FOOD COMPOSITION AND ADDITIVES

Liquid Chromatographic Determination of Tocopherols and Tocotrienols in Vegetable Oils, Formulated Preparations, and Biscuits

JOSEP SERRA BONVEHI
Research and Development Department of Nederland, PO Box 34, 08890 Viladecans, Barcelona, Spain
FRANCESC VENTURA COLL
Nabisco Iberia, PO Box 2022, 08080 Barcelona, Spain
IMMA ALSINA RIUS
Agricultural and Food Laboratory (Generalitat of Catalonia), 08348 Cabrils, Barcelona, Spain

A precise and selective liquid chromatographic procedure for determining tocopherol and tocotrienol isomers in vegetable oils, formulated preparations, and biscuits was developed and validated. The proposed method quantitates vitamin E in better conditions of recoverability and reproducibility than the standard saponification procedure. Tocopherols and tocotrienols were extracted in hexane from vegetable oils, passed through a silica Sep-pak, chromatographed on a μ-Bondapak C_{18} column with a mobile phase of methanol–water (95 + 5, v/v), identified at 292 nm, and detected with fluorescence procedure (excitation 296 nm, and emission 330 nm). The correlation coefficient on the calibration curve was 0.9995 over the range of 0.1 to 100 μg/mL. Overall recovery of vitamin E isomers was 93%; coefficients of variation for intra- and interday precision, <2.25%. The results obtained from extraction methods 1 (with saponification) and 2 (without saponification) were compared by ANOVA test. Significant differences appeared between vitamin E isomers ($p \leq 0.05$).

Vitamin E is a fat-soluble vitamin which is naturally present in small amounts in vegetable oils and biological fluids. Vitamin E comprises 2 vitamer types, tocopherols and tocotrienols. Tocopherols are capable of scavenging free radicals and, thus, together with the action of other natural antioxidants like ascorbic acid and β-carotene, prevent or delay the onset of the lipid peroxidation process (1). Vitamin E functions as an antioxidant to protect fat in membranes around cells (such as nerves, heart, muscles, and red blood cells) from damage by oxygen (2). They have also attracted the attention of nutritionists and clinicians because of their possible role in prevention of cancer and atherosclerosis (1, 3, 4). The problem of many vitamin E assays is the presence of a series of related tocopherols (α, β, γ, and δ) and their corresponding unsaturated tocotrienols.

The chemistry of the vitamin E group is complicated still further by the existence of optical and geometric isomers, each with different physiological responses (5). Current interest in the tocotrienols as aids in decreasing cardiovascular disease, and as possible anticarcinogenic agents, creates a need to further optimize liquid chromatographic (LC) separation systems to incorporate the tocotrienols. Tocotrienols have been found in palm oil, coconut oil, and cereal grains such as wheat, rye, oats, and barley. α-Tocotrienol is also able to suppress the elevation of the cholesterol level in blood (in some hypercholesterolemic subjects), and δ-tocotrienol is able to prevent aggregation of blood platelets (6, 7).

Tocopherol occurs in different active forms; isomers α, with higher biological activity, and γ are the most abundant in vegetable foods. A good preservation of both carotenes and α-tocopherol during the processing of oils is a significant parameter in assessing the quality of the technology (8–10). Application of LC coupled with fluorescence detection has been successful for quantitation of α-carotene, α-, β-, γ-, and δ-tocopherols and the corresponding tocotrienols (11–15). The reversed-phase approach is preferred over normal-phase in more than 70% of recently published procedures (16, 17). The reason is that reversed-phase chromatography offers certain practical advantages (e.g., column stability, reproducibility of retention times, and quicker equilibration). On the other hand, reversed-phase systems, with water and methanol in the mobile phase, have the disadvantages of pronounced deterioration of the peak shape of lipophilic compounds and may cause partial precipitation of lipophilic compounds. Normal-phase columns would have major resolution of vitamin E isomers if the problems of column deactivation could be solved.

An amount of water in the parts-per-million range in all organic solvents is sufficient to deactivate normal-phase columns during long-term usage. Bredeweg et al. (18) applied 2,2-dimethoxypropane (DMP) for chemical reactivation of silica columns. The procedure is based on the chemical reac-

Received July 29, 1999. Accepted by JL December 23, 1999.

tion of DMP with water to form 2 moles of methanol and 1 mole of acetone in presence of an acid catalyst. Currently, AOAC INTERNATIONAL (19) provides an official method based on saponification for analysis of vitamin E in foods and feeds. It is generally recognized that most samples do not lend themselves to such direct determination methods, and prior saponification is necessary to effect sample cleanup (20). Direct assay without saponification has been claimed for vegetable oils and fortified medical food, in which both reversed and normal-phase columns were used except for tocopherol esters (all-rac-α-tocopheryl acetate and retinyl palmitate) (17, 21).

The specific objectives of our study, therefore, were to quantitate tocopherol isomers with different extraction procedures, based on direct solvent extraction of vitamins or alkaline hydrolysis. In general, alkaline hydrolysis is the process usually used to hydrolyze ester forms of vitamins. Thus, the purpose of this study was to simultaneously analyze tocopherol and tocotrienol isomers in best conditions of recoverability and reproducibility using a reversed-phase LC system without saponification.

Experimental

Reagents and Solutions

(**a**) *Chemicals*.—LC grade ethanol (EtOH), hexane, and methanol (MeOH) were from Acros (Geel, Belgium). Ultrapure water (Milli-Q, Millipore Corp., Bedford, MA) was prepared for chromatographic use. Analytical grade pyrogallol, potassium hydroxide, sodium sulfate, isopropyl alcohol, and methylene chloride were obtained from E. Merck (Darmstadt, Germany).

(**b**) *Mobile phase*.—A, MeOH; B, Ultrapure Millipore water.

(**c**) *Reference standards*.—(+)-α-tocopherol, (+)-β-tocopherol, (+)-γ-tocopherol, and (+)-δ-tocopherol (Sigma Chemical Co., St. Louis, MO). DL-α-tocotrienol, DL-β-tocotrienol, DL-γ-tocotrienol and DL-δ-tocotrienol (Merck). According to the literature, the fluorescence intensity of tocotrienols is the same as the corresponding tocopherol, and the UV absorbance is similar (17).

(**d**) *Individual stock solutions*.—Individual stock solutions were prepared in ethanol. The exact concentrations of these solutions were determined spectrophotometrically by using the extinction coefficients listed in Table 1.

(**e**) *Calibration standard solutions*.—Individual stock solutions were diluted with a mixed mobile phase (EtOH–Millipore water, 95 + 5) and then mixed proportionally to make calibration standard solutions of 5, 12.5, 25, 50, and 100 μg/mL for all tocopherols and tocotrienols. A series of calibration standard solutions (n = 5) was prepared by diluting the above solutions with a mixed mobile phase. Solutions containing the target compounds, either standards or extracts, were always protected from light to avoid degradation.

(**f**) *Calculation*.—Concentrations (μg/mL) of all vitamin E isomers in sample extracts were determined from linear regression analyses of standard curves generated for each analysis of vitamin E isomers.

Table 1. Extinction coefficients and maximum wavelengths for tocopherols and tocotrienols in 96% (v/v) ethanol solutions[a]

Compound	Wavelength, nm	Extinction coefficient, 1%, 1 cm
(+)-α-tocopherol	292	75.8
(+)-β-tocopherol	297	87
(+)-γ-tocopherol	298	91.4
(+)-δ-tocopherol	297	91.2
DL-α-tocotrienol	292.5	91
DL-β-tocotrienol	294	87.3
DL-γ-tocotrienol	296	90.5
DL-δ-tocotrienol	297	89.1

[a] *See* references 14 and 22.

Apparatus

(**a**) *LC system*.—Waters 590 pump, 715 autoinjector, 996 photodiode array detector (DAD), and 470 fluorescence detector (all from Waters Chromatography Division, Milford, MA). The system was equiped with NEC 486/66i computer (NEC Technologies, Inc., Boxborough, MA) and Waters Millennium software, version 2.1 for data processing. The system was operated at ambient temperature.

(**b**) *LC column*.—μBondapak-C_{18} column, 300 × 3.9 mm id, 10 μm particle size with a μBondapak-C_{18} guard column (Waters). The system was operated at 25°C with a flow rate of 1 mL/min; fluorescence detector, sensitivity 0.3; excitation wavelength, 296 nm; emission wavelength, 330 nm; slit, 7 nm; DAD detector, 180–380 nm.

(**c**) *Silica Sep-pak for solid-phase extraction* (*500 mg*).—Shandon Hypersep (Shandon Scientific Ltd., Runcorn, UK).

Sample Preparation

Optimization of the method in relation to the saponification procedure was considered. The proposed method quantitates vitamin E in best conditions of recoverability and reproducibility. Extraction recoveries were evaluated by the method of standard additions. Known amounts of isomers (+)-γ-tocopherol, (+)-δ-tocopherol and (+)-α-tocopherol were added to samples of oil. The amounts added were equal to the amounts already present in the samples. The spiked samples (n = 5) were extracted. Method precision was assessed by analyzing samples on the same day (intraday) and on different days (interday). Analytical precision was determined by measuring standard solutions; method precision was determined by measuring spiked sample solutions.

The described procedure is suitable for assays of vitamin E isomers in oils, cocoa butter, formulated preparations, and biscuits. Solid cocoa butter at ± 40°C was gently melted before analysis and melted cocoa butter was homogenized. Biscuits were cut into small lumps and mixed in a homogenizer.

Vegetable oils and fats were collected after industrial processing (palm oil, cocoa butter, olive oil, sunflower oil, walnut oil, hazelnut oil, coconut oil, and corn oil). Samples were analyzed without saponification within 3 to 4 weeks after collection. Five formulated preparations containing complex matrixes of vitamins, amino acids, and organic acids were also analyzed without saponification. Palm oil, sunflower oil, cocoa butter, and biscuits with 10% palm oil and 10% sunflower oil containing tocopherol isomers added as antioxidant were analyzed by both direct solvent extraction of vitamins and alkaline hydrolysis of the products.

Method Validation

(**a**) *Calibration and linearity*.—A calibration curve was established by measuring vitamin E isomer peak areas over a 5-fold range of concentrations. The slope, intercept, and correlation coefficient were calculated by linear regression analysis.

(**b**) *Recovery*.—Two sets of samples were analyzed. The amount of vitamin E isomers added to the sample corresponded to 50–150% of expected vitamin E.

(**c**) *Analytical precision*.—Intraday precision was determined by analyzing 3 concentrations of standard vitamin E isomers ranging from 49.7 to 50.6 μg/mL in 5 replicates in 1 day. Interday precision was determined by measuring the same controls in duplicates for 3 days.

(**d**) *Method precision and accuracy*.—Precision and accuracy were determined oil sample spiked with vitamin E isomers at 3 concentrations ranging from 50 to 150 μg/mL (n = 5 for each concentration).

The following equations were used:

$$\text{Accuracy, \%} = \frac{\text{Measured concn} - \text{Actual concn}}{\text{Actual concn}} \times 100$$

$$\text{Precision, \%} = \frac{\text{Standard deviation}}{\text{Mean concn}} \times 100$$

Oil Extraction in Formulated Preparations and Soy Extracts

To ensure a representative sample, at least 20 tablets or capsules were weighed and ground to a fine powder. A working sample with weight equivalent to 5 tablets was transferred to a 100 mL amber centrifuge tube; 25 mL dimethyl sulfoxide was added, and the mixture was sonicated at 40°C for 20 min. Then, 40 mL hexane was shaken vigorously for 10 min and centrifuged at 3000 rpm for 5 min. This was repeated with 3 additional 40 mL portions of hexane. The resulting extract was evaporated to dryness under vacuum at a maximum of 40°C.

Oil Extraction in Biscuits

Approximately 20 g homogenized product was extracted with petroleum ether (40°–60°C)–diethyl ether (1 + 1) for 4 h using a Soxhlet apparatus. Oil was determined as the difference in weight of dried samples before and after extraction.

During all the steps that follow, assays were protected from light and atmospheric oxygen. Three replicates of each sample were run.

(**a**) *With saponification*.—Approximately 10 g oil or extracted oil from biscuits was saponified with 5 mL 50% ethanol KOH (w/v), 25 mL ethanol, and 50 mg pyrogallol for 30 min in a water bath at 56°C (23). Samples were then cooled, 20 mL distilled water was added, and the mixture was extracted 3 times with hexane to remove the esterified product. The combined extracts were washed with water to neutrality to remove fatty acid soaps, and desiccated on anhydrous sodium sulfate. The extract was evaporated to dryness under vacuum at a maximum of 40°C. The residue was dissolved in 5 mL methanol. Standard solutions were processed as sample.

(**b**) *Without saponification*.—The sample (0.2 g oil from formulated preparations and soy extracts, 1.5 g vegetable oil or 1.5 g oil extracted from biscuits) was dissolved in 10 mL hexane. A 1 mL aliquot of this solution was passed through a Shandon Hypersep silica Sep-pak (500 mg) column activated with 10 mL isopropanol and 15 mL hexane. Tocopherols were eluated with 2 mL methanol. Silica Sep-pak columns were used to remove interfering triglycerides from the extract. The extract was stabilized with 0.3 mL methylene chloride.

Chromatographic Conditions

LC was performed following these steps: guard-pack μBondapak-C_{18} (10 μm) precolumn; μBondapak-C_{18} (10 μm; 300 × 3.9 mm id) reversed-phase column; use of a photodiode array detector (DAD) at 292 nm coupled in-line with fluorescence detector included sensitivity 0.3, excitation wavelength 296 nm, emission wavelength 330 nm, and slit 7 nm [Model 470 scanning fluorescence detector (Waters)]; mobile phase [CH_3OH–H_2O (95 + 5 v/v)]; flow rate 1 mL/min. LC Model 590 (Waters) LC pumping units; Model 712 Ultra WISP valve loop injector fitted with a 20 μL loop. Chromatographic data from LC were processed on NEC 486/66i computing integrator.

Statistical Analysis

Analysis of variance was performed using an ANOVA program of Statgraphics Statistical packet, version 6.0 (SAS Institute, Inc., Cary, NC) (24) on the data set to determine the tocopherol levels that varied significantly with analytical procedures. Significant differences among and by analytical procedures means were determined by Duncan's Multiple Range test, Waller-Duncan k-ratio t-test, and Tukey's studentized range test. Group differences were considered statistically significant at a level of $p \leq 0.05$.

Results and Discussion

The vitamin E isomers were identified by coelution with a spike of standard isomers and the spectrum for the chromatographic peak, working with negative samples and enrichment

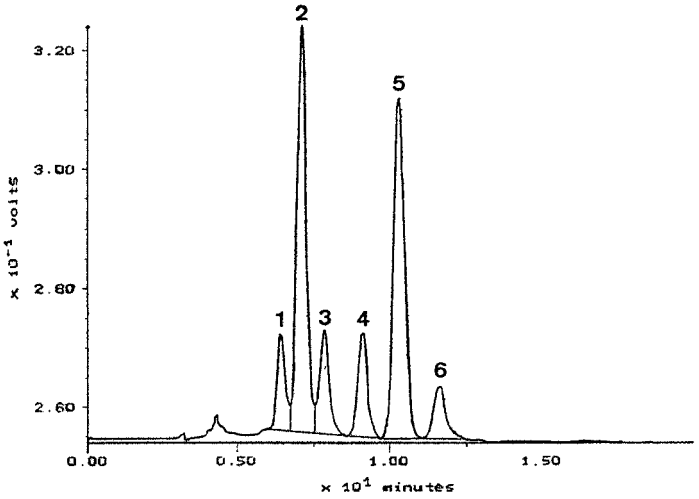

Figure 1. Chromatographic separation of vitamin E isomers. Peaks: 1, δ-tocotrienol; 2, γ-tocotrienol; 3, α-tocotrienol; 4, δ-tocopherol; 5, γ-tocopherol; 6, α-tocopherol.

samples. Figure 1 illustrates separation of the most important vitamin E isomers determined with the method using UV and fluorescence detection without saponification. With this procedure, α-, β-, γ-, and δ-tocopherols and tocotrienols are detected in the same chromatogram. Methanol as mobile phase reduced retention times, sharpened peaks, and improved recovery of tocopherols. However, γ-tocopherol is insufficiently separated from β-tocopherol. The inability of the LC method to separate β- and γ-tocopherol was not inconvenient because the majority of vegetable oils did not contain β-tocopherol. Tan and Brzuskiewicz (25) found that β- and γ-tocopherols could not be resolved by reversed-phase chromatography. Retention times were 13.9 min for α, 11.8 min for γ, 11.2 min for β, and 10.1 min for δ-tocopherols. The tailing factor for β/γ peaks was <1. The mobile phase was modified compared to reported procedures (26) because of the potent quenching effect of chloroform on tocopherol fluorescence.

Stabilities of tocopherol stock solutions were tested regularly by injecting standard working solutions into the LC system. Concentrations were calculated and compared with those of freshly prepared stock solutions. In the use of nonaqueous extraction procedures without saponification to estimate vitamin E levels in food products (21, 27–29), the use of Sep-pak chromatography is obligatory unless extraneous lipid material is first removed from the sample. In this study, silica Sep-pak was used to obtain a more complete extraction of isomers from foods matrixes. Peak purity and UV spectra at 292 nm of peaks were checked by spectral analysis and comparison with standard spectral data. The fluorescence emission had the advantages of greater sensitivity and selectivity than UV absorption. Vitamin E standards were analyzed at various excitation and emission wavelengths to obtain maximum response conditions. The emission was set initially at 340 nm and the excitation varied between 200 and 300 nm (in 5 nm increments). The emission wavelengths were then scanned at the optimum excitation wavelength.

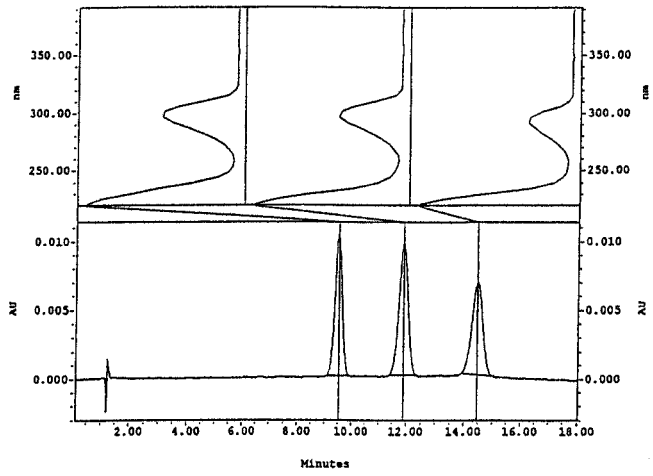

Figure 2. δ-, γ-, and α-tocopherol homogeneity determined by spectral overlay.

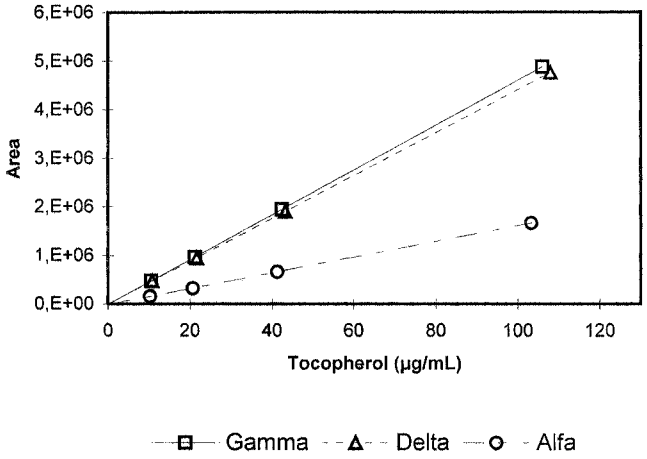

Figure 3. Representative standard curve for tocopherol isomers.

Table 2. Absolute recoveries of vitamin E isomers from spiked samples (μg/mL)

Product name	α-Isomer added	α-Isomer found	Rec., %	γ-Isomer added	γ-Isomer found	Rec., %	δ-Isomer added	δ-Isomer found	Rec., %
Palm oil	50.1	49.1	98	49.9	46.8	93.8	50.2	47.3	94.2
	102.1	100.2	98.1	101.3	109.1	107.7	101.1	111.2	110
	149.1	152.2	102.1	150.9	157.9	104.6	151.2	149.2	98.7
Cocoa butter	70.1	68.1	97.1	69.6	65.6	94.3	69	67.2	97.4
	140.2	138.2	98.6	138.4	141.8	102.5	138.1	140.1	101.5
	209.1	207.1	99	212.3	205.8	96.9	211.3	208.2	98.5
Olive oil	49.8	49.1	98.6	50.4	47.5	94.2	50.1	47.8	95.4
	101.2	100.3	99.1	101.8	96.2	94.5	100.5	97.8	97.3
	150.7	147.8	98.1	150.1	144.8	96.5	149.7	146	97.5
Sunflower oil	50.2	50.9	101.4	50.8	47.3	93.1	50.5	47.9	94.9
	101.7	99.8	98.1	100.1	103.8	103.7	100.5	103.1	102.6
	151.3	148.6	98.2	150.7	145.5	96.5	150.2	147.4	98.1
Coconut oil	69.9	71.8	102.7	69.9	66.2	94.7	69.5	67.4	97
	140.5	141.7	100.9	143.4	134.6	93.8	140.7	137.9	98
	210.3	208.1	99	215.7	205.5	95.3	210.5	216.5	102.9

Table 3. Analytical precision of the method for determination of vitamin E isomers

α-Isomer expected, μg/mL	α-Isomer, μg/mL	Precision, %	γ-Isomer expected, μg/mL	γ-Isomer, μg/mL	Precision, %	δ-Isomer expected, μg/mL	δ-Isomer, μg/mL	Precision, %
Intraday								
50.4	50.1 ± 0.72	1.44	50.2	50.7 ± 0.78	1.54	50.3	50.6 ± 0.71	1.40
49.7	50.2 ± 0.77	1.53	50.4	50.6 ± 0.84	1.66	50.1	50.8 ± 0.82	1.61
50.6	50.3 ± 0.80	1.59	50.5	50.2 ± 0.74	1.47	50.5	50.1 ± 0.69	1.38
Interday								
50.4	49.9 ± 1.05	2.10	50.2	50.5 ± 1.10	2.18	50.3	50.5 ± 1.12	2.22
49.7	50.1 ± 1.03	2.06	50.4	50.1 ± 0.98	1.96	50.1	49.8 ± 0.99	1.99
50.6	50.8 ± 1.08	2.13	50.5	50.2 ± 1.05	2.09	505	50.1 ± 1.02	2.04

Table 4. Method precision and accuracy for determination of vitamin E isomers[a]

α-Isomer added, μg/mL	α-Isomer found, μg/mL	Error, %	Precision, %	γ-Isomer added, μg/mL	γ-Isomer found, μg/mL	Error, %	Precision, %	δ-Isomer added, μg/mL	δ-Isomer found, μg/mL	Error, %	Precision, %
50.1	51.8 ± 1.41	3.39	2.72	50.2	51.8 ± 1.96	3.19	3.78	51.9	50.3 ± 1.97	3.08	3.92
100.6	97.8 ± 2.07	2.78	2.11	101.5	97.6 ± 3.11	3.84	3.19	100.4	96.3 ± 3.44	4.08	3.57
149.5	145.4 ± 3.21	2.74	2.21	151.1	145.1 ± 5.27	3.97	3.49	152.9	146.2 ± 5.86	4.38	3.87

[a] Values are means ± standard deviations.

Table 5. Tocopherols in 4 formulated preparations

Product name (source)	Type	Tocopherols, mg/tablet, capsule, or extract: Label claim	Assay	Agreement, %
Vitamin research products	Tablet	24.71	25.44	103
Vitamin research products	Tablet	15.86	16.67	105.1
Vitamin research products	Tablet	8.93	8.53	95.5
Vitamin research products	Capsule	20.32	21.67	106.6
Tocopherol soy extract	Fluid	70	66.61	95.2

Detection sensitivities were evaluated by serial dilutions. Linearity checks were performed using standard solutions and oils containing various predetermined quantities of added vitamin. Data were identical in both cases, and peaks were spectrally homogeneous (Figure 2). The response of LC system was linear at the concentration range 0–100 μg/mL for all isomers (correlation coefficient > 0.9999) (Figure 3). The limits of detection and quantitation of an analytical procedure are determined by analyzing a number of low concentration samples. The concentration of these samples should span the lowest quarter of the range established during the linearity study (30). Detection limits (signal-to-noise ratio ≥ 3) of the LC method are 0.15 μg/mL for vitamin E isomers and quantitation limits are 0.5 μg/mL.

Vitamin recoveries were quantitated for α-, γ-, and δ-tocopherols. Good recoveries of spiked samples demostrated that the method has no significant matrix interference. The acceptable recovery range for the levels determined is 93.1–110% (Table 2), meeting requirements of the AOAC Peer-Verified Methods Program (31). Analytical precisions were <1.70% (intraday) and <2.25% (interday) (Table 3). Method precision and accuracy were 3.95 and 4.40%, respectively (Table 4).

This method was used to quantitate tocopherols in 5 formulated preparations. These preparations usually contain raw material vitamins and other matrix material. A suitable extraction system must permit simultaneous extraction of vitamins and minimize extraction of interfering components. Partion of dimethyl sulfoxide with hexane resulted in reproducible phase extraction and separation (29). The amounts of tocopherols claimed for each product varied from 8.93 mg/tablet to 70 mg/soy extract (Table 5). The amounts of tocopherols detected were 8.53 mg/tablet to 66.61 mg/soy extract, and agreements with label claims ranged from 95.2 to 106.6%. These values are acceptable for the levels studied according to the International Union of Pure and Applied Chemistry. Table 6 shows the results of the vitamin determination by the 2 extraction methods assayed (oil saponification and direct solvent extraction). Vitamin content ranged from 6 μg/mL for δ- to 701 μg/mL for α-tocopherol isomers, and from 3 μg/mL for δ- to 378 μg/mL for γ-tocotrienol isomers. The close agreement of most duplicates indicates good repeatability.

The tocopherol concentrations, expressed as μg/mL (means ± SD; $n = 6$), were:

Method 1: α-tocopherol = 759 ± 20.9; γ-tocopherol = 166.5 ± 8.18; δ-tocopherol = 33.9 ± 0.86; total tocopherol = 958.5 ± 29.3; α-tocotrienol = 182.8 ± 10.5; γ-tocotrienol = 313.8 ± 17.2; δ-tocotrienol = 45.2 ± 2.3; total tocotrienol = 547 ± 29.2.

Method 2: α-tocopherol = 885.1 ± 17.71; γ-tocopherol = 204.7 ± 7.37; δ-tocopherol = 47.8 ± 0.93; total tocopherol = 1146.7 ± 25.02. α-tocotrienol = 229.3 ± 9.32; γ-tocotrienol = 365.2 ± 14.86; δ-tocotrienol = 55.9 ± 1.98; total tocotrienol = 658.7 ± 25.4.

All the levels of the isomers of vitamin E increased (about 20%) with method 2 (without saponification) in comparison with method 1. These results were compared by ANOVA

Table 6. Vitamin E values for raw materials and products measured by different methods[a]

Sample	Tocopherols, μg/mL						Tocotrienols, μg/mL					
	α	γ	δ	α	γ	δ	α	γ	δ	α	γ	δ
	Method 1			Method 2			Method 1			Method 2		
Palm oil	167	—	17	201	18	22	180	308	47	218	365	60
	161	—	14	197	16	19	187	317	43	226	378	53
Sunflower oil	584	—	—	672	—	—	—	—	—	—	—	—
	614	—	—	701	—	—	—	—	—	—	—	—
Cocoa butter	—	169	21	13	196	28	—	—	—	15	—	—
	—	158	18	15	182	25	—	—	—	11	—	—
Biscuit[b]	31	10	—	45	15	6	7	13	—	11	18	3
	29	8	—	42	17	8	9	11	—	13	16	4

[a] Method 1, with saponification; method 2, without saponification; (means; $n = 6$).
[b] Biscuit with 10% palm oil, and 10% sunflower oil, with tocopherols added as antioxidant.

Table 7. Tocopherol and tocotrienol content in different vegetable oils

	Tocopherols, μg/mL			Tocotrienols, μg/mL		
Sample	α	γ	δ	α	γ	δ
Palm oil	198	—	11	210	408	87
	232	—	9	237	425	78
Sunflower oil	765	—	—	—	—	—
	710	—	—	—	—	—
Cocoa butter	14	225	37	9	—	—
	9	187	31	7	—	—
Walnut oil	12	517	61	—	—	—
	15	569	72	—	—	—
Coconut oil	3	—	13	8	32	—
	5	—	15	11	27	—
Hazelnut oil	425	68	17	—	—	—
	478	74	14	—	—	—
Corn oil	263	1365	88	—	—	—
	245	1319	63	—	—	—

test. Significant differences appeared between tocopherols and tocotrienols isomers ($p \leq 0.05$). This method was selected to quantitate tocopherols and tocotrienols in different oils to evaluate its acceptability. The data obtained indicate that the main form in both crude oils and their mixture were α- and γ-tocopherol, whereas δ-tocopherol content was very limited (Table 7). The results of tocopherol separation in sunflower oil were in good accordance with the data already published (32). Palm oil is unique as it contains α-, γ-, and δ-tocotrienols. Tocotrienols were identified in palm oil, coconut oil, and cocoa butter. In hazelnut oil, the isomers present in our samples were α-, γ-, and δ-tocopherol. As expected, γ-tocopherol was the predominant homolog in walnut oil and corn oil. The values obtained are in agreement with those published by other authors (4, 15, 32, 33). The proposed LC method for determining vitamin E isomers in oils and in products containing oils (e.g., biscuits) is simple, accurate, and selective. The assay is valid for determining vitamin E without any interference from triglycerides.

Conclusions

The direct injection procedure was highly acceptable for quantitating tocopherol homologs in oils into the reversed-phase LC system. This technique allowed good resolution of α-, γ-, and δ-tocopherols, and tocotrienols. Significant differences ($p \leq 0.05$) between the procedure with saponification and without saponification were evidenced.

References

(1) Haliwell, B., & Gutteridge, J.M.C. (1996) in *Free radicals in Biology and Medicine,* B. Halliwell & J.M.C. Gutteridge (Eds), Oxford University Press, New York, NY

(2) Serbinova, E.A., Tsuchiya, M., Goth, S., Kagan, V.E., & Packer, L. (1993) in *Vitamin E in Health and Disease*, L. Packer & J. Fuchs (Eds), Marcel Dekker, Inc., New York, NY, pp 235–243

(3) Rim, E.B., Stampfer, M.J., Ascherio, A., Giovannucci, E., Colditz, G.A., & Willet, W.C. (1993) *N. Engl. J. Med.* **328**, 1450–1456

(4) Souci, S.W., Fachman, W., & Kraut, H. (1994) In *Food Composition and Nutrition Tables*, Medpharm, CRC Press, Stuttgart, Germany

(5) Parrish, D.B. (1980) *Food Sci. Nutr*. **13**, 161–187

(6) Esterbauer, H., Dieber-Rotheneder, M., Striegel, G., & Waeg, G. (1991) *Am. J. Clin. Nutr.* **53**, 314S–321S

(7) Gey, K.F., Puska, P., Jordan, P., & Moser, U.K. (1991) *Am. J. Clin. Nutr.* **53**, 326S–334S

(8) Ludwicki, J., Tayeb, I.B., & Dillon, J.C. (1986) *Sci. Aliments* **6**, 287–299

(9) Desai, I.D., Bhagavan, H., Salkeld, R., & Oliveiras, J.D. (1988) *J. Food Comp. Anal.* **1**, 231–238

(10) Satue, M.T., Hung, S.W., & Frankel, E.N. (1995) *J. Am. Oil Chem. Soc.* **72**, 1131–1137

(11) Hakansson, B., Jägerstad, M., & Öste, R. (1987) *J. Micronutr. Anal.* **3**, 307–312

(12) Olmedilla, B., Granado, F., Blanco, I., & Rojas-Hidalgo, E. (1992) *Food Chem.* **45**, 205–213

(13) Yao, F., Dull, G., & Eitenmiller, R. (1992) *J. Food Sci.* **57**, 1194–1196

(14) Epler, K.S., Ziegler, R.G., & Craft, N.E. (1993) *J. Chromatogr. B Biomed. Appl.* **619**, 37–48

(15) Lavedrine, F., Ravel, A., Poupard, A., & Alary, J. (1997) *Food Chem.* **58**, 135–140

(16) Shin, T.S., & Godber, J. S. (1993) *J. Am. Oil. Chem. Soc.* **70**, 1289–1291

(17) Eitenmiller, R.R., & Landen, W.O. (1998) in *Vitamin*

Analysis for the Health and Food Sciences, CRC Press, Boca Raton, FL
(18) Bredeweg, R.A., Rothman, L.D., & Pfeiffer, C.D. (1979) *Anal. Chim.* **51**, 2061–2064
(19) *Official Methods of Analysis* (1995) 16th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, **sec 45.1.23-28**
(20) Indyk, H.E. (1990) *Analyst* **115**, 1525–1530
(21) Chase, G.W. Jr, Eitenmiller, R.R., & Long, A.R. (1999) *J. AOAC Int.* **82**, 107–111
(22) *The Merck Index* (1996) 12th Ed., Merck Research Laboratories, Division of Merck & Co., Inc., Whitehouse Station, NJ
(23) Thompson, J.N., & Hatina, G. (1980) *J. Liq. Chromatogr.* **63**, 327–344
(24) SAS Institute, Inc. (1990) *SAS/STAT User's Guide*, SAS Institute, Inc., Cary, NC
(25) Tan, B., & Brzuskiewicz, L. (1989) *Anal. Biochem.* **180**, 368–372
(26) Woollard, D.C. (1987) *Food Technol. Aust.* **39**, 250–253
(27) Elton-Bott, R.R., & Stacey, C.I. (1981) *Anal. Chim. Acta* **127**, 213–218
(28) Landen, W.O. (1982) *J. Assoc. Off. Anal. Chem.* **65**, 810–816
(29) Sun, J. (1999) *J. AOAC Int.* **82**, 68–72
(30) Brittain, H.G. (1997) *J. Validation Technol.* **3**, 275–280
(31) *Peer-Verified Methods Program*, Manual on Policies and Procedures (1993) AOAC INTERNATIONAL, Gaithersburg, MD
(32) Deldime, P., Lefebvre, G., Sadin, Y., & Wybauw, M. (1980) *Rev. Fr. Corps Gras* **27**, 279–282
(33) Hogarty, C.J., Ang, C., & Eitenmiller, R.R. (1989) *J. Food Comp. Anal.* **2**, 200–209-