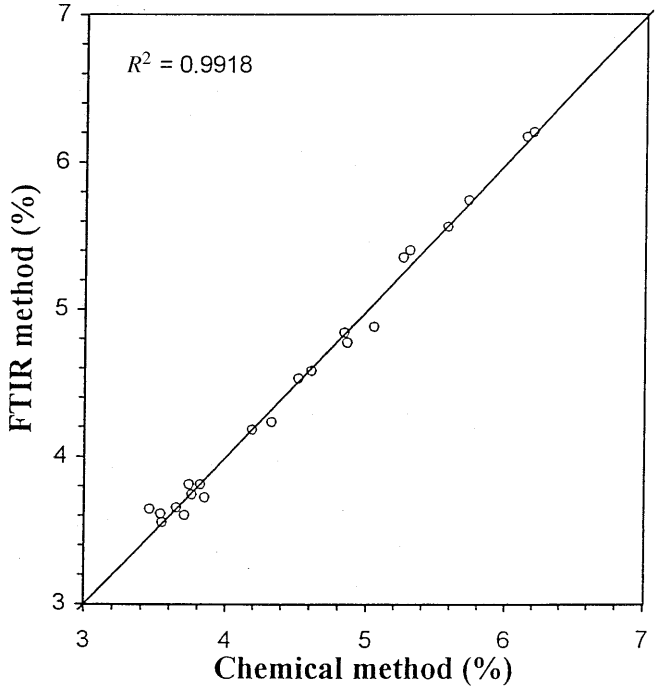

FIG. 4. Free fatty acid (FFA) calibration plot constructed from CPO calibration standards. See Figures 1 and 2 for abbreviations.

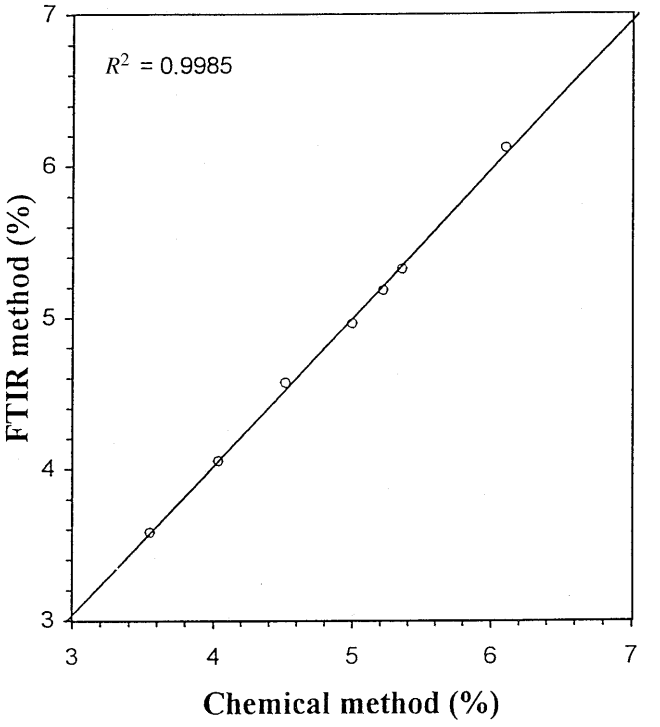

FIG. 6. FFA validation plot for CPO samples. See Figures 1, 2, and 4 for abbreviations.

0.001). These results reveal that the direct FTIR method generally performs as well as the titration method and is independent of the analyzed oil. In terms of accuracy, predictions from the FTIR method are as good as the overall chemical values. However, the latter have some major drawbacks in real-time analysis, i.e., accuracy in standardization of basic reagent and determination of endpoint. The FTIR method has greater potential, since procedures are simpler once a proper calibration model is developed. The FTIR method in this study proves that one can determine FFA in CPO to within 0.01% FFA.

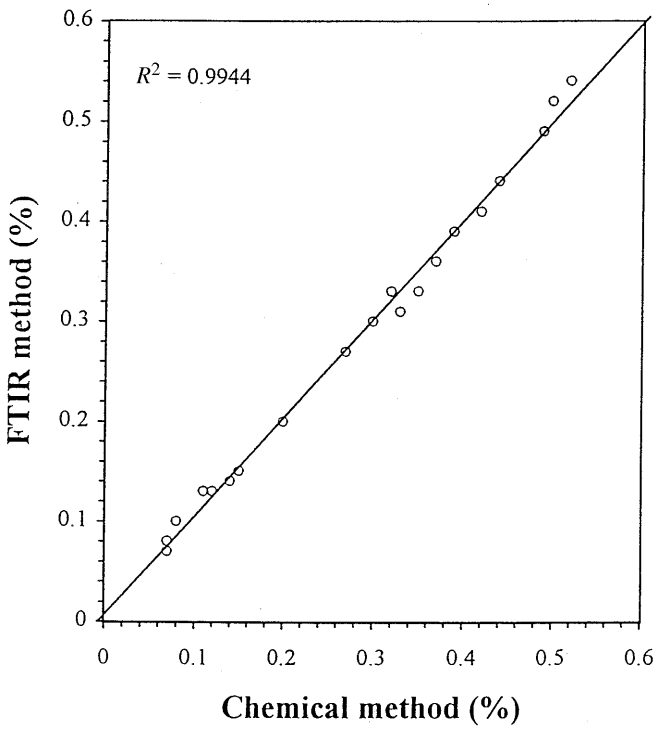

FIG. 5. FFA calibration plot constructed from RBD palm olein calibration standards. See Figures 1, 3, and 4 for abbreviations.

Validation of calibration for RBD palm olein samples. The sensitivity of the 100 μ transmission cell below 0.1% FFA was demonstrated using RBD palm olein. Figure 7 illustrates the plot of the mean duplicate obtained from the FTIR-predicted vs. FFA concentration determined by chemical method (0.07–0.5%) of the validation set of the RBD palm olein samples. The calibration equation is:

$$\text{RBD} - \text{FTIRFFA} = 0.9876\ \text{RBD} - \text{CHEMFFA} + 0.0029 \quad [2]$$

TABLE 2
Statistical Comparison of FFA (%) of CPO and RBD Palm Olein Obtained by AOCS Reference and the FTIR Methods[a]

	CPO		RBD palm olein	
Statistic	AOCS reference method	FTIR method	AOCS reference method	FTIR method
Max value	6.2	6.21	0.52	0.54
Min value	3.45	3.61	0.07	0.07
Mean	4.6	4.59	0.28	0.28
MD_r	–0.0039	–0.011	0.0001	–0.0004
SDD_r	0.004	–0.0078	–0.0004	–0.0005
MD_a	0.0071		–0.0004	
SDD_a	0.0081		0.0014	

[a]MD, mean difference; SDD, standard deviation of the difference; *r*, reproducibility; *a*, accuracy. See Table 1 for other abbreviations.

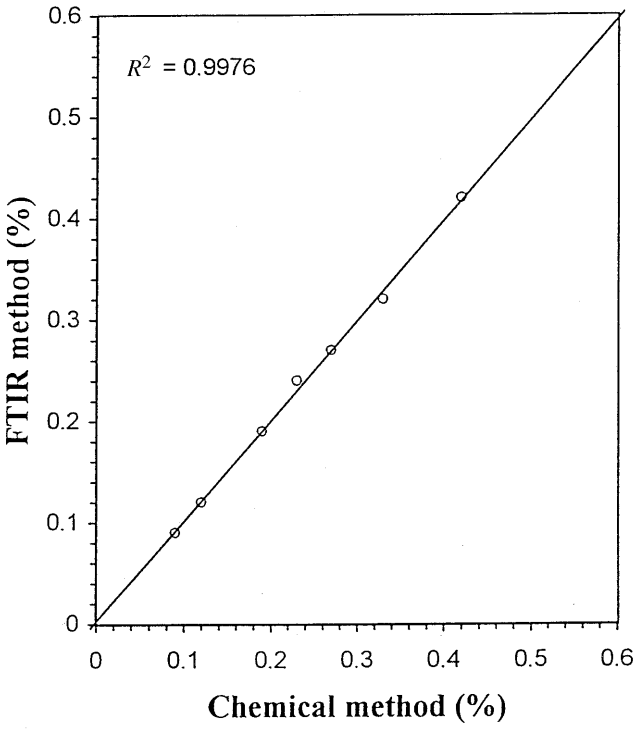

FIG. 7. FFA validation plot for RBD palm olein samples. See Figures 1, 3, and 5 for abbreviations.

Excellent predictions were obtained in this calibration, with $R^2 = 0.998$ and SEP of 0.006% FFA. In terms of reproducibility, the overall MD_r and SDD_r for FTIR and chemical methods were similar (Table 2). A paired comparison test showed the chemical and FTIR results were not significantly different ($P < 0.001$). This indicates that the FTIR method produces consistent results too. In terms of accuracy, FTIR results were 0.0004% FFA higher overall, with a SDD_a of ~0.0014% FFA being better compared to the SEC. Judging from the excellent standard curve obtained, it is likely that FTIR results are more reliable than the chemical results when determining FFA concentrations lower than 1%. Instead of preparing the basic reagent to 0.01 N, a transmission cell with 100 µ pathlength is considered a better choice in FFA analysis for both crude and RBD oils.

Results from this study show that FTIR spectroscopy coupled with a flow-through transmission cell is a useful technique for determining FFA in palm oil. The sensitivity of the 100 µ cell can measure FFA below 0.1%; that was as low as 0.07% FFA in this study. Once the spectrometer has been precalibrated, it can be used as a routine analytical tool with the ability of measuring hundreds of samples daily. It is a major improvement over the chemical method, with the total analysis taking less than 2 min per sample and yet still meeting the trading specification. In addition, by applying this technique, the amount of solvents can be reduced dramatically, as well as the cost of labor.

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