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Original Article

FT-IR based methodology for quantitation of total tocopherols, tocotrienols and plastochromanol-8 in vegetable oils

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Abstract

Fingerprint infra-red spectra of oils from several samples of canola, flax, soybean and sunflower seeds were recorded. The tocopherols, tocotrienols and plastochromanol-8, cumulatively called chromanols, amounts were determined with high-pressure liquid chromatography (HPLC). The sum of tocopherols, tocotrienols and plastochromanol-8 amounts and the infra-red spectra were subjected to partial least-squares analysis. Excellent correlation was obtained between the calculated and experimental values. The developed methodology may provide an alternative method for rapidly scanning vegetable oils for vitamin E type molecules.

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1. Introduction

Tocopherols, tocotrienols and plastochromanol-8 are natural antioxidants found in vegetable oils. They are characterized by a substituted benzopyrane ring structure (Fig. 1) in which R₁, R₂ and R₃ can take up four distinct values of (CH₃, CH₃, CH₃), (H, CH₃, CH₃), (CH₃, H, CH₃) and (H, H, CH₃). The resulting compounds are referred to as alpha (α), beta (β), gamma (γ) and delta

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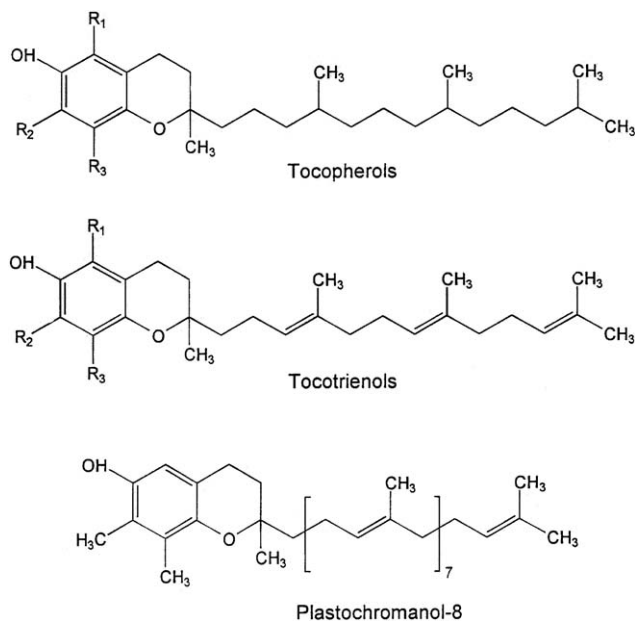


Fig. 1. Structures of tocopherols, tocotrienols and plastochromanol-8. R₁, R₂ and R₃ have combinations of (CH₃, CH₃, CH₃), (H, CH₃, CH₃), (CH₃, H, CH₃), and (H, H, CH₃) in α , β , γ and δ tocopherols/tocotrienols, respectively.

(δ) tocopherols/tocotrienols, respectively. These compounds possess vitamin E activity. Consumption of vitamin E has been shown to reduce the risk of coronary heart disease (Stampfer et al., 1993; Rimm et al., 1993) and heart attacks (Stephens et al., 1996). Tocopherols, tocotrienols and plastochromanol-8 in vegetable oils have been analyzed (Bourgeois, 1992) with high-pressure liquid chromatography (HPLC) or gas chromatography (GC). These methods are relatively complex, expensive and time consuming. In this paper we report a rapid, simple and inexpensive Fourier transform infra-red (FT-IR) methodology for quantification of total tocopherols, tocotrienols and plastochromanol-8 in canola, solin (low linolenic acid flax seed), soybean and sunflower oils. Our method avoids the use of toxic solvents and extends the applicability of FT-IR to the cases where a laboratory cannot afford a wide range of apparatus.

2. Materials and methods

2.1. Materials

Seventeen (four canola, five solin, four soybean, and four sunflower) oil samples were obtained from Canadian oil processors. α , δ and γ tocopherols were purchased from Sigma (St. Louis, USA) and had a stated purity of 98%.

2.2. Determination of tocopherols

Tocopherols, tocotrienols and plastochromanol-8 were analyzed by normal phase HPLC with fluorescent detection. A silica column, 5 μm , 3.2 mm \times 25 cm (Prodigy, Phenomenex, Torrance, CA) was used with *t*-butylmethyl ether in hexane (5% v/v) as the mobile phase. The flow rate was 0.7 mL/min and the column was held at 30°C. The fluorescent detector was set at 290 and 330 nm, respectively, for excitation and emission, and peak area used for quantification. External calibration was used for quantification of tocopherols, tocotrienols and plastochromanol-8. Each isomer of mentioned chromanols was calibrated separately from 5 to 1000 ppm. Content of plastochromanol-8 was calculated using γ -tocotrienol calibration. For all calibrated amounts of chromanol isomers coefficient of correlation of 0.99 was achieved.

2.3. Infra-red measurements

FT-IR spectra were recorded on a BIO-RAD (Cambridge, MA) FTS-135 spectrometer equipped with a KBr beamsplitter and a mercury cadmium telluride (MCT) nitrogen cooled detector. The spectra were recorded in the attenuated total reflection (ATR) mode with a Horizontal ATR accessory from PIKE Technologies, INC. (Madison, WI). The accessory was equipped with a ZnSe ATR crystal of a trapezoid shape and was 800 mm long, 10 mm wide and 4 mm thick. The crystal provided an angle of incidence of 45° and was enclosed in a stainless-steel cuvette. To record spectra, about 50 μL of oil or tocopherol sample was dissolved in 2 mL of hexane and the solution was poured on the ATR crystal and allowed to dry. This produced a uniform film on the ATR crystal whose single beam spectrum was recorded and then ratioed against the single beam spectrum of the bare crystal to obtain the absorbance spectrum. Spectra were recorded with a resolution of 4 cm^{-1} and 128 scans were averaged for each spectrum. The spectrometer's optics were sealed from the atmosphere but its compartment was not purged during measurements.

The spectra were run and processed with a BIO-RAD Win-IR™ program for Microsoft Windows. Partial least-squares (PLS) procedure was also performed using the Win-IR™ program. Absorbance spectra were converted to second derivative spectra prior to PLS analysis. Second derivative spectra were obtained with the Savitsky–Golay method (Savitzky and Golay, 1964). A fourth degree polynomial was utilized for derivative spectra with 15 convolution points.

3. Results and discussion

Representative fingerprint (1800–600 cm^{-1}) infra-red spectra of oils are shown in Fig. 2. The major peaks in these spectra arise from C=O stretching vibrations (1746 cm^{-1}), CH₃ and CH₂ scissoring vibrations (1377 and 1467 cm^{-1}), C–O stretching vibrations (1118, 1163 and 1238 cm^{-1}) and CH₂ rocking modes (723 cm^{-1}). These assignments are based upon previous work on vegetable oils (Guillen and Cabo, 1997). Although the fatty acid composition of oils is very different, these differences are not apparent in the infra-red spectra because the alteration in the fatty acid composition does not alter the population of non-equivalent functional groups significantly.

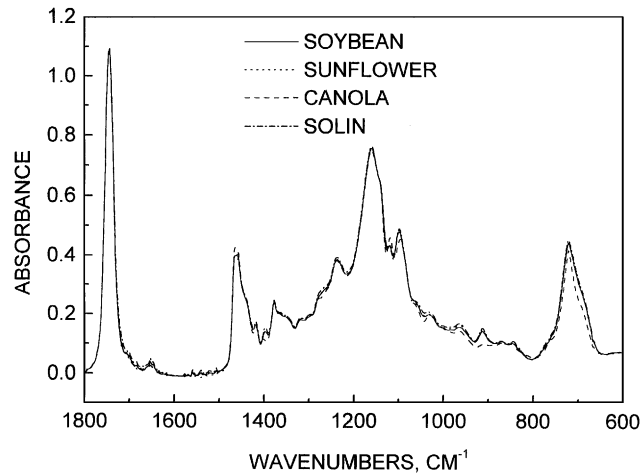


Fig. 2. Infra-red spectra of representative vegetable oils.

Table 1

Tocopherols, tocotrienols and plastochromanol-8 values ($\mu\text{g/g}$; ppm) in some vegetable oils obtained via HPLC

Sample	αT	γT	δT	P-8	$\alpha\text{-T3}$	Total
Canola	226	182	0.0	28	0.0	436
Canola	306	246	8	38	0.0	598
Canola	253	232	6	66	0.0	557
Canola	313	287	7	74	0.0	681
Solin	18	209	8	111	29	375
Solin	8	202	5	43	1	259
Solin	18	209	8	111	29	375
Solin	18	254	7	57	2	338
Solin	11	130	9	115	22	287
Soybean	118	567	248	0.0	0.0	933
Soybean	92	620	469	0.0	0.0	1181
Soybean	86	589	423	0.0	0.0	1098
Soybean	75	396	215	0.0	0.0	686
Sunflower	1000	5	1	0.0	0.0	1006
Sunflower	1097	11	0.0	0.0	0.0	1108
Sunflower	802	9	0.0	0.0	0.0	811
Sunflower	639	9	0.0	0.0	0.0	648

T = tocopherols, P = plastochromanol-8, T3 = tocotrienol.

The total amount of chromanols (Table 1) and the second derivative spectra (smoothing factor = 15) of oils were subjected to PLS analysis by utilization of the “leave-one-out” cross-validation technique. Various spectral regions were tried in the PLS procedure but only the 880–785 cm^{-1} region with 12 factors produced a reasonable correlation ($R^2 = 0.951$, $\text{RMSD} = 66.41$, Fig. 3). Examination of infra-red spectra of α , δ and γ tocopherols revealed that they all possess

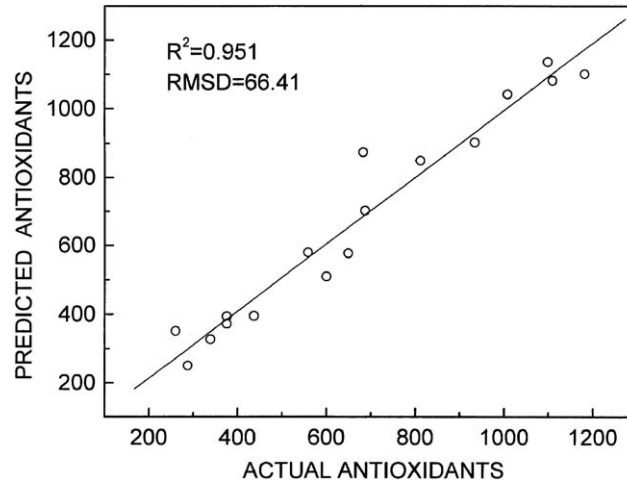


Fig. 3. PLS predicted and actual total antioxidant values in vegetable oils.

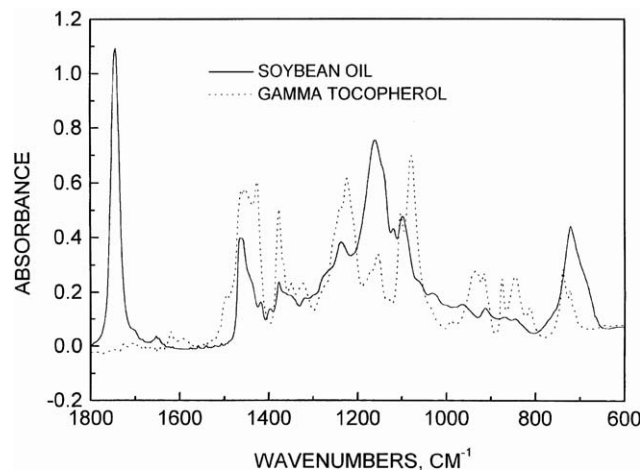


Fig. 4. Comparison of infra-red spectra of soybean oil and γ tocopherols.

bands in the $880\text{--}785\text{ cm}^{-1}$ region possible arising from vibrations of the benzopyrane ring structure. Infra-red spectra of tocopherols contained strong absorption throughout the $1500\text{--}1000\text{ cm}^{-1}$ region.

The reason that only the $880\text{--}785\text{ cm}^{-1}$ region succeeded in producing a reasonable PLS fit was found by comparing the spectra of oils with those of tocopherols. It is clear from Fig. 4 that the oil and γ -tocopherol vibrations overlap strongly in all regions except the $880\text{--}785$ and $1500\text{--}1800\text{ cm}^{-1}$ regions where the overlap is minimum. However, since tocopherols do not absorb strongly in the $1500\text{--}1800\text{ cm}^{-1}$ region only the $880\text{--}785\text{ cm}^{-1}$ region was successful in production of a calibration.

Tocopherols, tocotrienols and plastochromanol-8 are natural antioxidants and play an important role in vegetable oils by inhibiting lipid oxidation. Oxidation of lipids in oils affects their nutritional quality, physical appearance, flavor and safety, which are of concern to consumers (Rathjen and Steinhart, 1997). Because of this, efficient methods for determining antioxidants in oils are needed. Infra-red spectroscopy might serve as an alternative to HPLC and GC methods for quantification of total tocopherols, tocotrienols and plastochromanol-8; however, quantification of individual isomers would require separation with chromatographic methods. The FT-IR method might be used as a rapid screening tool, where it also has the additional advantage of not requiring the use of large quantities of hazardous solvents.

Sunflower oil does not contain δ -tocopherol or plastochromanol-8 (Table 1) and has only negligible amounts of γ -tocopherols. In this case infra-red spectroscopy could provide a direct measure of α -tocopherols in the sample.

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