Analytical, Nutritional and Clinical Methods

Determining α-tocopherol in refined bleached and deodorized palm olein by Fourier transform infrared spectroscopy

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Abstract

Fourier transform infrared (FTIR) spectroscopy was developed for the determination of α-tocopherol in refined bleached and deodorized (RBD) palm olein. The calibration and validation samples were prepared by spiking known amount of α-tocopherol to produce a wide range of α-tocopherol up to 2000 ppm. The method was based on the sodium chloride (NaCl) windows, and the transmission path was fixed at 50 μm at room temperature. The partial least squares (PLS) calibration models for predicting α-tocopherol were developed by using the FTIR spectral region at 3100–2750 cm^{-1}. The accuracy of the method was comparable to that of the high-performance liquid chromatography (HPLC), with coefficients of determination (R^2) from calibration samples of 0.9922. The models were validated and the R^2 of validation and the standard errors of prediction (SEP) computed. The standard deviation of difference for repeatability (SDD$_r$) for the method was comparable to that for HPLC. With its speed and ease of data manipulation by the computer software, FTIR spectroscopy is advantageous as a simple and rapid quantitative determining analytical tool for α-tocopherol in RBD palm olein.
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1. Introduction

Tocopherols are important minor components of oils and fats because of their antioxidant properties. They are especially important in RED palm olein because of the uniquely long shipment and storage times for the cooking oil (Wong, Timms, & Goh, 1988). Tocopherols are an effective antioxidant in lipids and lipid-containing food, because it is effectively scavenges peroxy radicals to form relatively stable products, which interrupt the propagation stage of the oxidative chain reaction (Fang & Wada, 1993; Michael, Kourimska, & Kourimska, 1995). Vitamin E, especially α-tocopherol, which has a highly important antioxidant function in vivo the cell membrane and is biologically the most active (Michael et al., 1995; Wong, Hashimoto, & Shibamoto, 1995). α-Tocopherol is a faster scavenger for peroxy radicals formed during auto-oxidation than γ-tocopherol and available for commercial used (Belitz & Grosch, 1999). Also it was more effective in increasing the oxidative stability and in reducing the relative rate of oxidation as the temperature increased (Gordon, 2000). On the contrary, α-tocopherol can act as a pro-oxidant rather than an antioxidant when present at high concentration (Belitz & Grosch, 1999; Makinen, Kamal-Eldin, Anna Maija, & Hopia, 2000). However, it was reported that no pro-oxidant effect was found for α-tocopherol when present at concentrations between 500 and 2000 ppm in purified oil (Makinen et al., 2000).

Current methods used for determining α-tocopherol are by gas chromatography (GC), high-performance liquid chromatography (HPLC) and spectrophotometry (Hammond, 1998). The increasingly widespread used

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of Fourier transform infrared (FTIR) in food industry indicate that it could be the technique of choice (Belton et al., 1995). The rationale for turning to use FTIR for quantitative analysis due to the generally rapid (1–2 min) method, can be automated and reduce the need for solvents and toxic reagents (van de Voort, Ismail, Sedman, & Emo, 1994). Furthermore, FTIR has been used to determine iodine value (Che Man, Setiowaty, & van de Voort, 1999), peroxide value in thermally oxidized palm olein (Setiowaty, Che Man, Jinap, & Moh, 2000), moisture content in palm oil and crude palm oil (Che Man & Mirghani, 2000) and aflatoxins in groundnut and groundnut cake (Mirghani, Che Man, Jinap, Baharin, & Bakar, 2001).

Multivariate calibration methods are more precise, more informative and faster than previous data analysis methods. In the process of calibration, the methods also estimate the precision of analysis, and yield important qualitative and diagnostic information, while the partial least squares (PLS) model created in the calibration was found to provide excellent results for a set of validation samples (Fuller, Ritter, & Drapper, 1988).

In this study, the PLS statistical methods were applied to FTIR spectra from a set of refined bleached and deodorized (RBD) palm olein samples to investigate the possibility to determine vitamin E concentration by focussing in one single isomer α-tocopherol.

2. Materials and methods

2.1. Sample and sample preparation

RBD palm olein without antioxidant was purchased from a local palm oil refinery. α-Tocopherol (99%) was purchased from Fluka Chemie AG, Buchs, Switzerland. All reagents were of analytical grade. α-Tocopherol in the oil was removed by passing it three times through a column of sand purified by acid fine (40–100 mesh, BDH, Poole, England) and silica gel 60 (70–230 mesh ASTM, Merck, Darmstadt, Germany), by modifying AOCS method Cd 20–91 (Aocs, 1993). The efficiency of this procedure was assessed by HPLC on the fresh oil and stripped oil was reduced from 178 to 80 ppm. Samples of α-tocopherol stripped oil were spiked by known amounts of α-tocopherol to concentration up to 2000 mg/kg (ppm) and used as standards for calibration and cross-validation.

2.2. Instrument analysis

2.2.1. HPLC analysis
The HPLC was used to determined α-tocopherol for RBD palm olein in the spiked samples using IUPAC method 2.432 (1992). The HPLC system used was equipped with UV visible detector with the wavelength 292 nm (SPD-10 AV, Shimadzu, Tokyo, Japan), HPLC pump (LC-10AT), a column oven (CTO-10A), Silica Column Shim-CLO-SIL 4×250 mm. The mobile phase was hexane/iso-propanal (99:1 v/v) with a flow rate of 1 mL/min.

2.2.2. FTIR analysis
Perkin–Elmer FTIR Spectrometer, Series 1725 X (Perkin–Elmer Corporation, Norwalk, CT, USA), with a room temperature deuterated triglycine sulfate (DTGS) detector controlled by a Perkin–Elmer Professional Computer, Model 7300 was used. The software used for FTIR data collection was the Infrared data management (IRDM) system. The instrument was maintained with two automatic dehumidifiers to minimize interference from water vapor.

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

2.3. Statistical multivariate analysis

All experiments and measurements were done in duplicate. The relationships between each of FTIR spectrum parameter and data from HPLC method were determined using the software program Nicolet Turbo Quant IR-Calibration and Prediction Package, Version 1.1 (Nicolet Instrument Co., Madison, WI).

2.4. Calibration development and validation

Fifty stripped oil samples spiked by known amounts of α-tocopherol to concentrations up to 2000 mg/kg (ppm) were separated into two sets, with 38 samples in the calibration sets and remaining 12 samples in the prediction set. PLS regressions were used to analyze the concentration data from the samples. Only the concentrations need to be known.

In PLS, the HPLC data and spectral data were correlated and correlation coefficients (R^2) were taken as estimates of the factor scores, which then were used as regressors to model both the spectral and chemical data.

3. Results and discussion

The samples of α-tocopherol stripped oil were spiked by commercial α-tocopherol for the FTIR calibration and HPLC analysis, and the data were used to estimate the calibration range.

3.1. Spectral analysis

Fig. 1 illustrates five overlayed FTIR absorbance spectra of samples containing α-tocopherol in RED palm olein in different concentrations. On the graphs shown, the only noticeable spectral differences are in 3700–3250 cm^{-1} as –OH stretching band. Fig. 2(a) shows the spectrum of α-tocopherol. The α-tocopherol spectrum exhibited absorption band at wavelengths 3473 cm^{-1} for OH (Che Man & Mirghani, 2000), 2927 and 2868 cm^{-1} for asymmetric and symmetric stretching vibrations of the CH_2 and CH_3 as reported by Guillen and Cabo (1997), 1461 cm^{-1} for phenyl skeletal (1450 cm^{-1}) and methyl asymmetric bending (1460 cm^{-1}), 1378 cm^{-1} for methyl symmetric bending, 1262 cm^{-1} for $–CH_2$, 1086 cm^{-1} for plane bending of phenyl and 919 cm^{-1} for trans $=CH_2$ stretching (Nakanishi & Solomon, 1977). Fig. 2(b) shows the different spectrum of spiked oil after it was subtracted by α-tocopherol stripped oil that reflects nearly the spectral features of α-tocopherol in Fig. 2(a). Three spectral regions, (3700–3250, 3100–2750, and 1470–910 cm^{-1}, respectively) were used to investigate in building the calibration models for α-tocopherol determination.

Fig. 2. (a) FTIR spectra of α-tocopherol. (b) The difference spectrum of spiked oil by α-tocopherol was subtracted from stripped oil.

3.2. Development of calibration and validation models

The calibration standards were designed to obtain data for the PLS regression, that is, as free as possible from interference by other component. In the case of mixtures, one of the major difficulties is the interference and overlapping of the absorption bands. This problem may be overcome by using powerful multi-component quantitative analysis as PLS (Wojciechowski, Dupury, Ta, Huvenne, & Legrand, 1998). Table 1 shows the result obtained from the PLS calibration in term of coefficient of determination (R^2), standard errors of calibration (SEC) and standard errors of prediction (SEP) used in choosing the best regions for determining α-tocopherol in RBD palm olein. It was found that FTIR spectral regions 3100–2750 cm^{-1} (CH_2 and CH_3 region) were the best for determining α-tocopherol with lowest SEC (53.54 ppm) and SEP (63.59 ppm) and highest R^2 (0.9922).

A correlation plot was established in building the model using that regions 3100–2750 cm^{-1} as shows a prediction in Fig. 3 by using the actual data from spiked value of striped oil that give the coefficient of determination (R^2) of 0.9900. While Fig. 4 shows a correlation plot
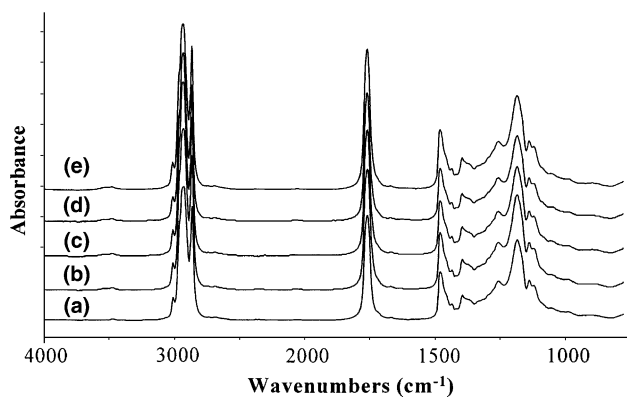

Fig. 1. FTIR spectra of: (a) α-tocopherols stripped oil. The known amount of α-tocopherol was spiked in stripped oil: (b) 500 ppm; (c) 1000 ppm; (d) 1500 ppm; (e) 2000 ppm.
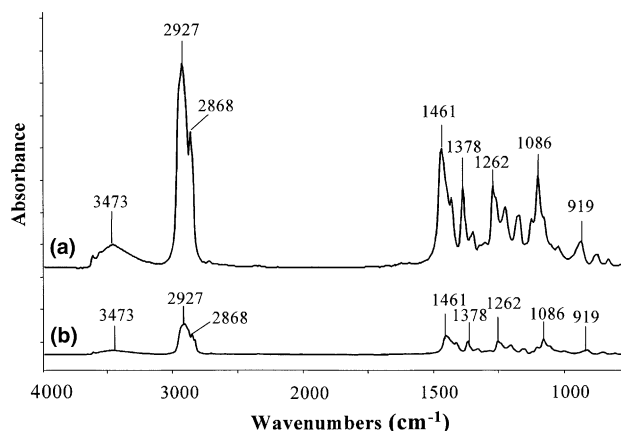
Table 1
Effect of different wavelength regions in developing calibration model for determining α-tocopherol content in RBD palm olein[a]

Region no.	Wavelength region (cm^1)	R^2	SEC	SEP
1	3700–3250	0.9673	104.90	123.90
2	3100–2750	0.9922	53.54	63.59
3	1490–910	0.9842	76.62	116.10
1 + 2	3700–3250 + 3100–2750	0.8989	178.90	133.10
2 + 3	3100–2750 + 1490–910	0.2023	495.40	305.80
1 + 3	3700–3250 + 1490 + 910	0.8072	250.80	115.00
1 + 2 + 3	3700–3250 + 3100–2750 + 1490–910	0.7463	287.7	186.80

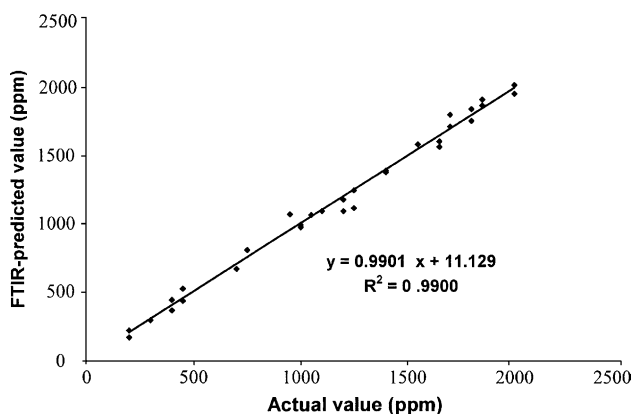[a] R^2, coefficient of determination; SEC, standard errors of calibration.

Fig. 3. Calibration plot of the actual data from 38 samples versus the PLS FTIR predicted values.
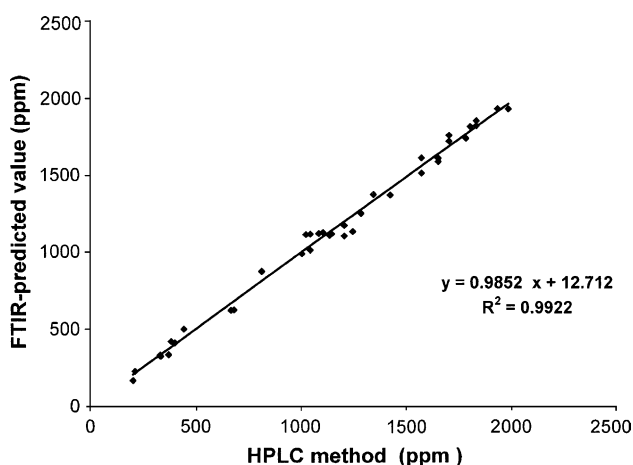

Fig. 4. Calibration plot of the HPLC analysis data from 38 samples versus the PLS FTIR predicted values.
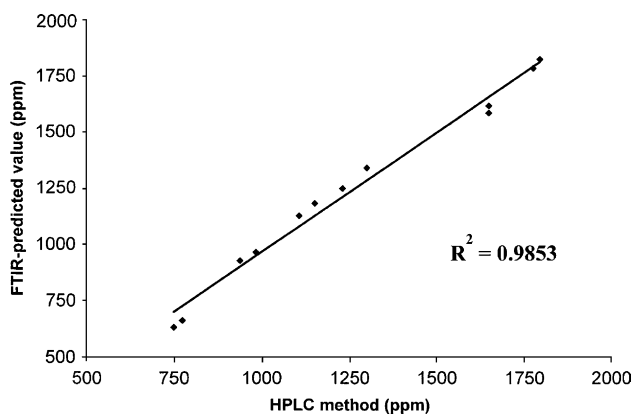

Fig. 5. Validation plot of the HPLC analysis data from 12 samples versus the PLS FTIR predicted values.

using the HPLC data set that give the coefficient value higher than actual data set with (R^2) of 0.9922. Fig. 5 shows a validation plot that test the calibration validity. In general, for n components to be analyzed, at least ($2n + 2$) standards are needed for the calibration (Wojciechowski et al., 1998). Table 2 shows the mean and standard deviation of the data obtained from the calibration and validation data set by HPLC analysis, the values were 1128.34, 16.86 and 1258.08, 17.42, respectively. While for the FTIR method the values were 1124.69, 15.34 and 1239.87, 16.63, respectively. The results of statistical analysis (Table 3) show the mean difference for repeatability (MD_r) were very low for both HPLC and FTIR methods, implying that there was little difference between them in the two series of analyses. The standard deviation of difference for repeatability (SDD_r), which measures variability around the mean difference (MD), was also very low in both FTIR and HPLC. The result in terms of the mean difference for accuracy (MD_a) and the standard deviation of difference for accuracy (SDD_a) indicated that α-tocopherol in RBD palm olein could be measured with good accuracy by FTIR spectroscopy. SEC relative to the mean value is expressed as the coefficient of variation (CV). The CV obtained using the PLS statistical method to predict FTIR results from the actual were 1.37%. A CV of <20% is sufficient for most analytical purpose as quoted by Che Man and Mirghani (2000).

From this study, we conclude that a new analytical method was developed for determination α-tocopherol in RBD palm olein by FTIR spectroscopy. To predict

Table 2

Calibration and validation statistics for α-tocopherol in RBD palm olein determined by HPLC analysis and FTIR spectroscopic methods using PLS of wavelength regions 3100–2750 (cm^{-1})[a]

Data set	HPLC method		FTIR method	
	Mean	SD	Mean	SD
Calibration	1128.34	16.86	1124.69	15.34
Validation	1258.08	17.42	1239.87	16.63

[a] HPLC, high performance liquid chromatography, FTIR, Fourier transform infrared spectroscopy; SD, standard deviation; all the data were the means from two replicates.

Table 3

Calibrations statistics for α-tocopherol content from data obtained by HPLC analysis and FTIR spectroscopy[a]

Statistic	HPLC method	FTIR method
MD_r	4.10	3.82
SDD_r	−1.78	−1.50
Min. value	158	160
Max. value	1983	1998
MD_a	3.65	
SDD_a	1.52	

[a] HPLC, high-performance liquid chromatography; MD, mean difference; SDD, standard deviation of difference; r, repeatability; a, accuracy.

the α-tocopherol content, it could be measured by utilization of $-CH_2$, $-CH_3$ absorption regions at 3100–2750 cm^{-1} by constructing the calibration model for good prediction of α-tocopherol content in RBD palm olein samples. From a practical viewpoint, FTIR quantitative analysis of the completed test can be performed in less than 2 min. In addition, FTIR analysis can reduced the need for solvents, hence environmental friendly.

Acknowledgements

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