

A Fourier Transform Infrared Spectroscopic Method for Determining Butylated Hydroxytoluene in Palm Olein and Palm Oil

W. Ammawath^a, Y.B. Che Man^{b,*}, R.B. Abdul Rahman^b, and B.S. Baharin^b

^aDepartment of Agro-industry, Rajamangala University of Technology Lanna, Amphur Muang, Lampang 52000, Thailand, and ^bDepartment of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43300 UPM, Serdang, Selangor, Malaysia

ABSTRACT: A simple, rapid, and direct FTIR spectroscopic method was developed for the determination of BHT content in refined, bleached, and deodorized (RBD) palm olein and RBD palm oil. The method used sodium chloride windows with a 50-mm transmission path. Fifty stripped oil samples of both RBD palm olein and RBD palm oil were spiked with known amounts of BHT concentrations up to 300 mg/kg (ppm). The data were separated into two sets for calibration and validation using partial least squares models. FTIR results for both oils correlated well with results obtained by the IUPAC HPLC-based method. For RBD palm olein, the coefficient of determination (R^2) was 0.9907 and the SE of calibration (SEC) was 8.47 ppm. For RBD palm oil, an R^2 of 0.9848 and an SEC of 10.73 ppm were achieved. Because of the significant decrease in analysis time and reduction in solvent usage, this FTIR method for BHT is especially well suited for routine quality control applications in the palm oil industry.

Paper no. J10982 in *JAOCs* 83, 187–191 (March 2006).

KEY WORDS: BHT, FTIR spectroscopy, RBD palm olein, RBD palm oil, PLS.

Lipid oxidation is one of the processes associated with the deterioration of quality in foods, especially in fat products. The change in quality is manifested by the formation of a number of volatile secondary products, by adverse changes in physico-chemical properties, and by the possible production of toxic compounds (1). Various synthetic and natural antioxidants are used to prevent or retard lipid oxidation (2). BHT is one of the most widely used antioxidants in the food industry, especially for oils and fatty foods. BHT is colorless, odorless, tasteless, highly active, and inexpensive (3). Noor and Augustin (4) studied the effectiveness of BHA and BHT in improving the stability of banana chips and found that chips fried in refined, bleached, and deodorized (RBD) olein containing BHA or BHT were more stable than chips fried in RBD olein without antioxidants. Generally, BHT can cause some darkening in the presence of iron, but the degree of change is not a serious issue (5). However, the uses of BHT in foods are limited for various reasons, and only a restricted amount is permitted as a food additive by international bodies (6).

Quantitative determination of synthetic phenolic antioxidants is an issue because incomplete extraction of antioxidants and co-extraction of potentially interfering substances may occur. For example, Saag (7) found that extraction into acetonitrile was not optimal because BHT recovery was low and high levels of interfering compounds were co-extracted.

The analysis techniques used for food products are crucial in food processing, as they enable manufacturing processes to be adjusted, if needed, while production is underway. FTIR spectroscopy has come of age in terms of price, performance, and ease of use (8). However, in complex mixtures, serious spectral interferences (overlapped signals) may result in non-linear correlations between the measured signal and the property of interest. To circumvent this problem, the application of multivariate analysis, together with the development of new methods for rapid acquisition of multisignal spectra, takes full advantage of the entirety of analytically relevant information in the FTIR spectra (9). Many authors have used multivariate regression to develop calibration models based on FTIR. Mirghani *et al.* (10) used partial least squares (PLS) to determine the residual soap in refined vegetable oil. Setiowaty and Che Man (11) described PLS and principal component regression to determine the cloud point in palm oil samples. Malaysia is the leading producer of palm oil and a major exporter of processed palm oil products. Palm oil is available in crude form, RBD form, and fractionated form as olein, a mid-fraction, and stearine (12).

The aim of the present study was to develop a rapid FTIR spectroscopic method combined with PLS regression for analysis of BHT in RBD palm olein and RBD palm oil.

EXPERIMENTAL PROCEDURES

Sample and sample preparation. RBD palm olein and RBD palm oil, without antioxidants added, were purchased from a local refinery. BHT (99%) was purchased from Sigma-Aldrich Chemie (Steinheim, Germany). All reagents were of analytical grade. To remove the existing tocopherols and tocotrienols in RBD palm olein and RBD palm oil, the oil was passed three times through a glass chromatographic column prepared by modifying AOCS method Cd 20-91 (13). The glass column (21 mm i.d., 450 mm in length; Wilmad-Labglass, Buena, NJ) was packed with 25 g silica gel 60 (70–230 mesh ASTM; Merck,

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

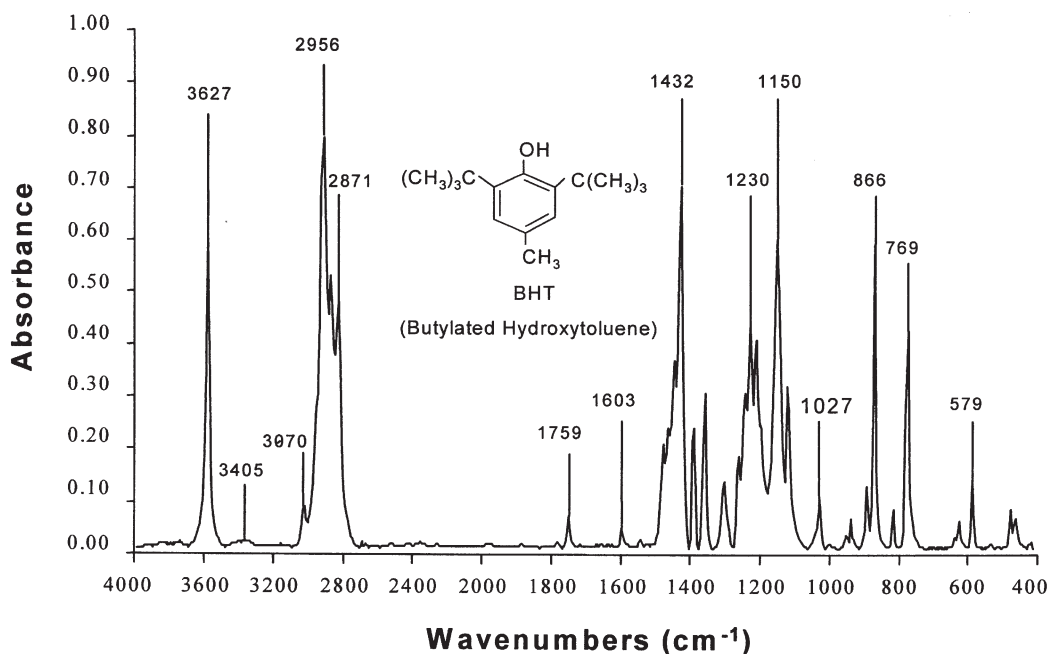


FIG. 1. FTIR spectra of BHT.

Darmstadt, Germany), and 4 g of sand purified by acid (40–100 mesh; BDH, Poole, United Kingdom) was added on the top of the silica gel packing. The efficiency of this cleanup step was assessed by HPLC. The total tocopherols and tocotrienols in RBD palm olein and RBD palm oil were reduced from 447 to 92 ppm and from 416 to 89 ppm, respectively. Samples of stripped oil were spiked with known amounts of BHT in both oils to concentrations of up to 300 mg/kg (ppm) and were used as standards for calibration and validation.

Instrumental analysis. (i) **HPLC analysis.** HPLC was used to determine BHT for RBD palm olein and RBD palm oil in the spiked samples using IUPAC method 2.642 (14). The stripped oil samples were spiked with known amounts of BHT to concentrations of up to 300 mg/kg (ppm). The samples (5 g) were dissolved in 25 mL hexane, and BHT was extracted from the solution with 150 mL acetonitrile. After evaporating the extracted fraction to 4 mL, 1 mL acetonitrile and 5 mL iso-

propanol were added prior to HPLC analysis. An HPLC system equipped with a UV-vis detector (SPD-10 AV; Shimadzu, Tokyo, Japan) was used to measure absorbance at 280 nm, and the column oven (CTO-10A) was set at 60°C. A column (250 × 4.6 mm) packed with microparticulate spherical silica (5 μm) bonded with octadecyldimethylsilyl (Merck) was used for the separation. The mobile phases were solution A (water/acetic acid, 95:5 vol/vol) and solution B (acetonitrile/acetic acid, 95:5 vol/vol) with a flow rate of 1 mL/min and a linear gradient (from 30 to 100%) of solution B over 7 min, followed by a 5-min hold at 100% solution B.

(ii) **FTIR analysis.** A Perkin-Elmer Spectrum BX (Norwalk, CT) with a room temperature deuterated triglycine sulfate detector was used for FTIR analysis. Spectral data manipulation was performed using Perkin-Elmer Spectrum software. The instrument was maintained with two automatic dehumidifiers to minimize interference from water vapor.

TABLE 1
Partial Least Squares (PLS) Calibration Statistics for Determining BHT Content in Refined, Bleached, and Deodorized (RBD) Palm Olein and RBD Palm Oil Using Different Wavelength Regions^a

Region no.	Wavelength region (cm ⁻¹)	RBD palm olein			RBD palm oil		
		R ²	SEC	SEP	R ²	SEC	SEP
1	3720–3550	0.9907	8.47	10.63	0.9848	10.73	12.92
2	3110–2740	0.3278	55.39	68.79	0.2830	53.85	67.62
3	1550–1400	0.5676	54.32	70.50	0.5078	46.13	73.93
4	1275–1063	0.4153	41.53	75.36	0.3483	51.34	70.40
1 + 2	3720–3550 + 3110–2740	0.4172	58.38	71.22	0.4154	47.67	66.28
1 + 3	3720–3550 + 1550–1400	0.8237	33.74	39.74	0.7124	21.99	29.76
1 + 4	3720–3550 + 1275–1063	0.5284	53.84	64.87	0.4629	56.14	64.46

^aR², coefficient of determination; SEC, SE of calibration; SEP, SE of prediction.

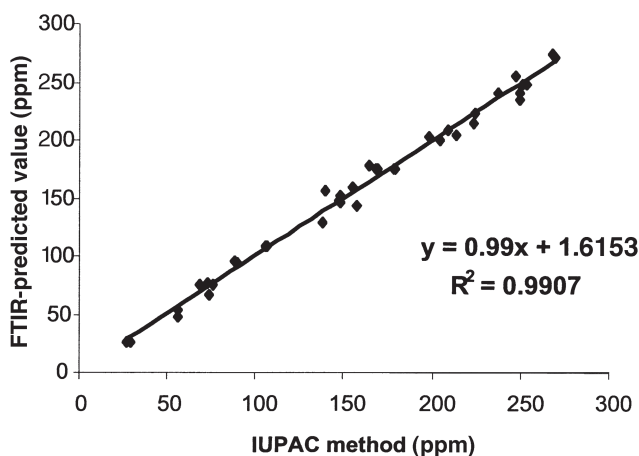


FIG. 2. Calibration plot of the data from the IUPAC method compared with the FTIR-predicted partial least squares (PLS) values for 38 samples of refined, bleached, and deodorized (RBD) palm olein.

Melted drops of each sample were placed between the sodium chloride (NaCl) windows, and the transmission path was fixed at 50 mm by adjusting the polytetrafluoroethylene spacer. The cell was then placed in the cell holder and the sample was scanned. After each sample was scanned, the NaCl windows of the transmission cell were rinsed three times with acetone and then dried with soft tissue in preparation for the next sample.

Statistical multivariate analysis. All the experiments and measurements were done in duplicate. Spectral parameters and data from the IUPAC method were determined, and a spectral data analysis was conducted using the Nicolet Turbo Quant IR-Calibration and Prediction package (version 1.1; Nicolet Instrument Co., Madison, WI).

Calibration development and validation. The two sets of 50 stripped RBD palm olein and RBD palm oil samples were spiked with known amounts of BHT concentrations up to 300 mg/kg (ppm). Each set of oil samples was divided into two sets, with 38 samples in each calibration set and the remaining 12 samples in the prediction set.

The correlation between the HPLC results (IUPAC standard method) and data obtained using the new FTIR method was assessed by PLS. The resulting coefficient of determination (R^2) was then taken as a measure of agreement between the two methods.

RESULTS AND DISCUSSION

Spectral analysis. Figure 1 illustrates the absorbance spectrum of BHT. This spectrum shows the dominant spectral features at 3627 cm^{-1} for the band of free hydroxyl, which is sharp and appears at a position well separated from other absorptions. Nakanishi and Solomon (15) have reported this single band to be useful for analysis of the hydroxyl group content. The bands at 2956 and 2871 cm^{-1} were attributable to asymmetric and symmetric C–H stretching vibrations of the CH_2 group (16,17)

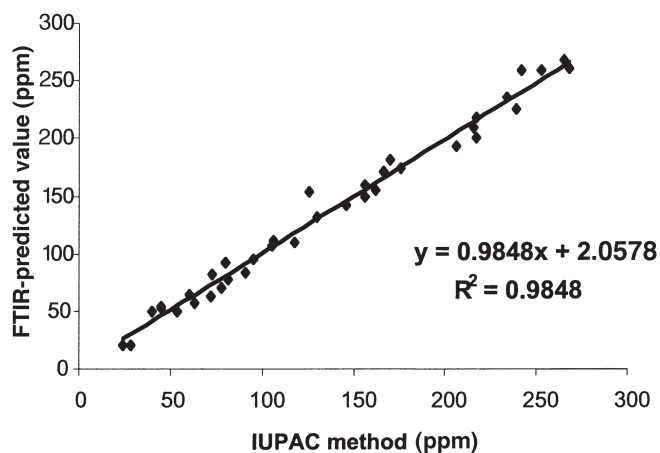


FIG. 3. Calibration plot of the data from the IUPAC method compared with the FTIR-predicted PLS values for 38 samples of RBD palm oil. For abbreviations see Figure 2.

and the band at 1432 cm^{-1} was attributable to the methyl bending vibration (18). The bands at 1230 and 1150 cm^{-1} were attributable to the in-plane –CH of 1,2-substituted phenyl (19), 866 cm^{-1} was attributable to the –CH of *p*-disubstituted phenyl, and 769 cm^{-1} was attributable to the out-of-plane –CH and ring bending (20).

Development of calibration and validation models. The FTIR spectrum of pure BHT was used to select the spectral regions for analysis. Four spectral regions (3720 – 3550 , 3110 – 2740 , 1550 – 1400 , and 1275 – 1063 cm^{-1}) were investigated for building the calibration models for BHT determination. Table 1 presents the results obtained from the PLS calibration in terms of the R^2 , the SE of calibration (SEC), and the SE of prediction (SEP) used in choosing the best regions. From these results, we postulated that the 3720 – 3550 cm^{-1} region could be used to develop a calibration model to predict the BHT content in RBD palm olein and RBD palm oil. The spectral regions (or combinations of spectral regions) that produced the highest R^2 value also produced the lowest SEC and SEP values for both palm oil and palm olein. The influence of interferences and overlapping of the absorption bands on FTIR measurement can be minimized by using multicomponent quantitative analysis such as PLS (21). In PLS, variables that

TABLE 2
Calibrations Statistics for BHT Content from Data Obtained by the IUPAC Method and FTIR Spectroscopy^a

Statistic	RBD palm olein		RBD palm oil	
	HPLC	FTIR	HPLC	FTIR
MD_r	1.95	0.17	3.21	3.31
SDD_r	2.89	0.85	1.01	0.43
Min. value	27.23	25.63	28.16	25.32
Max. value	279.37	273.50	268.24	267.90
MD_a		0.55		0.94
SDD_a		0.33		0.69

^aMD, mean difference; SDD, SD of difference; *r*, repeatability; *a*, accuracy; for other abbreviation see Table 1. Values are in ppm.

TABLE 3
Calibration and Validation Statistics for BHT in RBD Palm Olein and RBD Palm Oil Determined by the IUPAC Method and FTIR Spectroscopy Using PLS of the Wavelength Regions 3720–3550 (cm⁻¹)^a

Data set		HPLC method			FTIR method		
		Mean	SD	CV (%)	Mean	SD	CV (%)
RBD palm olein	Calibration	159.61	2.94	1.84	160.16	2.61	1.63
	Validation	158.67	5.51	3.47	162.19	3.47	2.14
RBD palm oil	Calibration	135.61	2.92	2.15	136.55	3.61	2.64
	Validation	145.83	4.96	3.40	137.87	3.90	2.83

^aAll data were the means of two replicates. Values are in ppm. For abbreviations see Table 1

show a high correlation with the response variables are given extra weight because they will be more effective at prediction (22).

Figures 2 and 3 show excellent correlations between FTIR (using the 3720–3550 cm⁻¹ region) and HPLC for RBD palm olein ($R^2 = 0.9907$) and RBD palm oil ($R^2 = 0.9848$), respectively. The corresponding R^2 values for the PLS model predictions for the validation samples with known concentrations were 0.9879 and 0.9803 for RBD palm olein and RBD palm oil, respectively.

Table 2 shows the statistical results for the mean difference for accuracy (MD_a), the SD of difference for accuracy (SDD_a), the mean difference for repeatability (MD_r), the SD of difference for repeatability (SDD_r) and minimum and maximum values for the IUPAC and FTIR methods in both RBD palm olein and RBD palm oil samples. The accuracy was evaluated by the difference between the MD IUPAC and MD FTIR values (expressed as MD_a), and the SDD IUPAC and SDD FTIR values (expressed as SDD_a). The results suggested good agreement between the FTIR predictions and IUPAC data for BHT content in RBD palm olein and RBD palm oil. In most analytical experiments the accuracy of the results is expressed as the deviation from the true value (23).

Table 3 shows the statistical results obtained with the IUPAC and FTIR methods in terms of the mean, SD for reproducibility between duplicates (DF = 36), and CV for BHT contents in both the calibration and validation sets of RBD palm olein and RBD palm oil samples. For both RBD palm olein and RBD palm oil, the FTIR spectroscopic analysis gave mean and SD values (for the calibration and validation data sets) comparable to those of the IUPAC method. The SD relative to the mean value for the reference IUPAC method was expressed as CV and was used to assess the accuracy of the FTIR calibration and validation sets, as shown in Table 3.

This study demonstrates that an FTIR spectrometer equipped with a transmission cell can be used to determine the BHT content in RBD palm olein and RBD palm oil. The BHT content in both oils was predicted well from absorbance measurements over a single spectral region from 3720–3550 cm⁻¹. Because FTIR allows the analysis to be carried out directly on neat samples of palm oil without prior extraction, the analysis time is reduced and the solvent waste disposal problems are minimized.

ACKNOWLEDGMENT

We would like to thank Universiti Putra Malaysia (UPM) for providing funding Intensification of Research in Priority Areas (IRPA) Grant No. 09-02-04-0457-EA001.

REFERENCES

- Pinho, O., I.M.P.L.V.O. Ferreira, M.B.P.P. Oliveira, and M.A. Ferreira, Quantification of Synthetic Phenolic Antioxidants in Liver Pates, *Food Chem.* 68:353–357 (2000).
- Kovatcheva, E.G., I.I. Koleva, M. Ilieva, A. Paulov, M. Mincheva, and M. Konushlieva, Antioxidant Activity of Extracts from *Lavandula vera* MM Cell Cultures, *Food Chem.* 72:295–300 (2001).
- Zhang, C.X., H. Wu, and X.C. Weng, Two Novel Synthetic Antioxidants for Deep Frying Oils, *Food Chem.* 84:219–222 (2004).
- Noor, N., and M.A. Augustin, Effectiveness of Antioxidants on the Stability of Banana Chips, *J. Sci. Food Agric.* 35:805–812 (1984).
- O'Brien, R.D., Fats and Oils Processing, in *Fats and Oils: Formulating and Processing for Applications*, edited by E.G. Perkin, and M.D. Erickson, Technomic Publishing, Lancaster, PA, 1998, pp. 47–180.
- Nenadis, N., I. Zafiropoulou, and M. Tsimidou, Commonly Used Food Antioxidants: A Comparative Study in Dispersed Systems, *Food Chem.* 82:403–407 (2003).
- Saag, K., Determination of Food Additives by HPLC, in *HPLC in Food Analysis*, edited by R. Macrae, Academic Press, London, 1988, pp. 185–258.
- Belton, P.S., E.K. Kemsley, M.C. McCann, S. Ttofis, R.H. Wilson, and I. Delgadillo, The Identification of Vegetable Matter Using Fourier Transform Infrared Spectroscopy, *Food Chem.* 54:437–441 (1995).
- Zagonel, F.G., P. Peralta-Zamora, and L.P. Ramos, Multivariate Monitoring of Soybean Oil Ethanolysis by FTIR, *J. Talanta* 63:1021–1025 (2004).
- Mirghani, M.E.S., Y.B. Che Man, S. Jinap, B.S. Baharin, and J. Bakar, FTIR Spectroscopic Determination of Soap in Refined Vegetable Oils, *J. Am. Oil Chem. Soc.* 79:111–116 (2002).
- Setiowaty, G., and Y.B. Che Man, Multivariate Determination of Cloud Point in Palm Oil Using Partial Least Squares and Principal Component Regression Based on FTIR Spectroscopy, *Ibid.* 81:7–11 (2004).
- O'Brien, R.D., Raw Materials, in *Fats and Oils: Formulating and Processing for Applications*, CRC Press, Boca Raton, FL, 2003, pp. 1–51.
- AOCS, *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., edited by D. Firestone, AOCS Press, Champaign, 1993.

14. IUPAC, *Standard Methods for Analysis of Oils, Fats, and Derivatives*, 1st supplement to the 7th edn., International Union for Pure and Applied Chemistry, Commission on Oils, Fats and Derivatives, Blackwell Scientific Publications, Osney Mead, Oxford, United Kingdom, 1992.
15. Nakanishi, K., and P.H. Solomon, *Infrared Absorption Spectroscopy*, Holden-Day, San Francisco, 1977.
16. Adhikari, C., A. Proctor, and G.D. Blyholder, Diffuse Reflectance Fourier Transform Infrared Spectroscopy of Oleic Acid Adsorption on Silica Acid, *J. Am. Oil Chem. Soc.* **71**:201–210 (1994).
17. Setiowaty, G., Y.B. Che Man, S. Jinap, and M.H. Moh, Quantitative Determination of Peroxide Value in Thermally Oxidized Palm Olein by Fourier Transform Infrared Spectroscopy, *Phytochem. Anal.* **11**:74–78 (2000).
18. Che Man, Y.B., W. Ammawath, and M.E.S. Mirghani, Determining α -Tocopherol in Refined Bleached and Deodorized Palm Olein by Fourier Transform Infrared Spectroscopy, *Food Chem.* **90**:323–327 (2005).
19. Roeges, N.P.G., Normal Vibrations and Absorption Regions of CX_3 , in *A Guide to the Complete Interpretation of Infrared Spectra of Organic Structures*, John Wiley & Sons, New York, 1994, pp. 10–47.
20. Mirghani, M.E.S., and Y.B. Che Man, A New Method for Determining Gossypol in Cottonseed Oil by FTIR Spectroscopy, *J. Am. Oil Chem. Soc.* **80**:625–628 (2003).
21. Wojciechowski, C., N. Dupury, C.D. Ta, J.P. Huvenne, and P. Legrand, Quantitative Analysis of Water-Soluble Vitamins by ATR-FTIR Spectroscopy, *Food Chem.* **63**:133–140 (1998).
22. Miller, J.N., and J.C. Miller, Multivariate Analysis, in *Statistics and Chemometrics for Analytical Chemistry*, Pearson Education, Harlow, Essex, 2000, pp. 214–238.
23. Miller, J.N., and J.C. Miller, Introduction, in *Ibid.*, pp. 1–19.

[Received November 3, 2004; accepted November 30, 2005]