Tocol Levels in Milling Fractions of Some Cereal Grains and Soybean

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ABSTRACT: Tocol levels in the milling fractions of rice, barley, corn, wheat, and soybeans were analyzed by HPLC with a fluorescence detector. Among all milling fractions tested in this study, rice germ had the highest total tocol levels. In the four milling fractions of barley, except pearling flour, all eight tocol isomers were detected, and they were more uniformly distributed than in any other cereal grains measured in this study. The total tocol and α-tocopherol levels of wheat germ were significantly ($P < 0.05$) higher than the other wheat milling fractions. A significantly ($P < 0.05$) higher proportion of γ-tocopherol was obtained from corn germ (71.5%) and endosperm (50.3%) than from corn hulls. Only four tocol isomers (α-, β-, γ-, and δ-tocopherol) were detected in soybean milling fractions; no tocotrienol isomers were detected. The δ-tocopherol level of soybean endosperm, although minor, was significantly higher than those in milling fractions of other cereal grains in this study.

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KEY WORDS: Barley, cereal grains, corn, milling, rice, soybean, tocol, tocopherol, tocotrienol, wheat.

Tocopherols and tocotrienols, which are lipid antioxidants, are vitamin E-active substances derived from a chromanol structure. The close relationship of these homologs and isomers depends respectively, on the number and position of methyl groups on the aromatic ring of the tocol backbone in tocopherols. The unsaturated analogs of tocopherol are tocotrienols, in which the carbon-2 triterpenyl side chain contains double bonds at the carbon-3′, -7′, and -11′ positions (1). Tocols (tocopherol + tocotrienol) are known to have a protective effect against approximately 80 disease conditions including cancer, cardiovascular diseases, cell membrane and DNA damage by free radicals, oxidation of LDL and disorders of the skin, eye, lungs, and other lipid-rich body constituents (2–8). α-Tocopherol has been called the most efficient antioxidant for breaking free radical-driven chain reactions. However, a recent study using a chemiluminescence assay in a liposome membrane system indicated that α-tocotrienol is at least threefold more efficient as a scavenger of peroxyl radicals than α-tocopherol (9).

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Since, in general, tocols occur in plants in varying abundance, plant oils are common sources of these lipid antioxidants. Common cereal grains, such as corn, wheat, soybean, rice, and oat, are major sources of tocols. However, the level and relative composition of tocols among the eight possible isomers vary considerably among plant sources. Grain milling, by which process the hull, bran, and germ are removed, reduces the tocol level of the grain because most tocols are concentrated in the outer part of the grain. The tocol composition of a wide range of cereal grains has been reported (10–14). However, most studies of tocols were performed with selected milling fractions, and only limited information is available on the composition of tocols in all milling fractions..

In this study, we determined the composition of individual tocol isomers as well as the content of oil in the milling fractions of soybean and four cereal grains, which are consumed worldwide.

EXPERIMENTAL PROCEDURES

Materials. All milling fractions used in this study were obtained from commercial milling companies: rice from Kimpo Agricultural Cooperative Federation (Kimpo, Korea); wheat from Daesun Flour Mills Co. (Seoul, Korea); barley from Jeongwon Industry, Co. (Seoul, Korea); corn from Samyang Genex Corp. (Inchon, Korea); and soybeans from Dr. Chung's Food Co. Ltd. (Chungju, Korea). Tocopherol isomers and tocotrienol isomers were purchased from Merck (Darmstadt, Germany) and Calbiochem (Calbiochem-Novabiochem, San Diego, CA), respectively. Other chemicals used in this study were purchased from Sigma (St. Louis, MO) and were of analytical grade unless mentioned otherwise.

Lipid extraction. All milling fractions were ground with an electric coffee grinder (HR2185; Philips, Grongingen, The Netherlands) and dried at 60°C and 50 mm Hg for 5 h with a vacuum drying oven (VO-10X; Jeiotec, Seoul, Korea). Lipids containing tocols were extracted with a Soxhlet apparatus by using *n*-hexane as the extraction solvent for 6 h. *n*-Hexane then was evaporated under vacuum at 45°C. The extracted oils were stored at –20°C under nitrogen prior to use. Extraction and analysis were performed in triplicate.

Determination of tocol levels. The tocopherol and tocotrienol isomers of each milling fraction of the individual

grains were analyzed by HPLC according to the previous method (15). A 0.5 g aliquot of the extracted oil, 2 mL 5% pyrogallol solution in ethanol, 30 mL ethanol, and a few boiling chips were placed in a 120-mL flat-bottomed flask fitted with a reflux condenser and heated on a hot plate. When the mixture started boiling, the condenser was removed and 1 mL of 50% aqueous potassium hydroxide was added. The sample was saponified for 5 min under the same conditions. After saponification, the flask was cooled. The mixture was transferred to a 250-mL separatory funnel. Extraction of the sample with 50 mL diethyl ether was repeated twice. The pooled diethyl ether layers were washed five times with 20 mL distilled water, filtered through anhydrous sodium sulfate, and then evaporated at 30°C. The residue was diluted with 10 mL *n*-hexane and filtered through a Millipore 0.45 μm membrane before injection into the HPLC. The LC system consisted of an HPLC (PU-1580; JASCO, Tokyo, Japan) connected to a Rheodyne (Cotati, CA) injector with a 20-μL sample loop and a fluorescence detector (FP-1520; JASCO) with excitation set at 298 nm and emission set at 325 nm. A Lichrospher Si-60 column (5 μm, 250 × 4.6 mm i.d.; Merck, Darmstadt, Germany) was used. The mobile phase was *n*-hexane/2-propanol (99:1) at 1.0 mL/min.

Statistical analysis. Each reported value is the mean of determinations for triplicate samples prepared from each milling fraction, and the data were analyzed by ANOVA and Duncan's multiple range test. Statistical significance was accepted at a level of $P < 0.05$ (16).

RESULTS AND DISCUSSION

The fractional proportions of germ, endosperm, bran, and hull obtained from rice milling were 0.2, 75.1, 7.2, and 17.5%, respectively. The oil contents and tocol levels in rice milling fractions are given in Table 1. The oil contents were much higher in rice germ and bran than in the endosperm and hull. Seven tocol isomers, i.e., α-, β-, γ-, and δ-tocopherol and α-, γ-, and δ-tocotrienol, were detected in all milling fractions except hulls, whereas no β-tocotrienol was detected in any of the four fractions. A significant difference ($P < 0.05$) in the level of all tocol isomers was observed between germ and bran fractions, but no significant difference was found between hull and endosperm fractions. Among the milling fractions, total tocol level was highest in rice germ and lowest in rice hull. As reported previously (15), the major tocol isomer in rice germ was α-tocopherol. The tocotrienols were found predominantly in rice bran. The major tocopherol isomer in all fractions was α-tocopherol, whereas the major tocotrienol isomers in all fractions except hull were α- and γ-tocotrienol.

The fractional proportions of germ, endosperm, pearling flour, bran, and hull obtained from barley milling were 0.3, 72.9, 4.1, 12.6, and 10.1%, respectively. Table 2 presents the oil contents and tocol levels of barley milling fractions prepared during commercial milling processing. The oil content of the milling fractions varied from 0.7% in the barley endosperm to 13.0% in barley germ. In the four milling fractions (except pearling flour), all eight tocol isomers were detected, and they were more uniformly distributed than in other cereal grains reported in this study. However, γ- and δ-tocopherol and tocotrienol were not found in the barley pearling flour fraction. The level of total tocols was highest in the pearling flour, followed by germ, bran, hulls, and endosperm. These results suggested that removal of the hulls, aleurone layer, and germ during the milling process influenced the level of tocols in final milling products such as starchy endosperm and flour. Wang *et al.* (10) reported that the pearling flour fraction of barley milling had the highest level of tocopherol and tocotrienols. α-Tocopherol was the predominant isomer in germ and pearling flour, whereas α-tocotrienol was the predominant isomer in endosperm, bran, and hulls. Peterson (11) reported that α-tocopherol was the predominant isomer in barley germ, but that α-tocotrienol was not detected in the fraction. However, in this study, a substantial quantity of α-tocotrienol was found in barley germ. These differences may be attributable to different genotypes and to different milling processes.

The fractional proportions of germ, endosperm, short, red dog, and hulls obtained from wheat milling were 0.5, 73.0, 1.0, 0.5, and 25.0%, respectively. Wheat germ had the highest oil content of all wheat milling fractions, whereas wheat flour (endosperm) had the least oil content (Table 3). β-Tocotrienol and γ-tocotrienol were not detected in any wheat

TABLE 1
Oil Contents and Tocol Levels[a] in Milling Fractions of Rice

Milling fractions	Oil content (%)	Tocopherol				Tocotrienol				Total
		α-T[b]	β-T	γ-T	δ-T	α-T3[b]	β-T3	γ-T3	δ-T3	T + T3
Germ	25.5 ± 0.1[a]	342.8[a] (80.8[a])	13.3[a] (3.1[a])	22.0[a] (5.2[b])	1.0[a] (0.2[c])	24.8[b] (5.9[c])	ND	18.8[b] (4.4[c])	1.8[b] (0.4[b])	424.5[a]
Endosperm	0.4 ± 0.0[c]	1.7[c] (32.7[b])	0.1[c] (1.9[b])	0.3[c] (5.8[b])	0.1[c] (1.9[a])	1.1[c] (21.2[b])	ND	1.7[c] (32.7[a])	0.2[c] (3.8[a])	5.2[c]
Bran	17.6 ± 0.1[b]	87.3[b] (36.7[b])	2.7[b] (1.1[c])	9.5[b] (4.0[b])	0.4[b] (0.2[c])	64.2[a] (27.0[a])	ND	69.1[a] (29.1[b])	4.4[a] (1.9[b])	237.6[b]
Hull	0.5 ± 0.0[c]	2.4[c] (80.4[a])	0.1[c] (3.3[a])	0.4[c] (13.2[a])	0.1[c] (1.5[b])	ND	ND	ND	0.1[c] (1.6[b])	3.1[c]

[a]Results (mg/kg of fraction) are expressed on a dry weight basis, and means within columns followed by the same letter are not significantly different ($P < 0.05$). Values in parentheses are the percent distribution of each tocol.
[b]T = tocopherol; T3 = tocotrienol; ND, not detected.

TABLE 2
Oil Contents and Tocol Levels[a] in Milling Fractions of Barley

Milling fractions	Oil content (%)	Tocopherol				Tocotrienol				Total
		α-T	β-T	γ-T	δ-T	α-T3	β-T3	γ-T3	δ-T3	T + T3
Germ	13.0 ± 0.4^{a}	91.6^{b} (68.4^{b})	4.6^{b} (3.4^{b})	21.8^{a} (16.2^{a})	1.7^{a} (1.2^{a})	7.7^{c} (5.8^{d})	2.5^{d} (1.9^{e})	3.7^{c} (2.8^{c})	0.4^{c} (0.3^{d})	134.0^{b}
Endosperm	0.7 ± 0.0^{e}	1.6^{c} (13.8^{d})	0.1^{b} (0.9^{c})	0.2^{d} (1.7^{d})	0.1^{c} (0.9^{b})	4.7^{d} (40.5^{b})	2.9^{c} (25.0^{a})	1.7^{d} (14.6^{b})	0.3^{c} (2.6^{b})	11.6^{c}
Pearling flour	10.7 ± 0.1^{b}	169.4^{a} (70.5^{a})	51.8^{a} (21.5^{a})	ND	ND	5.2^{d} (2.2^{e})	13.9^{b} (5.8^{c})	ND	ND	240.3^{a}
Bran	5.6 ± 0.0^{c}	12.1^{c} (10.4^{e})	0.6^{b} (0.5^{c})	4.6^{b} (3.9^{c})	1.1^{b} (0.9^{b})	57.5^{a} (49.4^{a})	15.7^{a} (13.5^{b})	21.0^{a} (18.0^{a})	4.0^{a} (3.4^{a})	116.6^{b}
Hull	2.6 ± 0.0^{d}	10.7^{c} (31.5^{c})	0.2^{b} (0.6^{c})	2.4^{c} (7.0^{b})	0.1^{c} (0.2^{c})	13.5^{b} (39.6^{c})	1.7^{e} (4.8^{d})	4.8^{b} (14.1^{b})	0.8^{b} (2.2^{c})	34.1^{c}

[a]Results (mg/kg of fraction) are expressed on a dry weight basis, and means within columns followed by the same letter are not significantly different ($P < 0.05$). Values in parentheses are the percent distribution of each tocol. For abbreviations see Table 1.

milling fraction. In contrast, Piironen *et al.* (17) and Morrison *et al.* (18) reported the presence of β-tocotrienol in wheat germ and bran. Davis *et al.* (19) detected only four tocopherol isomers, i.e., α-, β-, γ-, and δ-tocopherol, in eight different wheat species, but no tocotrienol isomer. The differences in results of these studies might be due either to different analytical conditions or to actual tocol level differences among the samples; the latter may be related to growth conditions and to genetic variation. The total tocol and α-tocopherol levels of wheat germ were significantly ($P < 0.05$) higher than in other milling fractions. On the other hand, the γ-tocopherol proportion was much higher in wheat endosperm than in other fractions. α-Tocotrienol was a major tocotrienol isomer in all wheat milling fractions. δ-Tocopherol and δ-tocotrienol were detected in wheat milling fractions, although the levels were very low.

The fractional proportions of germ, endosperm, and hull obtained from corn milling were 11.1, 82.9, and 6.0%, respectively. The oil contents and tocol levels of the three milling fractions of corn are given in Table 4. Corn germ showed the highest oil content of all milling fractions, 17.3 and 55.4 times greater than that in corn hulls and endosperm, respectively. Seven tocol isomers—α-, β-, γ-, and δ-tocopherol, and α-, γ-, and δ-tocotrienol—were detected in all milling fractions; no β-tocotrienol was detected. These results were similar to those for rice. The total tocol level of corn germ was the highest of the three fractions. A significantly ($P < 0.05$) higher proportion of γ-tocopherol was obtained from the corn germ (71.4%) and endosperm (50.3%) compared with the hulls (6.4%). This result was similar to that of Moreau *et al.* (14), who reported that the relative percentages of γ-tocopherol in germ, grits, and bran in the dry milling fraction of corn were 65, 28, and 4%, respectively. In contrast, the tocotrienols were located predominantly in the hull fraction. γ-Tocotrienol represented the largest proportion of all tocotrienol isomers. Cooney *et al.* (20) suggested that γ-tocopherol might be a more effective inhibitor of neoplastic transformation than α-tocopherol because of its superior ability to scavenge and chemically reduce nitrogen dioxide without forming a nitrosating intermediate.

The fractional proportions of germ, endosperm, and hull obtained from soybean milling were 2.3, 89.9, and 7.8%, respectively. Unlike most cereals, in which the highest oil content was in the germ fraction, the highest oil content of soybeans was in the endosperm (Table 5). Only four tocol isomers,

TABLE 3
Oil Contents and Tocol Levels[a] in Milling Fractions of Wheat

Milling fractions	Oil content (%)	Tocopherol				Tocotrienol				Total
		α-T	β-T	γ-T	δ-T	α-T3	β-T3	γ-T3	δ-T3	T + T3
Germ	8.4 ± 0.0^{a}	181.6^{a} (70.8^{a})	65.6^{a} (25.5^{a})	6.2^{c} (2.5^{e})	0.1^{b} (0.0^{c})	3.0^{c} (1.2^{d})	ND	ND	0.1^{c} (0.0^{c})	256.5^{a}
Endosperm	0.8 ± 0.0^{d}	2.9^{c} (17.3^{a})	1.4^{c} (8.4^{e})	10.8^{c} (64.7^{a})	0.1^{b} (0.6^{a})	1.4^{c} (8.4^{c})	ND	ND	0.1^{b} (0.6^{a})	16.7^{c}
Short	3.5 ± 0.0^{b}	33.2^{b} (44.1^{b})	15.0^{b} (19.9^{b})	20.5^{b} (27.2^{d})	0.1^{b} (0.1^{c})	6.3^{b} (8.4^{c})	ND	ND	0.2^{a} (0.3^{b})	75.3^{b}
Red dog	3.6 ± 0.0^{b}	22.7^{b} (40.3^{c})	9.7^{b,c} (17.2^{c})	17.4^{b} (31.0^{c})	0.1^{b} (0.1^{c})	6.2^{b} (11.1^{b})	ND	ND	0.2^{a,b} (0.3^{b})	56.2^{b}
Hull	2.3 ± 0.1^{c}	20.1^{b,c} (31.4^{d})	8.0^{b,c} (12.5^{d})	26.5^{a} (41.4^{b})	0.3^{a} (0.4^{b})	9.0^{a} (14.0^{a})	ND	ND	0.2^{a} (0.3^{b})	64.0^{b}

[a]Results (mg/kg of fraction) are expressed on a dry weight basis, and means within columns followed by the same letter are not significantly different $P < 0.05$). Values in parentheses are the percent distribution of each tocol. For abbreviations see Table 1.

TABLE 4
Oil Contents and Tocol Levels[a] in Milling Fractions of Corn

Milling fractions	Oil content (%)	Tocopherol				Tocotrienol				Total
		α-T	β-T	γ-T	δ-T	α-T3	β-T3	γ-T3	δ-T3	T + T3
Germ	55.4 ± 0.1[a]	98.8[a] (21.3[a])	4.2[a] (0.9[a])	331.1[a] (71.4[a])	12.6[a] (2.8[a])	5.5[b] (1.2[c])	ND	10.7[b] (2.3[c])	0.5[a] (0.1[c])	463.4[a]
Endosperm	1.1 ± 0.1[c]	2.3[b] (15.7[b])	0.1[b] (0.7[b])	7.4[b] (50.3[b])	0.4[c] (2.7[a])	1.7[c] (11.5[b])	ND	2.7[c] (18.0[b])	0.7[a] (1.1[b])	15.3[c]
Hull	3.2 ± 0.0[b]	1.6[b] (3.2[c])	0.1[b] (0.2[c])	3.2[b] (6.4[c])	1.0[b] (1.9[b])	16.7[a] (33.2[a])	ND	26.4[a] (52.5[a])	1.3[a] (2.6[a])	50.3[b]

[a]Results (mg/kg of fraction) are expressed on a dry weight basis, and means within columns followed by the same letter are not significantly different ($P < 0.05$). Values in parentheses are the percent distribution of each tocol. For abbreviations see Table 1.

TABLE 5
Oil Contents and Tocol Levels[a] in Milling Fractions of Soybeans

Milling fractions	Oil content (%)	Tocopherol				Tocotrienol				Total
		α-T	β-T	γ-T	δ-T	α-T3	β-T3	γ-T3	δ-T3	T + T3
Germ	8.2 ± 0.3[b]	90.1[a] (27.1[b])	1.4[a] (0.4[b])	222.5[a] (67.0[a])	18.3[b] (5.5[c])	ND	ND	ND	ND	332.2[a]
Endosperm	19.7 ± 0.3[a]	17.3[b] (8.3[c])	1.1[a] (0.5[b])	140.5[b] (67.2[a])	50.1[a] (24.0[a])	ND	ND	ND	ND	209.0[b]
Hull	0.4 ± 0.0[c]	2.7[c] (31.5[a])	0.2[b] (2.3[a])	4.1[c] (48.0[b])	1.6[c] (18.2[b])	ND	ND	ND	ND	8.6[c]

[a]Results (mg/kg of fraction) are expressed on a dry weight basis, and means within columns followed by the same letter are not significantly different ($P < 0.05$). Values in parentheses are the percent distribution of each tocol. For abbreviations see Table 1.

i.e., α-, β-, γ-, and δ-tocopherol, were detected in all soybean milling fractions; no tocotrienol isomers were detected. The oil content in endosperm was 2.4 times higher than in germ. However, the level of total tocols in endosperm was lower than that in germ. As shown in the milling fractions of corn, the major tocopherol of soybean germ, endosperm, and hulls was γ-tocopherol, with quantitative values being 2.5, 8, and 1.5 times greater than α-tocopherol in those fractions, respectively. The δ-tocopherol level for soybean endosperm, although minor, was significantly higher than in the milling fractions of other cereal grains in this study.

In conclusion, oil contents and total tocol levels were higher in germ than in other milling fractions of grains tested in this study except for soybeans and barley. Unlike soybeans, the central region of the starchy endsperm of the grains tested showed a low content of oil and tocols compared with other milling fractions.

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