FTIR Spectroscopic Determination of Soap in Refined Vegetable Oils

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ABSTRACT: A new analytical method was developed for the determination of soap in palm and groundnut oils by FTIR spectroscopy. Soap from 0 to 80 mg/kg oil was produced *in situ* in the oils by adding sodium hydroxide. The FTIR spectroscopy was with a sodium chloride transmission cell, and the partial least-squares statistical method was used to calibrate a model for each oil. The accuracy of the method was comparable to that of AOCS Method Cc17-95, with coefficients of determination (R^2) of 0.98 and 0.98 for both palm and groundnut oils. The standard errors of calibration were 1.84 and 1.36 for the two oils, respectively. The calibration models were cross-validated, and the R^2 of cross-validation and standard errors of cross validation were computed. The standard deviation of the difference for repeatability of the FTIR method was better than that for the chemical method used for determining soap in palm and groundnut oils. With its speed and ease of data manipulation by computer software, FTIR spectroscopy is a possible alternative to the standard wet chemical methods for rapid (2 min) and accurate routine determination of soap in chemically refined vegetable oils.

Sodium hydroxide (NaOH) is used in caustic refining of vegetable oils to neutralize FFA to produce soap. Although largely removed, the traces of soap remaining in the refined oil contribute to its breakdown and reduce its shelf life. In shortening, for example, soap contamination leads to foaming and an off-flavor (1), although the soap itself is not toxic unless in very high concentration (2). The soap also affects the rate of hydrogenation and may increase the concentration of toxins in the reaction system (3). The soap content must be <50 ppm (0.005%) to maintain oil quality.

The soap content in oil can be determined either by the AOCS Conductivity Procedure, Cc 15-60, or the Titration Method, Cc 17-95 (4). Other methods for soap determination are atomic absorption (5), flame photometry (6), and neutron activation (5). Wolff as cited in Nelson (5) earlier suggested direct titration of oils, and Nelson (5) proposed direct titra-tion of vegetable oils in isopropyl alcohol. The Palm Oil Research Institute of Malaysia (PORIM, now the Malaysian Palm Oil Board) (7) has developed a method for assessing palm oil with soap determined as sodium palmitate. The sodium content in oil and other foods can also be determined by an ion-selective electrode (8).

All the methods for determining soap are based on the sodium ion content in the oil, as the metal ion is a constituent of soap. This biases the results as the metal ion also can be associated with nonsoap compounds, such as phospholipids. However, none of the methods, including the new method described here, can differentiate between the sodium in soap and free alkali. Excess alkalinity is, therefore, included in the "soap" analysis. Biwald and Gavlick (9) used total organic carbon analysis and FTIR spectroscopy to determine the residues of cleaning agents on surfaces. FTIR spectroscopy has been used by Che Man and co-workers to analyze palm oil and its products for iodine value (10), anisidine value (11), moisture content (12), PV (13), and aflatoxins in groundnut (14).

The objective of this study was to develop a rapid method of soap determination in chemically refined vegetable oils for continuous quality monitoring of the oil.

MATERIALS AND METHODS

Samples and chemicals. All chemicals were of analytical grade. Palm oil was obtained from a local refinery. Groundnuts were purchased from local retailers, and the oil was solvent extracted from the ground nuts. Soap was produced *in situ* to 0–80 mg soap/kg oil by adding a NaOH solution to the oils. The oil samples were tightly covered and shaken vigorously to homogeneity on an Autovortex SA1 mixer (Stuart Scientific, Redhill, United Kingdom) and then oven dried.

Chemical analysis. Thirty-five groundnut oil calibration samples were analyzed in triplicate for their soap content using AOCS Method Cc 17-95, which determines soap as sodium oleate (4). PORIM method p2.13, Soap Content Method (7), was used to measure the soap content (as sodium palmitate) in 35 spiked palm oil samples.

Instrumental analysis. The infrared spectra were recorded at room temperature with a PerkinElmer FTIR Spectrometer, Model 1750 (PerkinElmer Corporation, Norwalk, CT), equipped with a deuterated triglycine sulfate detector and

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controlled by a PerkinElmer 7300 PC. The software used for FTIR data collection was the Infrared Data Management system. The instruments were maintained at constant humidity to minimize water vapor interference.

Melted drops from each sample were placed in a sodium chloride (NaCl) cell, and the transmission path was set at 100 μm by adjusting the PTFE spacer. The pathlength was calculated according to Smith (15) and Stuart (16) by estimating the interference fringes from the spectrum of an empty cell (ratioed against the empty beam of the instrument) by the equation:

$$\text{pathlength} = n/2 \times 10/(f_1 - f_2) \qquad [1]$$

where pathlength is the cell thickness in mm, f_1 and f_2 the frequencies (cm^{-1}) of two maxima for the interference fringes from the spectrum of the empty cell, and n the number of maxima between f_1 and f_2.

After each scan, the NaCl cell windows were rinsed three times with acetone and dried with soft tissue before filling with the next sample. Calibration spectra were obtained from 64 scans at a resolution of 4 cm^{-1} and gain of 2.0 with strong apodization through 4,000–600 cm^{-1} frequency. The spectra were ratioed against the background air spectrum. All scans were done in triplicate with the spectra recorded as absorbance and stored on a disk as JCAMP-DX (Joint Committee on Atomic and Molecular Physical Data—Data Exchange) files (17) for subsequent chemometric analysis.

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

Partial least-squares (PLS) regression was used to derive the soap contents in the oils. The optimal number of factors employed in the calibration models was indicated by the predicted residual error sum of squares (PRESS) values and was 6 and 8 for the palm and groundnut oils, respectively. An example of the PRESS plot is shown in Figure 1, which also shows the *F*-test significance for the method ($P < 0.05$). The *F* statistic for each PRESS value was calculated for all the factors up to the number with the smallest PRESS value. The optimal number of factors was empirically chosen as that giving the smallest PRESS value such that the *F* ratio probability dropped below 0.75. A Microsoft Excel 97 spreadsheet was used to correlate the FTIR-predicted and chemical data. The good correlation obtained for the 35 calibration samples indicated the adequacy of the FTIR calibration. Accuracy was assessed based on the smallest standard error of calibration (SEC) and the highest coefficient of determination (R^2) (18).

Validation. The "leave-one-out" cross-validation technique was used to verify the calibration model. The PRESS values were computed from the errors in prediction from the standards and plotted as a function of the number of factors employed in the calibration. The accuracy was assessed by the standard error of cross validation (SECV) and R^2. Further verification was obtained by the mean difference (MD) and standard deviation of difference (SDD) for repeatability (r) and closeness of fit (accuracy, a) between the chemical data and FTIR-predicted values.
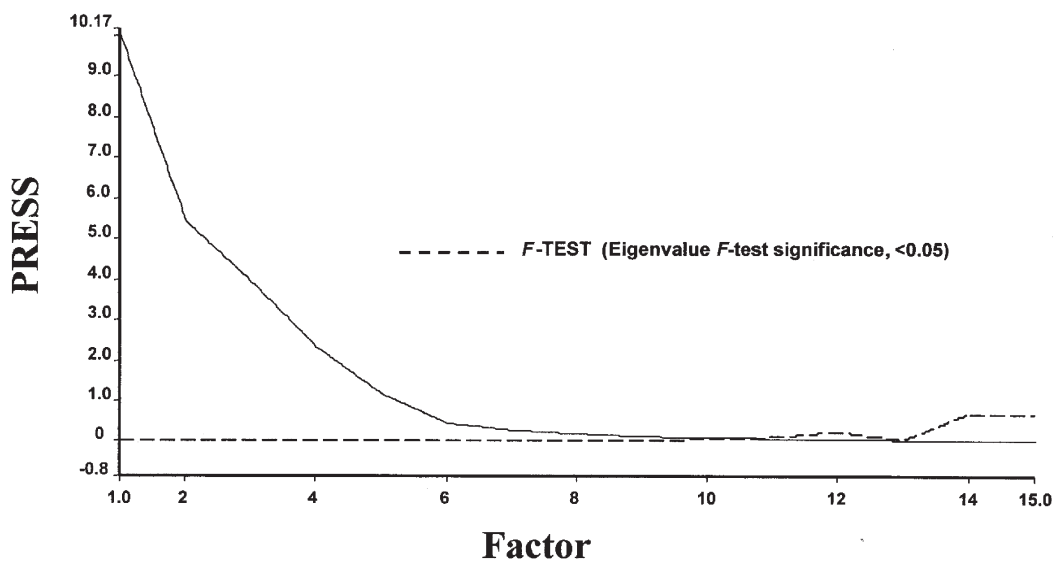

FIG. 1. Predicted residual error sum of squares (PRESS) plot from cross-validation of partial least-squares (PLS) calibration for determination of soap in groundnut oil showing the number of factors (eight) included in the calibration for minimum PRESS value. The *F*-test and significance (<0.05) for the PRESS were also plotted.

TABLE 1
Calibration and Cross-Validation for Soap Content (mg/kg) in Palm and Groundnut Oils by the FTIR Method in Comparison with Chemical Methods[a]

Data set	Palm oil		Groundnut oil	
	Mean	SD	Mean	SD
FTIR calibration	46.53	4.05	45.20	3.69
FTIR validation	44.17	3.75	45.82	3.85
AOCS method	—	—	43.55	4.72
PORIM method	45.69	5.18	—	—

[a]PORIM, Palm Oil Research Institute of Malaysia (now the Malaysian Palm Oil Board, MPOB). All data sets were obtained from three replications.

RESULTS AND DISCUSSION

Chemical and FTIR-predicted results. Table 1 shows the FTIR predicted values by the PLS statistical method as means and SD of the chemically analyzed data (AOCS and PORIM methods) (4,7) for soap contents in the oils. The means and SD for palm oil were 46.53 ± 4.05 and 44.17 ± 3.75 mg/kg for calibration and cross-validation, respectively. For groundnut oil, they were 45.20 ± 3.69 and 45.82 ± 3.85 mg/kg, respectively. The wet chemical method results for means and SD were 45.69 ± 5.18 and 43.55 ± 4.72 mg/kg for the palm and groundnut oil samples, respectively.

Spectra. Figure 2 shows the spectra for (A) NaOH-spiked palm oil, (B) pure palm oil, and (C) the difference between them in the frequency range 4000–600 cm^{-1}. The palm oil spectrum B showed the absorption bands characteristic of vegetable oils (19). With soap, there were additional bands between 1616 and 1507 cm^{-1} (Figs. 2C and 3), which can be assigned to FA–sodium salts (R–COONa). Painter *et al.* (20)

assigned the 1564 cm^{-1} vibration, as well as the weak residual band at 1699 cm^{-1} in the spectra of coal samples soaked in NaOH, to structures of the type COO$^-$M$^+$, although the 1700 cm^{-1} band may be related to COOH (19). Nakanishi and Solomon (21) related the band at 1549 cm^{-1} and (1610 to 1550 cm^{-1}) to the carboxylate ion of sodium benzoate. Rosado *et al.* (22) assigned the two vibrational bands of strong and very strong intensity in the glycine alkaline salt (NH$_2$CH$_2$COO$^-$Na$^+$) at 1597 cm^{-1} and 1561 cm^{-1}, respectively, to COO$^-$. An alternative, indirect analytical method was developed by Ismail *et al.* (23) based on the extraction of FFA from oils with KOH/MeOH. This method simultaneously extracts the FFA and converts them to their ionized potassium salts, which absorb at 1570 cm^{-1}.

Selecting the optimal frequency region for prediction. Figure 4 represents the correlation spectrum, which is calculated by multiplying the difference between each standard spectrum and the mean spectrum at each wavelength by the difference between the corresponding property concentration and the mean property concentration, and summing over all the standards. Peaks that do not correlate with the change in concentration are summed to zero, producing a spectrum that highlights the peaks that change with the change in concentration, i.e., the peaks that relate to soap content. Thus, the correlation spectrum was used to choose the 1675–1500 cm^{-1} and 2990–2800 cm^{-1} regions for calculating soap contents of the oil samples as described by Fuller *et al.* (24). High variances (25) were seen in the same regions (3000–2800 cm^{-1} and 1700–1500 cm^{-1}) for C–H and C=O in R–COONa (21). Hence, the correlation and variance spectra were used to select the best region (1675–1500 cm^{-1}) for prediction using the
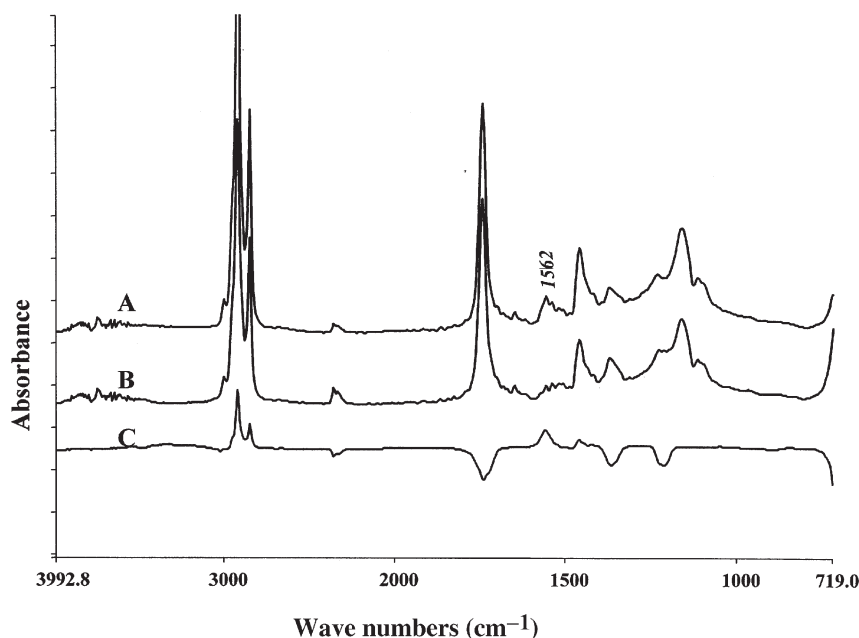

FIG. 2. Spectra of (A) NaOH-spiked palm oil, (B) pure palm oil, and (C) the difference between A and B (A–B).
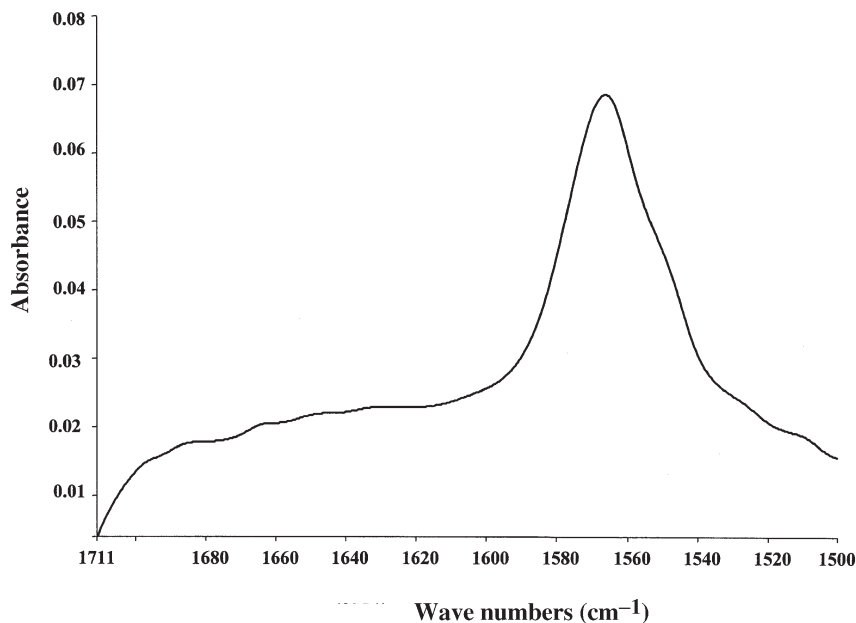
FIG. 3. Enlargement of the difference spectrum (C) (in Fig. 2) at wave number 1500–1711 cm^{-1}.

PLS statistical technique to develop calibrations from the FTIR spectral and chemical data. The R^2 and SEC from the PLS calibration were used to choose the best region for determining soap in palm and groundnut oils, and 1675–1500 cm^{-1} was found to be the best for both oils.

Statistical analysis. The results from PLS calibration for the data from the chemical method against the PLS FTIR-predicted values for soap content in the palm and groundnut oil samples are as follows. The best correlation between chemical and FTIR methods for determination of soap in palm oil was obtained (highest R^2 at 0.98 and lowest SEC at 1.84), and the cross-validation analysis resulted in R^2 = 0.96 and SECV = 1.45. The chemical data determined against the PLS FTIR

spectroscopy data predicted soap contents in groundnut oil showed good correlation of R^2 = 0.98 and SEC = 1.36, and the cross-validation analysis resulted in R^2 = 0.97 and SECV = 1.64. Because of the strong correlations between the FTIR spectroscopic and standard chemical determinations of soap in oils, linear regression equations between them were established. The slopes of 1.0165 and 1.0183 for cross-validation plots for palm and groundnut oil samples were not significantly different ($P > 0.05$), from 0.0 to 1.0, respectively.

Statistical analysis showed the MD$_r$ to be smaller for FTIR determinations than the chemical methods, implying that there was little difference between them in the two analyses. SDD$_{r,}$ which measures variability around the MD, was also
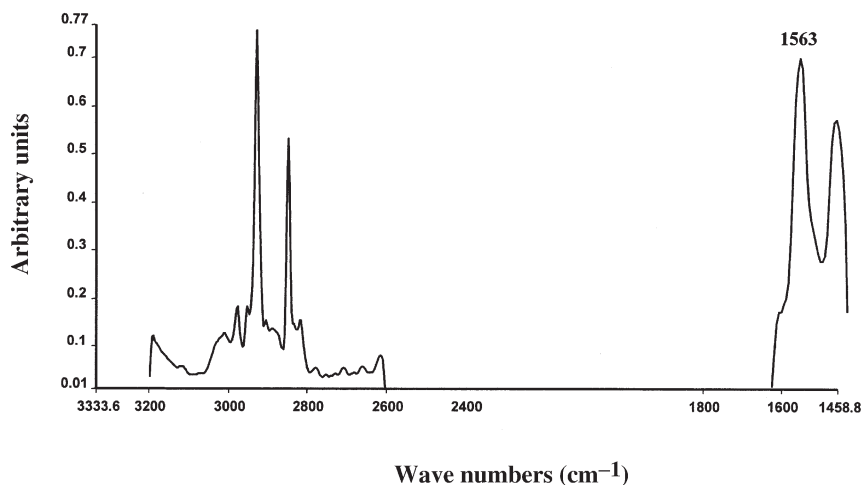

FIG. 4. The correlation spectrum showing spectral changes in the regions 1675–1500 cm^{-1} and 2990–2800 cm^{-1} from the soap contents of palm oil.

TABLE 2
Statistical Analysis for Soap Content in Palm and Groundnut Oils from Data Obtained by Chemical and FTIR Spectroscopic Methods[a]

Statistic	Palm oil		Groundnut oil	
	PORIM method	FTIR method	AOCS method	FTIR method
MD_r	1.86	0.43	1.25	0.27
SDD_r	0.60	−0.10	0.40	−0.08
MD_a	−0.84		−1.65	
SDD_a	1.13		1.03	

[a]MD, mean difference; SDD, standard deviation of difference; r, repeatability; a, accuracy; see Table 1 for other abbreviations.

very low but showed the FTIR method to have better repeatability.

There was good agreement between the FTIR predictions and chemically analyzed data for soap content in the NaOH-spiked oil samples. Table 2 shows the assessment of accuracy (as MD_a and SDD_a) for the FTIR method. The SD relative to the mean for the analytical 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 the PLS FTIR predictions using data from the AOCS and PORIM methods were 8.16 and 8.70% for groundnut and palm oils, respectively.

Thus, FTIR spectroscopy with PLS regression can be an important tool for analyzing soap content in palm and groundnut oils as it gives better repeatability than the present chemical method in use. It is a possible alternative for rapid, accurate, low-cost, and environmentally friendly routine determination of soap in chemically refined vegetable oils.

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