

Determination of Hexane Residues in Vegetable Oils with FTIR Spectroscopy

M.E.S. Mirghani and Y.B. Che Man*

Department of Food Technology, Faculty of Food Science and Biotechnology,
Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor DE, Malaysia

ABSTRACT: An FTIR spectroscopy method was developed for the determination of hexane residues in palm and groundnut (peanut) oils. The method was based on horizontal attenuated total reflectance with a ZnSe crystal at 45° at room temperature, and partial least squares (PLS) statistics were used to derive calibration models. The accuracy of the method was comparable to that of the AOCS Method Ca 3b-87, with coefficients of determination (R^2) of 0.9866 and 0.9810 for palm and groundnut oils, respectively, and SE of calibration of 3.83 and 4.91, respectively. The calibration models were validated, and the R^2 of validation and the SE of prediction computed. The SD of the difference for repeatability for the method was comparable to that for the standard AOCS method when used for palm and groundnut oils. With its speed and ease of data manipulation by computer software, FTIR spectroscopy has an advantage over present chemical methods, which require preparation of the oil using toxic solvents before GC.

Paper no. J10225 in *JAACS* 80, 619–623 (July 2003).

KEY WORDS: ATR, FTIR spectroscopy, hexane residue, PLS.

Hexane has been used for oilseed extraction since the 1930s (1). Commercial hexane is a mixture of several isomers of six-carbon paraffins, mainly *n*-hexane, and is considered to be toxic (2). In addition to its innate toxicity, hexane is volatile and can be photo-oxidized in the atmosphere to form ozone (3). The toxicity of hexane and its products has prompted the search for alternative solvents, but no totally benign solvent has yet been identified. Solvents other than hydrocarbons have been investigated with varying degrees of success (4), but the desirable solvent characteristics of hydrocarbons and their ready availability still make hexanes the preferred solvents in the oilseed industry (5). The U.S. Environmental Protection Agency (EPA) has published some health data from the American Petroleum Institute on commercial hexane, but the knowledge remains incomplete (6). There is considerable risk in using hexane, and the chemical is best avoided if possible (7).

In oilseed extraction, more than 99.9% of the hexane used is recycled. Of the unrecovered material, about 30% is lost in the oil and meal (8). Despite the low hexane loss in the products, the toxicity of the chemical has required its content to be determined. Several methods exist for doing so, for exam-

ple, IUPAC Method 2.607 (9), which measures hexane as a free volatile hydrocarbon by using GC. AOCS Method Ca 3b-87 is derived from the IUPAC method and determines hexane as a volatile hydrocarbon residue in fats and oils after solvent processing (10). However, both methods are tedious and involve reactions in the preparation of chemical reagents.

Increased environmental concern over the use of large volumes of solvents and reagents in quality control laboratories has prompted interest in methods that use automated instrumentation and dispense with the need for additional chemicals and ones that are faster, more efficient, and accurate (11).

FTIR spectroscopy is a fast, efficient, and accurate analytical technique for fats and oils (12–14). As a spectroscopic process, it dispenses with the need for chemicals altogether. The quantitative analysis of a mixture is made relatively simple through the identification of isolated absorption peaks for each component (15). The attenuated total reflectance (ATR) technique, which is used for examining liquids, has eliminated the problem of specifying the sample thickness, which affects the intensity of the spectral bands. ATR is easy to use in analyzing liquid samples with the accessories specially designed for this purpose. ATR FTIR has been used to record consistent spectral intensities in neat fats and oils due to the very small penetration of the evanescent waves into the oil film using an internal reflection element (16).

The objective of this research was to develop a rapid FTIR method for identifying and quantifying hexane residues in fats and oils as an alternative to the present chemical methods.

MATERIALS AND METHODS

Samples and chemicals. All chemicals were of analytical grade. *n*-Hexane was purchased from Merck KGaA Co. (Darmstadt, Germany), and freshly refined, bleached, and deodorized palm olein was obtained from a local refinery. Groundnuts (peanuts) were purchased from a local retailer, ground, and subjected to oil expression in the laboratory. Solvent-free oil samples were spiked with known amounts of *n*-hexane to 0–1,500 mg/kg (ppm).

Hexane analysis by GC. AOCS official method Ca 3b-87 for determination of free volatile hexane residues in fats and oils was adopted. A gas chromatograph (Model 5890; Hewlett-Packard, Palo Alto, CA), equipped with a FID, Hewlett-Packard model 3392 A integrator, and a BPX70 polar capillary column (SGE, Victoria, Australia; 0.32 mm i.d.,

*To whom correspondence should be addressed.
E-mail: yaakub@fsb.upm.edu.my

30 m length; 0.25 μm film thickness) was used. Helium (99.99%) at approximately 23 mL/min was used as the carrier gas. The hexane peak was identified by comparison with the retention time of the authentic standard. Peak areas were computed, and the amounts of hexane in mg/kg (ppm) were obtained. All analyses were carried out in duplicate.

Before quantification could be done using the FTIR spectroscopy, the IR band intensity was first calibrated with hexane. The hexane content of the sample could then be read off the calibration curve of the IR intensity, provided that (i) the spectrum from the sample was recorded under the same conditions as for the calibration, and (ii) the standards used were representative of the sample and covered the same range.

Spectrum acquisition. The mid-band IR ATR spectrum was obtained with a FT spectrometer (Series 1725; PerkinElmer Ltd., Beaconsfield, Buckinghamshire, United Kingdom) equipped with a deuterated triglycine sulfate detector and a ZnSe horizontal trough top-plate for Overhead ATR, with a 45° accessory of 66 mm length, 9 mm width, and 4 mm thickness (Specac Ltd. Orpington, Kent, United Kingdom). Two automatic dehumidifiers were used to protect against interference by water vapor. The sample was placed in contact with the ATR element (ZnSe crystal with 45° ends) at room temperature. Spectra were collected by co-adding 81 scans from 4000 to 600 cm^{-1} wavenumbers at 4 cm^{-1} resolution. A strong apodization was used. After each measurement, the crystal was cleaned three times with acetone and dried. The cleaned crystal was checked spectrally to ensure that no residue remained from the previous sample. Duplicate readings were taken for each of the 35 samples and stored in JCAMP DX (Joint Committee on Atomic and Molecular Physical Data—Data Exchange) files (17) on diskettes for subsequent analysis.

Mathematical and statistical analysis. The Nicolet Turbo Quant IR calibration and prediction software package, Version 1.1 (Nicolet Instrument Co., Madison, WI), based on par-

tial least square (PLS) regression, was used to obtain the calibration. Validation by the model as described by Fuller *et al.* (18) was then carried out to assess the predictive capability of the model. The calibration was further improved (14,19) by using the mean difference (MD) and the SD of the difference (SDD) between the predicted and actual values, or values determined by other instruments, such as a gas chromatograph (AOCS method) (10).

RESULTS AND DISCUSSION

Two sets of 35 samples of palm oil and groundnut oil were spiked by *n*-hexane and used for the FTIR prediction and calibration. After outliers were eliminated (four samples), samples with various levels of hexane contamination were analyzed by GC with AOCS method Ca 13b-87 (10), and the data were used to estimate the predictability of calibration.

Ratio comparison of the spectrum from a sample spiked with *n*-hexane with that of a solvent-free sample, and/or the spectrum from the pure solvent reveals the spectral features of *n*-hexane. Figure 1 shows the IR spectrum of *n*-hexane, which matches that shown by Nzai and Proctor (15). The hexane spectrum had the major peaks of alkanes: a C–H stretch at 3000–2875 cm^{-1} , a CH_2 bending absorption at 1465–1463 cm^{-1} , a CH_3 bending absorption at 1379–1361 cm^{-1} , and a CH_2 bending (rocking) motion at 725–722 cm^{-1} (20). Figure 2 shows the spectra of (A) spiked palm oil and (B) pure palm oil; hence, the difference spectrum (C), has all the spectral features of hexane (see Fig. 1).

Development of calibration models. The calibration standards were designed to obtain data for the PLS regression that were as free as possible from interference by other components. Thus, only data from regions that showed good correlation with the features of interest were abstracted by the PLS software to obtain a calibration standard(s) spectrally repre-

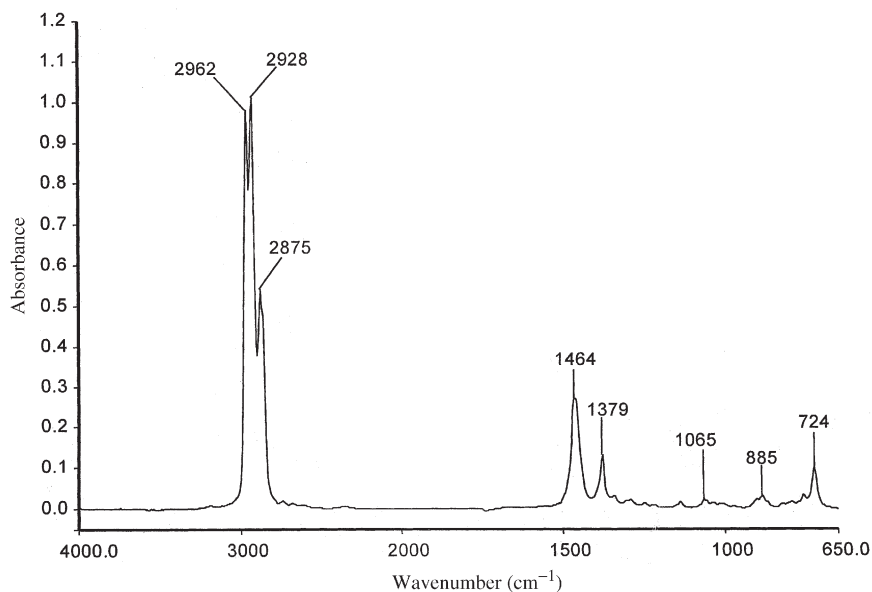


FIG. 1. IR spectrum of *n*-hexane.

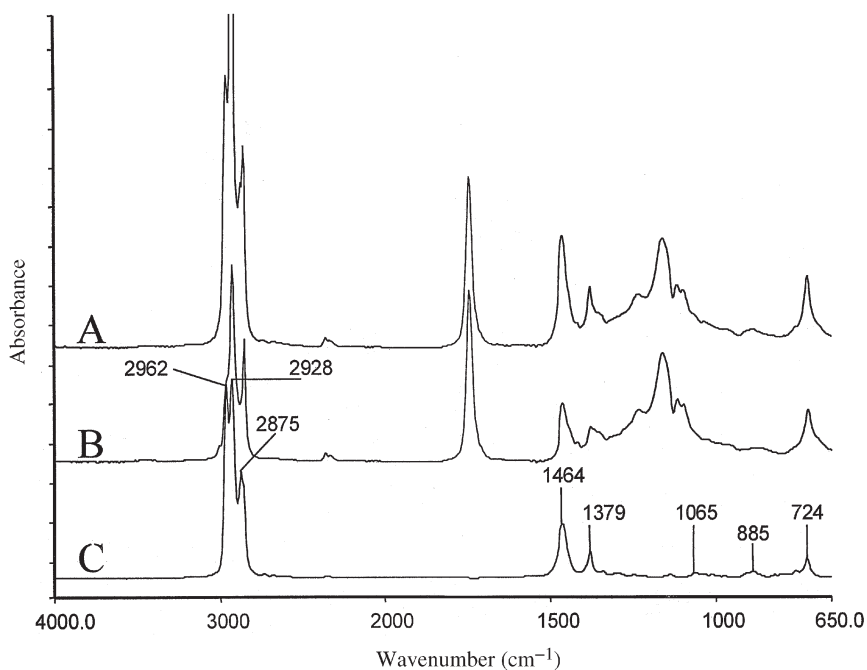


FIG. 2. Spectra of (A) hexane-spiked palm oil, (B) pure palm oil, and (C) the difference between A and B.

sentative of the samples to be analyzed (21). Table 1 shows the results (from duplicate readings) obtained from the derived calibrations and SD analysis of the data. The mean was calculated from the predicted results as the average of two replicates of hexane content for each set of oil samples using a Microsoft Excel spreadsheet.

The variance and correlation spectra were used to optimize the spectral data (Fig. 3). The correlation spectrum was used to select the best spectral regions for analysis, and the variance spectrum displayed the regions where absorbance was changed over the calibration. For the calibration in Figure 3, the spectral

regions with the highest correlation between concentration and spectral response (21) were set to include all the data from 2995 to 2841, 1449 to 1335, and 1246 to 1075 cm^{-1} for *n*-hexane. Table 2 shows the results obtained from the PLS calibration in terms of R^2 and standard error of calibration (SEC) used in choosing the best region(s) for determining hexane in palm and groundnut oils.

A correlation plot established (Fig. 4) by using the actual test readings and calibration data gave the highest coefficient of determination (R^2) of 0.9866 and lowest SEC (3.83) for hexane in palm oil. The equation was ($y = 1.0365x + 8.9313$),

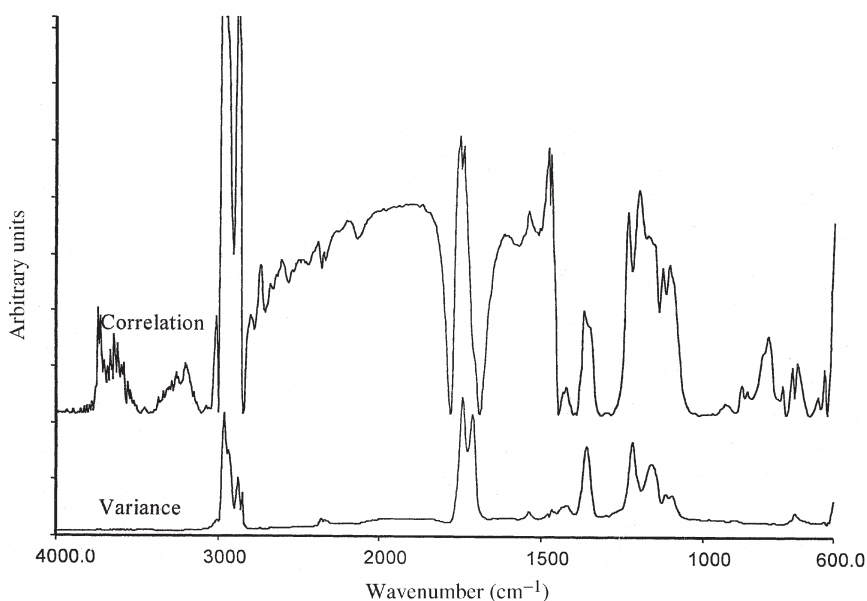


FIG. 3. Correlation and variance spectra.

TABLE 1
Calibration and Cross-validation for Hexane Content (mg/kg) in Palm and Groundnut Oils by the FTIR Method^a

Data set	Palm oil		Groundnut oil	
	Mean	SD	Mean	SD
Calibration	289.65	19.43	295.44	20.97
Validation	284.66	20.17	289.79	22.05

^aAll data represent the means of two replicates.

with the intercept and slope not significantly different ($P > 0.05$) from 0.0 and 1.0, respectively. For groundnut oil, the R^2 and SEC were 0.9810 and 4.91, respectively (Fig. 5). The equation was ($y = 1.0625x + 10.537$), and the intercept and slope were not significantly different ($P > 0.05$) from 0.0 and 1.0, respectively. The calibration was evaluated by a set of known spiked and GC-analyzed samples, which also were used for the validation in Table 3. The R^2 and SE of predictions were 0.9712 and 0.9784, and 3.96 and 4.60 for the palm and groundnut oils, respectively.

GC peak areas for hexane were computed by relating them to the actual amount of hexane spiked in the standard samples. GC data are presented in Table 4 in the form of mean

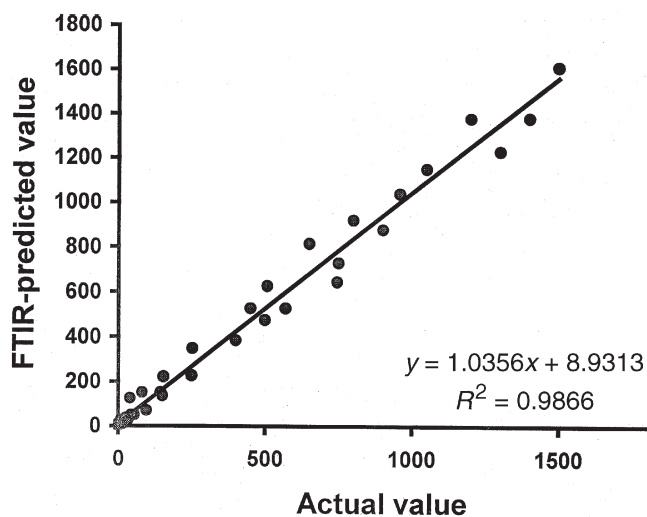


FIG. 4. Correlation plot of actual data vs. FTIR-predicted data for calibration (palm oil).

TABLE 2
Effects of Different Wavelength Regions in the Calibration Model for Determining Hexane Residues in Palm and Groundnut Oils^a

Region	Wavelength (cm ⁻¹)	Palm oil		Groundnut oil	
		R^2	SEC (%)	R^2	SEC (%)
1	2995–2841	0.9529	11.34	0.9337	13.22
2	1449–1335	0.8152	9.72	0.7744	8.58
3	1246–1075	0.7554	6.90	0.7127	7.68
1 + 2	2995–2841 + 1449–1335	0.8311	7.76	0.8105	8.43
1 + 3	2995–2841 + 1246–1075	0.8720	6.33	0.8692	7.16
2 + 3	1449–1335 + 1246–1075	0.8039	7.04	0.8097	8.13
1 + 2 + 3	2995–2841 + 1449–1335 + 1246–1075	0.9866	3.83	0.9810	4.91

^a R^2 , coefficient of determination; SEC, standard error of calibration.

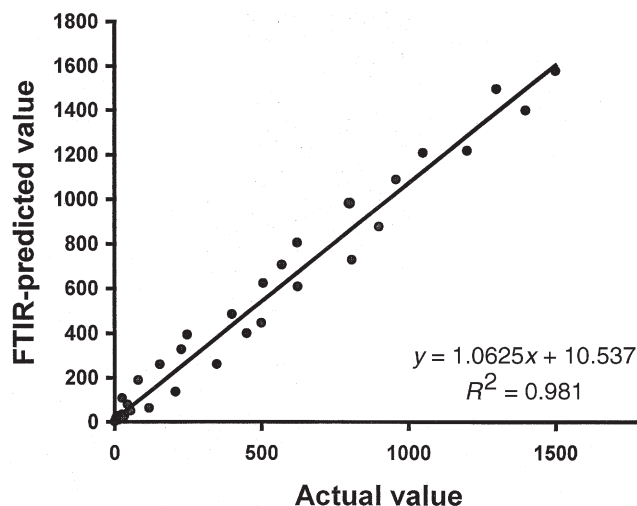


FIG. 5. Calibration plot of GC vs. FTIR data for validation (groundnut oil).

difference (MDr), SD of the difference (SDDr) for repeatability, and minimum and maximum values. The accuracy was evaluated by subtracting MD chemical from MD FTIR, to be expressed as MDa, and SDD chemical from SDD FTIR, which is expressed as SDDa.

The SD relative to the mean value is expressed as the CV. A CV of <20% arguably suffices for most analytical purposes (22). The CV obtained using the PLS statistical method to predict FTIR results from the actual and AOCS method data were 6.71–14.50 and 7.09–16.24% for the palm and groundnut oil samples, respectively.

From this study, we conclude that an FTIR spectrometer equipped with an ATR element can be used to derive equations for determining the hexane content in palm and groundnut oils. In determining hexane content in oils by using ATR FTIR, the absorbance of the $-\text{CH}_3$, $-\text{CH}_2$, and $-\text{CH}$ absorption bands is measured at 2995–2841, 1449–1335, and 1246–1075 cm^{-1} . The data for each sample can be obtained in approximately 2 min. The speed and convenience of the method make it suitable and practical for on-line quality control laboratories. A further advantage of the method is that it is environmentally friendly, as only acetone was used for cleaning the ATR cell.

TABLE 3
Results from Calibration Models Using PLS of Wavelength Regions 2995 to 2841, 1449 to 1335, and 1246 to 1075 cm⁻¹ to Determine the Hexane Contents in Palm and Groundnut Oils^a

Oil	Calibration		Validation	
	R ²	SEC (%)	R ²	SEP (%)
Palm oil	0.9866	03.83	0.9712	03.96
Groundnut oil	0.9810	04.91	0.9784	04.60

^aPLS, partial least squares; SEP, standard error of prediction. For other abbreviations, see Table 2.

TABLE 4
Calibration Statistics for Hexane Content from Data Obtained by GC Analysis and FTIR Spectroscopy^a

Statistic	Palm oil		Groundnut oil	
	AOCS method	FTIR method	AOCS method	FTIR method
MDr	13.94	16.43	14.88	18.02
SDDr	04.28	-3.37	05.01	-4.29
Min. value	0.00	2.95	0.00	3.27
Max. value	1566.40	1580.04	1572.26	1590.65
MDa		-8.07		-9.38
SDDa		-2.57		-3.17

^aMD, mean difference; SDD, SD of difference; r, repeatability; a, accuracy.

ACKNOWLEDGMENT

We would like to thank Universiti Putra Malaysia (UPM) for funding this study with IRPA Grant 09-02-04-0457-EA001.

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[Received January 22, 2002; accepted February 26, 2003]