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Application of Fourier transform infrared spectroscopy to determine free fatty acid contents in palm olein

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Abstract

A simple and rapid method for the quantitative determination of free fatty acid (FFA) contents in palm olein by Fourier transform infrared (FTIR) transmission spectroscope is described. A set of palm olein samples is used as the calibration set. This set was prepared by spiking increasing amounts of oleic acid into a series of palm oleins that covers a wide range of FFA (0.08–1.04%). A partial least squares (PLS) calibration model for the prediction of FFA contents was developed, based on the spectral range 1728–1662 cm^{-1}. This model was tested by cross-validation steps to minimize standard error of the model. The coefficient of determination (R^2) and standard error were 0.997 and 0.017% of a FFA unit. Accuracy of the method was determined by comparing the FFA of a series of oleic acid-spiked palm oleins predicted by a PLS model to values obtained by the AOCS titration method. For accuracy, the difference between the mean FFA determined by the chemical method and the mean FFA determined by the FTIR method (MD_a) gave FFA contents of a value of 0.00016, with the FTIR method giving a higher prediction of palm olein than the AOCS method. For reproducibility, the mean differences between duplicates (MD_r) of the chemical and FTIR methods were close to zero (−0.0064 and −0.0046, respectively). The implementation of such a method as a quality control tool would eliminate the use and disposal of hazardous solvents required by the chemical method, and drastically reduce analysis time to less than 2 min/sample. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

The acid value (AV) is expressed by the KOH necessary (in mg) for titration of free fatty acids (FFA) contained in 1g oil (Pritchard, 1991). The AV determination by titration technique (AOCS, 1989) requires substantial personnel time, glassware and accurate preparation of reagents and is dependent on a visual end point. It uses toxic solvents, such as diethylether and methyl-isobutyl ketone, which are sensitive to carbon dioxide contamination from the atmosphere.

A variety of chemical and instrumental methods are available that attempt to measure FFA contents without titration. These include a pH-metric technique (Tur′yan, Berezin, Kuselman, & Shenhar, 1996), chromatographic techniques (Ackman, 1992; Ballesteros, Gallego, & Valeareel, 1993; Ballesteros, Cardenas, Gallego, & Valcarcel, 1994; AOAC, 1993), and a calorimetric technique (Kwon & Rhee, 1986). Some of the methods developed for FFA have been shown to be quite sensitive, quantitative, reproducible, rapid, precise and well-correlated to compounds associated with the development of FFAs in many oils and fats.

Infrared (IR) spectroscopy has been recognized as a powerful analytical technique in the food industry for many years (van de Voort, 1992) and it has been employed to measure some quality parameters of oils (van de Voort, Sedman, Emo, & Ismail, 1992; Dubois, van de Voort, Sedman, Ismail, & Ramaswamy, 1996). For determination of FFAs in fats and oils, many methods have been reported. A successful measurement of the FFA content in extracted crude oils by FTIR through deconvolution of the carbonyl bands at 1710 cm^{-1} (for FFA) and 1746 cm^{-1} (for triglyceride ester) has been carried out (Snyder et al. in Lanser, List, Holloway, & Mounts, 1991). Lanser et al. (1991) have estimated FFA from mixtures of oleic acid and soybean oil samples by deconvoluting absorption bands between 2000 and 1600 cm^{-1} . The peak height of the oleic acid carboxyl C=O absorption band at 1711 cm^{-1} to determine FFA in oils by using transmission and attenuated total reflectance (ATR) approaches has been successfully applied (Ismail, van de Voort, Emo, & Sedman,

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1993). Gee (1996) has applied FTIR spectroscopy to determine FFA contents in palm oil and palm kernel oil using the absorption region of 1715 to 1708 cm^{-1}. Recently, Che Man and Moh (1998) investigated application of near infrared (NIR) spectroscopy for determining FFA in palm oil. Their work gave a satisfactory result with correlation coefficient squared (R^2) being close to 1.

In this report, we describe and evaluate application of FTIR spectroscopy in the determination of FFA contents palm olein to be the most efficient technique without titration because of its simplicity, rapidity, and efficiency. Further, we investigate the possibility of the improved FTIR spectroscopy method that is capable of replacing the titration method.

2. Materials and methods

2.1. Instrumentation

The instrument used for this work was a Perkin–Elmer 1600 series FTIR spectrorneter (Perkin–Elmer Corporation, Norwalk, CT, USA), and a room temperature deuterated triglycine sulfate (DTGS) detector was used for the analysis. This instrument was connected to a Perkin–Elmer model 7300 professional computer equipped with its related Infrared Data System Software (IRDM). To minimize water vapour interference, the instrument was maintained with two automatic dehumidifiers.

2.2. Standards

The oleic acid purchased from Aldrich Chemical Company (Milwaukee, WI) was used for preparing calibration standards. Palm olein was purchased from a local retail outlet. Thirty samples for calibration standards were prepared by spiking oleic acid to the palm olein in known amounts for covering a range of FFA contents of 0.08 to 1.04% (Table 1). The FFA contents of the standards were analyzed in duplicate according to the American Oil Chemists' Society (AOCS, 5a-40) titration method (AOCS, 1989).

Table 1
Mean duplicate readings of FFA content (%) by AOCS reference method for the RBD[a] palm olein

Oleic acid (%)	FFA (%)
0.000	0.08
0.030	0.11
0.060	0.16
0.101	0.20
0.133	0.24
0.165	0.25
0.210	0.26
0.230	0.29
0.263	0.34
0.304	0.39
0.332	0.42
0.359	0.44
0.401	0.47
0.434	0.52
0.459	0.54
0.498	0.57
0.528	0.58
0.557	0.64
0.600	0.69
0.631	0.72
0.659	0.75
0.697	0.79
0.726	0.81
0.756	0.84
0.795	0.88
0.824	0.91
0.854	0.94
0.894	0.98
0.922	1.01
0.951	1.04

[a] RBD, refined-bleached-deodorized.

2.3. Collecting spectra

For this work, a few drops of each standard were sandwiched between sodium chloride (NaCl) windows that are the parts of the demountable cells. A spacer of polytetrafluoroethylene (PTFE) was placed between the windows to give a fixing pathlength of 200 μ. The demountable cells were mounted in a cell holder in the FTIR spectroscope and the scanning of each standard was carried out. Prior to scanning of each sample, the NaCl windows were taken apart and rinsed three times with acetone to remove the oil sample and wiped with soft tissue. All spectra were collected from 16 scans at a resolution of 8 cm^{-1}. These spectra were ratioed against a background air spectrum. The background single-beam spectrum of the air was recorded by 32 scans at 8 cm^{-1} resolution after the standards. All standards were scanned in duplicate in the region of 4000 to 400 cm^{-1}.

2.4. Calibration/validation

The calibration step was carried out by using the Nicolet Turbo Quant IR-Calibration and Prediction Package version 1.1 (Nicolet Instrument Co., Madison, WI) which includes a partial least square (PLS) calibration routine. The calibration model was developed by studying the spectral features for regions that correlated with FFA contents. The calibration model was tested by a cross-validation step to assess the predictive accuracy of the calibration model (van de Voort, Ismail, Sedman, Dubois, & Nicodemo, 1994). The cross-validation step was further refined by using the mean difference (MD) and standard deviation of the difference (SDD) for reproducibility and accuracy between the predicted and chemical FFA contents as a measure of improved performance of the calibration model.

3. Results and discussion

3.1. Spectral basis for analysis

Fig. 1 illustrates the spectrum of olelc acid-spiked palm olein samples in the region 4000 to 600 cm^{-1} because the NaCl windows stop transmitting at approximately 600 cm^{-1}. The spectrum showed characteristic absorption bands associated with common oil. The absorption band at around 3550 cm^{-1} was given by the OH stretching vibration mode of monoglycerides or diglycerides or water in samples (van de Voort et al., 1994). The band to be observed at 3473 cm^{-1} was the overtone of the triglyceride ester carbonyl absorption (1748 cm^{-1}), being approximately double its frequency (van de Voort et al., 1994). The frequency of band at 3006 cm^{-1} was assigned to the =CH cis stretching vibration (Guillen & Cabo, 1997). The absorption region between 2900 and 2800 cm^{-1} was assigned to CH_3 and CH_2 asymmetric stretching vibration, respectively (Stuart, 1996). There was a fermi resonance vibration mode of C=O of the carbonyl ester group in the 2677 cm^{-1} region. The C=C cis stretching gave an absorption band of 1648 cm^{-1}. Two bands at 1465 and 1377 cm^{-1} were due to the CH absorption bending vibration of CH_2 and CH_3, respectively. The band at 723 cm was assigned to a CH_2 rocking vibration (Nakanishi & Solomon, 1977). The C=O absorption of free fatty acid lies on the shoulder of the COOR band of triglyceride esters (Ismail et al., 1993). However, no apparent C=O absorption band was discernible in the spectra. The very low concentrations of FFA of palm oil samples means that FTIR spectroscopy can only detect the presence of C=O of FFA as flat lines, not as bands. A band rising at 1711 cm^{-1} was discernible with increasing concentration of FFA (Ismail et al., 1993).

3.2. Statistical analysis

The spectral regions used for the analysis can be optimized with the use of variance and correlation plots. Fig. 2 shows the variance spectrum for the calibration data in the region from 4000 to 600 cm^{-1}. The variance spectrum can be very useful for identification of active versus inactive spectral regions. The variance plot was generated by calculating the average absorbance at each wave number position over the entire calibration data set and then calculating the square root of the variance about that mean for the entire data set (Fuller, Ritter, & Draper, 1988). High variances were seen to occur at 3000–2800, 1800–1700 and 1500–1000 cm^{-1} which were the spectral regions of OH, C=O and CO stretching of

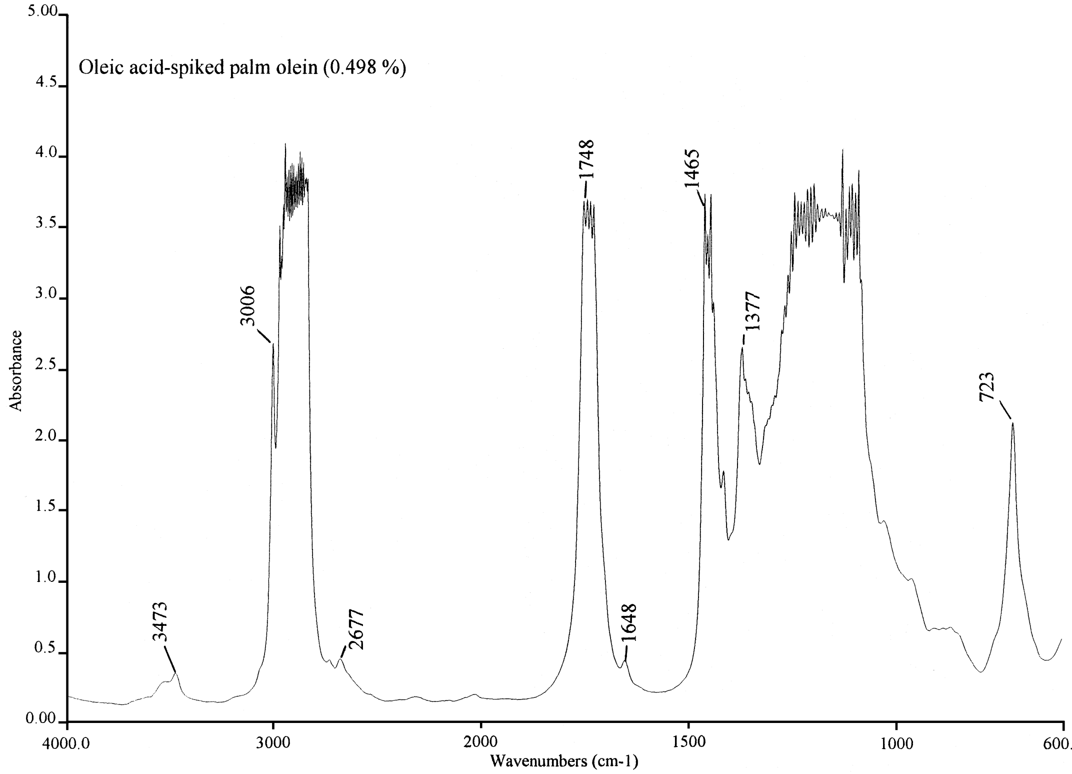

Fig. 1. Fourier transform infrared spectrum of the 4000–600 cm^{-1} region of oleic acid-spiked palm olein (oleic acid 0.498%).

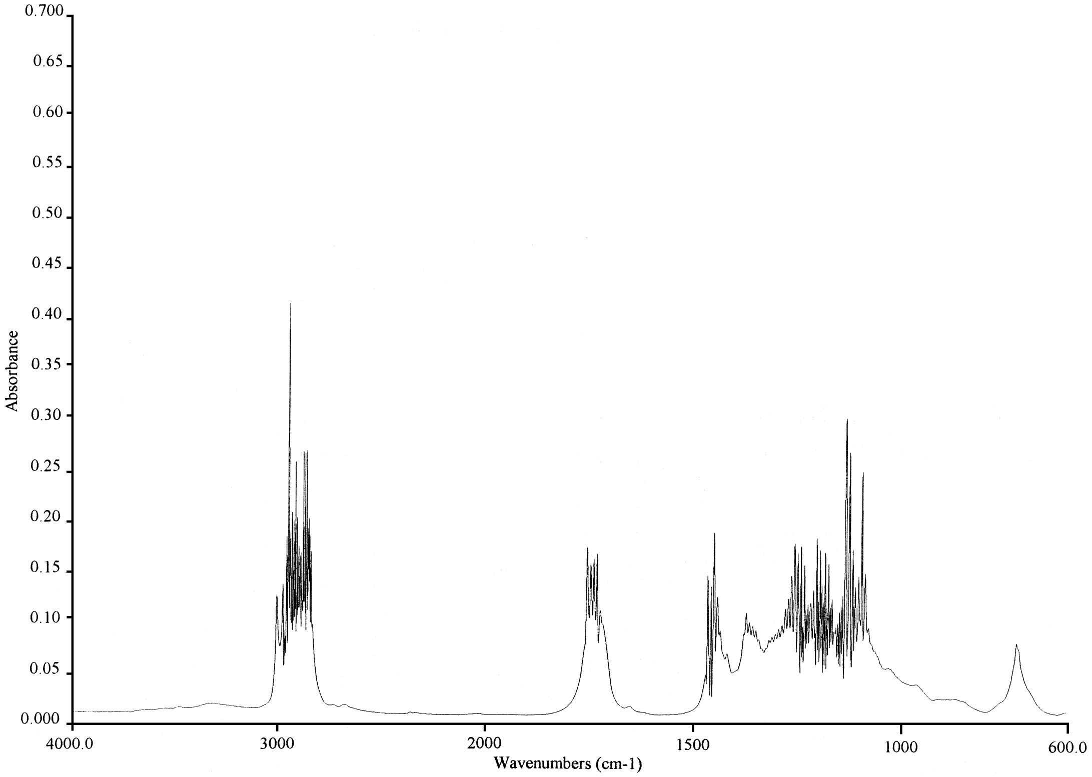

Fig. 2. The variance spectrum for the calibration samples.

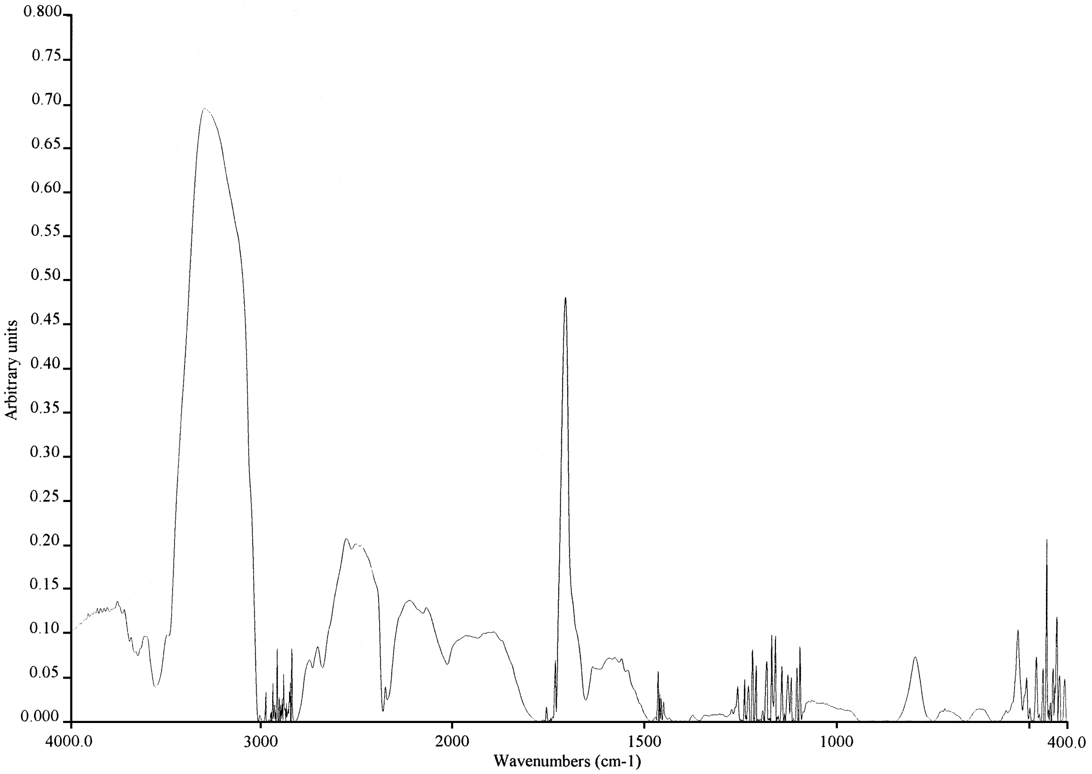

Fig. 3. The correlation spectrum for the calibration samples.

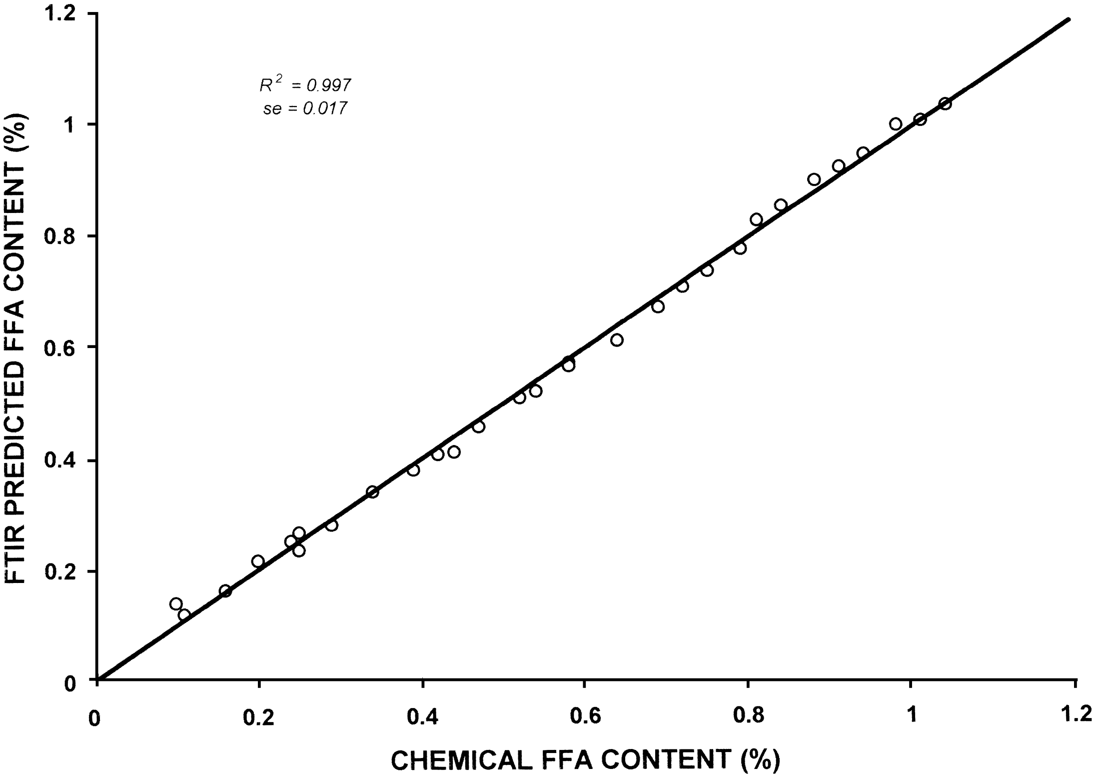

Fig. 4. Calibration plot of the predicted free fatty acids versus the chemical free fatty acid content in percentage as derived from the partial least squares calibration (PLS). R^2, correlation coefficient; SE, standard error; FTIR, Fourier transform infrared.

FFA, respectively (Stuart, 1996). Fig. 3 presents the correlation spectrum of the samples that are useful for selection of the best spectral region for the analysis. The spectral region used for the model should be restricted to those regions showing the highest correlation between FFA concentration information and spectral response. The spectral region used in the calibration was set to include all the data from 1728 to 1662 cm^{-1}, with 1586 cm^{-1} as a single point baseline, as suggested by the variance and correlation plots.

A plot of the means duplicate of predicted versus chemical FFA contents, obtained from the PLS calibration model is presented in Fig. 4 along with regression equation and associated statistics. Excellent predictions were obtained in this calibration, with a standard error (SE) of 0.017% FFA units and a standard deviation (SD) of 0.288 FFA units. The regression of the cross-validation results of the FFA PLS model against the calculated chemical FFA, produced the following equation:

$$FFA_p = 0.9964 \;\; FFA_c + 0.0037 \tag{1}$$

where FFA_p = PLS predicted FFA; FFA_c = chemical FFA, with coefficient of determination (R_2) of 0.995.

Comparing these data in terms of mean difference (MD_r) and standard deviation of difference (SDD_r) for reproducibility between duplicates of the chemical and instrumental results gives comparable mean differences (Table 2). As such, the overall reproducibility of the FTIR method is better than that of the chemical method. In terms of accuracy, the FTIR results were ≅ 0.0016 FFA units higher than the chemical values overall, with an SDD_a of 0.0003 FFA units. The overall standard error of prediction was 0.022 FFA. Based on an average of FFA of 0.5643 for these samples, an SE of 0.022 FFA represents a coefficient of variation (CV) of 3.90%, compared to an overall CV of 3.91% for duplicate chemical analyses of these samples.

Table 2
Statistical comparison of FFA content (%) of RBD palm olein obtained by AOCS reference and FTIR methods[a]

Statistic	Standard method	FT-IR method
MDr	−0.0046	−0.0027
SDDr	0.0032	0.0013
MDa	−0.0016	
SDDa	0.0003	

[a] FFA, free fatty acid; RBD, refined-bleached-deodorized; MD_r, the mean differences between duplicates of the chemical and FTIR methods; SDD_r, standard deviation of the MD_r; MD_a, the difference between the mean FFA determined by the chemical method and the mean FFA determined by the FTIR method (MD_a); SDD_a, standard deviation of the MD_a; FTIR, Fourier transform-infrared.

In this work, we have succeeded in our objective of describing calibration that allows one to directly predict the FFA of palm olein from its FTIR spectrum. The method described here provides a practical procedure that can be implemented in a straightforward manner for quality control applications in the fats and oils industry.

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