Tocol-Derived Minor Constituents in Selected Plant Seed Oils

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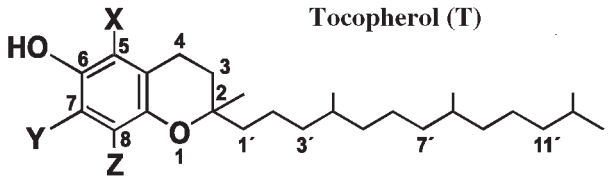
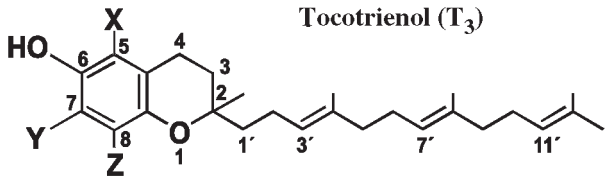
ABSTRACT: Various crude and processed seed oils were analyzed for tocopherols (T) and tocotrienols (T_3) by reversed-phase HPLC with fluorescence detection (FL). The oils included processed canola oil, crude corn oil, crude milkweed oil, crude palm oil, crude/processed rice bran oils, crude/processed soybean oil, crude/processed sunflower oil, and related modified oil varieties. The HPLC system consisted of a pentafluorophenylsilica (PFPS) column and a mobile phase of methanol and water. The results of comparative methodological studies with rice bran oils and milkweed oils indicated that the reversed-phase PFPS-HPLC method in conjunction with the use of less hazardous solvents proved to be superior and a viable alternative to the conventional normal-phase HPLC method. Unlike the traditional nonpolar octadecylsilica phase, which fails to resolve β-γ pairs of T and T_3, HPLC with the unique polar PFPS column enables separations of all compounds of interest. Except for palm oil, βT and γT were detected in all other crude oils. Although most milkweed oils contained moderate levels of βT and γT, the βT species was present in relatively low abundance in edible oils despite the observation of fairly high concentrations of γT in the latter oils. βT_3 and γT_3 were detected along with αT_3 and δT_3 only in palm and rice bran oils. Tocol-derived antioxidant distribution data for zero-time processed oils provided potential utility in correlation studies of frying quality and stability. The variable distribution data for crude oils shed some light on market profitability of oilseeds with rich sources of vitamin E-related minor constituents.

Paper no. J10414 in *JAOCS 80,* 327–333 (April 2003).

KEY WORDS: Content, distribution, edible oil, pentafluorophenylsilica column, plant seed oil, reversed-phase high-performance liquid chromatography, tocopherols, tocotrienols.

Tocol-related compounds, tocopherols (T) and tocotrienols (T_3), are important minor bioactive constituents in vegetable oils. They belong to a family of vitamin E-active substances derived from a chromanol structure (Scheme 1). The compounds occur in plants as closely related homologs/ring position isomers and are known to offer multifaceted health benefits. In physiological systems, the vitamin-active chemicals are essential for mitochondrial electron transport function. Owing to their lipid-soluble antioxidative properties, these compounds inhibit lipid peroxidation processes of PUFA and other compounds in cell membranes (1). Biological activities of the individual lipid antioxidants vary, and their relative





(A) α, X = CH_3, Y = CH_3, Z = CH_3
(B) β, X = CH_3, Y = H, Z = CH_3
(C) γ, X = H, Y = CH_3, Z = CH_3
(D) δ, X = H, Y = H, Z = CH_3

SCHEME 1

stabilities in frying oils are a function of oil varieties: in soybean oil, αT > δT > βT > γT; in corn oil, αT > γT > δT > γT_3; and in palm oil, αT > δT_3 > αT_3 > γT_3 (2). The structural complexity and the wide variation in antioxidative activity of the title compounds necessitate reliable analytical techniques for quantification of individual components in mixtures derived from various sample matrices.

T and T_3 occur in plants in variable abundance. Vegetable oils provide the best sources of these lipid antioxidants. Based on the large numbers of publications available in the literature, most researchers have preferred normal-phase (NP)-HPLC techniques (3–12) for separations of lipid antioxidants because isomeric βT and γT and T_3 have been separated. However, RP-HPLC methods (9,12–15) with an octadecylsilica (ODS) column have so far failed to resolve the β-γ isomers of interest. We report in this paper a polar RP-HPLC column (pentafluorophenylsilica) system suitable for the complete separation of the β- and γ-isomeric tocol-derived compounds. The column procedure, entailing no hazardous solvents, can serve as a viable alternative to conventional normal-phase HPLC. In addition, the content and composition of the tocol-derived oil constituents provide useful information on baseline distribution data for zero-time frying oils (16) currently being used in our ongoing frying oil studies to evaluate the effects of T/T_3 levels on the oxidative stability of stripped frying oils.

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EXPERIMENTAL PROCEDURES

Materials. Tocopherol standards (α-, β-, γ-, and δT) were obtained from Matreya, Inc. (Pleasant Gap, PA). Tocotrienol standards (α-, β-, γ-, and δT_3) were obtained from VWR Scientific (Batavia, IL). Crude canola oil and sunflower oil were obtained from the Northern Crop Science Laboratory, USDA, ARS (Fargo, ND). Pressed, unrefined corn oil was obtained from Spectrum Naturals (Petaluma, CA). Cold-pressed milkweed oils stored under different conditions and milkweed seeds of different harvest years were supplied by Natural Fibers Corporation (Ogallala, NE). Both the oil and seed samples were obtained from various production locations. Fresh milkweed oils were prepared from the seeds by milling in a coffee grinder and extracting in a Soxhlet extractor with petroleum ether followed by evaporation of solvent. Crude palm oil was obtained from Malaysian Palm Oil Board (Selangor, Malaysia). Crude and refined, bleached, and deodorized (RBD) rice bran oils, RBD soybean oil, and high-oryzanol rice bran oil were obtained from Riceland Foods (Stuttgart, AR). RBD cottonseed oil (CSO), RBD high-oleic sunflower oil (HOSUN), RBD mid-oleic sunflower oil (MOSUN), RBD sunflower oil (SUN), RBD soybean oil (SBO), RBD high-oleic soybean oil (HOSBO), and RBD low-linolenic soybean oil (LLSBO) were obtained from commercial oil processors.

General saponification solvents and reagents (i.e., potassium hydroxide, sodium chloride, hexane, ethyl acetate, and methanol) were obtained from Fisher Chemicals (Fairlawn, NJ). Absolute ethanol and pyrogallol were obtained from Aaper Alcohol & Chemical Company (Shelbyville, KY) and Sigma-Aldrich (Milwaukee, WI), respectively. Chromatography solvents were HPLC-grade high-quality products of Fisher Chemicals. Ultrapure HPLC water was obtained by purification of in-house distilled water through a Millipore (Bedford, MA) Milli Q water purifier.

Methods. All HPLC assays were performed on a Thermo Finnigan (San Jose, CA) Spectra System chromatograph consisting of a Model P4000 quaternary pump, a Model AS3000 autosampler, and a membrane degasser. The chromatographic instrument was interfaced with a Applied Biosystems (Foster City, CA) Model 980 Programmable fluorescence (FL) detector and Thermo Finnigan ChromQuest software for handling chromatographic data. Aliquots (5–10 μL) of assay samples in mobile phase solvents with concentrations ranging from 50 to 100 μg/mL were injected on the column *via* a Rheodyne (Cotati, CA) Model 7125 injector fitted with a 20-μL loop. The column effluents were detected at an excitation wavelength of 298 nm and an emission wavelength of 345 nm. Quantification of analytical samples was accomplished by using the automated computer data acquisition/processing system and by calibration with known concentrations of T and T_3 standards. Different modes of HPLC measurements required different sets of calibration plots. All oil samples and standards in amber vials were stored in a freezer, and analytical solutions were freshly prepared prior to assays.

Saponification of selected oil samples. Several oil samples were subjected to saponification for the removal of interfering materials. A literature method (17) was modified as follows: Samples were analyzed in duplicate. To a stirring mixture of an oil sample (2.0 g), 5,7-dimethyltocol (50–100 ppm) and absolute ethanol (70 mL) under a stream of nitrogen, a fresh solution (5 mL) of 5% (w/w) pyrogallol in absolute ethanol, and a 76% (w/w) potassium hydroxide solution (20 mL) were added in sequence. The mixture was heated to 70°C in a water bath and stirred continuously at this temperature for 30 min. After cooling, the saponified matter was treated with a solution (25 mL) of 2.5% (w/w) sodium chloride in water and extracted three times with 50-mL portions of hexane/ethyl acetate (85:15, vol/vol). Solvents were evaporated and the residue was dissolved in methanol (0.5 mL). Upon filtration through a 0.45 μm polypropylene microfilter (Chrom Tech, Apple Valley, MN), the methanolic solution was diluted to exact volume. Aliquot samples were analyzed by RPHPLC. 5,7-Dimethyltocol was used as internal standard.

RP-HPLC-FL detection of T and T_3. The FL detector inlet was connected to an Ansys Technologies, Inc. (Lake Forest, CA) HPLC column (250 × 4.6 mm i.d.) packed with 3 μm pentafluorophenylsilica (PFPS) commercialized under the trade name Taxsil. A mobile phase of methanol/water (9:1) was pumped isocratically through the column at a flow rate of 0.5 mL/min. Normally, the column was ready for sample injections after it had been equilibrated with the mobile phase for about 30 min, as indicated by the steady pump pressure readings. Triplicate injections were made for all methanolic solutions of duplicate analytical samples. For quantitation, calibration curves were constructed with the eight antioxidant standards analyzed under RP-HPLC conditions.

NP-HPLC-FL detection of T and T_3. A published NP-HPLC-FL detection procedure (11) was used with modifications for the analysis of the oil samples. The HPLC instrument described earlier in the general procedure was coupled with an Ansys Technologies, Inc. HPLC column (250 × 4.6 mm i.d.) packed with 3 μm silica. The column was eluted with a mobile phase consisting of hexane/isopropanol (98:2) at a flow rate of 1 mL/min until equilibration was attained. HPLC of samples with complex matrices containing low levels of β- and γ-components often necessitated the use of a hexane/*t*-butyl methyl ether (94:6) mobile phase to identify the analytes of interest unequivocally. As to the NP-HPLC system conditioning, the mobile-phase solvents were prepared by manually mixing them well and then pumped through the column until steady readings of pressure and retention times were obtained. The conditioning process was necessary because changes in column conditions occurred upon disruption of assays due to solvent evaporation and adsorption on the column. It usually required 2–3 h to get the column well conditioned before assays. Subsequently, solutions of samples in hexane were injected in triplicate onto the column and quantified for T and T_3 by calibration with standards analyzed under NP-HPLC conditions. Samples were analyzed in duplicate.

Statistical analysis. Data from duplicate sample analyses

were computed for SD and reported in the tables as averages of relative standard deviation (RSD).

RESULTS AND DISCUSSION

In conventional RP-HPLC, an ODS column commonly has been used for the analysis of tocol-derived antioxidants in vegetable oils. The ODS-HPLC method has been very convenient and useful for studies where separations of β-γ pairs of T and their 3′,7′,11′-unsaturated analogs (Scheme 1), T_3, are not critical for specific study objectives. However, oil chemical investigations often require complete resolution of these isomeric compounds as distinguishable chemical entities. Under the RP conditions, neither the βT-γT nor the βT_3-γT_3 isomeric pairs can be resolved with the ODS phase because of the close similarity in hydrophobicity of the 5,8-dimethyl- and 7,8-dimethyl-substituted tocol structures depicted in Scheme 1. On the other hand, the sufficient polarity difference in these compounds facilitates their separation by NP-HPLC on silica. Hence, the NP separation methodology has enjoyed immense popularity despite the use of environmentally hazardous nonaqueous volatile solvents in mobile phases.

In view of the relatively easy equilibration/operation of an RP-HPLC system, we have examined the general applicability of a polar phenyl-bonded silica stationary phase (18,19), pentafluorophenylsilica (PFPS), for the analysis of the title T and T_3 compounds in the RP mode. An eight-component mixture of T and T_3 standards αT, βT, γT, δT, αT_3, βT_3, γT_3, and δT_3 were separated on PFPS with a mobile phase of methanol and water. Normally, a complete assay run requires about 35 min. Baseline separations of all components can be achieved by increasing the water content of the mobile phase at the expense of longer analysis times. As shown in Figure 1, the components eluted in the following order: $\delta T_3 \rightarrow \beta T_3 \rightarrow \gamma T_3 \rightarrow \alpha T_3 \rightarrow \delta T \rightarrow \beta T \rightarrow \gamma T \rightarrow \alpha T$. The four unsaturated-side-chain T_3 compounds as a group emerged from the column before the more hydrophobic T component group. Fortuitously, the β-, and γ-position isomers of T- and T_3-antioxidants had the same retention sequence as that observed in NP-HPLC.

Table 1 summarizes the content of T and T_3 in seven vegetable oils analyzed by RP-HPLC-FL detection with a PFPS column. In practice, experimental procedures for analytical sample preparation varied depending on sample matrices of different oils. Among the oils investigated, all but rice bran samples could be assayed by direct injection of oil samples in mobile phase solvents without purification by virtue of the detection specificity of the FL detector. Rice bran oils contained complex endogenous substances that interfered with the antioxidant assays and therefore required sample cleanup prior to HPLC analyses. However, the tocol-derived antioxidant data in Table 1 were obtained for comparison of different oils assayed under the same conditions. Therefore, for the purpose of procedural consistency, all samples were saponified and purified in the same manner. Examples of RP-PFPS-HPLC separations of some oil samples are given in Figures 2–4.

The data in Table 1 show a wide variability in the distribution of tocol-derived antioxidants in various oils. Of the oils analyzed, three oils, i.e., crude canola, crude soybean, and crude sunflower, contained only tocopherols at total tocopherol levels ranging from 1323 to 430 ppm. Although all eight components of T and T_3 were present in variable amounts in all five crude rice bran oils tested, βT and βT_3 were absent in both the RBD rice bran oils assayed. Significant losses in amounts of T and T_3 antioxidants during RBD processing were evident when compared to those in crude oils. Thus, the content of total antioxidants in the investigated crude rice bran oils ranged from 457 to 1425 ppm, which appeared to be more than twice that of the RBD rice bran oils (197–239 ppm). A

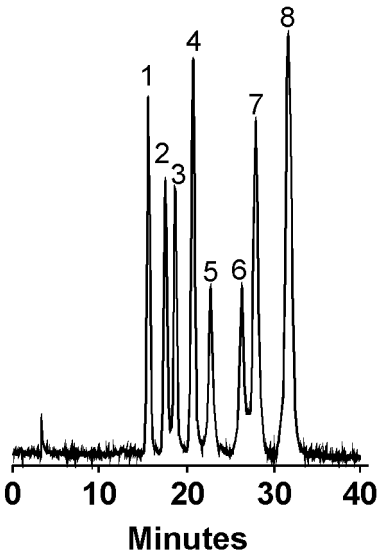

FIG. 1. RP-HPLC-fluorescence (FL) separation of tocopherol (T) and tocotrienol (T_3) standards on a pentafluorophenylsilica (PFPS) column. Mobile phase, methanol/water (9:1). Peaks (1) δT_3, (2) βT_3, (3) γT_3, (4) αT_3, (5) δT, (6) βT, (7) γT, (8) αT.

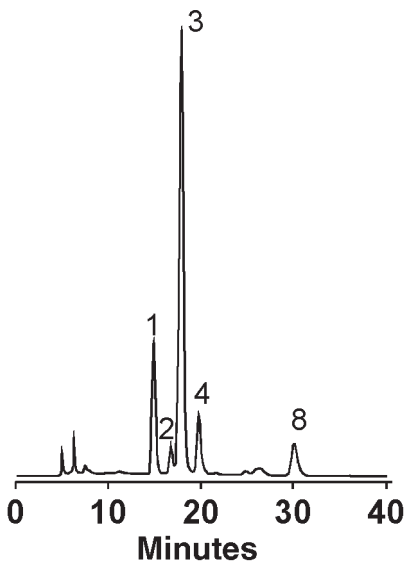

FIG. 2. RP-PFPS-HPLC-FL separation of a crude palm oil. Conditions, peak assignments, and abbreviations are the same as in Figure 1.

TABLE 1
Content of Tocopherols (T) and Tocotrienols (T_3) in Selected Commercial Edible Oils (ppm)[a]

	Component								
Sample	δT_3	βT_3	γT_3	αT_3	δT	βT	γT	αT	Total
Crude canola oil	ND	ND	ND	ND	11.8	1.44	370	47.0	430
Unrefined corn oil	9.33	ND	57.0	ND	45.2	8.11	1345	51.4	1516
Crude palm oil	81.0	26.1	398	78.3	ND	ND	ND	28.0	611
Crude rice bran oil-1	31.4	2.40	352	45.2	15.0	2.39	58.0	38.3	545
Crude rice bran oil-2	37.1	3.33	331	32.0	10.4	6.42	98.0	10.4	529
Crude rice bran oil-3	42.0	5.10	416	41.2	6.11	4.30	69.4	26.3	610
Crude rice bran oil-4	51.4	8.00	868	142	23.2	4.44	203	125	1425
Crude rice bran oil-5	27.0	3.17	272	22.3	18.4	9.23	89.1	16.0	457
RBD rice bran oil-1	17.3	ND	154	13.3	15.4	ND	34.0	5.09	239
RBD rice bran oil-2	15.4	ND	127	12.3	6.44	ND	14.3	21.1	197
RBD PHsoybean oil	ND	ND	ND	ND	74.3	5.38	432	14.0	526
RBD soybean oil	ND	ND	ND	ND	13.3	Trace	495	52.0	560
Crude soybean oil	ND	ND	ND	ND	211	9.07	1081	22.4	1323
Crude sunflower oil	ND	ND	ND	ND	7.26	18.3	69.3	401	496
Av. RSD[b]	4.5	8.3	2.6	3.3	6.9	9.0	5.4	6.0	5.8

[a]Analyzed by RP-HPLC with a pentafluorophenylsilica column. ND, none detected; RBD, refined, bleached, deodorized; PHsoybean oil, partially hydrogenated soybean oil. All samples were purified by saponification.
[b]Av. RSD, average relative SD.

similar trend was observed from the data for soybean oils (Table 1). The total tocol-derived antioxidant contents in RBD soybean oil, RBD partially hydrogenated soybean oil, and crude soybean oil were 560, 526, and 1323 ppm, respectively.

Inspection of the data (Table 1) for canola oil, corn oil, and soybean oils revealed that γT was the most abundant species found in these oils. However, αT was the most predominant tocol-derived antioxidant constituent in sunflower oil. For the two T_3-rich oil varieties, i.e., palm and rice bran, γT_3 was detected in these oils at the highest levels. Generally, βT and βT_3 were found to exist in edible oils in fairly low abundance (βT, 0.00–18.3 ppm; βT_3, 0.00–8.00 ppm, Table 1). Thus, except for sunflower oil, the β-antioxidant species were either absent or present at smallest amounts. Although sunflower oil contained δT as the least abundant species, βT was identified in other test oils at trace or minimal concentrations. Careful examination of the data for rice bran oils (Table 1) indicated that the content of the two minor components βT and βT_3 in some rice bran oils (samples 2 and 5, Table 1) could shift levels of low abundance. In these two samples, βT_3 was the least abundant species.

Table 2 includes some data for T in a number of zero-time modified oils used as baseline references for our frying oil studies (16). Since there were few interferences from sample matrices of the listed samples, RP-PFPS-HPLC-FL assays were carried out by direct injection of analytical aliquots in a

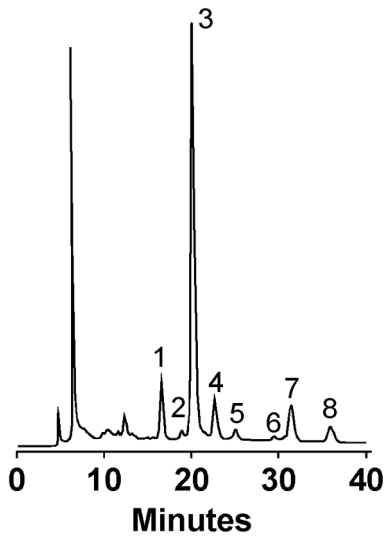

FIG. 3. RP-PFPS-HPLC-FL separation of a crude rice bran oil. Conditions, peak assignments, and abbreviations are the same as in Figure 1.

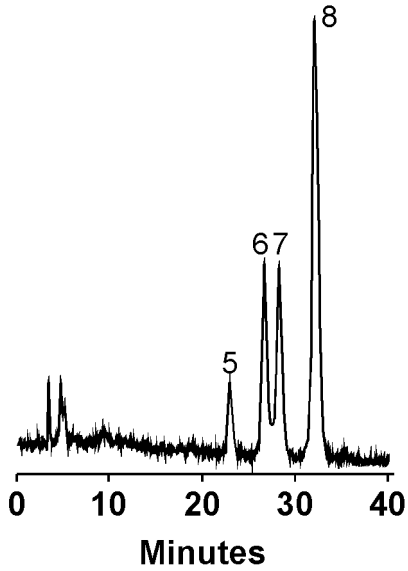

FIG. 4. RP-PFPS-HPLC-FL separation of a crude sunflower oil. Conditions, peak assignments, and abbreviations are the same as in Figure 1.

TABLE 2
Content of T in Various RBD Zero-Time Modified Frying Oils (ppm)[a]

Sample	Component				Total
	δT	βT	γT	αT	
CSO-1	Trace	ND	102	205	307
CSO-2	Trace	ND	120	289	409
LoLnSBO	60.3	ND	229	18.0	307
HOSBO	123	ND	271	22.2	416
SUN	Trace	12.4	36.3	233	282
MOSUN-1	Trace	14.7	29.4	255	299
MOSUN-2	ND	7.20	19.2	87.2	114
MOSUN-3	Trace	12.8	24.1	217	254
MOSUN-4	Trace	16.0	24.4	243	283
HOSUN-1	Trace	11.4	19.3	325	356
HOSUN-2	Trace	Trace	22.9	189	212
Av. RSD	2.5	8.8	2.9	3.6	4.5

[a]Analyzed by RP-HPLC with a pentafluorophenylsilica column. All samples were analyzed by direct injections without saponification. CSO, cottonseed oil; LoLnSBO, low-linolenic soybean oil; HOSBO, high-oleic soybean oil; SUN, sunflower oil; MOSUN, mid-oleic sunflower oil; HOSUN, high-oleic sunflower oil; for other abbreviations see Table 1.

mobile phase solvent without purification. The frying oil gold standard, cottonseed oil (CSO), had γT and αT as the major species. βT was not found in either variety of CSO, nor in soybean oils [low-linolenic soybean oil (LoLnSBO) and HOSBO], but was present in small amounts in all sunflower oil varieties—SUN, MOSUN, and HOSUN. Generally, genetic modification of oilseeds caused little modulation in the T distribution of oils. Thus, as observed in normal oils (Tables 1 and 2), γT and αT were the most abundant tocol-derived antioxidants in modified SBO and modified SUN strains, respectively.

Table 3 compares the content of T and T_3 in various rice bran oils evaluated by RP-PFPS-HPLC and NP-silica-HPLC. The results from the two HPLC methods were in fairly close agreement. Rice bran oils, which normally contain moderate levels of T and T_3, were chosen for the comparative methodological study because oil samples of variable storage times and sampling sources were more accessible than other oils. Since different sets of samples of various origins were used in the two tables, the rice bran oils listed in Table 3 had significantly greater total amounts tocol-derived antioxidants (crude oils, 1649–1867 ppm; RBD oils, 689–770 ppm) than those (crude oils, 457–1425 ppm; RBD oils, 197–239 ppm) in Table 1, reflective of longer storage and sampling times for the latter old rice bran oils (aged for 1–2 yr). As mentioned earlier, rice bran oil sample matrices were quite complex and required saponification prior to HPLC analyses. Although there were often some early-eluting unidentifiable peaks preceding the T- and T_3-components observed in NP-HPLC, much cleaner HPLC chromatograms were obtained with the samples run by RP-PFPS-HPLC (Figs. 2–4). In the RP-HPLC system, the undesirable nonpolar peaks posing a nuisance to assays were usually absent on the chromatograms, as they were either retained by the PFPS column or eluted far beyond anlysis times. Whenever necessary, the RP-HPLC method has been employed in our laboratory to corroborate NP-HPLC results.

In search for a new value-added crop, it was deemed appropriate to include nonedible milkweed oils (20) in this study in order to compare their antioxidant contents with those of food-grade commercial edible oils. Table 4 shows the content of T in milkweed oils analyzed by both RP-HPLC and NP-HPLC. The PFPS-RP-HPLC results were in good agreement with those obtained by silica-NP-HPLC. A stability study on the same sets of samples showed that storage of samples in a freezer for a short period (3 mon) caused few changes in T levels in all samples. T_3 were detected in none of the samples tested. Contingent on sample sources and variations in extraction methods, the total T occurring in milkweed oils varied from 0.00 to 552 ppm. In most cases, the antioxidant levels in freshly solvent-extracted samples (484–683 ppm) (samples 1–7, Table 4) appeared to be higher than those in cold-pressed samples (average 295 ppm) (samples 8–12, Table 4) provided by industrial suppliers.

Similar to sunflower oil but contrary to most of the edible

TABLE 3
Content of T and T_3 in Various Rice Bran Oils (ppm)[a]

Sample	Component															
	αT		$αT_3$		βT		γT		$γT_3$		δT		$δT_3$		Total	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Crude	243	240	330	334	16.0	15.1	110	107	1075	1080	35.9	36.3	57.0	58.4	1867	1871
RBD	120	118	150	148	ND	ND	45.2	46.4	411	414	11.5	11.8	31.9	30.2	770	768
Crude	215	220	218	206	16.1	17.0	115	118	1000	1005	25.9	30.4	58.7	61.2	1649	1658
RBD	119	120	121	115	ND	ND	33.0	35.5	369	371	18.0	16.3	28.9	29.9	689	688
Crude	213	215	310	306	14.5	13.3	99.2	100	1011	1017	30.9	31.7	68.0	70.5	1747	1754
RBD	117	116	138	140	ND	ND	49.8	50.3	400	396	15.9	17.7	34.5	36.6	755	757
Crude	252	269	308	299	18.3	15.8	111	109	1006	998	46.2	45.0	63.7	66.3	1805	1802
RBD	102	106	136	133	ND	ND	57.0	57.5	355	352	20.0	22.7	33.5	32.2	704	703
HORBO	135	132	22.9	25.0	ND	ND	134	139	193	192	14.8	15.5	32.6	33.0	532	537
Av. RSD	4.2	4.6	2.9	3.1	8.9	9.0	6.0	5.5	2.5	2.7	7.3	6.9	7.0	6.8	5.5	5.5

[a]A, reversed-phase method; B, normal-phase method. HORBO, RBD high-oryzanol rice bran oil. For other abbreviations see Table 1.

TABLE 4
Content of T in Various Milkweed Oils (ppm)

	Component									
	αT		βT		γT		δT		Total	
Sample no.[a]	A	B	A	B	A	B	A	B	A	B
1	340	345	71.3	70.4	75.6	74.3	65.0	64.4	552	554
2	279	278	81.2	82.0	129	128	83.7	82.2	573	573
3	253	251	76.9	77.5	107	105	74.7	75.9	512	509
4	321	324	55.6	55.1	46.8	46.3	60.4	60.2	484	486
5	281	279	79.1	79.4	62.8	65.0	69.2	69.4	492	493
6	386	388	74.3	75.0	151	152	71.3	70.1	683	685
7	351	348	89.6	89.3	134	135	77.1	76.3	652	649
8	28.5	29.0	44.9	46.2	120	121	78.4	78.1	272	274
9	413	417	64.9	65.4	223	222	ND	ND	701	704
10	192	188	ND	ND	ND	ND	ND	ND	192	188
11	61.8	61.0	56.9	57.4	107	108	81.9	83.3	308	400
12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Av. RSD	3.9	4.3	6.6	7.3	6.9	7.0	7.1	6.7	6.1	6.3

[a]Samples 1–7 were petroleum ether extracts prepared freshly in house. Samples 8–12 were cold-pressed milkweed oils provided by industrial suppliers. For abbreviations and sample codes, see Tables 1 and 3.

oils listed in Table 1, the most abundant tocol species found in solvent-extracted milkweed oils (Samples 1–7, Table 4) was αT, at levels of 253–386 ppm. Unlike the T distribution patterns of the edible oils shown in Table 1, the less abundant components βT, γT, and δT of the food-grade oils were present in these milkweed oils at nearly comparable concentrations (βT, 71.3–89.6 ppm; γT, 46.8–151 ppm; δT, 60.4–83.7 ppm, Table 4). The relatively high content of βT in the solvent-extracted milkweed oils was particularly noteworthy in consideration of the low abundance of this species invariably observed in most common edible oils. For cold-pressed milkweed oils (samples 8–12), the data in Table 4 show somewhat erratic T distributions different from those of solvent-extracted oils. In general, milkweed oils might have the potential of providing vitamin E-related neutraceuticals if the cost of removing toxic metabolites from the oilseed would not be too expensive to be of practical value.

The results of this study represent the first report on the use of a PFPS column in simultaneous determinations of T and T_3 in various seed oils. The RP-PFPS-HPLC-FL technique can be utilized not only as a viable alternative but also as a complement to the conventional NP-HPLC procedure to evaluate distributions of T and T_3 in plant seed oils. The method is particularly useful for the antioxidant assays dealing with oil samples that contain interfering endogenous substances such as in rice bran oil varieties. For analysts who prefer nonvolatile mobile phase solvents, the RP-HPLC approach is the method of choice as it can facilitate separations of β-γ isomers and skip the use of volatile hazardous solvents routinely employed in NP-HPLC. Distribution data for tocol-derived antioxidants in zero-time processed edible oils provide useful information on oxidation stability and quality of frying oils. In addition, market profitability of oilseeds relies on the availability of distribution data for the identification of rich sources of minor oil constituents such as vitamin E.

ACKNOWLEDGMENTS

The author thanks Kathy A. Rennick for technical assistance.

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[Received August 7, 2002; accepted December 13, 2002]