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Determination of Tocopherol Contents in Refined Edible Oils Using an HPLC Method

– Research Note –

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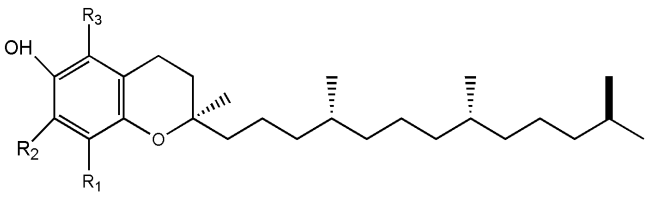
Abstract

A high-performance liquid chromatography method was applied to determine the contents of tocopherols in edible oils using a LiChrosorb DIOL HPLC column and hexane fortified with 0.1% acetic acid in an isocratic mode. The validation of the method included tests for linearity, sensitivity, accuracy, precision, and recovery. All calibration curves showed good linear regression (r^2>0.9995) within the tested ranges. The established method offered good precision and accuracy with overall intra-day and inter-day variations of 0.94～4.27 and 1.77～4.88%, respectively. The tocopherol recoveries ranged from 91.44～108.90%. Subsequently, the method was successfully applied to qualitatively and quantitatively determine the total contents of α, γ, and δ-tocopherols in 12 selected refined edible oils, showing a range of 0.92 to 188.71 mg/100 g.

Key words: refined edible oils, tocopherols, HPLC separation

INTRODUCTION

Recently, tocopherol compounds have been well recognized as effective antioxidants both endogenously and as additives in most vegetable oils and fats (1-3). Different concentrations of tocopherol compounds in oils are reported to have diverse functions for the oxidative stability of the oils (4,5). There are eight different tocopherol compounds in vegetable oils, including α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols, with varying contents, of which α, γ, and δ-tocopherols are considered the most effective forms (6). Fig. 1 shows their chemical structures. Besides their antioxidant effects, tocopherols have also demonstrated activities against cardiovascular diseases (7), cancer (8), protein cross-linking and DNA mutations (9), inflammation (10), and hemostasis function (11).



Tocopherols	R_1	R_2	R_3
α-tocopherol	$-CH_3$	$-CH_3$	$-CH_3$
γ-tocopherol	$-CH_3$	$-CH_3$	-H
δ-tocopherol	$-CH_3$	-H	-H

Fig. 1. Chemical structure of tocopherol compounds.

Due to their contribution to oil oxidative stability and health benefits, the determination of tocopherol compounds in vegetable oils has importance. To date, some chromatographic determinations of tocopherol compounds have been conducted in oils using HPLC methods with either normal-phase systems or reversed-phase systems (12-14). Normal-phase systems are only suitable for the direct analysis of cooking oils and fats, since apolar normal-phase eluents are good solvents for these samples (15). Usually, an alkane (hexane, heptane, isooctane), an ether (tetrahydrofuran, methyl, *tert.*-butyl, isopropyl), or a chlorohydrocarbon (dichloromethane, chloroform) selectively make up normal-phase eluent systems. Cyano, amino, and silica columns are most commonly used (16). Reversed-phase systems for tocopherol compounds primarily use pure methanol or methanol-water mixtures containing up to 10% water as the mobile phase besides other solvents such as acetonitrile, isopropanol, and ethanol (17). The Octadecylsilane (ODS, C18)-modified silica column as well as the C30 stationary column are generally employed in the reverse phase system (18). The reverse-phase HPLC method usually resolves most of the analytical problems related to tocopherol compound analysis in a more simple and rapid manner. Compared to the normal-phase HPLC method, the main advantages of the reverse-phase HPLC method are its fast equilibration time and better reproducibility of retention times (19).

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Although many methods have been reported for the determination of tocopherol compounds in oils, few studies have validated an analytic method using commercial oil products (18,19). Gliszczyńska-Świgło et al. attempted to validate a reverse-phase HPLC method for the determination of tocopherols in edible oils. However, accuracy and recovery tests were not completely achieved in their report (19). In the present study, an efficient HPLC-UV method was completely validated. Subsequently, the validated method was applied to qualitatively and quantitatively determine the total contents of α, γ, and δ-tocopherols in 12 selected refined edible oils.

MATERIALS AND METHODS

Materials and reagents

The following 12 refined edible oils were purchased from local markets in South Korea: rice bran oil, canola oil, soybean oil, rapeseeds oil, olive oil, corn oil, coconut oil, wheat germ oil, apricot kernel oil, macadamia oil, avocado oil, and almond oil. HPLC-grade hexane was purchased from Fisher Scientific (Norcross, GA, USA). α, γ, and δ-tocopherol standards and pyrogallol were provided by Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade.

Sample preparation

Six percent pyrogallol in ethanol (5 mL) and 60% KOH in distilled water (1.25 mL) were added to 1.0 g of each oil sample in a 25-mL vial. Once flushed under N_2 gas flow, the screwed vial was placed in a water-bath shaker at 70°C and 200 rpm. After 30 min, the sample was cooled at room temperature. The tocopherols were extracted 3 times with 5 mL of 2% NaCl solution and 5 mL of solvent (hexane : ethyl acetate, 85:15) containing 0.05% BHT. The upper layer was combined and blown to dryness under N_2 gas and then the residue was dissolved in 5 mL of hexane. The solution was filtered through a membrane syringe filter (13 mm, 0.45 μm) before injecting 10 μL into the HPLC system.

The standard stock solutions of α, γ, and δ-tocopherols were prepared in hexane and further diluted to appropriate volumes in ranges to be used as working standard solutions. All prepared standard solutions were stocked at -10°C prior to use and for no longer than 1 week.

HPLC-UV analytic method

The quantitative analysis of α, γ, and δ-tocopherols in each sample was performed using a Hewlett Packard HPLC series 1100 (Agilent Technologies, Little Falls, DE, USA), equipped with a quaternary pump, vacuum degasser, autosampler, and UV detector. The HPLC system was connected to Agilent Chemstation Software. A LiChrosorb DIOL HPLC column (100×3 mm, 5 μm, Varian, CA, USA) was used for the separation. The isocratic elution system consisted of hexane fortified with 0.1% acetic acid. The flow rate was 0.8 mL/min. The detection wavelength was set at 295 nm and column temperature was kept at 30°C.

Method validation

The validation procedure was performed according to the method of Zhang et al. with slight modification (20). Briefly, to verify the linearity, calibration standards of six concentrations for the α, γ, and δ-tocopherols, ranging from 8.29 to 265.5, 8.98 to 287.5, and 9.76 to 312.5 μg/mL, respectively, were prepared. The calibration standard curves were then constructed. Meanwhile, the limit of detection (LOD) and limit of quantitation (LOQ) under the present chromatographic conditions were defined at signal to noise (S/N) ratios of 3 and 10, respectively. The precision of the proposed method was determined by intra- and inter-day assays using the authentic standards with three different concentrations. The intra-day accuracy and precision were assessed from the results of 5 replicate analyses of the standards with three different concentrations on a single day. The inter-day accuracy and precision were determined from the same three analytes on 5 consecutive days. The precision was expressed as the % relative standard deviation (RSD), and the accuracy was defined as the detected mean value/actual value×100%. The recovery experiments were performed by adding the spiked standard solutions of three different concentrations into rapeseed oil, from which the tocopherols were extracted by the same method mentioned above.

Statistic analysis

The values are expressed as means±standard deviations (SD). The significance of differences among the means was analyzed using Statistical Analysis System Software (SAS, 2000 Cary, NC, USA). The tested significance level was set at $p<0.05$.

RESULTS AND DISCUSSION

Method development

Tocopherols are integral components of the unsaponifiable matter present in most vegetable oils and fats. Saponification is carried out by the treatment of a sample in a strong alkali environment. This decreases the interference of other materials that are extracted together with the tocopherols into the organic phase (18). Considering that tocopherols are easily oxidized during such a saponification process, adding an antioxidant is

necessary to avoid further oxidation. In this study, 6% pyrogallrol was used as the antioxidant. The mobile condition in HPLC was also studied, indicating that hexane fortified with 0.1% acetic acid provided excellent separation with a short time. The UV wavelength set at 295 nm obtained a maximum absorbance. Fig. 2 shows the stable baselines of the completely saponified samples and no impurity interfered with the tocopherol separation according to the chromatogram. Based on the similar retention times with each spiked standard peak, the peaks of α, γ, and δ-tocopherols in the oil samples were well identified. As shown in Fig. 2(E), after complete saponification, the rapeseed oil showed only three peaks (α, γ, and δ-tocopherol) on the chromatogram. From these results, it was proven that the method conditions were suitable to determine the tocopherol contents of the oil samples.

Method validation

The calibration curves were achieved from 6 different concentrations of spiked standards ranging from 8.29 to 265.50 μg/mL for α-tocopherols, 8.98 to 287.50 μg/mL for γ-tocopherols, and 9.76 to 312.50 μg/mL for δ-tocopherols, respectively. A high regression coefficient ($r^2 \geq 0.9995$) value for each calibration curve indicated excellent linearity in this study (Table 1). To reach a detectable probability, the α, γ, and δ-tocopherol standards should be diluted to low concentrations until the signal-to-noise (S/N) ratio reaches the requirement of 3 (for LOD) or 10 (for LOQ) (17). Under our analytical conditions, the LODs of the α, γ, and δ-tocopherols were determined to be 0.19, 0.10, and 0.48 μg/mL while the LOQs were 0.78, 0.48, and 0.97 μg/mL, respectively (Table 1). Therefore, the proposed method provided a satisfying linearity and good sensitivity.

Table 2 summarizes the intra- and inter-day accuracy and precision for the α, γ, and δ-tocopherol assays. The intra-day RSD values of the α, γ, and δ-tocopherols were 3.77, 4.27, and 4.18%, while the inter-day RSD values were 4.69, 4.88, and 4.74%, respectively. The accuracy values offered an acceptable range, suggesting that this HPLC-UV method is accurate and precise.

The extraction efficiency of α, γ, and δ-tocopherols from the refined edible oils was evaluated by recovery tests. Table 3 shows the absolute recoveries of each tocopherol from the rapeseed oil after spiking different amounts of the standards. The recovery of α-tocopherol ranged from 96.16 to 108.90% within an RSD value of 0.62%, while the recovery of γ-tocopherol ranged from

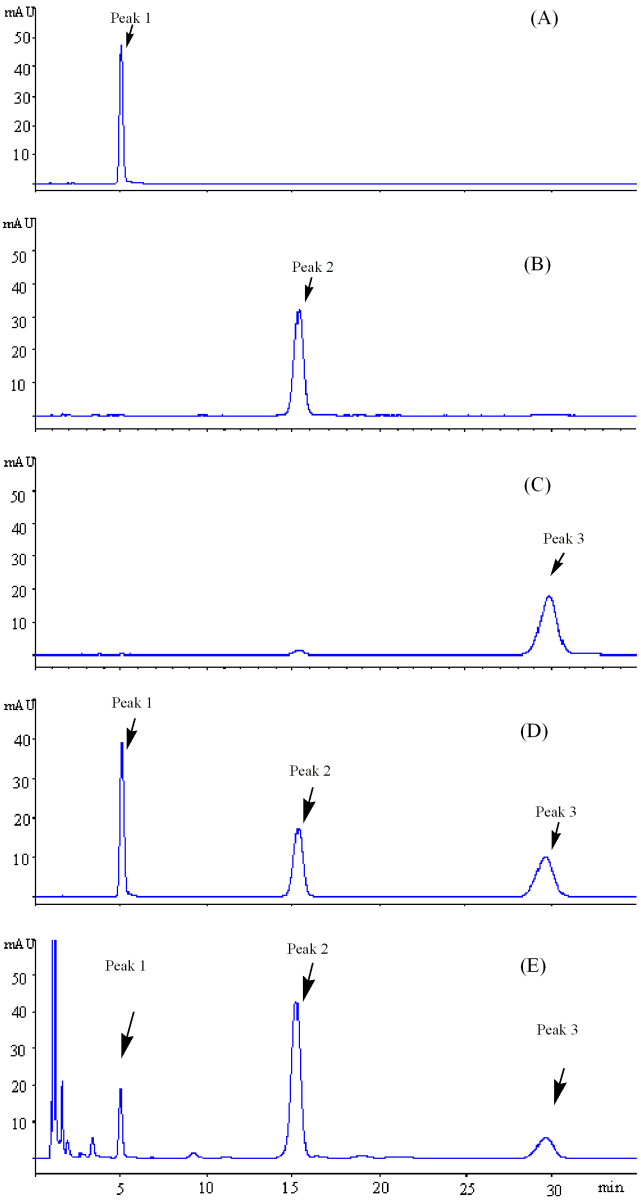

Fig. 2. HPLC chromatograms of α, γ, and δ-tocopherols. (A), α-tocopherol standard; (B), γ-tocopherol standard; (C), δ-tocopherol standard; (D), mixed standards; and (E), rapeseed oil as a representative. Peak 1, α-tocopherol; Peak 2, γ-tocopherol; and Peak 3, δ-tocopherol.

Table 1. Linearity data for the determination of α, γ, and δ-tocopherols with the proposed HPLC-UV method

Tocopherols	Retention time (min)	Calibration curves	Regression coefficient (r^2)	Test range (μg/mL)	Limit of detection (μg/mL)	Limit of quantitation (μg/mL)
α-tocopherol	5.00	y=5.5996x+23.8124	0.9996	8.29～265.50	0.19	0.78
γ-tocopherol	13.21	y=6.4355x+32.0858	0.9995	8.98～287.50	0.10	0.48
δ-tocopherol	29.24	y=4.6174x+25.8872	0.9995	9.76～312.50	0.48	0.97

Table 2. Intra- and inter-day variability for the assay of α, γ, and δ-tocopherols by the HPLC-UV method

Tocopherols	Concentration (μg/mL)	Intra-day (n=5)			Inter-day (n=5)		
		Detected (μg/mL)	RSD (%)	Accuracy (%)	Detected (μg/mL)	RSD (%)	Accuracy (%)
α-tocopherol	31.25	30.83±1.16	3.77	98.68	32.37±1.52	4.69	103.57
	155	156.53±2.59	1.73	100.98	158.06±4.12	2.61	101.97
	325	321.51±3.02	0.94	98.92	325.71±8.70	2.67	100.22
γ-tocopherol	40	40.50±1.73	4.27	103.58	40.73±1.98	4.88	104.16
	200	195.25±4.89	2.50	97.62	195.25±4.89	2.51	97.62
	410	401.58±5.49	1.36	97.94	404.23±7.15	1.77	98.59
δ-tocopherol	40	41.06±1.17	4.18	102.64	41.37±1.96	4.74	103.43
	200	202.11±5.66	2.80	101.05	203.17±7.03	3.46	101.59
	410	420.16±12.86	3.06	102.47	420.68±14.16	3.37	102.60

Table 3. Determination of the recoveries of α, γ, and δ-tocopherols in rapeseed oil

Tocopherols	Original amount (mg/100 g)	Spiked amount (mg/100 g)	Total amount detected (mg/100 g)	RSD (%)	Recovery (%)
α-tocopherol	14.51	5.66	19.96±0.10	0.50	96.16
		11.34	26.87±0.15	0.55	108.90
		22.59	37.68±0.23	0.62	102.56
γ-tocopherol	27.12	4.93	31.62±0.46	1.45	91.44
		9.86	36.18±0.72	1.98	96.97
		19.64	45.29±0.38	0.83	92.50
δ-tocopherol	0.92	5.17	6.07±0.22	3.70	99.70
		10.36	10.88±0.34	3.10	98.50
		20.62	20.21±0.82	4.00	93.56

91.44 to 96.97% within an RSD value of 1.98%. δ-tocopherol also showed a high recovery at 93.56% within an RSD value of 3.70%.

Quantitative analysis of α, γ, and δ-tocopherols in refined edible oils

Table 4 shows the contents of α, γ, and δ-tocopherols in 12 selected refined edible oils. Among these 12 oils, wheat germ oil contained the highest amount of α-tocopherol (127.42 mg/100 g), comprising 67.52% of the total tocopherols (188.71 mg/100 g), followed by apricot kernel oil with 49.60 mg/100 g. High levels of γ-tocopherol, which is reported to have strong activity in plant seeds (21), were found in the soybean oil (61.23 mg/100 g) and wheat germ oil (55.99 mg/100 g). However, γ-tocopherol was not detected in the avocado and macadamia oils. Meanwhile, the soybean oil contained 18.23 mg/100 g of δ-tocopherol and the rapeseed oil, corn oil, wheat germ oil, and avocado oil contained limited amounts. With respect to the total tocopherols, the coconut oil showed the lowest amount (0.92 mg/100 g) while the highest amount was found in the wheat germ oil (188.71 mg/100 g). Interestingly, no tocopherols were de-

Table 4. Determination of α, γ, and δ-tocopherol contents (mg/100 g) in refined edible oils[1)]

Refined oils	α-tocopherol	γ-tocopherol	δ-tocopherol	Total tocopherols
1. Rice bran oil	17.59±1.21^{c}	17.38±2.18^{e}	ND[2)]	34.98±3.39^{e}
2. Canola oil	14.11±0.15^{d}	24.90±0.41^{d}	ND	39.02±0.26^{de}
3. Soybean oil	10.18±0.66^{e}	61.23±3.13^{a}	18.23±1.98^{a}	89.65±4.97^{b}
4. Rapeseed oil	14.51±0.34^{d}	27.11±0.74^{d}	0.91±0.03^{c}	42.55±1.11^{d}
5. Olive oil	13.78±1.63^{d}	0.29±0.15^{f}	ND	14.07±1.78^{g}
6. Corn oil	17.71±0.28^{c}	33.91±1.12^{c}	1.28±0.26^{c}	52.92±1.66^{c}
7. Coconut oil	0.09±0.03^{g}	0.82±0.01^{f}	ND	0.92±0.01^{h}
8. Wheat germ oil	127.42±2.57^{a}	55.99±2.01^{b}	5.29±0.13^{b}	188.71±4.45^{a}
9. Apricot kernel oil	49.60±1.47^{b}	3.45±0.09^{f}	ND	53.06±1.56^{c}
10. Macadamia oil	ND	ND	ND	ND
11. Avocado oil	2.86±0.07^{f}	ND	0.78±0.07^{c}	3.65±0.15^{h}
12. Almond oil	16.31±0.69^{cd}	3.33±0.01^{f}	ND	19.65±0.69^{f}

[1)]The values are expressed as the means±SD of duplicates. Values with different letters in the same column are significantly different ($p<0.05$).
[2)]ND: not detected.

tected in the macadamia oil. According to a report by Kaijeser et al., less than 0.11 mg/100 g of tocopherols were found in macadamia nuts grown in different areas (22). The probable reason was due to the loss of the tocopherols during the production process. Our results are in good agreement with other references, which presented similar tocopherol amounts in refined oils (18,19,23).

CONCLUSION

There are many reports of assays of tocopherols in natural plants using HPLC-UV methods; however, to our knowledge, few studies have completely validated an analytic method with commercial oil products. In the present study, an HPLC-UV method was validated as an effective approach for the qualitative and quantitative determination of α, γ, and δ-tocopherol concentrations in refined edible oils. The assay was sensitive, precise, and reproducible. By the examination of 12 refined edible oils obtained from Korea markets, we found the highest level of α-tocopherol in wheat germ oil (127.42 mg/100 g) while soybean oil was a rich source of γ and δ-tocopherols, presenting 61.23 and 18.23 mg/100 g, respectively. Alternatively, no traces of tocopherols were found in macadamia oil.

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