

Quantitative Determination of Peroxide Value in Thermally Oxidized Palm Olein by Fourier Transform Infrared Spectroscopy

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A simple and rapid quantitative method to determine peroxide value (PV) of palm olein has been developed using transmittance Fourier transform (FT) IR spectroscopy. Palm olein was oxidized and blended with unoxidized palm olein to generate samples with PVs in the range 3.52–9.86. These samples were used in the calibration and validation steps. A calibration model based on partial least-squares analysis was constructed using the spectral and chemical data of the calibration set. Evaluation of the calibration model was carried out by cross-validation. The standard error of prediction and coefficient determination obtained from the cross-validation equation were 0.172 PV and 0.996, respectively. The standard deviation of the difference for reproducibility of the FTIR method was found to be better than that of the chemical method. The FTIR method would be suitable for PV determinations in the palm olein industry and takes an average of less than 2 min per sample. Copyright © 2000 John Wiley & Sons, Ltd.

Keywords: Peroxide value; palm olein; FT infra red spectroscopy; partial least-squares analysis.

INTRODUCTION

The autoxidation of lipids and oils is the major cause of their deterioration, and hydroperoxides (generally referred to as peroxides) formed by reaction between oxygen and the unsaturated fatty acid moieties are the primary products of this process. Therefore, it seems reasonable to determine the concentration of peroxides as a measure of the extent of oxidation. The peroxide concentration, usually expressed as a peroxide value (PV), therefore gives a measure of the early steps of lipid oxidation (Hamilton, 1989). The PV is the concentration of substances, in terms of milli-equivalents of peroxides per 1000 g of sample, that oxidize potassium iodide.

Numerous analytical procedures have been described in the literature for the measurement of PV (for a review see Gray, 1978). For routine laboratory use, standard methods for the determination of PV of fats and oils have been developed. One of these is the American Oil Chemists' Society method (AOCS, 1989), this being a frequently used iodometric method which employs acetic acid:isooctane as a solvent. Fiedler (1974) has carried out the PV determination of fats and oils by a coulometric method, which is a modification of the iodometric method involving replacement of the titration step with an electrochemical technique. In this method, the iodine liberated is reduced at a platinum electrode maintained at a constant potential. Yamamoto *et al.* (1985) measured the amount of hydroperoxide using a chemiluminescence method and observed that there was an excellent linear

relationship between total chemiluminescence and concentration of hydroperoxides. Some iodometric methods to determine PV by conventional, difference or difference-derivative spectrophotometry have also been developed (Lezerovich, 1985; Lovaas, 1992). Miyazawa *et al.* (1994) estimated the PV of soybean oil by measuring thermoluminescence and showed that there was a linearity of the thermoluminescence intensity with the PV of soybean oil. van de Voort *et al.* (1994a) developed a Fourier transform (FT) IR method for the quantitative determination of PV of oils. These authors claimed that the reproducibility of the FTIR method was better than that of the chemical method. Recently, Moh *et al.* (1999) investigated the applicability of a near-IR method to predict the PV of crude palm oil. An excellent linear relationship between the near-IR and the AOCS methods was generated in this work. In the present study, we have developed and examined the applicability of a FTIR method for the rapid estimation of PV of palm olein.

EXPERIMENTAL

Chemicals and oil samples. All chemicals and reagents used in this study were of analytical grade. Refined-bleached-deodorized (RBD) palm olein was purchased from a local refinery and a sample was thermally oxidized in a Beto (Padova, Italy) deep fryer, model EPD 8B. RBD palm olein (4 kg) was used for frying 20 batches of French fries (100 g/batch), each batch requiring 3 min frying time with 15 min being required to stabilize the temperature of frying which was set at 180°C. This treatment aimed at accelerating the oxidation process of the palm olein. For every five batches, 100 g of RBD

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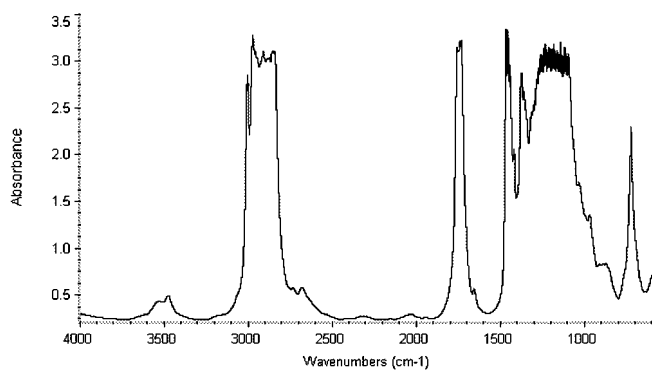


Figure 1. Typical absorbance spectrum of thermally oxidized palm olein in its neat form, obtained by ratio comparison of the single beam spectrum of the oil against the single beam spectrum of air.

palm olein was analysed for the peroxide value (PV) in duplicate according to the AOCS method (AOCS, 1989), and frying was discontinued after 20 batches when a high PV was obtained. The oxidized RBD palm olein was blended with unoxidized palm olein in appropriate amounts in order to create a broad range of PV. These blends were analysed to determine their PV values based on the AOCS method.

The reference oil was obtained by purifying the RBD palm olein by passing it twice through a column of activated silica gel (60 mesh; Aldrich, Milwaukee, WI, USA) in order to remove any carbonyl compounds and hydroperoxides that may have been present (Dubois *et al.*, 1996). The efficiency of this procedure was assessed by carrying out the PV test on the unpurified and purified palm oleins: the PV was reduced from 3.5 to <1.0.

Measurement of FTIR spectra. A Perkin-Elmer (Norwalk, CT, USA) model 1600 series FTIR spectrometer was interfaced to a Perkin-Elmer model 7300 professional computer running Infrared Data Management System (IRDM) software. This instrument was also connected to a deuterated triglycine sulphate (DTGS) detector operating at room temperature. Two automatic dehumidifiers was used to protect the instrument from interference by water vapour.

The sample spectra were measured using a demountable cell which consisted of a pair of IR transmitting windows (of sodium chloride) with a polytetrafluoroethylene (PTFE) spacer placed between them in order to establish a sample thickness (pathlength) of 200 (Coleman, 1993). A few drops of each sample were placed on the bottom sodium chloride window, the spacer and the top window were positioned, and the cell was clamped into the mount of the FTIR spectrometer. Prior to scanning each sample, the cell was taken apart and the windows rinsed three times with acetone to remove the oil sample and wiped dry with a soft tissue.

Emittance spectra in the range 4000–400 cm^{-1} were collected in 16 scans at a resolution of 8 cm^{-1} with Norton–Beer strong apodization and a gain of 2.0: all samples were scanned in duplicate. The spectra were ratioed against the emittance spectrum of the reference oil (which was recorded in 64 scans at 8 cm^{-1} resolution) to produce differential spectra. One emittance spectrum of each sample was also ratioed against an air emittance background to yield a typical spectrum of the oil. Spectra

were stored on diskette in JCAMP files for subsequent partial least-square (PLS) analysis.

Calibration and validation. The calibration was carried out using the Nicolet (Madison, WI, USA). Turbo Quant-IR calibration and prediction software package. A PLS method was chosen to develop a calibration model: the routine includes a validation procedure which provides statistical parameters indicative of the predictive capability of the calibration model (Fuller *et al.*, 1988a). The calibration was developed by studying the spectral features of the difference spectra between the samples and the reference oil in the absorbance regions that correlated with PV. The calibration model was examined in a validation step. The accuracy of the validation step was assessed according to the standard error of prediction (SEP) and the coefficient of determination (R^2). The correlating spectral regions were further refined using the mean difference (MD) and standard deviation of the difference (SDD) between the predicted and chemical PV as a measure of improved performance of calibration (van de Voort *et al.*, 1992).

RESULTS AND DISCUSSION

Spectroscopy of thermally oxidized palm olein

Oxidative deterioration involves the uptake of oxygen through the formation of hydroperoxides that subsequently break down by various mechanisms to produce a variety of aldehydes, fatty acids, and alcohols (Patterson, 1989). Hydroperoxide moieties exhibit characteristic absorption bands between 3600 and 3400 cm^{-1} due to their —OO—H stretching vibrations, with the peak maximum being a function of the polarity of the medium and the extent of hydrogen bonding (van de Voort *et al.*, 1994b). Figure 1 illustrates the absorbance spectrum of thermally oxidized palm olein in its neat form, obtained by ratio comparison of the single beam spectrum of the oil against the single beam spectrum of air. This spectrum shows the dominant spectral features associated with edible oils: the CH stretching absorption in the region from 3050 to 2800 cm^{-1} (*cis* C=CH, CH₂, CH₃ and CH₂/CH₃ stretching bands), the carbonyl absorption of the triglyceride ester linkage at 1746 cm^{-1} (Guillen and Cabo, 1997), and the bands associated with the fingerprint region (1500–1000 cm^{-1} ; Ismail *et al.*, 1993). The band observed at 3468 cm^{-1} is the overtone of the triglyceride ester carbonyl absorption. No apparent hydroperoxide absorption band is discernible in this spectrum showing that it is difficult to detect the presence of the hydroperoxide by simple inspection.

One of the strengths of FTIR spectroscopy is its ability to ratio out common features in the spectra of two samples that differ in a constituent. The ratio technique is a sensitive means of detecting relative spectral changes as long as the signal at the detector is strong enough to be measured accurately. Ratio comparison of the spectrum of the oxidized palm olein against that of the reference oil (Fig. 2) permits the spectral features of hydroperoxide to become apparent. The broad band centred at 3444 cm^{-1} is the OH stretching vibration of hydroperoxides (van de Voort *et al.*, 1994b). The remaining peak (3550 cm^{-1}) throughout the experiment may be due to the OH

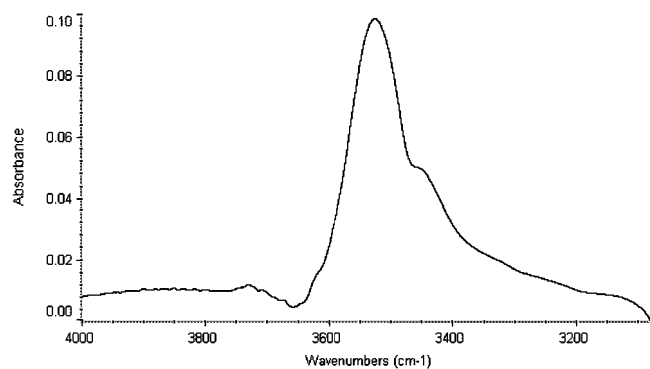


Figure 2. The difference spectrum obtained by ratio comparison of the single beam spectrum of the oxidized palm olein against that of the reference palm olein.

stretching absorption of alcohol and water, which occurs in the same vicinity ($3700\text{--}3500\text{ cm}^{-1}$; van de Voort *et al.*, 1994b).

Development of calibration model

Chemometric software based on partial least-square (PLS) is an alternative which allows the whole spectrum to be investigated with respect to potential correlations. The regions that correlate with the feature of interest are readily determined by this type of software, and PLS is capable of accounting for interfering compounds that are external to the features in question, as long as the

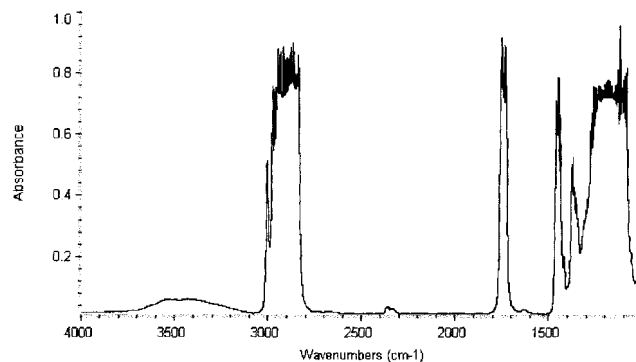


Figure 3. The variance spectrum obtained from the calibration standards.

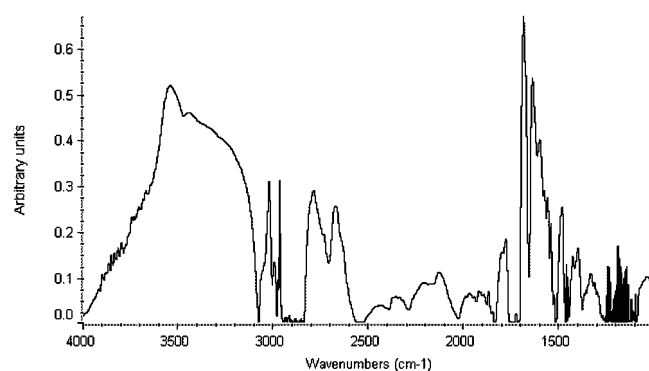


Figure 4. The correlation spectrum obtained from the calibration standards.

Table 1. Results of mean duplicate analyses of RBD palm olein obtained using the AOCS reference method^a

Ratio of fresh:oxidized palm olein (w/w)	PV value
100:0	3.52
98:2	3.74
95:5	3.95
91:9	4.17
89:12	4.38
84:16	4.60
81:19	4.81
77:23	5.03
74:26	5.25
70:30	5.46
67:33	5.68
63:37	5.89
60:40	6.11
56:44	6.33
53:47	6.54
49:51	6.76
46:54	6.97
42:58	7.19
39:61	7.41
35:65	7.62
32:68	7.84
28:72	8.05
25:75	8.27
22:79	8.48
18:82	8.70
14:86	8.92
11:89	9.13
7:93	9.35
4:96	9.56
0:100	9.78

^a AOCS, 1989

calibration standards are spectrally representative of the samples to be analysed in the spectral regions associated with the component of interest (Fuller *et al.*, 1988a). The calibration standards were designed to meet the requirement for a valid PLS calibration such that no correlation should exist between the concentrations of the interfering components and the component of interest. Table 1 summarizes the 30 calibration standards prepared and used to develop the PLS calibration. Some calibration diagnostics were used to optimize the spectral data such as the variance spectrum and correlation spectrum diagnostics. The variance spectrum displays the regions of the spectrum where there are changes in the absorbance values over the calibration set and it is actually the square root of the variance at each frequency in the spectral regions that have been selected (Fig. 3). The correlation spectrum (Fig. 4) is 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 concentration information and spectral response (Fuller *et al.*, 1988b). For this calibration set, the spectral region used in the calibration was set to include all of the data from $3470\text{ to }3082\text{ cm}^{-1}$. A calibration plot (Fig. 5) was developed using the chemical PV of the calibration set. The calibration was optimised by the leave-one-out cross-validation procedure to minimize the prediction error and to provide an estimate of the overall accuracy of the prediction. The standard error of calibration (SEC) of the calibration model found was 0.148 PV. Figure 6

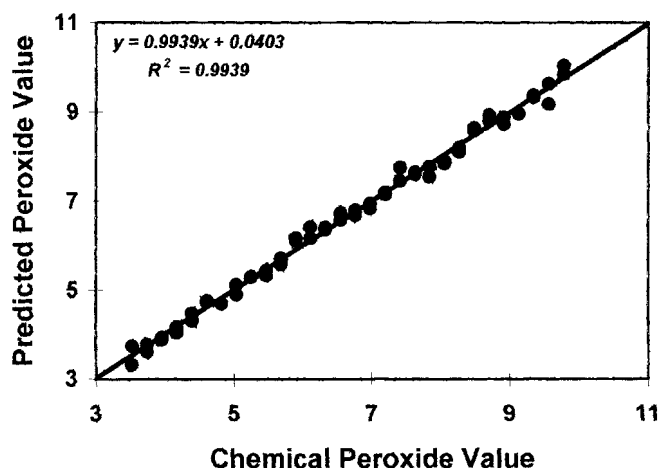


Figure 5. A calibration plot of FTIR-predicted PV vs chemically determined PV for 30 oxidized and unoxidized palm olein blends. [Regression equation and correlation coefficient (R^2) shown.]

presents a cross-validation plot of PV determined by FTIR versus PV determined by the chemical method based on the following equation:

$$PV_p = 0.9901PV_c + 0.0728$$

where PV_p = predicted PV (meq/kg), and PV_c = chemical PV (meq/kg). This plot was linear with a slope and correlation coefficient being close to 1.0. The standard error of prediction (SEP) obtained from this regression was 0.172 PV. The SEP obtained from the regression can be considered as a measure of the accuracy of the FTIR PV method because it relates predicted PV to chemical PV. This indicates that PV can be measured with good accuracy by FTIR spectroscopy when the calibration is based on representative oxidised samples. Based on an average PV of for these samples, an SEP of 0.172 PV represents a coefficient of variation (CV) of 2.58%, compared to an overall CV of 2.59% for duplicate chemical analyses of these samples. In terms of reproducibility, the FTIR method has a higher mean difference ($MDD_r = -0.055$) between the duplicates than does the chemical method ($MDD_r = 0.017$). The duplicate

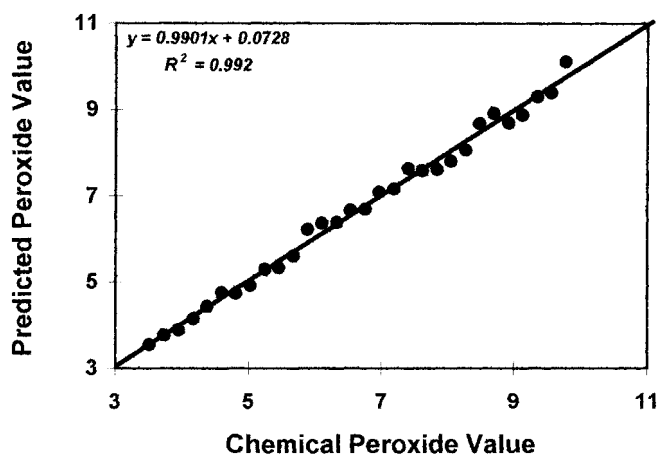


Figure 6. A cross-validation plot of FTIR-predicted PV vs chemically determined PV for 30 oxidized and unoxidized palm olein blends. [Regression equation and correlation coefficient (R^2) shown.]

FTIR analysis had a standard deviation of the difference for reproducibility (SDD_r) of 0.02 PV, compared to an SDD_r of 0.05 PV for duplicate chemical analyses. The standard deviation of the difference of the chemical method was higher, indicating that the FTIR method is superior in producing consistent results for any one sample.

From this study it is shown that the FTIR spectroscopy can be used as a means of determining the oxidative state of an oil as measured by its PV. The FTIR spectrometer allows the analysis to be carried out directly on neat samples (fats or oils) thus reducing both analytical time (<2 min/sample) and reagent use and disposal problems associated with the chemical PV method.

Acknowledgements

The authors thank the Universiti Putra Malaysia (UPM) for providing funds (IRPA no. 03-02-04-048) for this work. The authors are grateful to the Department of Chemistry UPM for allowing the use of the 1600 Series FTIR spectrometer.

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