

## Normal Phase High-Performance Liquid Chromatography Method for the Determination of Tocopherols and Tocotrienols in Cereals

GIANFRANCO PANFILI,\* ALESSANDRA FRATIANNI, AND MARIO IRANO

DISTAAM, Università degli Studi del Molise, via De Sanctis, 86100 Campobasso, Italy

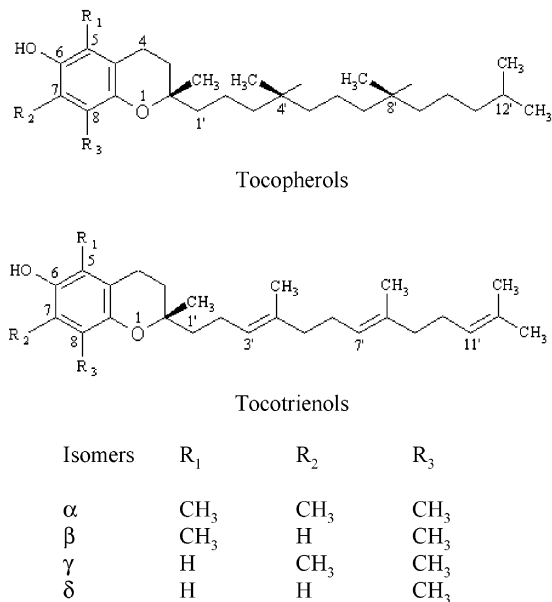
The eight vitamers of vitamin E ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols and -tocotrienols) have different antioxidant and biological activities and have different distributions in foods. Some cereals, especially oat, rye, and barley, are good sources of tocotrienols. A fast procedure for the determination of tocopherols and tocotrienols (tocols) in cereal foods was developed. It involves sample saponification and extraction followed by normal phase high-performance liquid chromatography (HPLC). The results have been compared with those found by direct extraction without saponification. The method is sensitive and selective enough to be tested on a wide variety of cereal samples. The highest tocol levels were found in soft wheat and barley ( $\sim 75$  mg/kg of dry weight).  $\beta$ -Tocotrienol is the main vitamer found in hulled and dehulled wheats (from 33 to 43 mg/kg of dry weight),  $\gamma$ -tocopherol predominates in maize (45 mg/kg of dry weight), and  $\alpha$ -tocotrienol predominates in oat and barley (56 and 40 mg/kg of dry weight, respectively).

**KEYWORDS:** Vitamin E; tocopherol; tocotrienol; HPLC; cereal; food

### INTRODUCTION

Tocopherols and tocotrienols (tocols) are a group of soluble lipid compounds recognized as a generic term for vitamin E, being well represented in vegetables, fruits, seeds, nuts, and oils (1). In animal products, such as dairy foods (2, 3),  $\alpha$ -tocopherol is the major vitamin E vitamer found. Although cereals are a modest source of lipids, they are a good source of tocols even if their concentration and composition vary considerably among the sources (4). Naturally occurring vitamin E is composed of eight vitamers:  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -T); and their four corresponding unsaturated tocotrienols ( $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -T3). Moreover, in certain cereals, together with the naturally occurring eight vitamers of tocols, other components have been identified, such as plastocho- manol-8 (5) and novel tocotrienols (6).

Biological activities of tocols are generally believed to be due to their antioxidant action by inhibiting lipid peroxidation in biological membranes.  $\alpha$ -Tocopherol has been labeled as the most efficient antioxidant for breaking free radical driven chain reactions. However, recent results indicate that  $\alpha$ -tocotrienol is at least 3-fold more efficient as a scavenger of peroxy radicals than  $\alpha$ -tocopherol (7). Tocotrienols were found to lower serum cholesterol levels in various animal models and humans (8, 9). The number and localization of the methyl groups of their chroman rings influence their biological activities,  $\delta$ -tocotrienol (8-methyl) being the most potent cholesterol inhibitor, followed by  $\gamma$ -tocotrienol (7,8-dimethyl) and  $\alpha$ -tocotrienol (5,7,8-trimethyl) (10) (Figure 1). Furthermore, the tocotrienols differ substantially in their capacity to suppress tumor cell proliferation (11).



**Figure 1.** Chemical structures of tocopherols and tocotrienols.

The structural complexity and the wide variation in biological activity of the different vitamin E vitamers necessitate reliable analytical techniques for the isolation, separation, differentiation, and quantification of individual components in mixtures derived from various sample matrices. Moreover, the increased interest in these compounds has created a need for routine analysis in which reproducible separation of all vitamers is obtained.

HPLC provides a convenient method for quantification and characterization purposes of vitamin E. Several chromatographic HPLC methods are available in the literature, and attempts to independently analyze tocopherols and tocotrienols have been performed on both normal and reversed phase columns (12). Although reversed phase columns are generally known to have the advantages of better stability and longer durability than normal phase columns, the latter are more efficient in separating  $\beta$  and  $\gamma$  isomers of tocopherols and tocotrienols. Moreover, they provide the possibility of operating with organic solvents, allowing a high solubility of lipids.

Considering the qualitative and quantitative variability of tocopherols and tocotrienols in plants, the use of normal phase columns is required for the analysis of food samples, like some cereals such as barley, which contain all of the vitamin E vitamers, including  $\beta$  and  $\gamma$  isomers of tocopherols and tocotrienols.

Among the several methods available in the literature that use normal phase columns, complete peak separation is not often achieved (13). Furthermore, methods have been applied to the characterization of cereal tocopherols (5, 14, 15), without any systematic comparison of different extraction procedures suitable for a quantitative recovery of tocopherols from cereal samples.

The aim of this study was to develop a normal phase HPLC method for a fast and reliable simultaneous determination and quantification of tocopherols and tocotrienols of cereals. Moreover, to achieve an extraction procedure that facilitates the isolation of cereal tocopherols with a reduced loss of tocol derivative compounds, a comparison among three different extraction methods was made. Finally, the proposed method has been tested on different cereal species.

## MATERIALS AND METHODS

**Chemicals.**  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols and  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocotrienols were from Merck (Darmstadt, Germany). All other reagents were of analytical or HPLC grade and were purchased from Carlo Erba (Milano, Italy).

**Samples.** Different cereal products were purchased in local stores (corn and oat) or supplied by research institutes (wheat, spelt, triticale, and barley). Cereal samples were collected according to standard sampling procedures and were stored at 4 °C. Immediately before analysis, the samples were ground in a Cyclotec 1093 laboratory mill with a 0.5 mm sieve (FOSS Italia, Padova, Italy) and carefully mixed.

**Equipment.** Chromatography was performed using an HPLC analytical system comprising a Waters model 510 solvent delivery system (Milford, MA) equipped with an injector with a 50  $\mu$ L loop (Rheodyne, Cotati, CA) and a programmable model 470 spectrofluorimeter. Results were evaluated by a Waters Millennium chromatography system.

**Sample Preparation. Saponification.** Tocopherols were extracted according to the method reported by Fratianni et al. (16), as follows: 2 g of cereal sample was saponified under nitrogen in a screw-capped tube with 2 mL of potassium hydroxide (600 g/L), 2 mL of ethanol (95%), 2 mL of sodium chloride (10 g/L), and 5 mL of ethanolic pyrogallol (60 g/L) added as antioxidant. The tubes were placed in a 70 °C water bath and mixed every 5–10 min during saponification. After alkaline digestion at 70 °C for 45 min, the tubes were cooled in an ice bath, and 15 mL of sodium chloride (10 g/L) was added. The suspension was then extracted twice with a 15 mL portion of *n*-hexane/ethyl acetate (9:1 v/v). The organic layer was collected and evaporated to dryness, and the dry residue was dissolved in 2 mL of isopropyl alcohol (1% in *n*-hexane).

**Extraction without Saponification.** The procedure for extraction without saponification was similar to that of the extraction with saponification except that 2 mL of distilled water instead of potassium hydroxide was added to the extracting mixture. After the second extraction with *n*-hexane/ethyl acetate, the suspension was further

extracted with 15 mL of *n*-hexane/ethyl acetate, and solvent and sample residue were separated by filtration. The upper layer was collected and evaporated to dryness, and the dry residue was dissolved in 2 mL of isopropyl alcohol (1% in *n*-hexane and filtered prior injection).

**Methanol Extraction.** Direct extraction of tocopherols was obtained according to the method of Peterson and Qureshi (17). Cereal sample (0.5 g) was extracted with 7 mL of methanol on a horizontal shaker for 15 min at 200 oscillations/min. After centrifugation (15 min at 1000g), the supernatant was collected and the solvent evaporated under vacuum. The residues were extracted a second time with 7 mL of methanol and, after the procedure described above, they were added to the extracts of the first extraction after most of the solvent had evaporated. The dry residue was redissolved in 2 mL of isopropyl alcohol (1% in *n*-hexane instead of *n*-hexane and filtered prior injection).

A sample volume of 50  $\mu$ L was injected for the chromatographic analysis. For the recovery procedure 2 g of a barley sample was spiked with 200  $\mu$ L of a solution of  $\alpha$ -tocopherol (37  $\mu$ g/mL),  $\beta$ -tocopherol (42  $\mu$ g/mL),  $\gamma$ -tocopherol (46  $\mu$ g/mL),  $\delta$ -tocopherol (35  $\mu$ g/mL), and  $\alpha$ -tocotrienol (140  $\mu$ g/mL). The sample was submitted in triplicate to the entire procedure of saponification, extraction, and chromatographic determination.

**HPLC Analysis.** The chromatographic separation of the compounds was achieved with the normal phase method of Shin and Godber (14) with a slight modification. A 250 mm  $\times$  4.6 mm i.d., 5  $\mu$ m particle size, Kromasil Phenomenex Si column (Torrance, CA) was used. The mobile phase was *n*-hexane/ethyl acetate/acetic acid (97.3:1.8:0.9 v/v/v) at a flow rate of 1.6 mL/min. The mobile phase was previously degassed by sonication for 10 min. Fluorometric detection of all peaks was performed at an excitation wavelength of 290 nm and an emission wavelength of 330 nm.

**Standards Preparation.**  $\alpha$ -,  $\gamma$ -, and  $\delta$ -tocotrienol standards were obtained from a saponified barley fraction enriched by pearling in a TM-05 Taka-Yama testing mill fitted with a 40P abrasive roller (from Italia srl Brughiero, Milano, Italy), whereas  $\beta$ -tocotrienol was obtained from a saponified sample of durum wheat. These standards were purified by an HPLC separation technique using the normal phase method of Shin and Godber (14) modified as follows: mobile phase, *n*-hexane/ethyl acetate/acetic acid (97.3:1.8:0.9 v/v/v); flow rate, 3.0 mL/min on a semipreparative 250 mm  $\times$  10 mm, 5  $\mu$ m particle size, Hichrom Si column (Kromasil, Reading, Berkshire, U.K.). Each tocotrienol was collected during several chromatographic runs, dried under vacuum, and dissolved in ethanol. The chromatographic purity of each tocotrienol, monitored by analytical HPLC (data not shown), as reported under HPLC Analysis, was >99%. The concentrations of purified tocotrienols were calculated from the optical density of each ethanol solution using the extinction coefficients reported by Podda et al. (18). Stock solutions of tocopherols (Merck) and tocotrienols (purified) were prepared to a concentration of nearly 500  $\mu$ g/mL in ethanol and stored under nitrogen at -20 °C in the dark. These solutions were diluted (1:19) in ethanol and were confirmed for potency against the known extinction coefficient of each vitamer (18). The working solution was prepared by pooling suitable volumes of each stock solution and diluting with isopropyl alcohol (1% in *n*-hexane to obtain concentrations ranging from 2.5 to 25  $\mu$ g/mL for each compound).

**Statistical Analysis.** Results were statistically evaluated by means of Student's *t* test.

## RESULTS AND DISCUSSION

Tocopherol and tocotrienol standards are available in commercial isomer kits but, on chromatography, the tocotrienol isomers showed double peaks (Figure 2A), most probably due to the formation of racemic mixtures of 2R and 2S stereoisomers during synthesis (19). Because our aim was to set up a reliable procedure to completely separate all eight isomers from cereals, a mixture containing extracts of purified  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocotrienols and commercial tocopherols was prepared and used for the testing of the chromatographic method studied.

The chromatographic separation of tocopherols in a solution of purified standards is reported in Figure 2B, whereas the

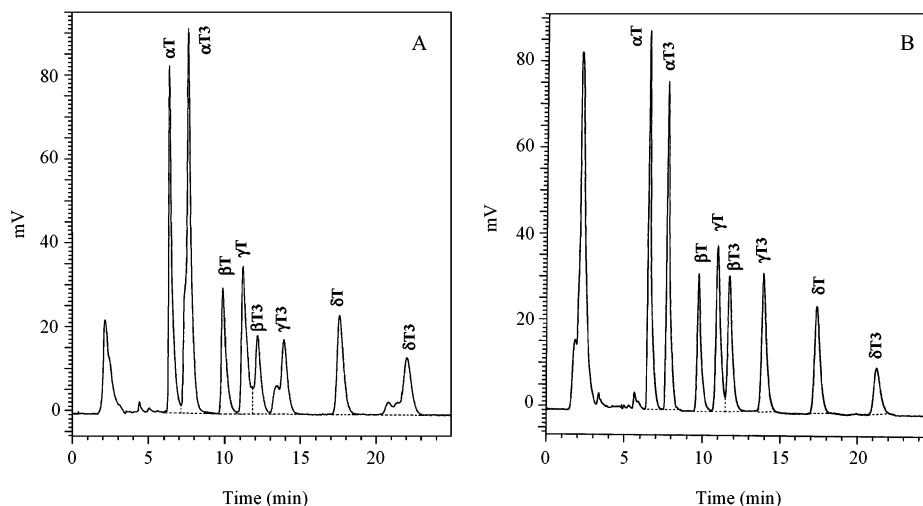


Figure 2. Typical chromatograms of a mixture of commercial standards (A) and purified tocots (B).

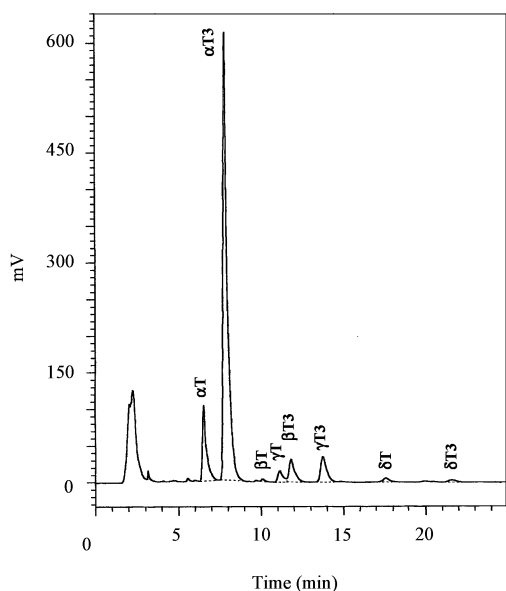


Figure 3. Chromatogram of tocots in a barley sample.

separation of tocots in a barley sample is shown in **Figure 3**. A typical run lasted  $\sim 25$  min, and the eight homologues of vitamin E were baseline separated and eluted as follows:  $\alpha$ -tocopherol,  $\alpha$ -tocotrienol,  $\beta$ -tocopherol,  $\gamma$ -tocopherol,  $\beta$ -tocotrienol,  $\gamma$ -tocotrienol,  $\delta$ -tocopherol, and  $\delta$ -tocotrienol.

With continued use, loss of column efficiency, resulting in particular in a reduction in peak size and inaccurate measurements of the components, was found. The problem of poor batch to batch reproducibility in cereal samples could be due to matrix effects in which interferences may bind analytes of interest (20). Thus, to improve the stability and the reproducibility of the silica column used, after every eight injections the column was reactivated with a solution of 10% isopropyl alcohol in *n*-hexane (v/v). This reactivation produced a constant retention time for each vitamer during several injections. Repeated calibrations with standard solutions during the analysis of a batch of samples were used to verify the assay precision. Results demonstrated a CV < 3% for every vitamin E vitamer (data not shown).

A linearity test with a standard solution was carried out over concentration ranges (micrograms per milliliter) close to the tocol amounts found in cereal samples as follows:  $\alpha$ -tocopherol, 1.0–8.1;  $\beta$ -tocopherol, 0.25–2.0;  $\gamma$ -tocopherol, 0.85–6.9;  $\delta$ -tocopherol, 0.25–2.2;  $\alpha$ -tocotrienol, 2.7–21.7;  $\beta$ -tocotrienol,

0.9–7.1;  $\gamma$ -tocotrienol, 0.45–3.6;  $\delta$ -tocotrienol, 0.2–1.7. Regression analysis of the plot of area response versus concentration of each vitamer revealed an excellent relationship, the correlation coefficients being nearly unity for all standard curves. The detection limit values, estimated according to the method of Kaiser (21) and expressed as amount injected, were 0.1 ng ( $\alpha$ -tocopherol,  $\gamma$ -tocopherol,  $\delta$ -tocopherol, and  $\delta$ -tocotrienol), 0.2 ng ( $\beta$ -tocopherol and  $\beta$ -tocotrienol), and 0.3 ng ( $\alpha$ -tocotrienol and  $\gamma$ -tocotrienol).

As lipophilic substances, tocots are intimately associated with lipid components of the sample matrix; therefore, the various sample preparation procedures serve to liberate vitamin E from the sample matrix and extract it into a solvent that is compatible with the chromatographic system. The sample preparation methods for vitamin E analysis fall into two categories: nonsaponification (solvent extractions) and saponification methods (alkaline hydrolysis) (22). More recently, the use of supercritical fluid extraction has been proposed as an alternative method for the determination of vitamin E vitamers from foods (16, 23). Saponification, involving the heating of the sample in a strong alkaline environment, reduces the amount of organic extractables in a sample. This is accomplished by hydrolysis of lipid esters to more hydrophilic fatty acids and alcohols, which, in a solvent system extraction, will remain in the aqueous phase. This decreases the load of material that will be extracted with tocots into the organic phase and injected into the analytical column.

To better assess the isolation procedure aimed at reducing the loss of tocol derivative compounds and suitable for the food matrix used, three different extraction techniques were evaluated: hot saponification followed by solvent extraction, extraction without saponification, and solvent extraction with methanol.

**Table 1** shows a comparison of the extraction yields of tocopherols and tocotrienols in a barley sample and the percentages of recovery obtained by testing the above-mentioned procedures. Hot saponification followed by solvent extraction provided significantly ( $p < 0.001$ ) higher tocots recoveries. In comparison with the saponification method, solvent extraction without saponification provided tocol recoveries varying from 68% for  $\beta$ -tocopherol to 100% for  $\gamma$ -tocotrienol and  $\delta$ -tocopherol. In methanol extraction these recoveries ranged from 67% for  $\alpha$ -tocotrienol and  $\gamma$ -tocotrienol to 93% for  $\alpha$ -tocopherol. The low recoveries may be attributed to the following factors: (i) extraction of unsaponified samples is more difficult than that

**Table 1.** Tocol Content<sup>a</sup> (Milligrams per Kilogram of Dry Weight) in a Barley Sample and Percentage of Recovery Using Different Extraction Procedures

tocol	hot saponification followed by solvent extraction (A)	solvent extraction without saponification (B)	% recovery (B/A × 100)	methanol extraction (C)	% recovery (C/A × 100)
α-tocopherol	6.2 ± 0.1	4.4 ± 0.3	71	5.8 ± 0.2	93
β-tocopherol	0.8 ± 0.1	0.5 ± 0.2	68	0.5 ± 0.1	68
γ-tocopherol	2.8 ± 0.1	2.3 ± 0.1	82	2.3 ± 0.1	82
δ-tocopherol	0.7 ± 0.0	0.7 ± 0.0	100	0.6 ± 0.0	86
α-tocotrienol	44.1 ± 1.9	35.7 ± 2.9	81	29.8 ± 0.7	67
β-tocotrienol	7.2 ± 0.1	6.6 ± 0.5	92	5.2 ± 0.2	72
γ-tocotrienol	13.6 ± 0.2	13.6 ± 0.6	100	9.1 ± 0.5	67
δ-tocotrienol	0.8 ± 0.0	0.7 ± 0.0	90	0.6 ± 0.0	75

<sup>a</sup> Mean of three determinations.**Table 2.** Analytical Recoveries of Tocols (Mean<sup>a</sup> ± SD) Added to a Barley Sample

tocol	theoretical (natural + added), μg	found, μg	% recovery
α tocopherol	20.03	19.37 ± 0.57	96.73 ± 2.30
β tocopherol	10.24	9.97 ± 0.30	97.38 ± 2.44
γ tocopherol	14.84	14.27 ± 0.24	96.15 ± 1.36
δ tocopherol	8.84	9.22 ± 0.13	104.38 ± 1.24
α tocotrienol	58.20	57.28 ± 2.46	98.41 ± 3.72

<sup>a</sup> Mean of three determinations.

of saponified samples, requiring several steps (filtration, SPE, and other purification techniques), owing to the formation of emulsions, the presence of sample particles, and the high amount of lipidic compounds extracted; (ii) some tocopherols may exist in ester forms or as compounds bonded to the matrix without saponification. These findings, in accord with those of previous works (16, 24, 25), show that, to quantify total tocopherols from cereals, a saponification procedure, which transforms the tocol esters into their corresponding alcohols, is needed. Moreover, the alkaline hydrolysis gave clearer extracts because free alcohols could be separated from the soaps and the interfering substances by extraction with organic solvents. In light of the above-reported results the saponification procedure was selected as an elective method for the extraction of tocopherols from cereals.

To standardize the extraction procedure and to study the accuracy of the proposed method, a barley sample was spiked with known quantities of tocopherols and tocotrienols. The results, given in **Table 2**, provide evidence of the quantitative recovery of all vitamers and of good reproducibility, the mean recovery values (percent) for α-, β-, γ-, and δ-tocopherols and α-tocotrienol always being >95% with a standard deviation ranging from 1.24 to 3.72. These results indicate that the assay procedure used provided a successful determination of tocopherols within an acceptable precision range.

Several cereal species were analyzed using the method developed. Results are shown in **Table 3**, where tocopherols are

reported as single isomers and as the sum of single tocopherols (total tocopherols). The table also provides the vitamin E activity, expressed by the tocopherol equivalents (TE), calculated as reported in Sheppard et al. (26), and the tocotrienols/tocopherols ratio (T3/T), which is an index of the different distributions of tocopherols within the kernel. Barley is the best source of vitamin E vitamers, all eight isomers being detected, with an average of total tocopherols of 75 mg/kg dw. For oats, α-tocotrienol is the major fraction (~40 mg/kg dw for barley and 56 mg/kg dw for oat), followed by α-tocopherol and γ-tocotrienol (~10 mg/kg dw). Unlike barley, no γ-tocotrienol or δ isomers were found in oats. Taking into consideration the vitamin E activity of particular tocopherols, in oats there is a higher level of TE (~34 mg/kg dw), followed by soft wheat and barley (~23 mg/kg dw). Considering the Recommended Dietary Allowance (RDA) of different countries (ranging from 8 to 10 mg of TE), these cereals can be considered good nutritional sources of vitamin E. Moreover, oats are characterized by the highest tocotrienols/tocopherols ratio (6.0). Only α and β isomers of tocopherols and tocotrienols are present in durum wheat, soft wheat, triticale, and spelt, β-tocotrienol being the most representative isomer (from 33 mg/kg dw in spelt to 42 mg/kg dw in soft wheat). With the exception of β-tocotrienol, all vitamin E vitamers were detected in maize, which is characterized by the lowest T3/T ratio (0.3), mainly due to the contribution of the lowest tocotrienol content and the higher proportion of γ-tocopherol (~45 mg/kg dw), which is the main representative isomer.

Our findings are in good agreement with several works previously reported (27, 28), even if cereal tocopherols vary in dependence on genotype and location. Some qualitative differences were found with other authors. In barley the presence of γ-tocotrienol is omitted by some authors (26, 29), whereas in spelt and durum wheat the presence of γ-tocopherol is reported by Grela (30). However, the lack of a standardized method for the extraction and analysis of tocopherols until recently has led to a wide variation in quantities and qualities of the various tocopherols reported. In particular, apart from reversed phase chromatography

**Table 3.** Tocol Content (Mean ± SD) in Different Cereal Species

species	no. of samples	tocopherols (mg/kg of dw)										TE <sup>a</sup>	T3/T
		α-T	α-T3	β-T	γ-T	β-T3	γ-T3	δ-T	δ-T3	total tocopherols			
oat	1	14.9	56.4	3.0	0.4	5.4					72.1	33.6	6.0
spelt	3	10.3 ± 2.0	5.5 ± 0.3	7.0 ± 0.9		32.7 ± 5.0					56.5 ± 7.8	17.2 ± 2.7	2.1
durum wheat	3	8.4 ± 1.0	6.9 ± 1.2	4.8 ± 0.8		39.6 ± 4.9					60.6 ± 7.1	14.7 ± 2.2	3.3
soft wheat	2	15.9 ± 1.6	6.4 ± 0.4	9.5 ± 0.5		42.5 ± 4.3					74.3	23.8	1.9
maize	1	3.7	5.3	0.2	45.0		11.3	1.0	0.4		66.9	9.8	0.3
barley	3	8.6 ± 2.8	40.3 ± 7.1	0.9 ± 0.1	5.6 ± 2.8	8.7 ± 1.7	10.4 ± 2.8	0.7 ± 0.2	0.9 ± 0.3		74.7 ± 13.2	23.3 ± 4.5	3.8
triticale	1	13.6	6.2	6.5		32.0					68.3	20.3	2.4

<sup>a</sup> Vitamin E activity, expressed as tocopherol equivalents, calculated as in Sheppard et al. (26).

graphic analysis, which is not able to resolve  $\beta$  and  $\gamma$  isomers of tocopherols and tocotrienols (12), also some normal phase methods are not able to separate the couple  $\gamma$ -tocopherol/ $\beta$ -tocotrienol, and this could lead to a misinterpretation in the identification of the different isomers.

In conclusion, the proposed method is sensitive and selective enough for the evaluation of the content of all eight isomers of vitamin E in cereal samples. Taking into consideration the different antioxidant and biological activities of the eight vitamin E vitamers, quantitative data on individual tocols may be useful in the evaluation of the healthy and nutritional values of a sample of interest. In particular, due to the variability in the content and in the distribution of tocols in different cereals, the proposed method could be considered also as a useful tool for the analysis of mixtures composed of different cereal species.

#### LITERATURE CITED

- Bramley, P. M.; Elmadfa, I.; Kafatos, A.; Kelly, F. J.; Manios, Y.; Roxborough, H. E.; Schuch, W.; Sheehy, P. J. A.; Wagner, K.-H. Vitamin E. *J. Sci. Food Agric.* **2000**, *80*, 913–938.
- Panfili, G.; Manzi, P.; Pizzoferrato, L. High performance liquid chromatographic method for the simultaneous determination of tocopherols, carotenes, and retinol and its geometric isomers in Italian cheeses. *Analyst* **1994**, *119*, 1161–1165.
- Manzi, P.; Panfili, G.; Pizzoferrato, L. Normal and reversed-phase HPLC for more complete evaluation of tocopherols, retinols, carotenes and sterols in dairy products. *Chromatographia* **1996**, *43*, 89–93.
- Burton, G. W.; Traber, M. G. Vitamin E: antioxidant activity, biokinetics, and bioavailability. *Annu. Rev. Nutr.* **1990**, *10*, 357–382.
- Balz, V. M.; Shulte, E.; Thier, H. P. Trennung von tocopherolen und tocotrienolen durch HPLC. *Fat Sci. Technol.* **1992**, *94*, 209–213.
- Qureshi, A. A.; Mo, H.; Packer, L.; Peterson, D. Isolation and identification of novel tocotrienols from rice bran with hypocholesterolemic, antioxidant, and antitumor properties. *J. Agric. Food Chem.* **2000**, *48*, 3130–3140.
- Packer, L. Nutrition and biochemistry of the lipophilic antioxidants; vitamin E and carotenoids. In *Nutrition, Lipids, Health, and Disease*; Ong, A. S. H., Niki, E., Packer, L., Eds.; American Oil Chemists' Society: Champaign, IL, 1995; pp 8–35.
- Qureshi, A. A.; Burger, W. C.; Peterson, D. M.; Elson, C. E. The structure of an inhibitor of cholesterol biosynthesis isolated from barley. *J. Biol. Chem.* **1986**, *261*, 10544–10550.
- Qureshi, A. A.; Bradlow, B. A.; Salser, W. A.; Brace, L. D. Novel tocotrienols of rice bran modulate cardiovascular disease risk parameters of hypercholesterolemic humans. *J. Nutr. Biochem.* **1997**, *8*, 290–298.
- Qureshi, N.; Qureshi, A. A. Tocotrienols, novel hypocholesterolemic agents with antioxidant properties. In *Vitamin E in Health and Disease*; Packer, L., Fuchs, J., Eds.; Dekker: New York, 1993; pp 247–268.
- He, L.; Mo, H.; Hadisusilo, S.; Qureshi, A. A.; Elson, C. E. Isoprenoids suppress the growth of murine B16 melanoma in vitro and in vivo. *J. Nutr.* **1997**, *127*, 668–674.
- Abidi, S. L. Chromatographic analysis of tocol-derived lipid antioxidants. *J. Chromatogr. A* **2000**, *881*, 197–216.
- Kamal-Eldin, A.; Gorgen, S.; Pettersson, J.; Lampi, A. M. Normal phase high performance liquid chromatography of tocopherols and tocotrienols. Comparison of different chromatographic columns. *J. Chromatogr. A* **2000**, *881*, 217–227.
- Shin, T. S.; Godber, J. Improved high-performance liquid chromatography of vitamin E vitamers on normal-phase columns. *J. Am. Oil Chem. Soc.* **1993**, *70*, 1289–1291.
- Shin, T. S.; Godber, J. Isolation of four tocopherols and four tocotrienols from a variety of natural sources by semi-preparative high-performance liquid chromatography. *J. Chromatogr. A* **1994**, *678*, 49–58.
- Fратиanni, A.; Caboni, M. F.; Irano, M.; Panfili, G. A critical comparison between traditional methods and supercritical carbon dioxide extraction for the determination of tocochromanols in cereals. *Eur. Food Res. Technol.* **2002**, *215*, 353–358.
- Peterson, D. M.; Qureshi, A. A. Genotype and environment effects on tocols of barley and oats. *Cereal Chem.* **1992**, *70*, 157–162.
- Podda, M.; Weber, C.; Traber, M. G.; Packer, L. Simultaneous determination of tissue tocopherols, tocotrienols, ubiquinols, and ubiquinones. *J. Lipid Res.* **1996**, *37*, 893–901.
- Drotleff, A. M.; Ternes, W. Separation and characterization of cis–trans isomers of  $\alpha$ -tocotrienol by HPLC using a permethylated  $\beta$ -cyclodextrin phase. *Z. Lebensm. Unters. Forsch.* **1998**, *206*, 9–13.
- Dolan, J. W.; Snyder, L. R. In *Troubleshooting LC Systems*; Humana Press: Clifton, NJ, 1989.
- Kaiser, H. Quantitation in elemental analysis. *Anal. Chem.* **1970**, *4*, 24A–59A.
- Lang, K. J.; Schillaci, M.; Irvin, B. Vitamin E. In *Modern Chromatographic Analysis of Vitamins*; De Leenheer, A. P., Lambert, W. E., Nelis, H. J., Eds.; Chromatographic Science Series 60; Dekker: New York, 1992; pp 153–190.
- Panfili, G.; Cinquanta, L.; Frатиanni, A.; Cubadda, R. Extraction of wheat germ oil by supercritical CO<sub>2</sub>: oil and defatted cake characterization. *J. Am. Oil Chem. Soc.* **2003**, *80*, 157–161.
- Wang, C.; Ning, J.; Krishnan, P. G.; Matthees, D. P. Effects of steeping conditions during wet-milling on the retentions of tocopherols and tocotrienols in corn. *J. Am. Oil Chem. Soc.* **1998**, *75*, 609–613.
- Ueda, T.; Igarashi, O. Determination of vitamin E in biological specimens and foods by HPLC—pretreatment of samples and extraction of tocopherols. *J. Micronutr. Anal.* **1990**, *7*, 79–96.
- Sheppard, A. J.; Pennington, J. A. T.; Weihrauch, J. L. Analysis and distribution of vitamin E in vegetable oils and foods. In *Vitamin E in Health and Disease*; Packer, L., Fuchs, J., Eds.; Dekker: New York, 1993; pp 9–31.
- Chung, O. K. Cereal lipids. In *Handbook of Cereal Science and Technology*; Lorenz, K. J., Kulp, K., Eds.; Dekker: New York, 1993; p 497.
- Jadhav, S. J.; Lutz, S. E.; Ghorpade, V. M.; Salunkhe, D. K. Barley: chemistry and value-added processing. *Crit. Rev. Food Sci.* **1998**, *38*, 123–171.
- Hakkarainen, R. V. J.; Typponen, J. T.; Bengtsson, S. G. Changes in the content and composition of vitamin E in damp barley stored in airtight bins. *J. Sci. Food Agric.* **1983**, *34*, 1029–1038.
- Grela, E. R. Nutrient composition and content of antinutritional factors in spelt (*Triticum spelta* L) cultivars. *J. Sci. Food Agric.* **1996**, *71*, 399–404.

Received for review January 6, 2003. Revised manuscript received April 7, 2003. Accepted April 8, 2003.

JF030009V