Application of FTIR Spectroscopy in Determining Sesamol in Sesame Seed Oil

M.E.S. Mirghani[a], Y.B. Che Man[a,*], S. Jinap[b], B.S. Baharin[a], and J. Bakar[a]

Departments of [a]Food Technology and [b]Food Science, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor DE, Malaysia

ABSTRACT: A new analytical method was developed for determining sesamol in sesame seed oil by FTIR spectroscopy. Sesamol was also spiked at 0 to 1000 mg/kg in freshly refined, bleached, and deodorized palm olein (RBDPOo) and groundnut (peanut) oil. FTIR spectra were recorded using a transmission (NaCl) cell accessory at room temperature, and the partial least squares regression statistical method was used to derive calibration models for each oil. The standard errors of calibration were 6.07, 5.88, and 4.24 mg/100 g for sesame, RBDPOo, and groundnut oils, with coefficients of determination (R^2) of 0.9947, 0.9940, and 0.9662, respectively. The calibration models were validated by the "leave-one-out" cross-validation method, and the R^2 of validation, the standard errors of prediction, and SD of the differences for repeatability and accuracy were computed. Our results support the premise that FTIR spectroscopy is an efficient and accurate method for determining minor components such as sesamol in edible oils.

Paper no. J10132 in *JAOCS 80,* 1–4 (January 2003).

KEY WORDS: FTIR spectroscopy, PLS, RBDPOo, sesame, sesamol.

Sesame seed oil, extracted mainly from *Sesamum indicum* Linn. (1), is unique because of its high oxidative stability and characteristic flavor (2). The use of sesame oil as an edible oil is, however, largely limited to the areas of production because of the high cost of the seed. This is due to the low yield of the crop and difficulties in mechanized harvesting because of the uneven ripening of the capsules (3,4). The combined internal consumption of sesame seed in the major producing countries (China, India, Sudan, Mexico, Myanmar, Nigeria, and Somalia) represented more than 60% of the total world production in 1987 (5). Sesame seed are mainly mechanically pressed to produce oil; however, sometimes they are mechanically prepressed followed by solvent extraction. The oil content, FA composition, and acyl lipid classes of different *Sesamum* species were reported by Kamal-Eldin and Appelqvist (6). The characteristic flavor and chemical and physical properties of the oil are due mainly to the presence of lipid-soluble lignans such as sesamin and sesamolin. Sesamol and sesaminol were reported (7) to be present in sesame oil in trace amounts as phenolic antioxidant factors. They also are liberated during the refining of oil from unroasted sesame seeds (8). Sesamol, also is reported to be liberated during refining of oil from roasted seeds (9). Alkali neutralization, washing, and deodorization diminish the release of sesamol (10), which makes the refined oil less stable to oxidation than crude oil.

Refined, bleached, deodorized palm olein (RBDPOo) and groundnut (peanut) oils are widely available in Africa and Asia, where sesame oil also is produced and consumed in food and folk medicine. Sesame oil is sometimes adulterated with RBDPOo and groundnut oils, neither of which contains any sesamol or compounds that may release sesamol.

Different chromatographic methods were used to analyze the unsaponifiable lignans in sesame seed oil (11), and normal-phase HPLC was able to provide a good separation of sesamol. Chromatographic techniques are very useful but time-consuming. Prior to chromatography, several steps are necessary including saponification or hydrolysis and cleanup. In addition, many of the chemicals used in chromatography are hazardous to the analyst as well as the environment.

The use of FTIR spectroscopy is increasing in many fields (12) including food studies (13). IR studies of edible oils generally use specific bands to evaluate traditional indices and other parameters of interest in relation to the composition of edible oils (14–17). The objective of this study was to use FTIR spectroscopy to determine the sesamol content of sesame seed oil and sesamol spiked in RBDPOo and groundnut oils.

MATERIALS AND METHODS

Materials. Sesame seeds were purchased from a local retailer, and the oil was hexane-extracted from a ground mixture of the seed and sea sand. Mechanically pressed sesame seed oil was obtained from the National Oilseed Processing Research Institute (NOPRI), Sudan. Sesamol [3,4-(methylenedioxy)phenol] was purchased from Sigma Chemical Company (St. Louis, MO). Groundnut oil was obtained by solvent extraction of ground samples. RBDPOo was purchased from a local refinery.

Samples. Four different sets of samples were prepared; 20 groundnut oil samples, 20 RBDPOo samples, 20 hexane-extracted sesame oil samples, and 20 mechanically pressed sesame oil samples. Each set of samples was spiked with sesamol in the range of 0 to 100 mg/100 g oil. All samples were prepared in triplicate. The amount of sesamol in the unspiked sesame oil samples was determined by HPLC accord-

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

ing to Kamal-Eldin *et al*. (11) (2.0–5.4 mg/100 g oil), and the samples were then spiked with sesamol up to 100 mg/100 g oil. It was confirmed that the unspiked RBDPOo and groundnut oil samples did not contain sesamol.

FTIR spectra. The IR spectra were recorded with a PerkinElmer (Beaconsfield, Buckinghamshire, United Kingdom) 1725 series FTIR spectrometer equipped with a deuterated triglycine sulfate detector controlled by a PerkinElmer 7300 PC. The software used for FTIR data collection was the Infrared Data Management system (PerkinElmer). The instrument was purged with dry nitrogen, and automatic dehumidifiers were used to protect from interference by CO_2 and water vapor, respectively. The prepared samples were placed between sodium chloride (NaCl) windows, and the transmission path was fixed at 100 μm with a polytetrafluoroethylene spacer. The cell was then placed in the cell holder and the sample scanned at room temperature. After each scan, the NaCl windows were rinsed three times with acetone and dried with soft tissue before filling with the next sample. After each measurement, the cleaned window was checked spectrally to ensure that no residue of the previous sample remained on it. Calibration spectra were obtained by co-addition of 40 scans at 4 cm^{-1} resolution and a gain of 2.0 with strong Beer–Norton apodization over the frequency region 4000–500 cm^{-1}. The spectra were ratioed against a background air spectrum. Two spectra were collected from each of the prepared samples and stored in Joint Committee on Atomic and Molecular Physical Data–Data Exchange (JCAMP) files on diskette for subsequent analysis.

Calibration models for the prediction of sesamol content in the different types of oil samples from FTIR spectral data were obtained by partial least squares (PLS) regression using Spectrum V 3.02 software (PerkinElmer). In the PLS analyses, spectral data (predicted by the software using the FTIR spectra) and actual data on sesamol content were correlated, and the correlation coefficients (r) were taken as estimates of the factor scores, which were then used as regressors to model both spectral and actual data.

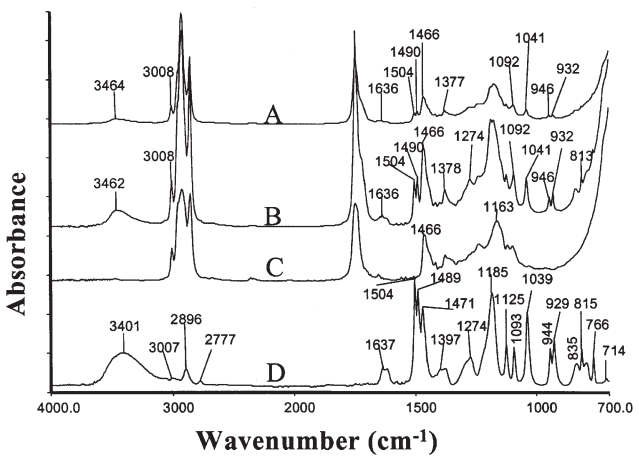

FIG. 1. FTIR spectra of (A) roasted sesame seed oil, (B) sesamol-spiked sesame oil, (C) sesame seed oil, and (D) sesamol.

The "leave-one-out" cross-validation technique was used to validate calibration models, and the accuracy of each model was assessed according to the standard error of prediction (SEP) and coefficient of determination (R^2). The FTIR method was further evaluated by computing the mean difference and SD of the differences for repeatability and accuracy between the predicted FTIR data and the actual sesamol content values in the oil samples.

RESULTS AND DISCUSSION

Figure 1 shows IR spectra of roasted sesame seed oil (A), sesamol-spiked sesame oil (B), sesame seed oil (C), and sesamol (D). The major peaks of sesamol are listed in Table 1. The sesamol-spiked sesame oil spectrum (A) is somewhat similar to the spectrum of the roasted sesame seed oil, which may be due to release of sesamol in roasted sesame seed (9).

The spectral response to changes in sesamol content was investigated by examination of the correlation and variance spectra. The correlation spectrum is calculated by multiplying the differences between each standard spectrum and the mean spectrum, at each wavelength, by the difference between the corresponding property concentration and the mean property concentration, and summing over all the standards. Peaks that do not correlate with the change in concentration are summed to zero, producing a spectrum that highlights the peaks that change with change in concentration, i.e., the peaks that relate to the property. The variance spectrum distinguishes between the active and inactive spectral regions. Thus, the correlation spectrum can be used to choose which

TABLE 1
Peak Assignments for Sesamol [3,4-(methylenedioxy)phenol] in Figure 1D

No.	Peak (cm^{-1})	Assignment	Remarks
1	3401	–OH	OH of phenolic O–H
2	3007	–CH	Unsaturated–CH
	2896	–CH	Saturated–CH
3	2777	$–CH_2$	Symmetric stretching
4	1637	Phenyl	Phenyl skeletal frequency
5	1504	Phenyl	Out-of-plane CH bending
	1489		
	1471	$–CH_2$	CH_2 bending (1480 cm^{-1})
6	1397	Methyl	Methyl symmetric bending
7	1274	C–O	C–O of phenolic OH
8	1185	C–H	In-plane bending of aromatic C–H
9	1125	C–O–C	Symmetric stretching
10	1093	–CH	Probably in-plane bending of phenolic CH enhanced by polar substituent
11	1039	=C–O–C	Symmetric stretching
12	944	C–O	Most characteristic for methylenedioxy (927 cm^{-1}), probably related to C–O stretching
	929		
13	835	–CH	CH of two adjacent hydrogens at 3,4 positions
14	815	–CH	Out-of-plane CH bending bands suggest adjacent hydrogen atoms
	796		
15	766		Di-substitution of phenyl
16	714	C–O–C	Weak bands, sometimes absent
	611		

TABLE 2
Calibration and Cross-Validation Statistics for the Calibration Models Developed for the Prediction of Sesamol Content of Sesame, Groundnut, and RBDPOo Oils[a]

	Calibration		Validation	
Method	R^2	SEC (mg/100 g)	R^2	SEP (mg/100 g)
MP sesame oil	0.9947	5.93	0.9897	5.30
Palm oil	0.9940	5.88	0.9598	4.31
Groundnut oil	0.9753	4.24	0.9586	3.96
SE sesame oil	0.9662	4.87	0.9527	4.17

[a]R^2, coefficient of determination; RBDPOo, refined, bleached, and deodorized palm olein; SEC, standard error of calibration; SEP, standard error of prediction for validation; MP, mechanically pressed; SE, solvent-extracted.

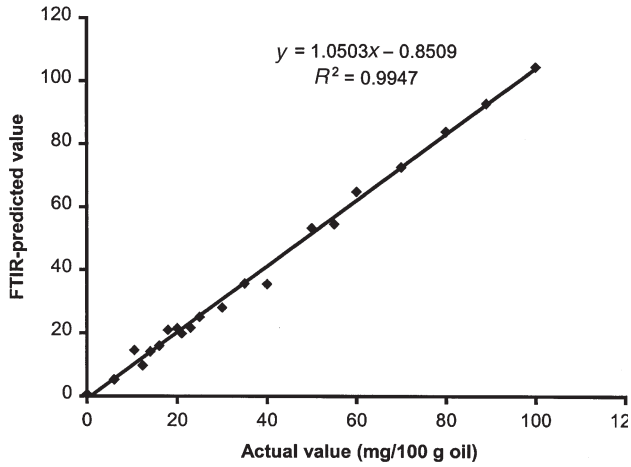

FIG. 2. A plot of the actual value of sesamol in sesame oil vs. FTIR-predicted values for the partial least squares calibration.

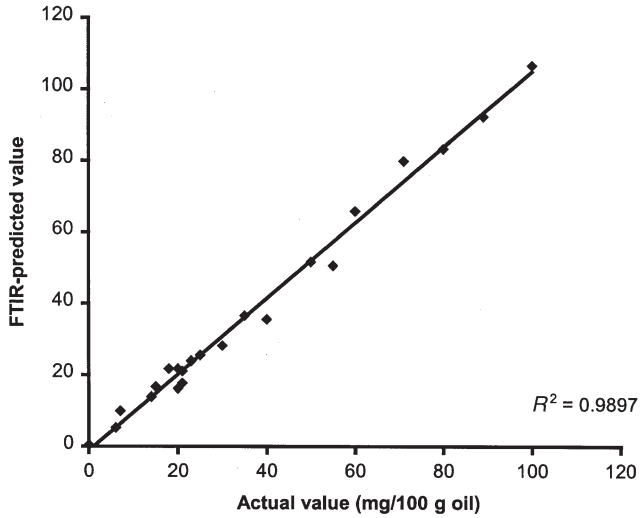

FIG. 3. Actual value vs. FTIR-predicted sesamol content for the cross-validation.

peak or region is selected for the calibration (18). The correlation spectrum was used to select regions that showed a mathematical correlation between spectral changes and the sesamol content in the oil samples, and the variance spectrum was used to distinguish between active and inactive spectral regions (19,20).

The spectral regions used in the calibration were set to include the data from 3650–3000, 1600–1450, and 1200–900 cm^{-1}, as suggested by the correlation and variance spectra. Table 2 shows the coefficients of determination (R^2), standard errors of calibration, and SEP obtained using these spectral regions. Figures 2 and 3 show plots for calibration and cross-validation, respectively, for the determination of spiked sesamol in sesame seed oil by FTIR spectroscopy using PLS statistical analysis. The intercepts and slopes of these plots are not significantly different ($P > 0.05$) from 0.0 and 1.0, respectively. The repeatability and accuracy of the FTIR method, as determined from the cross-validation data, are presented in Table 3.

This study shows that FTIR spectroscopy with the PLS statistical method can be used to determine the sesamol content in sesame seed oil. The analysis is rapid, requires only a minimal sample size (>2 mL), and avoids the use of chemicals.

ACKNOWLEDGMENTS

The authors thank the Universiti Putra Malaysia (UPM) for providing the funding (IRPA Grant 03-02-04-048) for this study. The first author is grateful to the National Oilseeds Processing Research Institute (NOPRI) of Sudan for allowing him the chance to read for his Ph.D. in UPM.

TABLE 3
Repeatability and Accuracy of FTIR Predictions of Sesamol Content Obtained by Cross-Validation[a]

Statistic	MP sesame oil	RBDPOo	Groundnut oil	SE sesame oil
MDr	2.46	1.38	1.45	2.03
SDD_r	–0.24	–0.09	–0.12	–0.18
MD_a	0.49	0.37	0.40	0.48
SDD_a	0.18	0.14	0.16	0.19

[a]MD, mean difference; SDD, SD of difference; r, repeatability; a, accuracy. For other abbreviations see Table 2.

REFERENCES

1. Tashiro, T., Y. Fukuda, T. Osawa, and M. Namiki, Oil and Minor Components of Sesame (*Sesamum indicum* L.) Strains, *J. Am. Oil Chem. Soc. 67*:508–511 (1990).
2. Latta, S., Gourmet Oils in the 1990s, *INFORM 2*:98–107 (1991).
3. Azhri, A., *Oil Crops of the World*, edited by G. Robbelen, R.K. Downey, and A. Azhri, McGraw-Hill, New York, 1989, p. 375.
4. Weiss, E.A., *Oilseed Crops, Tropical Agriculture Series*, Longman, London, 1983, p. 290.
5. Terrones, A., Sesame, Safflower Seek New Imputes in Mexico, *INFORM 1*:701–704 (1990).
6. Kamal-Eldin, A., and L.A. Appelqvist, Variation in Fatty Acid Composition of Different Acyl Lipids in Seed Oils from Four *Sesamum* Species, *J. Am. Oil Chem. Soc. 71*:135–139 (1994).
7. Fukuda, Y., T. Osawa, M. Namiki, and T. Ozaki, Studies on Antioxidative Substances in Sesame Seed, *Agric. Biol. Chem., 49*:301–306 (1885).
8. Fukuda, Y., M. Nagata, T. Osawa, and M. Namiki, Contribution of Lignan Analogues to Antioxidants Activity of Refined Unroasted Sesame Seed Oil, *J. Am. Oil Chem. Soc. 63*:1027–1031 (1986).
9. Fukuda, Y., M. Nagata, T. Osawa, and M. Namiki, Chemical Aspects of the Antioxidative Activity of Roasted Sesame Seed Oil, and the Effect of Using the Oil for Frying, *Agric. Biol. Chem. 50*:857–862 (1986).
10. Sudhir, S.D., U.S. Deshpande, and D.K. Salunkhe, Sesame Oil, in *Bailey's Industrial Oil and Fat Products*, 5th edn., Vol. 2, edited by Y.H. Hui, Wiley-Interscience, New York, 1996, pp. 466–474.
11. Kamal-Eldin, A., L.A. Appelqvist, and G. Yousif, Lignan Analysis in Seed Oils from Four *Sesamum* Species: Comparison of Different Chromatographic Methods, *J. Am. Oil Chem. Soc. 71*:141–147 (1994).
12. Ferraro, J.R., and L.J. Basile, *Fourier Transform Infrared Spectroscopy: Applications to Chemical Systems*, 4th edn., Academic Press, Orlando, 1985, 406 pp.
13. Gullin, M.D., and N. Cabo, Infrared Spectroscopy in the Study of Edible Oils and Fats, *J. Sci. Food Agric. 75*:1–11 (1997).
14. van de Voort, F.R., J. Sedman, G. Emo, and A.A. Ismail, Rapid and Direct Iodine Value and Saponification Number Determination of Fats and Oils by Attenuated Total Reflectance/Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc. 69*:118–1123 (1992).
15. Che Man, Y.B., and G. Setiowaty, Determination of Anisidine Value in Thermally Oxidized Palm Olein by Fourier Transform Infrared Spectroscopy, *Ibid. 76*:1–5 (1999).
16. Che Man, Y.B., and M.E.S. Mirghani, Rapid Method for Determining Moisture Content in Crude Palm Oil by Fourier Transform Infrared Spectroscopy, *J. Am. Oil Chem. Soc. 77*:631–637 (2000).
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. Mirghani, M.E.S., Y.B. Che Man, S. Jinap, B.S. Baharin, and J. Bakar, Multivariate Calibration of Fourier Transform Infrared Spectra for Determining Thiobarbituric Acid-Reactive Substance Content in Palm Oil, *J. Am. Oil Chem. Soc. 78*:1127–1131 (2001).
19. Fuller, M.P., G.L. Ritter, and C.S. Drapper, Partial-Least Squares Quantitative Analysis of Infrared Spectroscopic Data. Part I: Algorithm Implementation, *Appl. Spectrosc. 42*:217–227 (1988).
20. Fuller, M.P., G.L. Ritter, and C.S. Drapper, Partial-Least Squares Quantitative Analysis of Infrared Spectroscopic Data. Part II: Application to Detergent Analysis, *Ibid. 42*:228–236 (1988).

[Received October 24, 2001; accepted September 13, 2002]