Tocopherol and Tocotrienol Contents of Vegetable Oils, Margarines, Butters, and Peanut Butters Consumed in the Korean Diet

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Abstract The tocopherol and tocotrienol contents of 39 vegetable oils, margarines, butters, and peanut butters typically consumed in the Korea diet were determined by direct solvent extraction method followed by normal phase liquid chromatography. All samples were locally obtained in the Cheongju area in 2003 and 2004. The study examined data on 19 vegetable oils, 10 margarines, 6 butters, and 4 peanut butters. γ-Tocopherol (γ-T) and α-tocopherol (α-T) were detectable in all products except butters and were usually present in the vitamin E form in greater quantity than the other isomers. Usually, γ-T content was higher than α-T content in many samples. Tocotrienols were measurable in some samples but usually at levels less than the corresponding tocopherols. Three analytical method validation parameters, accuracy, precision, and specificity, were calculated to ensure the method's validity and were all confirmed to be reliable and satisfactory.

Keywords: vitamin E, tocopherol, tocotrienol, HPLC, α-tocopherol equivalent (α-TE)

Introduction

Vitamin E consists of four tocopherols (α-, β-, γ-, and δ-T) and the corresponding tocotrienols, in which RRR-α-T is the most biologically active form. Vitamin E is widely distributed in foods. It is synthesized only by plants and, therefore, is found primarily in plant products, the richest source being plant oils (1).

Many analytical methods have been used for the analysis of tocopherols and tocotrienols. At the present time, the method of choice for vitamin E analysis is high performance liquid chromatography (HPLC) because it is simpler, more precise, and more specific than other methods, and has the ability to separate all 8 homologs (2). Using HPLC, the most critical and time consuming step is the extraction of vitamin E from the samples. In order to release vitamin E, various extraction methods, depending on the nature of the sample, have been applied. This may involve saponification, enzymatic hydrolysis, Soxhlet extraction, or direct solvent extraction (3). Tocopherols and tocotrienols in animal and vegetable oils are directly determined after appropriate dilution of the sample with *n*-hexane or suitable solvents. Saponification has been widely used to extract tocopherols and tocotrienols from many samples including erythrocytes, animal tissues and organs, plants, foods, and animal feeds. Another extraction method, developed by Landen (4) for general application to fat-soluble vitamin extraction, has seen much recent use for analysis of vitamin E from a variety of foods. Landen's extraction has been adapted by several investigators in the development of methods for vitamin E analysis from various matrices (5-8).

Reports on vitamin E content in foods have been confusing because vitamin E activity was based either on a total tocopherol basis (mg/100 g) or only an α-tocopherol basis. The best way to report vitamin E activity would be to quantify all eight isomers and then convert to α-T equivalents (α-TE) (2). Tocotrienol should be quantified along with tocopherols since it also exhibits hypo-cholesterolemic activity and anticarcinogenic effects (9, 10). Moreover, since new chromatographic methods of analysis have been developed, advanced data on vitamin E composition in oils, fats and their processed products is required by researchers in nutrition, food science, dietetics, and other areas. Therefore, the object of this study was to provide reliable vitamin E data for oils, fats, and peanut butters in the Korean diet. This study reports data on 19 vegetable oils, 10 margarines, 6 butters, and 4 peanut butters. Three validation parameters, accuracy, precision, and specificity, were also calculated for the complete analytical methods.

Materials and Methods

Sampling and pretreatment All samples were obtained locally in the Cheongju area in 2003 and 2004. The samples were stored frozen at -60°C until assayed. Before analysis, the samples were thawed in the refrigerator at 2-8 °C overnight prior to mixing. The samples were removed from the refrigerator and allowed to sit for 12 hr. All assays were performed in duplicate.

Standard preparation Tocopherol and tocotrienol standards were obtained from Merck (Darmstadt, Germany). Purity and stability of the standards were monitored by $E^{1\%}_{1cm}$ values (11) measured by using a Spectrophotometer (DU® 650; Beckman Instruments Inc., CA, USA). Concentrations were calculated from the peak area determined by integrator (M807-IT; Jasco International Co., Japan) and linear regression.

HPLC quantitation The normal phase HPLC system

consisted of a solvent delivery pump (M930; Young Lin Instrument Inc., Korea) equipped with a spectrofluoro-metric detector (LC305; Thermo Separation Products Inc., CA, USA) and Lichrosorb Si60 column (250×4 mm, 5 μm, Hibar Fertigsaube RT, Darmstadt). The isocratic mobile phase contained 0.9% isopropanol in n-hexane (J.T. Baker Chemical Co., Phillipsburg, NJ, USA). The flow rate was 1.0 mL/min. The mobile phase was filtered using a 0.22 μm nylon membrane filter (MSI Inc., Westboro, MA, USA) and de-gassed by stirring under vacuum. The wavelengths were set at 290 nm for excitation and 330 nm for emission for the determination of tocopherols and tocotrienols. Tocopherol and tocotrienol peaks were identified by comparison of retention times to the standards (12).

Direct solvent extraction for peanut butter Peanut butter was accurately weighed to 0.4 g and added to a 125 mL, round bottom, glass bottle. Hot water (80°C, 4 mL) was added to the sample which was then mixed with a spatula. Ten milliliters of isopropanol were added to the mixture. Approximately 5 g of anhydrous magnesium sulfate was added followed by 25 mL extracting solvent (hexane: ethyl acetate, 90:10, v/v) containing 0.01% butylated hydroxytoluene (BHT). The mixture was homo-genized with Polytron® homogenizer for 1 min and the Polytron was rinsed with 5 mL of extracting solvent. The mixture was filtered through a medium porosity glass filter using a vacuum bell jar filtration apparatus (Knots, Vineland, NJ, USA). The vacuum was released and the filter cake was broken with a spatula, washed with 5 mL of extracting solvent and transferred to the same 125 mL round bottom glass bottle for the repeat extraction. Five milliliters of isopropanol and 30 mL of extracting solvent were added to the mixture followed by homogenization and filtration. The combined filtrate was transferred to a 100 mL volumetric flask and diluted to volume with extracting solvent followed by filtration using a 0.45 μm nylon membrane filter (MSI Inc.). A 1.0 mL aliquot of the combined filtrates was evaporated with nitrogen gas and then diluted to the appropriate concentration of analytes with mobile phase. All steps were carried out under yellow light and all solvents were HPLC grade (13).

Extraction method Margarine and butter (2.5 g) were weighed into 125 mL Erlenmeyer flask to which 40 mL of the extracting solvent was added, then sonicated with intermittent mixing until the sample material had dissolved. The sides of the flask were rinsed with 10 mL of the extracting solvent and 5 g of anhydrous MgSO₄ was added. After the mixture was allowed to stand for 3 hr, the solution was filtered by using medium porosity, fritted glass filter and bell jar filtration apparatus. The filter was washed with the extracting solvent and the filtrate was transferred to a 100 mL volumetric flask and diluted to volume with the extracting solvent (14). The vegetable oil sample was diluted with mobile phase containing 0.01% BHT and injected into the HPLC system.

Calculation of α-TE (α-tocopherol equivalent) α-TE values were determined by multiplying the amount (milligrams) of α-T by 1.0, of β-T by 0.5, of γ-T by 0.1, of

δ-T by 0.03, of α-T3 by 0.3, and of β-T3 by 0.05. γ-T3 and δ-T3 were not included in the α-TE calculation because their biological activities are unknown. α-, β-, γ-, and δ-T and α-, β-, γ-, and δ-T3 indicate the corresponding tocopherols and tocotrienols, respectively. Therefore, α-TE was determined as follows (2).

$$\alpha\text{-TE} = (\alpha\text{-T mg/100 g} \times 1.0) + (\beta\text{-T mg/100 g} \times 0.5) + (\gamma\text{-T mg/100 g} \times 0.1) + (\delta\text{-T mg/100 g} \times 0.03) + (\alpha\text{-T3 mg/100 g} \times 0.3) + (\beta\text{-T3 mg/100 g} \times 0.05)$$

Method validation Prior to data collection, the analytical methods were validated for use on margarine and peanut butter by determining the accuracy (recovery), precision (repeatability and reproducibility), and specificity (peak purity). For the recovery, the amount of vitamin E isomers was added to the sample corresponded to 50-150% of expected vitamin E. Recovery was calculated by the following equation:

$$R\% = [(C_s\text{-}C_p)/C_a] \times 100$$

where, R (%) = percentage recovery of added standard, Cs = tocopherol content in the spiked sample, Cp = tocopherol content in the sample, Ca = tocopherol standard added.

Peak purity was determined by the procedure described by Haroon et al. (15) for fluorescence response. Peak heights of the standard were determined at excitation wavelengths of 270, 280, and 290 nm, while keeping the emission wavelength constant at 330 nm. Then, the ratios of the peak heights from the samples were compared with the peak ratios of the standards obtained at the same wavelengths.
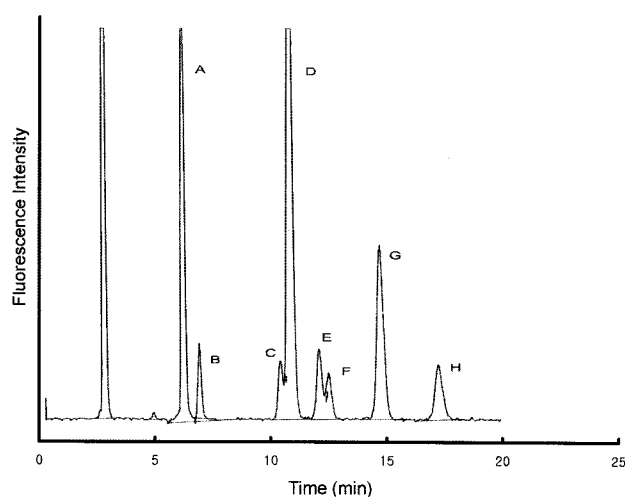

Fig. 1. Normal-phase LC chromatogram of the standard using fluorescence detector (Ex 290 nm, Em 330 nm), flow rate of 1.0 mL/min, injection volume of 20 μL, and mobile phase of 0.8% isopropanol in hexane. (A: α-tocopherol, B: α-tocotrienol, C: β-tocopherol, D: γ-tocopherol, E: β-tocotrienol, F: γ-tocotrienol, G: δ-tocopherol, H: δ-tocotrienol).
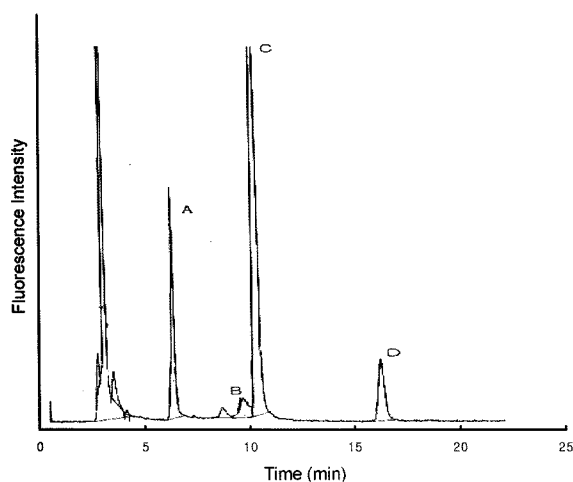
Fig. 2. Normal-phase LC chromatogram of margarine using fluorescence detector (Ex 290 nm, Em 330 nm), flow rate of 1.0 mL/min, injection volume of 20 μL, and mobile phase of 0.8 % isopropanol in hexane. (A: α-tocopherol, B: β-tocopherol, C: γ-tocopherol, D: δ-tocopherol).

Results and Discussion

Method validation Three analytical method validation parameters, accuracy, precision, and specificity were calculated to ensure the overall method's validity. The HPLC chromatograms of standard and sample are presented in Fig. 1 and 2, respectively. Repeatability and reproducibility of the assay were assessed for the samples of margarine and peanut butter (Table 1). The coefficient of variation (CV) of repeatability and reproducibility was less than or around 5% except for β-T. The relatively higher CV for β-T may have been due to its lower content in the samples. The accuracy was evaluated by measuring the recovery. The percentage mean recoveries ± standard deviation (SD, n=5) in margarine were 101.94 ± 1.91 for α-T, 99.98 ± 0.47 for β-T, 96.28 ± 3.29 for γ-T, and 91.42 ± 2.37 for δ-T. The percentage mean recoveries ± SD (n= 5) of peanut butter were 97.09 ± 4.01 for α-T, 97.29 ± 10.19 for β-T, 94.56 ± 2.51 for γ-T, and 106.72 ± 1.01 for δ-T (Table 1). The recovery values for most of the margarine and peanut butter components were above 90%

Table 1. Three parameters for the precision and accuracy of assay

Tocopherols	Parameters	Precision		Accuracy [1]
		Repeatability [2]	Reproducibility [3]	Recovery (%)
Margarine				
α-Tocopherol	Mean [4]	10.78	10.72	101.94
	SD [5]	0.22	0.28	1.91
	CV, % [6]	2.04	2.60	1.88
β- Tocopherol	Mean	0.10	0.13	99.98
	SD	0.01	0.02	0.47
	CV,%	5.27	18.23	0.47
γ- Tocopherol	Mean	35.34	35.20	96.28
	SD	0.96	1.40	3.29
	CV, %	2.72	3.98	3.42
δ- Tocopherol	Mean	4.95	4.94	91.42
	SD	0.29	0.29	2.37
	CV, %	5.81	5.86	2.60
Peanut butter				
α-Tocopherol	Mean	9.30	10.43	97.09
	SD	0.14	0.50	4.01
	CV, %	1.46	4.79	4.13
β- Tocopherol	Mean	0.85	1.00	97.29
	SD	0.13	0.16	10.19
	CV, %	15.65	16.01	10.48
γ- Tocopherol	Mean	7.46	8.58	94.56
	SD	0.28	0.09	2.51
	CV, %	3.75	1.01	2.66
δ- Tocopherol	Mean	0.47	0.57	106.72
	SD	0.02	0.04	1.01
	CV, %	4.46	6.24	0.95

[1] Accuracy is a measure of the closeness of the analytical result to the value evaluated by analyzing a spike sample.
[2] Repeatability refers to the results of independent determinations carried out on a sample by analyzing 5 replicates of the sample on the same day.
[3] Reproducibility refers to the results of independent determinations carried out a sample by analyzing 5 replicates of the sample at different periods of time.
[4] n=5 (mg/100 g).
[5] Standard deviation.
[6] Coefficient of variation.

Table 2. Evaluation of peak purity by fluorescence response ratio[1,2]

		Peak response ratios	
		Standard	Sample
α-T[3]	280/290	0.57	0.62
	270/290	0.24	0.25
	280/270	2.52	2.56
γ-T	280/290	0.55	0.57
	270/290	0.20	0.21
	280/270	2.70	2.76
δ-T	280/290	0.53	0.55
	270/290	0.20	0.20
	280/270	2.75	2.78

[1]Emission wavelength was constant (330nm).
[2]Fluorescence ratios shown were calculated by dividing the values for the two peak heights for each analyte obtained from separate chromatographic runs at two different excitation wavelengths.
[3]Corresponding tocopherols.

or close to 100%, indicating almost complete extraction. The results of peak purity test are presented in Table 2. The values obtained from the samples and standard were very close, indicating the purity of the tocopherol peaks. Generally, the results of the three validation parameters were reliable and satisfactory for the overall analytical method.

Determination of tocopherols and tocotrienols The tocopherol and tocotrienol composition of the 19 vegetable oils, 10 margarines, 6 butters, and 4 peanut butters are shown in Table 3. Each sample showed differences in all tocopherol and tocotrienol levels and α-TE levels. Vitamin E contents in foods may be affected by heating, storage, refining, and other processing. In this study, tocopherol isomers were detectable in all products except butters. In all of the vegetable oils, α- and γ -T occurred at high levels. Sunflower oil showed the highest α-T content (48.92 mg/100 g), followed by safflower oil (43.87 mg/

Table 3. Tocopherols and tocotrienols contents in vegetable oils, margarines, butters, and peanut butters (mg/100 g)[1]

	α-T[2]	β-T	γ-T	δ-T	α-T3	β-T3	γ-T3	δ-T3	Total	α-TE[3]
Vegetable oils										
Soybean A[4]	9.47	1.19	68.50	22.26	-[5]	-	-	-	101.42	17.58
B	5.14	0.8	66.61	23.19	-	-	-	-	95.74	12.90
Sunflower A	38.61	0.73	4.17	0.28	-	-	-	-	43.79	39.40
B	48.92	0.57	1.33	0.4	-	-	-	-	51.22	49.35
Safflower A	43.87	0.18	0.53	0.22	-	-	-	-	44.80	44.02
B	40.53	0.77	1.42	2.97	-	-	-	-	45.69	41.15
Corn A	16.17	0.46	54.43	2.09	-	-	-	-	73.15	21.91
B	14.91	0.27	32.19	1.10	-	-	-	-	48.47	18.30
Grape seed A	23.21	0.07	2.77	Tr[6]	14.86	-	10.69	Tr	51.60	27.98
Olive A	13.08	0.07	0.71	-	-	-	-	-	13.86	13.19
B	19.09	0.2	0.3	-	-	-	-	-	19.59	19.22
Rice bran A	15.89	0.23	4.93	-	5.08	-	-	-	26.13	18.02
Perilla A	3.20	0.34	120.69	1.86	-	-	-	-	126.09	15.49
B	3.02	0.29	121.07	1.04	0.55	-	0.91	4.91	131.79	15.47
Sesame A	3.15	-	40.2	1.46	-	-	-	-	44.81	7.21
Pepper seed A	8.58	0.20	85.33	0.70	-	-	-	-	94.81	17.23
B	16.56	0.14	97.08	1.12	-	-	-	-	114.90	26.37
Margarines										
Regular										
Vegetable A	9.83	0.45	25.70	7.52	-	-	-	-	43.50	12.85
B	10.89	0.40	35.13	10.75	2.74	0.63	3.52	0.72	64.77	15.78
Corn A	12.24	0.47	37.85	5.71	-	-	-	-	56.27	16.43
B	11.15	0.53	38.10	5.81	-	-	-	-	55.59	15.40
Low-fat										
Vegetable A	8.74	0.30	22.40	6.54	-	-	-	-	37.98	11.33
B	4.60	0.36	18.39	5.83	1.53	-	1.05	-	31.74	7.24
Corn A	10.85	0.32	33.96	3.39	-	-	-	-	48.52	14.51
B	11.35	0.36	37.80	5.43	0.37	-	0.53	-	55.83	15.58
Imported low fat A	5.61	-	2.89	1.07	6.98	0.62	9.79	1.09	28.04	8.05
B	5.87	0.12	52.92	13.89	-	-	-	-	72.79	11.64

Table 3. continued

	α-T [2]	β-T	γ-T	δ-T	α-T3	β-T3	γ-T3	δ-T3	Total	α-TE [3]
Butters										
Regular A	1.30	-	-	-	-	-	-	-	1.30	1.30
B	1.39	-	-	-	-	-	-	-	1.39	1.39
With salt A	1.38	-	-	-	-	-	-	-	1.38	1.38
B	1.49	-	-	-	-	-	-	-	1.49	1.49
Garlic A	1.33	-	-	-	-	-	-	-	1.33	1.33
B	1.63	-	-	-	-	-	-	-	1.63	1.63
Peanut butters										
Chrunky A	8.95	0.22	12.29	1.29	-	-	-	-	22.75	10.33
B	7.86	0.14	8.81	0.82	-	-	-	-	17.63	8.83
Creamy A	8.52	0.34	11.35	1.51	-	-	-	-	21.72	9.87
B	11.05	0.13	9.41	0.94	-	-	-	-	21.52	12.08

[1] All samples were assayed in duplicate.
[2] Corresponding tocopherols and tocotrienols.
[3] α-Tocopherol equivalent.
[4] A, B: different sample brand name.
[5] Not detected.
[6] Trace amount (less than 0.01 mg/100 g).

100 g), grape seed oil (23.21 mg/100 g), corn oil (21.91 mg/100 g), and olive oil (19.22 mg/100 g). The highest level of γ-T was found in perilla oil (121.67 mg/100 g), followed by soybean oil (68.50 mg/100g), and corn oil (54.43 mg/100g). α-TE levels in vegetable oils ranged from 7.21 to 49.35 α-TE/100 g. Similar results were reported by Eitenmiller and Lee (2). McLaughlin and Weihrauch (16) reported that total vitamin E content ranged from 3.58 in coconut oil to 390 mg/100g in orange flavedo palm oil. In margarines, γ-T was higher than α-T in all of the samples except imported low fat margarine. α-TE levels in margarines ranged for from 8.05 to 16.43 α-TE/100 g. The highest α-TE level was in corn margarine (16.43), followed by vegetable margarine (15.78) and low-fat corn margarine (15.58). Slover *et al.* (17) reported that margarines ranged from 0.3 to 24.3 mg/100g α-T and from 1.4 to 25.7 α-TE/100 g. In butters, α-T was detected only at low levels in all of the samples and ranged from 1.30 to 1.63 mg/100 g, with α-TE levels ranging from 1.30 to 1.63 α-TE/100 g. Garlic butter had the highest level at 1.63 α-TE/100 g. Peanut butters contained only tocopherol isomers and ranged from 8.83 to 12.08 α-TE/100 g.

This study has provided vitamin E values expressed for each isomer, total and α-TE for fats, oils, and peanut butters. The vitamin E isomers found in relatively high quantity were α-T and α-T. Fats, oils, and peanut butters provide good sources of vitamin E for the Korean consumer.

Acknowledgments

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