

# Determination of Iodine Value of Palm Oil by Fourier Transform Infrared Spectroscopy

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**ABSTRACT:** A rapid method for the quantitative determination of iodine value (IV) of palm oil products by FTIR transmission spectroscopy is described. A calibration standard was developed by blending palm stearin and superolein in specific ratios that covered a range of 27.9 to 65.3 IV units. The spectra of these standards was measured in the range between 3050 and 2984  $\text{cm}^{-1}$ , corresponding to the absorption band of  $=\text{C-H}$  *cis* stretching vibration. A partial least squares calibration model for the prediction of IV was developed to quantify the IV of palm oil products. A validation approach was used to optimize the calibration with a correlation coefficient of  $R^2 = 0.9995$  and a standard error of prediction of 0.151. This study concludes that the FTIR transmission approach can be used to determine the IV of palm oil products with a total analysis time per sample of less than 2 min for liquid samples.

Paper no. J8862 in *JAOCS* 76, 693–699 (June 1999).

**KEY WORDS:** FTIR spectroscopy, iodine value, palm oil, PLS.

Iodine value (IV) is a measure of the unsaturation of oils and fats. It is expressed as the number of grams of iodine absorbed by 100 grams of oil or fat under the test conditions (1). In the palm oil industry, the IV is an important parameter in quality control. It is often used to guide fractionation processes in order to achieve the desired product quality at optimum operating conditions.

Usually, the IV is determined by titration methods such as the Wijs method (2), which is used extensively, Hanus and Hubl (3), Hofmann and Green (4), Rosenmund-Kuhnhenh (1), or modifications of the Wijs method (5–7). All the above titration methods involve the use of highly toxic, carcinogenic, and environmentally unfriendly chemicals. These methods are also time consuming, costly, and largely dependent on the skills of the analyst. Instrumental methods may be a good substitute for IV determination using any of the titration methods. Many instrumental methods to determine IV have been reported, such as the use of gas liquid chromatography (8), Fourier transform infrared (FTIR) (9), differential scanning calorimetry, (10) and high-performance liquid chromatography (11). Refractive index has also been used to determine the IV of palm oil (12).

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Mid infrared (MIR) spectroscopy has also been used to determine the IV of fats and oils. Some authors have reported using MIR spectroscopy to estimate IV of fats and oils based on the number of double bonds. MIR spectroscopy can be used to identify organic compounds because some groups of atoms display characteristic vibrational absorption frequencies in this infrared region of the electromagnetic spectrum. Sinclair *et al.* in Guillen and Cabo (13) used a dispersive infrared method to show a linear relationship between the number of *cis* double bonds for a series of unsaturated fatty acid methyl ester (FAME) and the ratio between the absorbance at 2920  $\text{cm}^{-1}$ , assignable to the stretching vibration of the  $-\text{CH}_2-$  group, and the difference between the absorbance of the band at 2920  $\text{cm}^{-1}$  and that at 3020  $\text{cm}^{-1}$  of the *cis*-olefinic group  $=\text{CH}-$ . Arnold and Hartung (14) used the ratio of absorbances of the olefinic (3030  $\text{cm}^{-1}$ ) and aliphatic (2857  $\text{cm}^{-1}$ ) C-H stretching vibration bands in their infrared spectra to determine the degree of unsaturation (IV). Bernard and Sims in Guillen and Cabo (13) used the weak C=C stretching band at 1658  $\text{cm}^{-1}$  to calculate the total unsaturation. Muniategui *et al.* in Guillen and Cabo (13) used the intensity of the olefinic band at 3007  $\text{cm}^{-1}$  to estimate IV. Peak height ratios of the C-H stretching vibration bands at 3010 and 2854  $\text{cm}^{-1}$  were used to determine IV with attenuated total reflectance-FTIR spectroscopy (15). Van de Voort *et al.* (16) used pure triglycerides as calibration standards and spectral regions from 3200–2600  $\text{cm}^{-1}$  and 1600 to 1000  $\text{cm}^{-1}$ . The correlation between these regions and experimental IV were determined by the partial least square (PLS) methodology. Gee (9) reported that the good spectral regions to determine IV were 3100–2990  $\text{cm}^{-1}$  corresponding to *cis*  $-\text{CH}=\text{CH}-$  stretching and 1500–1300  $\text{cm}^{-1}$  corresponding to  $\text{CH}_2$  scissoring and  $\text{CH}_3$  symmetrical deformation. Gee (17) has also used the frequency regions for IV determination of palm oil product between 3020–2995  $\text{cm}^{-1}$  and 975–950  $\text{cm}^{-1}$  using FTIR.

Fourier transform infrared (FTIR) spectroscopy is amenable to quantitative IV of fats and oils because the double bonds contained in unsaturated fatty acids have absorption bands in useful spectral regions. The greater the absorption, the greater the IV. There is evidence that FT-IR spectroscopy may be a promising technique for the rapid, simple, and cost-effective for determination of the analysis of the IV of palm oil. In this study, the use of FTIR spectroscopy for IV determination was reexamined and improved.

## MATERIALS AND METHODS

**Calibration standards.** Malaysian refined, bleached, deodorized (RBD) palm stearin was purchased from a local refinery. RBD-superolein produced from the fractionation of palm olein with a high IV (IV 60 min) (18) was obtained from the Palm Oil Research Institute Malaysia (PORIM). All chemicals were of analytical grade. The IV of each oil was analyzed in duplicate by AOCS method (19). A set of 20 calibration standards were prepared by blending two oils to obtain oils with a wide range of iodine value (IV). The IV of the standards was calculated from the IV of the original oils multiplied by the percentage of the weight of their contributions (Table 1).

**Instrumentation/sample handling.** The instrument used for this work was a Perkin-Elmer model Paragon 1000 FT-IR spectrometer (Perkin-Elmer Instrument Corporation, USA) with a deuterated triglycine sulfate (DTGS) detector interfaced with a DEC 5150 Venturis FX PC, which operated under Windows-based Perkin-Elmer Spectrum Lite version 1.5 software. The instrument was also equipped with a heated sample handling accessory which consisted of inlet and outlet lines and a 100  $\mu\text{m}$  BaF<sub>2</sub> transmission flow cell. The inlet line was used to direct the oil flow and an outlet line that emptied into a collection vessel, which in turn was connected to a vacuum line, was used to aspirate the sample through the flow cell. All components of the accessory were set to 80°C so that the palm stearin fraction would be liquid and would flow without crystallization in the lines or cell. The instrument and sample compartment were purged with purified N<sub>2</sub> gas to minimize water vapor and carbon dioxide interference. Prior to starting any analysis, *n*-hexane was passed through the system to clean the cell and transfer lines. All samples were preheated to 80°C prior to loading to minimize temperature perturbations in the cell. The cell was rinsed with *n*-hexane before every sample to avoid oil build-up on the cell windows.

**Calibration/validation.** A background spectrum was recorded with an air emittance spectrum before every standard emittance spectrum. The standard emittance spectrum was ratioed against an air emittance spectrum to produce absorbance spectra which were displayed on the computer monitor and the spectra were stored to diskette as a JCAMP file for subsequent chemometric analysis. For all standards, emittance spectra were collected from eight scans at a resolution 4 cm<sup>-1</sup>, with strong apodization and a gain of 1. The calibration was developed using the Nicolet Turbo Quant-IR calibration and prediction software package (Nicolet Instrument Co., Madison, WI). A PLS method was chosen to develop a calibration model. Correlation spectra, which relate spectral changes to the value of the variable of interest, were generated and examined to identify spectral features that correlated with the IV data for palm oil samples. Calibration was derived and optimized to generate a predictive model for IV. The model was validated by the validation procedure and the optimal number of factors was selected on the basis of the predicted residual error sum of squares (PRESS) test. The evalu-

ation of the calibration of performance was estimated by computing the standard error of calibration (SEC). The standard error of prediction (SEP) was used as a validity criterion to check the calibration (20). The efficacy of the calibration to predict IV was assessed by comparing the FTIR predicted IV for samples of mixtures of palm stearin-superolein oils with those obtained by the AOCS method (19).

## RESULTS AND DISCUSSION

**General theory.** The absorption of infrared radiation can cause a molecule to vibrate. However, only those vibrations that are accompanied by a change in the electric dipole moment cause absorption of infrared light. Vibrations which do not change the dipole moment, do not result in the absorption of infrared radiation. The intensity of absorption depends on the square of the change in dipole moment, thus there can be large differences in absorption intensity. The vibration of polyatomic molecules is much more complicated. Fortunately, different parts of a molecule can vibrate independently (21). The concept of group frequencies is basic to the understanding of infrared spectra and their use in identification.

**Spectral analysis.** Figure 1 presents a typical spectrum of a mixture of palm stearin and superolein ranging from 4000–400 cm<sup>-1</sup>, using a 100  $\mu\text{m}$  BaF<sub>2</sub> transmission cell. The BaF<sub>2</sub> transmission technique allows the full spectrum to be examined to 800 cm<sup>-1</sup>. This spectrum illustrates the dominant spectral features associated with the oil absorption regions: the CH stretching absorption in region from 3050–2800 cm<sup>-1</sup> (*cis* C=CH, CH<sub>2</sub>, CH<sub>3</sub> and CH<sub>2</sub>/CH<sub>3</sub> stretching bands), the -C=O stretching absorption of the triglyceride ester linkage around 1800–1700 cm<sup>-1</sup>, and the fingerprint region (1500–1000 cm<sup>-1</sup>) (22). Regions with absorbance values greater than 2.0 absorbance units have a signal reaching the detector that is too small to be sampled properly, which leads to digitization noise in the spectrum (22). By replacing the cell with one with a shorter path length, one can reduce band intensity. However, that is done at the expense of sensitivity in other regions with low intensities, i.e., the absorption band around 1650 cm<sup>-1</sup> (C=C *cis* stretching). The absorption bands for IV determination due to -CH stretch in *cis* HC=CH, *cis* C=C stretch, and CH stretch in *trans* HC=CH regions at around 3006, 1650, and 968 cm<sup>-1</sup>, respectively (13).

**Calibration assessment.** Table 1 is a listing of the reference standards into calibration and validation groupings based on the IV sorting of the PLS algorithm. The lowest and highest IV are included in the calibration data set rather than in the validation data set. Each calibration standard that will be used in the modeling step must be identified both in terms of the spectral information and the IV information. The spectral regions used for the analysis can be optimized using the variances and correlation plots.

Figure 2 shows the variance spectrum for these standards. The variance spectrum is an attempt to display the regions of the spectra where there are changes in the absorbance values over the reference set. It can provide a guide in the selection

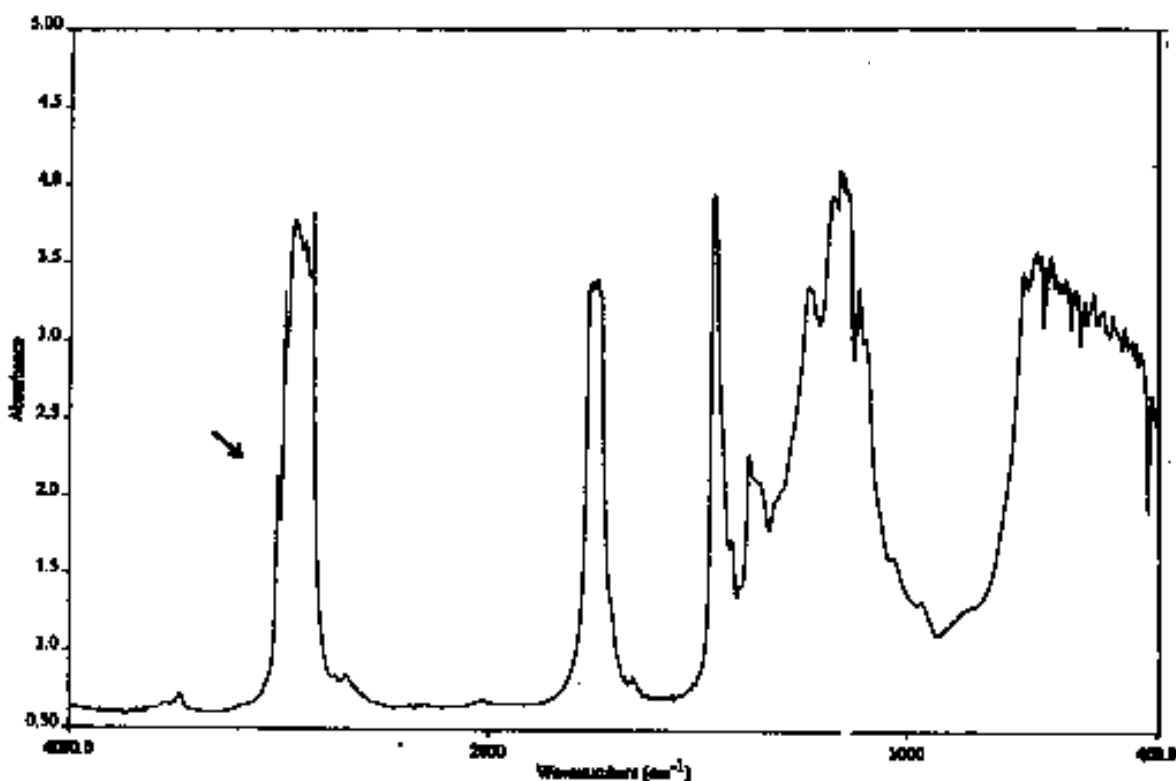


FIG. 1. A typical Fourier transform infrared (FT-IR) spectrum of mixture palm stearin and superolein. An arrow indicates the spectral region employed for calibration.

of the spectral regions where there may be significant information relating to the components of interest (23). The largest variance can be seen in the 1500–1000  $\text{cm}^{-1}$  region which is the absorption region of C-H stretching. The variance spectrum is dominated by features unrelated to the double bond.

The correlation spectrum in Figure 3 can be used to select the best spectral regions for the analysis (23). The correlation spectrum shows the correlation coefficient relating the reference spectra to the reference concentrations. For spectral regions where there are absorptions caused by the component of interest, there is a positive deflection of the correlation coefficient. Used together, both variance and correlation spectra provide clues to the spectral regions that provide the best calibration.

According to the correlation spectrum, the highest correlation is given by CH stretch in *cis* HC=CH at 3006  $\text{cm}^{-1}$  region and followed by *cis* C=C stretch region at 1650  $\text{cm}^{-1}$ . Further investigation to the variance spectrum obtained the high variance at around 3006  $\text{cm}^{-1}$  (the *cis* HC=CH stretch region). No variance is found at around 1650  $\text{cm}^{-1}$  for *cis* C=C stretch. Based on the correlation and variance spectra diagnostic, we conclude that the *cis* HC=CH stretching regions can provide the best region for constructing calibration model to predict the IV of palm oil blends. This region exhibits the characteristic absorption band with the medium peak as a function of the polarity of the extent of hydrogen bonding (21).

*PLS calibration and cross validation.* PLS is the chemo-

metric method of choice for developing a calibration model. The power of PLS is based on its ability to use spectral information from broad spectral regions and to correlate spectral changes with changes in the concentration of a component of

TABLE 1  
Actual Iodine Value for the RBD Palm Stearin, Superolein, and Their Mixtures<sup>a</sup>

Palm stearin and superolein ratios (w/w)	Iodine value	Usage
0:100	65.3	Calibration
5: 95	63.4	Calibration
10: 90	61.6	Validation
15: 85	59.7	Calibration
20: 80	57.8	Validation
25: 75	56.0	Calibration
30: 70	54.1	Validation
35: 65	52.2	Calibration
40: 60	50.3	Calibration
45: 55	48.4	Calibration
50: 50	46.4	Calibration
55: 45	44.7	Calibration
60: 40	42.9	Validation
65: 35	41.1	Calibration
70: 30	39.1	Calibration
75: 25	37.3	Validation
80: 20	35.4	Calibration
85: 15	33.5	Calibration
90: 10	31.7	Calibration
100: 0	27.9	Calibration

<sup>a</sup>RBD, refined-bleached-deodorized.

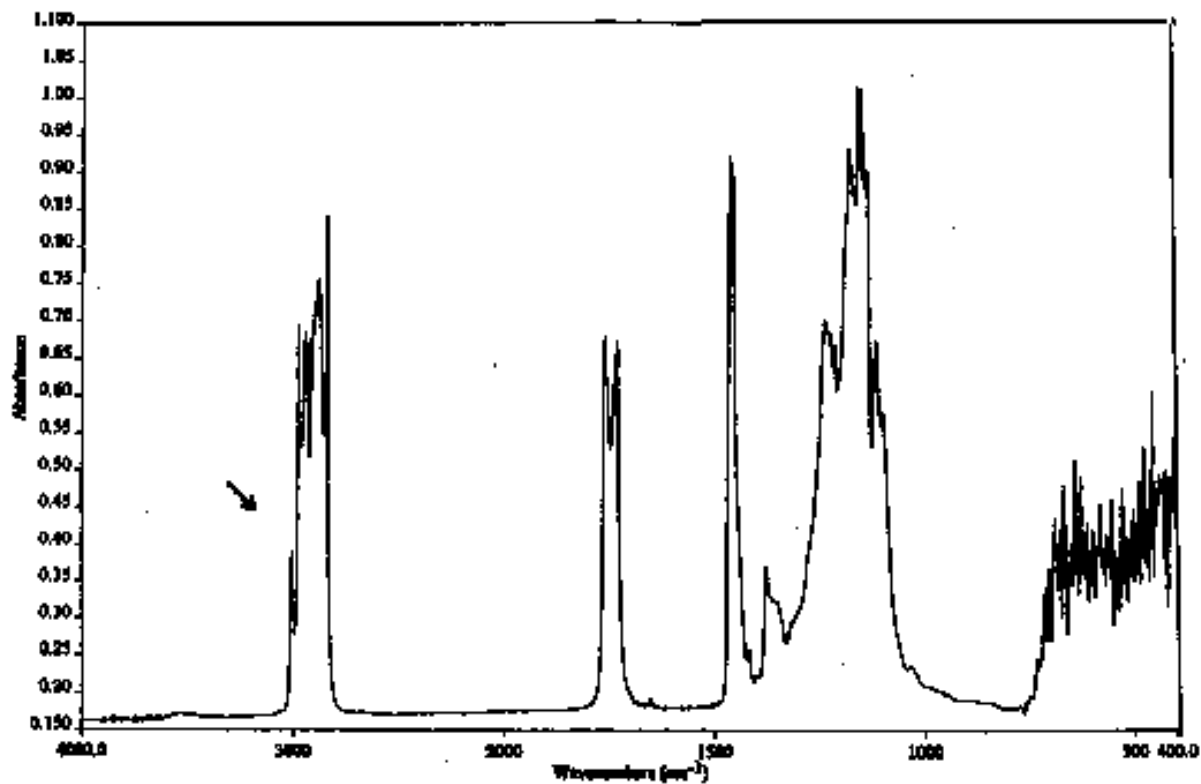


FIG. 2. A FT-IR variance spectrum obtained from the calibration standards. An arrow indicates the spectral region employed for calibration. For abbreviation see Figure 1.

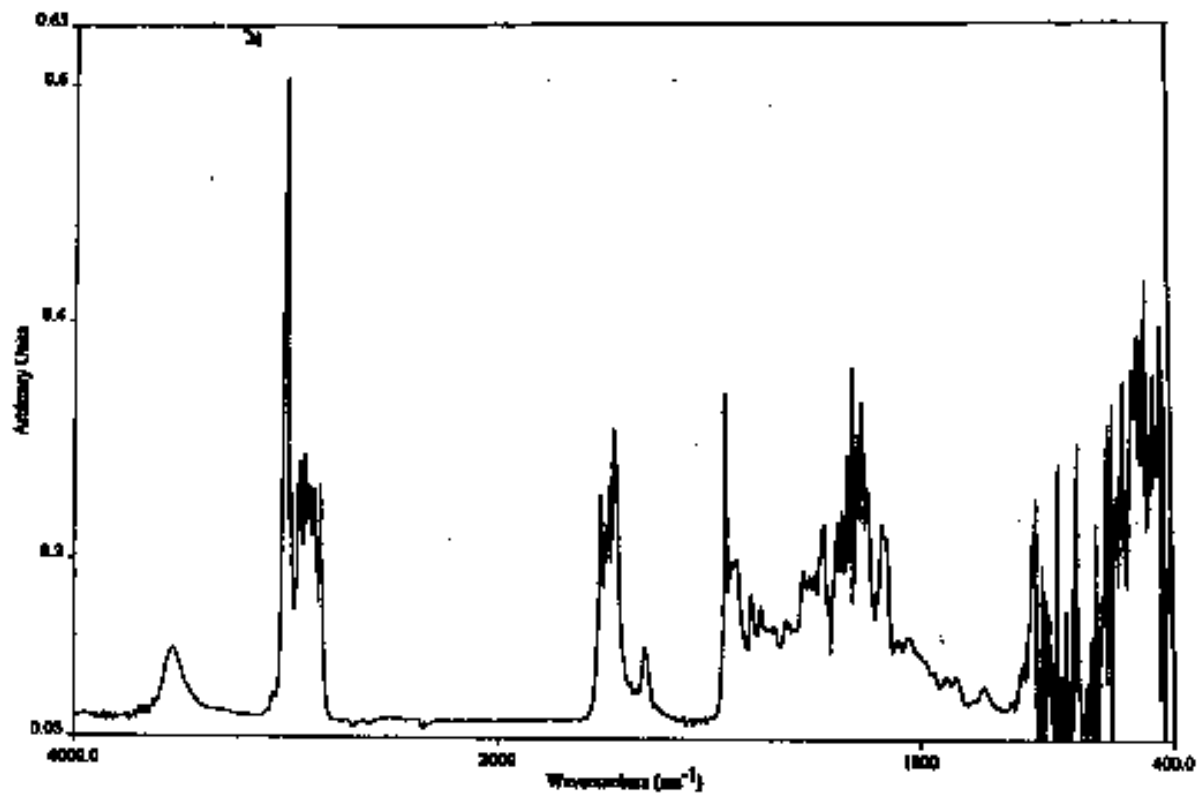


FIG. 3. A FT-IR correlation spectrum obtained from the calibration standards. An arrow indicates the spectral region employed for calibration. For abbreviation see Figure 1.

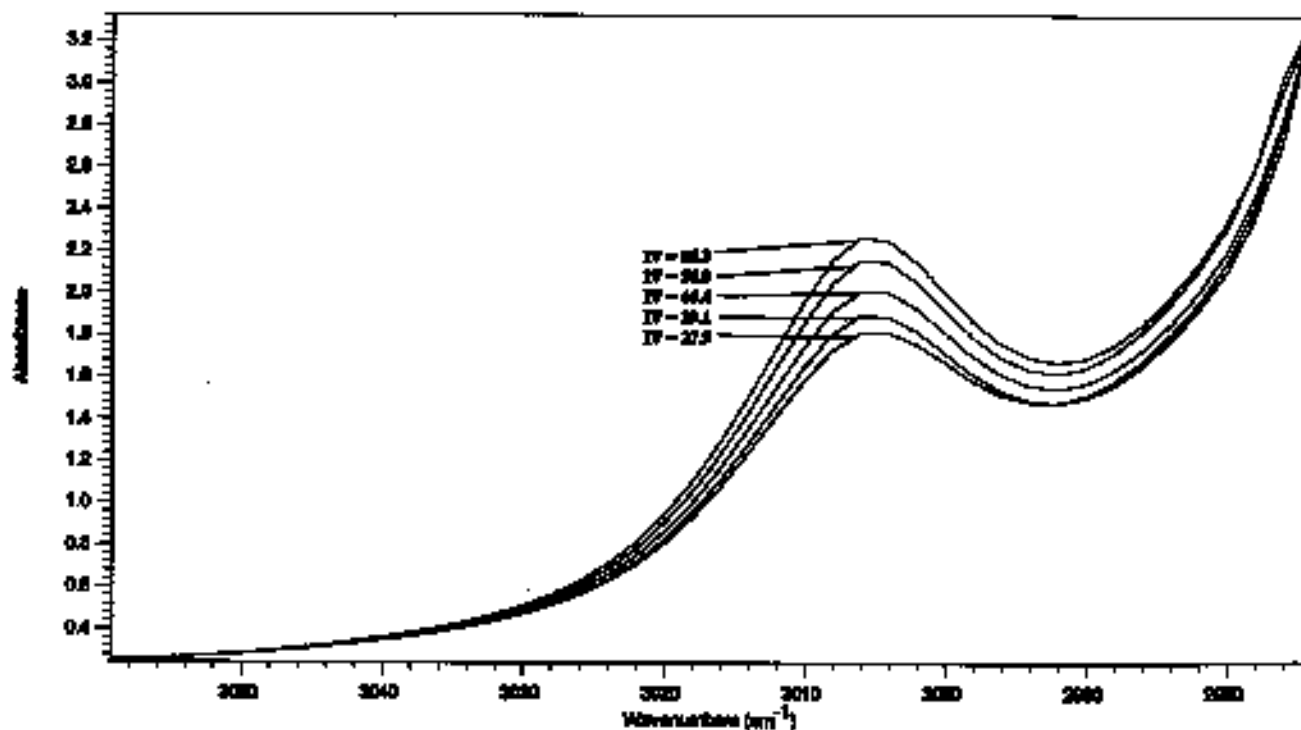


FIG. 4. The FT-IR spectral region that provided the best calibration. IV, iodine value. For other abbreviations see Figure 1.

interest while simultaneously accounting for other spectral contributions that may perturb the spectrum. A PLS calibration model is capable of delivering accurate results as long as the calibration spectra contain enough information that is representative of both the component of interest and the nonrelated spectral variations associated with the samples to be analyzed. A PLS calibration model was developed based on the calibration standards (Table 1) that included the different weighted amounts of blended superolein and palm stearin that covered a broad range of IV. A PLS calibration model is constructed using the broad spectral region of *cis* HC=CH stretching for determining the predicted IV of samples at 3050–2984  $\text{cm}^{-1}$  with 2984  $\text{cm}^{-1}$  as a single point baseline (Fig. 4).

Validation within a calibration set can be used to calculate PRESS values. PRESS values are an indication of how closely a model fits the calibration data, and are used to specify the optimum number of factors. The best model includes the fewest number of factors such that the PRESS for that model is not significantly greater than the minimum PRESS value (24).

A plot of duplicate means of the FTIR predicted IV against actual IV, obtained from PLS calibration based on palm oil blends, is presented in Figure 5. The plot is linear with coefficient of determination ( $R^2$ ) of 0.9995 and a standard error of calibration (SEC) of 0.170 IV units, using two factors.

After calibrating the model, the validation procedure is carried out to minimize the prediction error and provide an estimate of the overall accuracy of the predictions. A linear

regression of the optimized validation on FT-IR results, obtained for the predicted IVs against their standard IVs, yields the following equation:

$$IV_p = 0.9965IV_a + 0.1841 \quad [1]$$

where  $IV_p$  is the IV predicted by FT-IR and  $IV_a$  is the IV determined by the AOCS method, with  $R^2 = 0.9995$  and standard error of prediction (SEP) of 0.151 IV units. Figure 6 presents a validation plot of the duplicate means of the FTIR predicted vs. the AOCS method IV of the calibration set of the palm oil samples. This plot illustrates an excellent linear relationship between the IV predicted by FT-IR and that obtained from standard method with the slope and correlation coefficient being close to 1.0.

Table 2 compares the IV data between the duplicate FT-IR result and standard result in terms of the mean difference (MDa) and standard deviation difference (SDDa) for overall accuracy (a). In terms of accuracy, the predictions from FT-IR method are  $\sim 0.032$  IV units higher than the actual values overall. The SDDa indicates that IV can be measured with good accuracy by FT-IR spectroscopy to within 0.506 IV units of its actual value with 95% confidence.

From this study, we can estimate the degree of unsaturation of palm oil samples using FTIR equipped with transmission flow cell by means of measuring the absorbance of a single band such as the =C-H *cis* stretching band at 3050–2984  $\text{cm}^{-1}$ . The degree of unsaturation is closely related to the IV and can be determined by means of calibrations of actual IV

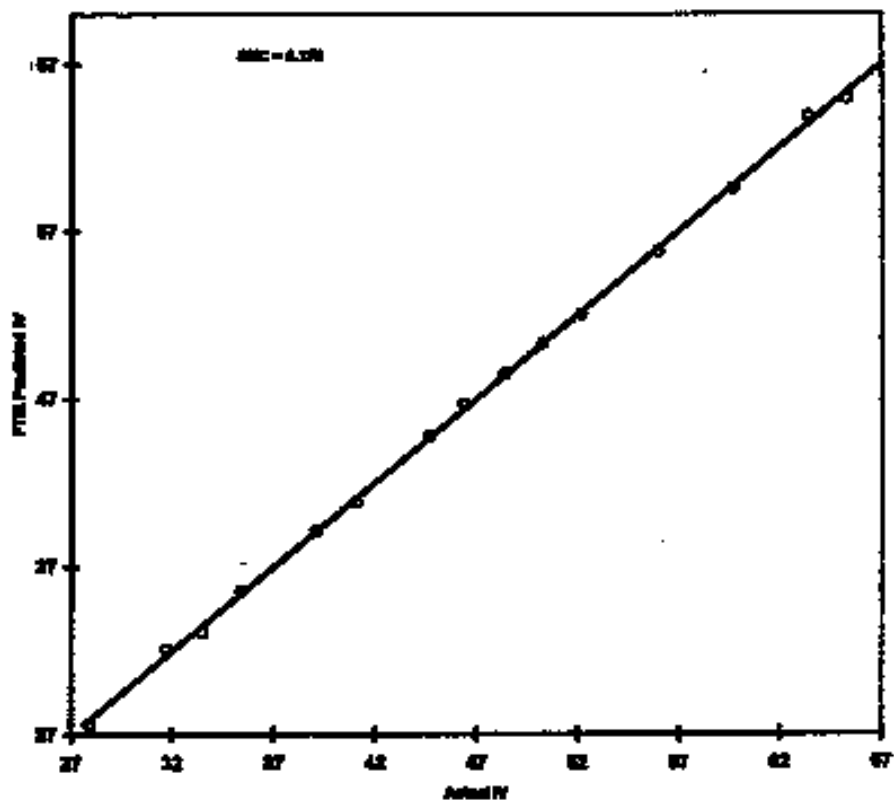


FIG. 5. IV calibration plot yielded from RBD palm stearin and superolein blends calibration standards. RBD, refined-bleached-deodorized; SEC, standard error of calibration; for other abbreviations see Figures 1 and 4.

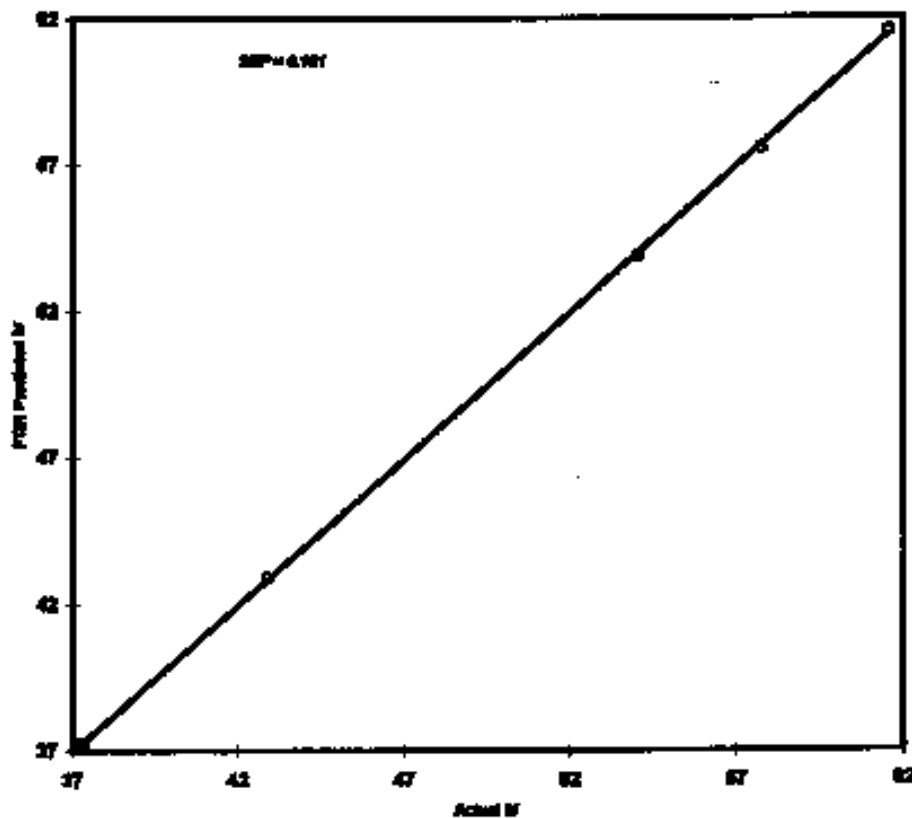


FIG. 6. IV validation plot yielded from RBD palm stearin and superolein blends calibration standards. SEP, standard error of prediction; For other abbreviations see Figures 1, 2, and 5.

**TABLE 2**  
**Statistical Comparison of IV of RBD Palm Stearin, Superolein and Their Mixtures Obtained by AOCS Reference and FT-IR Methods<sup>a</sup>**

Statistic	Standard method	FT-IR method
Max value	65.3	64.9
Min value	27.9	27.7
Mean	47.79	47.7
MD		-0.0318
SDD		0.5063

<sup>a</sup>IV, iodine value; RBD, refined-bleached-deodorized; MD, mean difference; SDD, standard deviation difference; a, accuracy; FT-IR, Fourier transform infrared.

applying PLS to a spectral region. The PLS model approach has been shown to provide excellent results for this set of mixture of palm stearin and superolein samples. The average time of analysis to determine IV of palm oil samples can be completed less than 2 min per sample; consequently, it is possible to analyze the IV of hundreds of palm oil samples daily. The rapid IV determination by FT-IR spectroscopy is therefore suitable and practical for process control. Another advantage of FT-IR method is that it is environmentally friendly as no chemical is needed except hexane for cell cleaning. By utilizing this method, chemical cost is negligible as compared to that incurred by the method using cyclohexane.

## ACKNOWLEDGMENTS

The authors thank the Universiti Putra Malaysia for providing the fund (IRPA No. 03-02-04-048) for this work. The authors are grateful to the Perkin-Elmer Sdn. Bhd. Malaysia for providing the Paragon 1000 FTIR spectrometer.

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[Received May 1, 1998; accepted January 8, 1999]