Application of Direct Solvent Extraction to the LC Quantification of Vitamin E in Peanuts, Peanut Butter, and Selected Nuts

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ABSTRACT

Direct solvent extraction with hexane:ethyl acetate (90:10, v/v), saponification, and Soxhlet extraction with hexane were evaluated for their usefulness as extraction methods to determine vitamin E in peanuts and peanut butters. The direct solvent extraction procedure yielded higher values for each tocopherol homolog in peanut and peanut butter compared to the other methods. Coupling of the rapid, direct solvent extraction with normal phase chromatography on Si 60 provides a highly reproducible procedure to quantitate vitamin E in peanuts and peanut products. The overall % recoveries (means ± S.D.) of this study for α-, β-, γ-, and δ-tocopherol were 99.9 ± 3.29, 100.4 ± 11.7, 98.9 ± 5.94, and 100.3 ± 4.87, respectively. Limits of detection and limits of quantitation were 0.21, 0.06, 0.11, and 0.08 ng and 0.39, 0.14, 0.23, and 0.13 ng, respectively, for α-, β-, γ-, and δ-tocopherol. Repeatability, reproducibility, and accuracy of the methods were excellent with % CVs for accuracy of 1.19 (α-T), 1.55 (γ-T), and 2.69 (δ-T). Accuracy was not acceptable for β-T due to its extremely low concentration in the peanut and because peak purity was not obtained for β-T. The method was applied to other seeds with good success. Major homologs were γ-T in peanuts, pecans, cashews, walnuts, and pistachio; α-T in almonds; and α-T3 in macadamia.

Key Words: HPLC, nutrition, saponification, seeds, Soxhlet extraction.

Vitamin E consists of a group of eight homologs, four tocopherols (α-T, β-T, γ-T, and δ-T), and the corresponding tocotrienols (α-T3, β-T3, γ-T3, and δ-T3), in which RRR-α-tocopherol is the most biologically active form. By definition, biological activity is given in terms of RRR-α-tocopherol equivalents (α-TE). One α-TE is equal to 1 mg of RRR-α-tocopherol. The factors to convert milligrams of the other tocopherols and tocotrienols to milligrams of RRR-α-tocopherol to get α-TE units are, for example, β-T, 0.5; γ-T, 0.1; δ-T, 0.03; α-T3, 0.3; β-T3, 0.05. The biological activities of γ-T3 and δ-T3 are unknown (Pryor, 1995). Vitamin E is essential for normal growth and development, and deficiency leads to clinical abnormalities. Vitamin E prevents cell damage by preventing *in vivo* peroxidation and is thought to be a preventative factor for inflammation, cardiovascular disease, cancer, various neurodegenerative diseases—including Alzheimer's disease and other disease states involving oxidative stress (Huang *et al.*, 1986; Meydani *et al.*, 1990; Byers and Perry, 1992; Gey, 1993; Evans, 1994; Bowling and Beal, 1995; Knight, 1997; Manton *et al.*, 1997; Sayre *et al.*, 1997).

The most significant sources of vitamin E in the U.S. diet are fats and oils. Eitenmiller (1997) recently cited USDA statistics that show that salad and cooking oils, margarine, salad dressings, mayonnaise, and shortening provide 26.9% of the vitamin E in the U.S. diet. Increased use of reduced fat products without vitamin E addition to such products could negatively affect vitamin E intake. Fruits and vegetables contain low to moderate levels of vitamin E, providing a significant and consistent source (more than 15%) of the vitamin E. Nuts and seeds are rich sources of vitamin E compared to fruits and vegetables, but they contribute less than 5% to the diet because they are less frequently consumed due to the consumer's perception that they are a significant source of fat (Bauernfiend, 1980; Murphy *et al.*, 1990). However, nuts and seeds account for only about 2.5% of the total fat intake in the U.S. diet (Dreher *et al.*, 1996). Nuts and seeds are generally low in saturated fatty acids and high in mono- and polyunsaturated fatty acids (Dreher *et al.*, 1996; Sabate and Hook, 1996). Favorable fatty acid composition, therefore, makes peanuts and other seeds

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desirable replacements for foods containing higher levels of saturated fatty acids.

Recent research suggests that there is an inverse relationship between frequent nut and seed consumption and coronary heart disease (Fraser *et al.*, 1992). The protective effect of nuts and seeds in relation to occurrence of coronary heart disease may be related to their fatty acid composition and other intrinsic components such as folate, vitamin B-6, trace elements including selenium, arginine, and vitamin E (Sabate and Hook, 1996). Vitamin E plays a role as an antioxidant and, therefore, prevents and/or delays lipid oxidation, including oxidation of low density lipoprotein (Esterbauer *et al.*, 1991). Due to the antioxidant effect of vitamin E in nuts and seeds and to increasing nutritional interest in these foods, more complete data on the vitamin E content of nuts and seeds are needed.

The objective of this study was to improve methodology to quantify tocopherols and tocotrienols in peanuts, peanut products, other seeds, and nuts. In this study, direct solvent extraction, saponification, and Soxhlet extraction methods were compared as preparatory steps for the LC quantification of tocopherols and tocotrienols in peanut and peanut butter samples; and the results have been evaluated to determine the optimal extraction procedure for vitamin E homologs. Validation parameters are provided for the analytical method.

Materials and Methods

Sampling and Pretreatment. Dry roasted, unsalted peanuts (*Arachis hypogaea* L.), regular and low fat peanut butter, and other seeds and nuts including almond, pistachio, macadamia, walnut, pecan, and cashew were purchased from retail stores in Athens, GA. Nuts (50 g) were ground in a coffee mill for 30-60 sec and analyzed immediately after grinding.

Standard Preparation. Tocopherol standards were obtained from Sigma (St. Louis, MO). Purity and stability of standards were monitored by $E^{1\%}_{1cm}$ values (Table 1) measured by using a DU-64 spectrophotometer (Beckman Instruments Inc., Fullerton, CA). Accurately, 25 mg of each homolog was weighed and dissolved in 25 mL of hexane containing butylated hydroxytoluene (BHT; 0.01%, w/v). Appropriate dilutions were made with the mobile phase to give a stock standard concentration of 18.54, 0.51, 20.70, and 3.30 μg/mL for α-, β-, γ-, and δ-T, respectively. For a daily working standard, 1 mL of the stock standard solution was diluted into a 50-mL volumetric flask with mobile phase. The working standard concentration of α-, β-, γ-, and δ-T was 0.37, 0.01, 0.41, and 0.07 μg/mL, respectively. Concentrations of the tocopherol homologs were calculated from peak area determined by the Waters 764 integrator (Millipore Corp., Cary, NC). Peak responses for tocopherols were used to quantify the corresponding tocotrienols (Thompson ans Hatina, 1979).

Table 1. Specific absorption coefficients ($E^{1\%}_{1cm}$) and maximum wavelengths (λmax) for tocopherols (Scott, 1978).

Homologs	λ_{max} nm	$E^{1\%}_{1cm}$
α-Tocopherol	294	71.0
β-Tocopherol	297	86.4
γ-Tocopherol	298	92.8
δ-Tocopherol	298	91.2

Direct Solvent Extraction. The direct solvent extraction procedure used in this study was modified from the method developed by Landen (1982). In the original method for infant formula, samples were dehydrated with anhydrous magnesium sulfate and the lipid-soluble components were extracted with isopropanol and methylene chloride. High performance gel permeation chromatography was used to fractionate fat-soluble vitamins from the lipid material. In the present study, samples of 0.4 g were accurately weighed into a 125-mL round-bottom glass bottle. Hot deionized water (80 C, 4 mL) was added to the sample and then mixed with a spatula. Ten milliliters of isopropanol was 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% BHT. The mixture was homogenized with a Polytron® homogenizer for 1 min at medium speed and the homogenizer was rinsed with 5 mL extracting solvent. The mixture was filtered through a medium porosity glass filter using a vacuum bell jar filtration apparatus (Kontes, Vineland, NJ). The vacuum was released and the filter cake was broken with a spatula and washed with 5 mL extracting solvent. The filter cake was 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., Westboro, MA). A 1.0-mL aliquot of the combined filtrates was evaporated with nitrogen gas and then made to the appropriate concentration of analytes with mobile phase. All steps were carried out under yellow light and all solvents were HPLC grade.

Saponification. Ten milliliters of ethanol containing pyrogallol (6% w/v) was added to each sample (0.2 g) in a saponification vessel and agitated to avoid agglomeration. After sonicating for 10 min, 2 mL of 60% potassium hydroxide in deionized water (freshly prepared) was added and the vessel was flushed with nitrogen gas for 1 min. After attachment of an air condenser, contents were digested at 70 C for 30 min in a shaker water bath. Following cooling in an ice bath, 20 mL of 2% sodium chloride in deionized water was added and the mixture was extracted three times with 10 mL of extracting solvent (hexane:ethyl acetate, 90:10, v/v) containing 0.01% BHT. The extracting solvents were collected into a 50-mL volumetric flask. The volume was adjusted to 50 mL with extracting solvent followed by filtration using 0.45-1m nylon membrane filter. A 1.0-mL aliquot of the combined filtrates was evaporated with nitrogen gas and then made to the appropriate concentration of analytes with mobile phase.

Soxhlet Extraction. A portion of the ground sample (3 g) was mixed with anhydrous sodium sulfate at a ratio of 1:4 (sample/sodium sulfate, w/w) and blended until mixed thoroughly. Five grams of the mixture was extracted in a Soxhlet apparatus with hexane containing 0.01% BHT. The extractions were done in the dark for 4 hr. The extract was used directly for vitamin E analysis (Hashim *et al.*, 1993 a).

HPLC Quantitation. The normal phase HPLC system consisted of a Shimadzu LC-6A pump equipped with a

Shimadzu RF-10A spectrofluorometric detector (Shimadzu Corp.), a Spectra series AS100 autosampler (Thermo Separation Products Inc., San Jose, CA), and a 25-cm × 4 mm, 5-1m Lichrosorb Si60 column (Hibar Fertigsaube RT. Darmstadt, F.R. Germany) equipped with a precolumn packed with Perisorb A 30-40-μm (Darmstadt, F.R. Germany). The isocratic mobile phase contained 0.6% isopropanol in *n*-hexane (J.T. Baker Chemical Co., Phillipsburg, NJ), and 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) 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 tocopherol and tocotrienol homologs. For recovery studies, 2 mL of the stock standard solution was added to the sample with the extracting solvent. The spiked levels of α-, β-, γ- and, δ-T were 18.55, 0.53, 20.70, and 3.30 mg/100-g sample, respectively.

Statistical Analysis. Statistical analysis (two-way ANOVA) was performed by using the Statistical Analysis System (SAS, 1990). Means were compared by the Duncan's test at α = 0.05.

Results and Discussion

Extraction. Saponification, direct solvent extraction, and Soxhlet extraction were studied for the extraction of vitamin E components from peanuts and peanut butter (Table 2). The tocotrienols were not detected in peanut samples. There were significant differences between the three methods for the determination of tocopherols ($P < 0.01$). The α-TE values from the three methods also were significantly different ($P < 0.01$). The highest values of α-TE were observed from direct solvent extraction and the lowest values from saponification. The α-TE values obtained by direct solvent extraction of the peanut samples were higher than the values from Soxhlet extraction and saponification by 20.0 and 35.8%, respectively. For peanut butter, the α-TE values from direct solvent extraction were higher than Soxhlet extraction and saponification by 3.3 and 21.8%, respectively. Lavedrine *et al.* (1997) compared three extraction methods for the determination of tocopherols in walnuts using reversed-phase LC with UV-visible detection at 292 nm. The three methods were saponification, Soxhlet extraction, and acid hydrolysis prior to extraction with a Soxhlet apparatus. Small differences in tocopherol levels were found by the three methods. The highest level of α-T was obtained from the acid hydrolysis and the lowest level of α-T was determined using Soxhlet extraction.

Table 2. Assay values of tocopherols in peanuts and peanut butter using three different extraction methods.

	Extraction Method[a]		
	Saponification	Direct solvent extraction	Soxhlet extraction
	mg/100 g sample[b] (% recovery)		
Peanut			
α-T[c]	2.59 a (86.7)	3.55 b (96.7)	2.94 c (91.3)
β-T	0.13 a (117.0)	0.14 a (91.3)	0.12 a (102.0)
γ-T	6.10 a (98.0)	8.04 b (98.7)	6.94 c (100.7)
δ-T	0.46 a (87.3)	0.59 b (101.7)	0.54 b (106.3)
α-TE[d]	3.27 a	4.44 b	3.70 c
Peanut Butter			
α-T	7.92 a (89.0)	9.54 b (97.0)	9.34 b (99.7)
β-T	0.19 a (97.0)	0.38 b (104.7)	0.21 a (106.0)
γ-T	7.85 a (94.0)	9.78 b (97.0)	9.36 c (101.7)
δ-T	0.68 a (93.0)	0.85 b (102.7)	0.78 c (101.7)
α-TE	8.82 a	10.74 b	10.40 c

[a]Values in the same row that are followed by the different letter are significantly different ($P < 0.01$).

[b]Data represent a mean (n = 3).

[c]Corresponding tocopherols.

[d]α-Tocopherol equivalent.

For the determination of vitamin E using HPLC, the most critical and time-consuming step is the quantitative extraction of all vitamin E forms from samples. Depending on the nature of the sample, various extraction methods have been applied to release the vitamins. In many cases, Soxhlet extraction and saponification have been used for the extraction of vitamin E from nuts and seeds (Piironen *et al.*, 1986; Fourie and Basson, 1989; Yao *et al.*, 1992; Hashim *et al.*, 1993a,b). Saponification has been applied to many matrices including erythrocytes, animal tissues and organs, plants, foods, and animal feeds (Eitenmiller and Landen, 1995, 1999). However, saponification can result in problematic emulsion formation which makes efficient analyte recovery difficult. Further, if saponification parameters and conditions are not properly controlled, analyte degradation can rapidly occur (Eitenmiller and Landen, 1995; 1999; Ye *et al.*, 1998). During saponification, care was taken to protect samples from light by using yellow light and from atmospheric oxygen by flushing containers with nitrogen gas at each step. The antioxidants, pyrogallol in the saponification media, and BHT in the extracting solvent were provided. However, in our study, the recoveries obtained from peanuts and peanut butter by saponification were slightly lower compared to both Soxhlet extraction and direct solvent extraction. Such lower recoveries may have been due to the effect of soap from fatty acids in the saponified media after saponification (Ueda and Igarashi, 1987).

Originally, Landen (1982) developed an LC method for the determination of retinyl palmitate and α-tocopheryl acetate in infant formulas using the direct solvent extraction method that we employed in this study. Recently, Chase *et al.* (1997) determined all-rac-α-tocopheryl acetate, tocopherols, and retinyl palmitate in fortified infant formula, SRM 1846, using a slight modification of Landen's procedure. In their method, the hexane was substituted for methylene chloride and the gel permeation chromatographic clean up step was eliminated. Data provided in Table 2 show that the direct solvent extraction method gave higher values for each tocopherol in peanuts and peanut butter; therefore, it was chosen for further application in this study.

Method Validation. Using the direct solvent extraction method and normal phase chromatography, analytical method validation parameters such as accuracy, precision, limit of detection, limit of quantitation, and speci-

ficity were calculated to prove the validity of the complete procedure for vitamin E analysis of peanuts and peanut products. A precision study was performed on the peanut butter and the results are given in Table 3. The repeatability and reproducibility (% CV) were usually less than or about 5% except for β-T. The higher % CV for the β-T may have been due to the very low but detectable content in the samples. The accuracy was evaluated by analyzing five samples of the peanut butter to which known concentrations of each vitamin were added prior to extraction. Table 3 shows recovery data based on five trials for tocopherol homologs. The % mean recoveries ± S.D. (n = 5) were 98.2 ± 0.01, 102.0 ± 0.12, 96.6 ± 0.01, and 100.8 ± 0.03 for α-, β-, γ-, and δ-T, respectively.

The limit of detection (LOD) and limit of quantitation (LOQ) were determined based on the detector's signal-to-noise (S/N) ratio. The standard deviation of the S/N ratio was calculated and multiplied by a factor of 3; then, this value was added to the average of the S/N ratio to obtain the LOD. For LOQ, 10 was chosen as a factor (Food Chemicals Codex, 1996). The LODs in ng/20 μL were 0.21, 0.06, 0.11, and 0.08, and the LOQs in ng/20 μL were 0.39, 0.14, 0.23, and 0.13 for α-, β-, γ-, and δ-tocopherol, respectively.

Peak purity was determined using peanut butter by the procedure described by Haroon *et al.* (1986) for fluorescence response. Peak heights of tocopherols were determined at excitation wavelengths of 270, 280, and 290 nm, while keeping the emission wavelength constant at 330 nm. The ratio of the peak heights from samples then was compared with the peak ratio of standard obtained at the same wavelengths (Table 4). Very close values were obtained from samples using the different extraction methods and standards for α-, γ-, and δ-T, indicating the purity of the peaks. For the δ-T in peanut butter, there was a big difference between standard ratios and the ratios using the three extraction methods. The peak response ratios for β-T from the three methods were almost 10 times higher than the ratios from standard. The peak purity for β-T was further determined for other samples containing β-T (Table 5). The peak response ratios from almond and pecan were almost identical to the standard; however, large differences were observed in peanut, peanut butter, and peanut butter-reduced fat as compared to the standard, indicating an unknown impurity. Therefore, caution is needed for the quantitation of β-T in peanut and peanut-containing foods. However, β-T in peanut and peanut butter contributes less than 2% for the total α-TE. Validation parameters generally showed the method to be excellent.

Quantitation of Tocopherols and Tocotrienols. The direct solvent extraction method was used to quantify the tocopherols and tocotrienols in nut samples and peanut butter. The typical HPLC chromatograms for tocopherols from peanut extracts and standard are shown in Fig. 1. These depict an injection of a concentrated sample extract and standard which gave an off-scale peak of α- and γ-T to quantitate β- and δ-T. The linearity test for quantitation was carried out over the range of 0.0 to 37.1, 0.0 to 1.0, 0.0 to 41.4, and 0.0 to 6.6 ng/20 μL injected for α-, β-, γ-, and δ-T, respectively. Regression analysis showed an excellent linear relationship (r^2 = 0.999). The level of isopropanol in hexane in the isocratic

Table 3. Precision and accuracy of assay of peanut butter sample.

Homolog	Parameters	Precision		Accuracy[a]
		Repeatability[b]	Reproducibility[c]	Recovery
		------- mg/100 g -------		%
α-tocopherol	Mean[d]	7.68	7.26	98.2
	S.D.[e]	0.23	0.23	1.17
	CV[f], %	2.96	3.12	1.19
β-tocopherol	Mean	0.19	0.17	102.0
	S.D.	0.02	0.02	11.76
	CV, %	8.96	9.34	11.53
γ-tocopherol	Mean	11.62	11.05	96.6
	S.D.	0.59	0.41	1.50
	CV, %	5.05	3.69	1.55
δ-tocopherol	Mean	0.84	0.86	100.8
	S.D.	0.06	0.05	2.71
	CV, %	6.97	6.02	2.69

[a]Accuracy is a measure of the closeness of the analytical result to the true value evaluated by analyzing a spiked sample.

[b]Repeatability refers to the results of independent determinations carried out on a sample by analyzing five replicates of the sample on same day.

[c]Reproducibility refers to the results of independent determinations carried out on a sample by analyzing five replicates of the sample at different periods of time.

[d]n = 5.

[e]Standard deviation.

[f]Coefficient of variation.

Table 4. Evaluation of peak purity by fluorescence ratio using peanut butter.

	Excitation wavelength	Peak response ratios[a]			
		Standard	Saponification	Direct solvent extraction	Soxhlet extraction
	nm				
α-T[b]	280/290	1.16	1.16	1.15	1.15
	270/290	0.82	0.83	0.82	0.82
	280/270	1.42	1.40	1.41	1.40
β-T	280/290	1.04	2.21	2.44	1.82
	270/290	0.69	6.07	6.76	4.13
	280/270	1.50	0.36	0.36	0.44
γ-T	280/290	0.99	0.98	0.97	0.97
	270/290	0.63	0.65	0.64	0.63
	280/270	1.58	1.51	1.53	1.54
δ-T	280/290	0.94	0.95	0.93	0.91
	270/290	0.57	0.56	0.60	0.56
	280/270	1.63	1.71	1.57	1.63

[a]Fluorescence ratios shown were calculated by dividing the values for the two peak heights for tocopherol homologs obtained from separate chromatographic runs at three different excitation wavelengths, with the emission wavelength constant at 330 nm.

[b]Corresponding tocopherols.

Table 5. Peak purity test for β-tocopherol in peanuts, peanut butter and selected nuts.

Sample	Excitation wavelength	Peak response ratios[a] Standard	Direct solvent extraction
	nm		
Peanut	280/290	1.04	1.81
	270/290	0.69	4.28
	280/270	1.50	0.43
Peanut butter	280/290	1.04	2.44
	270/290	0.69	6.76
	280/270	1.50	0.36
Peanut butter-reduced fat	280/290	1.04	2.00
	270/290	0.69	5.37
	280/270	1.50	0.37
Almond	280/290	1.04	1.02
	270/290	0.69	0.72
	280/270	1.50	1.46
Pecan	280/290	1.04	1.07
	270/290	0.69	0.69
	280/270	1.50	1.58

[a]Fluorescence ratios shown were calculated by dividing the values for the two peak heights for tocopherol homologs obtained from separate chromatographic runs at three different excitation wavelengths, with the emission wavelength constant at 330 nm.

mobile phase was kept less than or at 0.6% to get baseline separation especially for β-tocopherol since unknown impurity peaks are just prior to the β-T peak (Fig. 1). Chromatographic quality control parameters (Table 6) were calculated based on the equations given by Tuan *et al.* (1989) and Pomeranz and Meloan (1994).

The analytical values of tocopherols and tocotrienols in each product are listed in Table 7. Hashim *et al.* (1993a) reported the α-TE levels of peanuts in the range of 8.69 to 14.81. The α-TE level of 12.06 in peanut was reported by Piironen *et al.* (1986). In our study, the

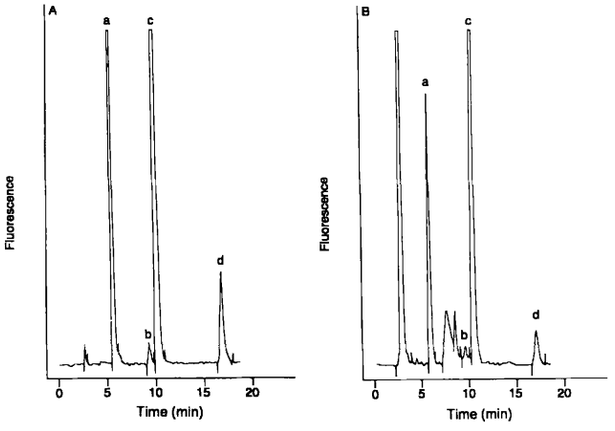

Fig. 1. Normal-phase LC chromatogram of tocopherols using fluorescence detection (Ex 290 nm, Em 330 nm), flow rate of 1.0 mL/min, injection volume of 20 μL, and mobile phase of 0.6% isopropanol in hexane. A is the standard and B is the extract of the peanut sample where (a) α-tocopherol, (b) β-tocopherol, (c) γ-tocopherol, and (d) δ-tocopherol.

Table 6. Chromatographic quality control parameters on Lichrosorb Si 60.[a]

	N[b]	k[c]	α[d]	R[e]
α-tocopherol	1497	1.335	2.297	7.946
β-tocopherol	6989	3.066	1.137	1.688
γ-tocopherol	3496	3.486	1.858	9.047
δ-tocopherol	6987	6.479	-	-

[a]Chromatographic conditions as in text.
[b]Theoretical plate number.
[c]Column capacity factor.
[d]Column selectivity.
[e]Resolution between adjacent peaks.

levels of α-TE in peanut, peanut butter, and low fat peanut butter were found in the range of 4.44 to 8.44, 9.29 to 13.68, and 5.10 to 9.41, respectively. The overall % recoveries (means ± S.D.) of this study for α-, β-, γ-, and δ-T were 99.9 ± 3.29, 100.4 ± 11.7, 98.9 ± 5.94, and 100.3 ± 4.87, respectively. There were differences in

Table 7. Tocopherol and tocotrienol concentrations in peanuts, peanut butter, and other nuts.[a]

Sample	Tocopherols and tocotrienols α-T[b]	α-T3[c]	β-T	γ-T	γ-T3	δ-T	α-TE[d]
	mg/100 g sample						
Dry-roasted peanuts							
A	3.55	-	0.13	8.04	-	0.59	4.44
B	7.22	-	0.15	11.18	-	0.93	8.44
C	4.41	-	0.16	9.48	-	0.84	5.46
D	7.15	-	0.20	11.34	-	0.96	8.41
Mean	5.58	-	0.16	10.01	-	0.83	6.69
Peanut butter							
A	9.51	-	0.38	9.76	-	0.86	10.70
B	12.27	-	0.68	10.40	-	0.85	13.68
C	9.53	-	0.20	9.98	-	0.75	10.65
D	8.15	-	0.18	10.32	-	0.50	9.29
Mean	9.87	-	0.36	10.12	-	0.74	11.08
Peanut butter - reduced fat							
A	8.46	-	0.25	8.02	-	0.82	9.41
B	7.06	-	0.11	6.72	-	0.30	7.80
C	4.24	-	0.20	7.49	-	0.39	5.10
D	6.78	-	0.22	7.30	-	0.61	7.64
Mean	6.64	-	0.20	7.38	-	0.53	7.49
Almond	29.49	0.40	0.17	1.02	-	-	29.80
Cashew	0.96	-	-	4.55	0.39	-	1.42
Macadamia	-	1.84	-	-	-	-	0.55
Pecan	1.39	-	0.11	30.38	-	-	4.48
Pistachio	2.34	0.87	Tr[e]	25.12	1.86	0.69	5.13
Walnut	1.23	-	-	23.53	-	1.82	3.64

[a]Four different brands were analyzed for peanut, peanut butter, and peanut butter-reduced fat and each sample was analyzed in triplicate (n = 3).
[b]Corresponding tocopherols.
[c]Corresponding tocotrienols.
[d]γ-Tocotrienol was not added to calculate α-TE.
[e]Trace amount (< 0.01 mg/100 g sample).

each tocopherol level and α-TE values for peanut, peanut butter, and low fat peanut butter. Hashim *et al.* (1993a) found significant differences in tocopherol content among two different peanut cultivars, and these differences were affected by the degree of maturation. The average α-TE values for peanut butter and low fat peanut butter were 11.08 and 7.49, respectively. α-TE levels in low fat peanut butter were reduced by 32.4% when compared to peanut butter due to reduced fat. γ-T was identified as the major homolog in peanut, pecan, walnut, and pistachio. α-T was the major homolog in almond. From a nutritional point of view, almond has the highest biological activity due to a higher level of α-T. From the stability point of view, peanut, pecan, pistachio, and walnut have the higher levels of γ-T which act as a more effective *in vitro* antioxidant to protect the stored fats (Lavedrine *et al.*, 1997).

In this study, three different extraction methods were compared and the direct solvent extraction method proved to be the best method for the determination of tocopherol homologs in peanuts and peanut products. Using the direct solvent extraction method, higher analytical values for vitamin E were obtained compared to those from other extraction methods. The method as presented is fast and accurate. Direct solvent extraction requires less time and provides a less harsh environment compared to other extraction procedures. We have incorporated the procedure into routine use for the quantification of vitamin E in peanut and peanut products.

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