

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

Y.B. Che Man* and G. Setiowaty

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

ABSTRACT: Fourier transform infrared (FTIR) spectroscopy with transmission cell is described to predict anisidine value of palm olein. The calibration set was prepared by mixing the thermally oxidized palm olein and the unoxidized palm olein with certain ratios (w/w) covering a wide range of anisidine values. A partial least square (PLS) regression technique was employed to construct a calibration model. This model was further accomplished by a validation step. The standard error of prediction found was 0.51. The precision of this method was shown to be comparable to the accuracy of the American Oil Chemists' Society method used for measurement of anisidine value, with coefficient of determination (R^2) of 0.99. The study showed that mid-band FTIR spectroscopy combined with a PLS calibration technique is a versatile, efficient, and accurate technique for the estimation of anisidine value of palm olein within about 2 min with less than 2 mL of sample.

Paper no. J8926 in *JAOCs* 76, 243–247 (February 1999).

KEY WORDS: Anisidine value, Fourier transform infrared spectroscopy, palm olein, partial least-square regression.

Several studies have attempted to measure the amounts of carbonyls produced by the decomposition of fatty acid hydroperoxides. The major volatile carbonyls formed in the autoxidation of edible oils are saturated and unsaturated aldehydes ranging in number of carbon atoms from 3 to 10. Although these volatile compounds are formed as relatively minor autoxidation products, they are the most important contributors to rancid and unpleasant flavors in oxidized oils (1). Therefore, the determination of aldehyde content is important in judging the quality of oils, and a variety of methods are available.

Several studies attempted to measure the amounts of carbonyls. Many were based on the colorimetric method (2–4). Others were based on gas chromatography of volatiles produced (5,6). Measurement of the ultraweak chemiluminescence which accompanies autoxidation was reported to be a potentially useful method (7). A simple spectrophotometric method for determination of volatile carbonyl compounds in edible oils was also reported (8). An attempt was also carried out to measure the monocarbonyl compounds from catalytic

decomposition of autoxidized unsaturated fatty acid esters by using thin-layer chromatography (9). The thiobarbituric acid value (10) and anisidine value (11,12) are also used to determine secondary oxidation products formed from peroxides. Anisidine value is a combined measure of mostly 2-alkenals, 2,4-dienals and, to a more limited degree, saturated aldehydes.

None of the methods mentioned above alone fulfills the criteria for a simple test for rancidity assessment, and the search for an alternative analysis is thus important. Recently, Dubois *et al.* (13) reported application of Fourier transform infrared (FTIR) spectroscopy to determine anisidine value in thermally stressed oils. This method was reported to be a very useful method for prediction of anisidine value of oils. In the present study, the FTIR method is established for replacing the common, laborious wet-chemical method to determine anisidine value of palm olein, thus minimizing the environmental concern associated with the use of organic reagents.

MATERIALS AND METHODS

Sample and sample preparation. All reagents were of analytical grade. Palm olein was purchased from a local refinery. Twenty calibration and 10 validation samples were derived from the blends of thermally oxidized and unoxidized palm olein. The calibration and validation samples were prepared by oxidation of palm olein samples in the oven at 120°C for 6 d. The anisidine value determination of these samples was carried out to monitor the extent of secondary oxidative products daily. These thermally oxidized palm olein samples were blended with fresh palm olein in certain ratios (w/w) to cover a range of anisidine value of samples.

Chemical analyses. The calibration samples were analyzed in duplicate for anisidine value using the spectrophotometric technique, as described by an AOCS method (12).

FTIR scanning and calibration. A few drops of each standard was sandwiched between sodium chloride (NaCl) windows that are the parts of the demountable cells. A polytetrafluoroethylene spacer was placed between the windows to give a fixed pathlength of 0.2 cm. The cells were mounted in a cell holder in the FTIR spectrometer, and the scanning of each sample was carried out. Prior to scanning each sample, the NaCl windows were taken apart and rinsed three times with acetone to remove the oil sample and wiped with a soft tissue.

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

Samples were scanned using a Perkin-Elmer 1600 series FTIR spectrometer (Perkin-Elmer Corporation, Norwalk, CT) with a deuterated triglycine sulfate detector connected to a Perkin-Elmer model 7300 professional computer. Infrared Data System (IRDM) software was used during FTIR data collection. To minimize water vapor interference, the instrument was maintained with two automatic dehumidifiers.

FTIR data were collected from 16 scans at a resolution of 8 cm^{-1} and a gain of 2.0 and strong apodization throughout the region $4000\text{ to }400\text{ cm}^{-1}$. These spectra were ratioed against 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 samples were scanned in duplicate. The spectra were recorded as absorbance values at each data point.

The software program Nicolet Turbo Quant IR-Calibration and Prediction Package version 1.1 (Nicolet Instrument Co., Madison, WI) was used to construct a model of entire samples for calibration development. A partial least square (PLS) approach was developed for mathematical treatments of FTIR data.

The adequacy of the FTIR calibration was first assessed on the goodness of fit between chemical data and FTIR predictions of anisidine value on the 20 samples used during the calibration process. For PLS equation the assessment of accuracy was based on the standard error (SE) and the coefficient of determination (R^2) (13).

Model validation. The validation procedure was used to test the calibration model, and the accuracy of the equation was assessed according to the standard error of performance (SEP) and R^2 . The validation step was further investigated by using the mean difference and standard deviation of the difference (SDD) for reproducibility and accuracy between the predicted and chemical anisidine values.

RESULTS AND DISCUSSION

Anisidine value measured by the reference method. The range of means of duplicates of anisidine values of palm olein samples for the calibration and validation data sets were from 0.133 to 17.097 and from 1.000 to 15.300, respectively. The means and standard deviations of anisidine values of calibration and validation data sets are presented in Table 1.

The absorption bands of aldehydes. The identification of aldehydes can usually be confirmed by a study of the C-H stretching frequency of the aldehydic group. By virtue of the strong influence of the carbonyl oxygen, this C-H frequency is virtually independent of the rest of the molecule, and is therefore highly characteristic. It is to be expected that aldehydes will show a characteristic absorption in this region arising from the valence vibration of the hydrogen atom attached to the carbonyl group (14). Colthup in Bellamy (14) quotes the range $2900\text{--}2700\text{ cm}^{-1}$ for this vibration for all types of aldehydes. Pozefsky and Coggeshall in Bellamy (14) and

TABLE 1
Calibration and Validation Statistics for Anisidine Value Prediction by the AOCS Method^a

Data set	Mean	SD
Calibration	8.684	5.072
Validation	8.288	4.771

^aAOCS, American Oil Chemists' Society; SD, standard deviation.

Nakanishi (15) found two bands in this region which were near $2720/2730\text{ cm}^{-1}$ and 2820 cm^{-1} , respectively. Dubois *et al.* (13) give the range $2820\text{--}2700\text{ cm}^{-1}$ for the C-H stretching region for saturated aldehydes. Further, they imply that the carbonyl (C=O) of saturated aldehydes has the absorption peak at 1727 cm^{-1} . However, this wavelength cannot be applied to measure saturated aldehydes because the C=O absorption area of saturated aldehydes is obscured by an apparent broadening of the triglyceride ester linkage absorption (1748 cm^{-1}). The broadening of the triglyceride ester linkage is due to hydrogen bonding of alcohols and hydroperoxides (other secondary oxidative products) with the C=O groups of triglyceride ester. Thus, the C=O frequency is not a practical and useful means for measurement of saturated aldehydes.

Unsaturated aldehydes with the double bond in the $\alpha\text{-}\beta$ position show a fall in the carbonyl frequency and absorb in the range $1705\text{--}1685\text{ cm}^{-1}$ (14). Dubois *et al.* (13) found the peaks of C=O of hexenal and decadienal at 1697 and 1689 cm^{-1} , respectively. Subsequent experimentation by using oxidized canola oil showed a shoulder developing between 1705 and 1680 cm^{-1} as the result of the formation of unsaturated aldehydes.

Figure 1 illustrates the mean spectrum of the oxidized palm olein. It can be pointed out that the C-H stretching absorption region of the aldehydes at 2820 cm^{-1} is overlaid by the CH_2 symmetric stretching region of palm olein at 2850 cm^{-1} (16). A broad band of C-H stretching absorption appears at around 2730 cm^{-1} . In the C=O region of unsaturated aldehydes ($1705\text{--}1680\text{ cm}^{-1}$), there is no absorption peak. This may be due to absence of unsaturated aldehydes being formed by oxidation of palm olein samples. Another possibility may be a little formation of unsaturated aldehydes in palm olein that gave weak intensity. Because this region was near the C=O absorption of triglyceride ester linkage (strong intensity), the C=O band of unsaturated aldehydes was obscured by the C=O of triglyceride ester.

Selection of the optimal region for anisidine value prediction. Figures 2 and 3 show the correlation spectra between 2800 and 2600 cm^{-1} and between 1750 and 1600 cm^{-1} , corresponding to the saturated and unsaturated aldehyde absorption regions, respectively. The correlation spectra presented show that there was a mathematical correlation between spectral changes with changes in the anisidine values as described by Fuller *et al.* (17). For these two regions, it appears that there is correlation between these regions and anisidine values obtained from the chemical method (12).

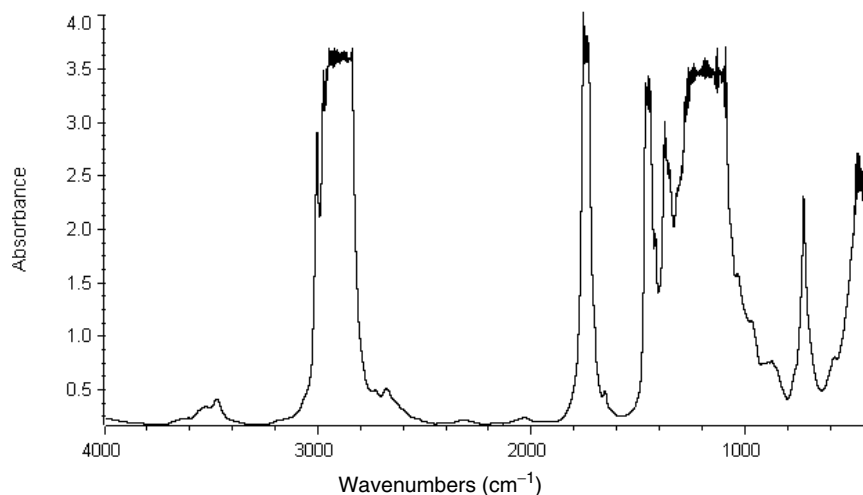


FIG. 1. Mean spectrum of calibration set.

Based on this investigation, we decided to use the regions 2747–2619 and 1715–1673 cm^{-1} individually and simultaneously to develop the calibration model for prediction of anisidine value of palm olein samples. Table 2 shows the results obtained from the PLS calibration in terms of R^2 and SE used in choosing the best region for determining anisidine values of palm olein.

Expanded FTIR calibration model for predicting anisidine value. An FTIR calibration model was obtained using the two wavelength regions described before and PLS for the prediction of anisidine value in palm olein samples. Using the calibration data set, the SE for the model was 0.24 and R^2 0.996. A good linear regression of chemically-determined anisidine value against FTIR-predicted anisidine value was obtained ($y = 1.0033x - 0.0577$) that gave an intercept and slope not significantly different from 0.0 and 1.0, respectively ($P > 0.05$)

(Fig. 4). Expressing SE of an FTIR calibration relative to the mean value of the reference analyses (covariance, CV) is a method of describing the accuracy of the FTIR calibration. Clark *et al.* (18) suggested that a CV less than 20% is acceptable for most analytical purposes. The CV of the equation we developed to predict anisidine value in palm olein was around 2.8%.

When the validation data set predicted with the calibration model, the SEP was 0.51, the R^2 was 0.990, and the mean difference and SDD for accuracy were 0.0864 and -0.0997 , respectively. The intercept and slope of the linear regression line (Fig. 5), plotting chemically determined vs. FTIR-predicted anisidine value for the validation samples ($y = 1.0157x - 0.2162$), were not significantly different from 0.0 and 1.0, respectively ($P > 0.05$). Table 3 compares these data in terms of MD_r and SDD_r for reproducibility between duplicates of

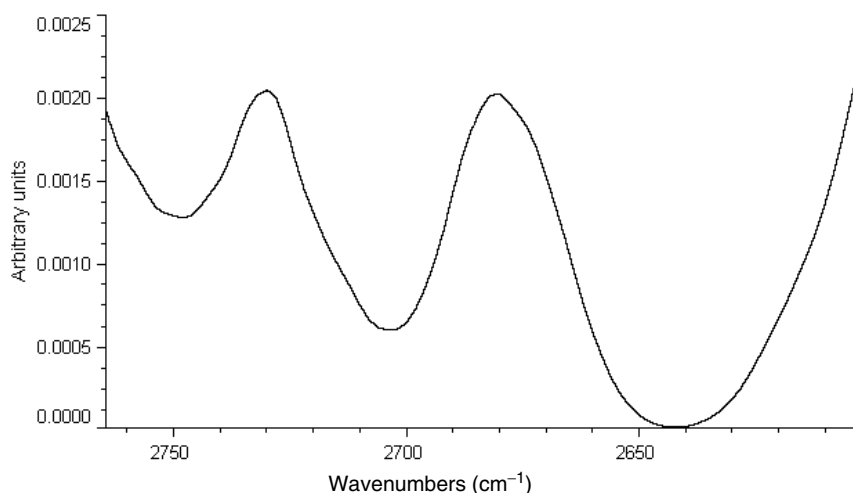


FIG. 2. Correlation spectrum at 2760–2600 cm^{-1} obtained from the calibration standards.

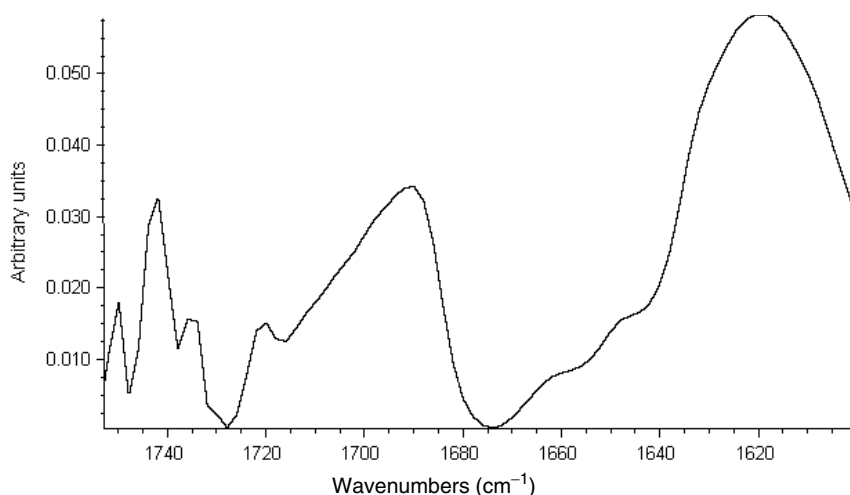


FIG. 3. Correlation spectrum at 1750–1600 cm^{-1} obtained from the calibration standards.

the chemical and FTIR results. In terms of reproducibility, both the chemical and FTIR results have comparable mean differences and standard deviations.

From the result in this study, we concluded that FTIR spectroscopy can be used to develop an equation to predict anisidine value in palm olein with satisfactory accuracy when anisidine value is no greater than 17. The use of FTIR spectroscopy to predict anisidine value in palm olein will allow more rapid determination than can be achieved using the chemical method.

ACKNOWLEDGMENTS

The authors thank the Universiti Putra Malaysia (UPM) for providing the funding (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.

TABLE 2
Effect of Different Wavelength Regions in Development of the Calibration Model in RBD Palm Olein Samples^a

Wavelength region (cm^{-1})	R^2	SE
1715–1673	0.985	0.61
2747–2619	0.873	1.77
1715–1673, 2747–2619	0.996	0.24

^aRBD, refined-bleached-deodorized; R^2 , coefficient of determination; SE, standard error.

TABLE 3
Statistical Comparison of Anisidine Value of Blends of Oxidized and Unoxidized RBD Palm Olein Obtained by AOCS Reference and FTIR Methods^a

Statistic	AOCS Method	FTIR method
MD_r	-0.1721	-0.1014
SDD_r	0.1338	0.1011

^aFTIR, Fourier transform infrared spectroscopy; MD, mean difference; SDD, standard deviation difference; r , reproducibility. See Table 1 for other abbreviation.

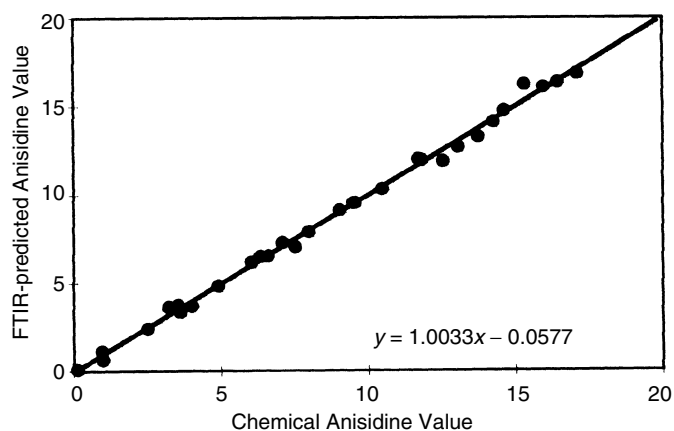


FIG. 4. Anisidine value calibration plot yielded from blends of oxidized and fresh palm olein. FTIR, Fourier transform infrared.

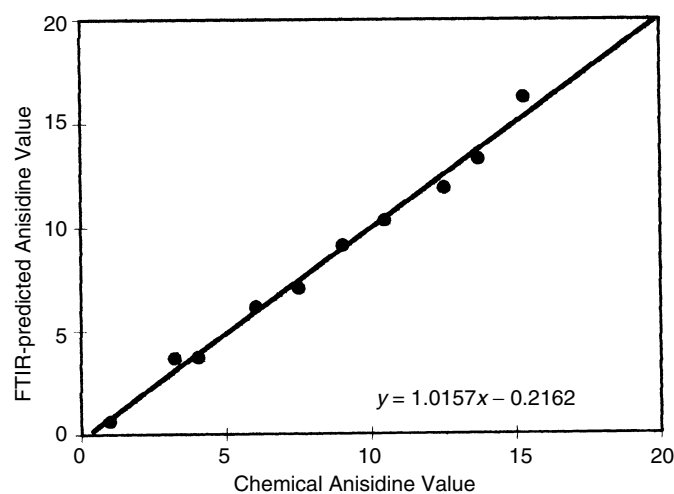


FIG. 5. Anisidine value validation plot yielded from blends of oxidized and fresh palm olein. For other abbreviation see Figure 4.

REFERENCES

1. Frankel, E.N., Chemistry of Autoxidation: Mechanism, Product and Flavor Significance, in *Flavor Chemistry of Fats and Oils*, edited by D.B. Min and T.H. Smouse, American Oil Chemists' Society, Champaign, 1985, p. 1.
2. Chiba, T., M. Takazawa, and K. Fujimoto, A Simple Method for Estimating Carbonyl Content in Peroxide-Containing Oils, *J. Am. Oil Chem. Soc.* 66:1588-1592 (1989).
3. Miyashita, K., K. Kanda, and T. Takagi, A Simple and Quick Determination of Aldehydes in Autoxidized Vegetable and Fish Oils, *Ibid.* 68:748-751 (1991).
4. Yukawa, N., H. Takamura, and T. Matoba, Determination of Total Carbonyl Compounds in Aqueous Media, *Ibid.* 70:881-884 (1993).
5. Pamela, J.W., and E.G. Hammond, Quantification of Carbonyl Compounds in Oxidized Fats as Trichlorophenylhydrazones, *Ibid.* 60:1769-1770 (1983).
6. Saidia, B., and E.G. Hammond, Quantification of Carbonyls Produced by the Decomposition of Hydroperoxides, *Ibid.* 66:1097-1102 (1989).
7. Burkow, I.C., P. Moen, and K. Overbo, Chemiluminescence as a Method For Oxidative Rancidity Assessment in Autoxidized Marine Oils, *Ibid.* 69:1108-1111 (1992).
8. Przybylski, R., and F.W. Hougen, Simple Method for Estimation of Volatile Carbonyl Compounds in Edible Oils and Fried Potato Chips, *Ibid.* 66:1465-1468 (1989).
9. Kimoto, H.I., and A.M. Gaddis, Monocarbonyl Compounds from Catalytic Decomposition of Autoxidized Unsaturated Fatty Acid Esters, *Ibid.* 51:307-311 (1974).
10. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., AOCS Press, Champaign, 1995, Method Cd 19-90.
11. List, G.R., C.D. Evans, W.F. Kwolek, K. Warner, and B.K. Boundy, Oxidation and Quality of Soybean Oil: A Preliminary Study of the Anisidine Test, *J. Am. Oil Chem. Soc.* 51:17-21 (1974).
12. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., AOCS Press, Champaign, 1995, Method Cd 18-90.
13. Dubois, J., F.R. van de Voort, J. Sedman, A.A. Ismail, and H.R. Ramaswamy, Quantitative Fourier Transform Infrared Analysis for Anisidine Value and Aldehydes in Thermally Stressed Oils, *J. Am. Oil Chem. Soc.* 73:787-794 (1996).
14. Bellamy, L.J., *The Infra-Red Spectra of Complex Molecules*, 3rd edn., John Wiley and Sons, Inc., New York, 1975, pp. 151-176.
15. Nakanishi, K., and P.H. Solomon, *Infrared Absorption Spectroscopy*, 2nd edn., Holden-Day, Inc., San Francisco, 1977, p. 205.
16. Stuart, B., *Modern Infrared Spectroscopy*, John Wiley & Sons, Chichester, 1996, p. 120.
17. Fuller, M.P., G.L. Ritter, and C.S. Drapper, Partial-Least Squares Quantitative Analysis of Infrared Spectroscopic Data. Part I: Algorithm Implementation, *Appl. Spectros.* 42:217-227 (1988).
18. Clark, D.H., H.F. Mayland, and R.C. Lamb, Mineral Analysis of Forages with Near Infrared Reflectance Spectroscopy, *J. Agron.* 79:485-490 (1987).

[Received July 1, 1998; accepted October 5, 1998]