Effects of Steeping Conditions During Wet-Milling on the Retentions of Tocopherols and Tocotrienols in Corn

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ABSTRACT: Vitamin E is a natural antioxidant that plays significant roles in food preservation and disease prevention. There are eight naturally occurring vitamin E isomers (tocols): α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols. Corn oil is a major source of vitamin E. Most of the corn oil produced in the United States is a co-product of corn wet-milling. There is limited knowledge about the effects of corn wet-milling on the retention of these vitamin E isomers. A high-performance liquid chromatography method was developed for simultaneous determinations of tocols in steeped corn samples. Effects of steeping conditions (steeping time and SO_2 concentration) on retention of tocols in corn were investigated. α-Tocopherol, γ-tocopherol, α-tocotrienol, and γ-tocotrienol are the predominant vitamin E isomers in the corn variety used in the study. Steeping conditions had little effect on the concentration of α-tocopherol and α-tocotrienol. However, a higher concentration of SO_2 and a shorter steeping time gave a slightly higher γ-tocotrienol content and lower γ-tocopherol content. Corn kernels steeped in a vitamin C solution had a much higher concentration of the tocols than those steeped in SO_2 solution.
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KEY WORDS: Corn, HPLC, oil, processings, tocopherols, tocotrienols, vitamin E, wet-milling.

Vitamin E is a natural antioxidant that plays significant roles in food preservation and disease prevention. As shown in Figure 1, there are eight naturally occurring vitamin E isomers (tocols): α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols (1). Vitamin E activity of α-tocopherol is the highest; the other tocols have 15–25% that of α-tocopherol. However, as antioxidants, β-, γ-, and δ-tocopherols and tocotrienols may be superior to α-tocopherol (2–4). Therefore, it is imperative to determine all tocols in evaluating these natural antioxidants.

Corn oil is a major source of vitamin E. Most of the corn oil produced in the United States is a co-product of corn wet-milling (Fig. 2). Corn wet-milling separates corn into its major chemical components: starch, gluten, fiber, and oil. Knowledge about the effects of corn wet-milling on the retention of vitamin E is limited. One study showed that only 27% of the total tocopherols were recovered after wet-milling (5). However, no details were offered regarding which step was most detrimental to the tocopherols. Therefore, each processing step needed to be evaluated for its impact on the tocols.

Analytical techniques for tocols have improved greatly, especially with the application of high-performance liquid chromatography (HPLC). However, several different extraction procedures have been used for tocol analysis. Saponification was used by some researchers and skipped by others (6,7). Furthermore, an extraction procedure for wet corn samples was not available. Therefore, the objectives of the study were: (i) to develop an analytical procedure for vitamin E isomers in steeped corn, and (ii) to investigate the effects of steeping conditions during wet-milling on the retention of vitamin E isomers.

MATERIALS AND METHODS

Materials. The solvents used for extraction and chromatography were HPLC-grade hexane, ethyl alcohol, and 2-propanol. All other reagents (ascorbic acid, sodium chloride, sodium sulfate, and potassium hydroxide) were of analytical grade (Fisher Scientific Co., Pittsburgh, PA). Standards employed for tocol identification and quantitation were obtained from Hoffman-La Roche (Nutley, NJ). The yellow dent corn sample in this study was obtained from the AgriPro Seed Company (Brookings, SD).

Extraction with saponification. Steeped corn samples were finely ground in an A-10 mill (Tekmar, Germany). The mill was cooled by running cold water. About 9.5 g ground corn was weighed and placed in each 250-mL Erlenmeyer flask, which contained 1.2 g ascorbic acid and 20 mL ethyl alcohol. Five milliliters of 60% KOH was quickly added. The flasks were covered and heated at different temperatures (26, 50, 70, and 90°C) for different lengths of time (5, 15, and 45 min). Then the flasks were quickly placed in an ice bath, and 45 mL ice cold water and 45 mL hexane were added. Each flask was shaken for 1 min. Sufficient time was allowed for the formation of a biphasic system. The hexane layer was separated in a separatory funnel and transferred to a 150-mL flask. The

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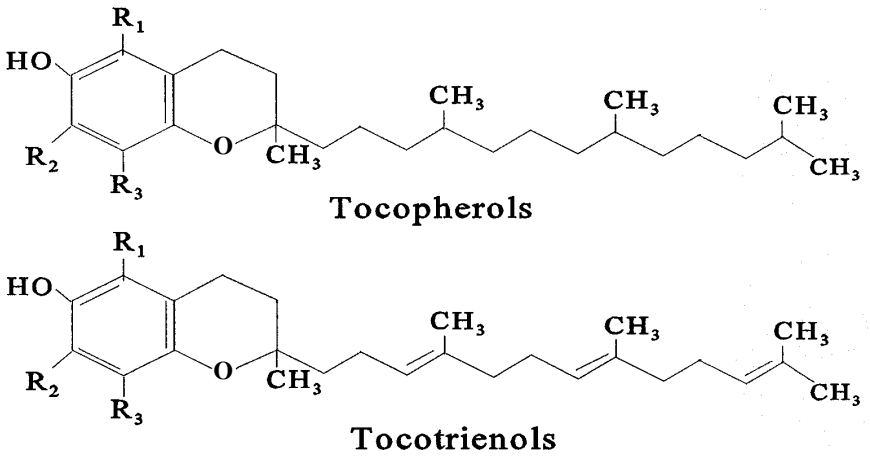

Isomers	R_1	R_2	R_3
α	CH_3	CH_3	CH_3
β	CH_3	H	CH_3
γ	H	CH_3	CH_3
δ	H	H	CH_3

FIG. 1. Chemical structures of tocopherols and tocotrienols.

sample was reextracted with an additional 45 mL hexane. The hexane extracts were then combined, washed three times with 45-mL portions of distilled water to remove residual KOH, and dried with anhydrous sodium sulfate. The extract was then transferred to a 250-mL flat-bottom rotary evaporator flask and evaporated at 50°C under vacuum. After the extract was reduced in volume, it was quantitatively transferred to a 10-mL volumetric flask with a Pasteur pipette and diluted to the mark with hexane. The extract was then covered with aluminum foil and stored at −10°C. HPLC determinations were carried out within the same day. All manipulations were done in a darkened laboratory.

Extraction without saponification. The procedures for extraction without saponification were similar to those of extraction with saponification, except that no KOH was added to the extracting mixture. Two incubating conditions were used: 75°C for 15 min and 25°C for 15 min.

Effects of steeping conditions. The effects of the concentration of the SO_2 solution and steeping time on each tocol isomer were determined by analyzing the samples at three different SO_2 concentrations and three different steeping times (18, 24, and 48 h). The results were also compared with samples steeped with vitamin C (experimental control).

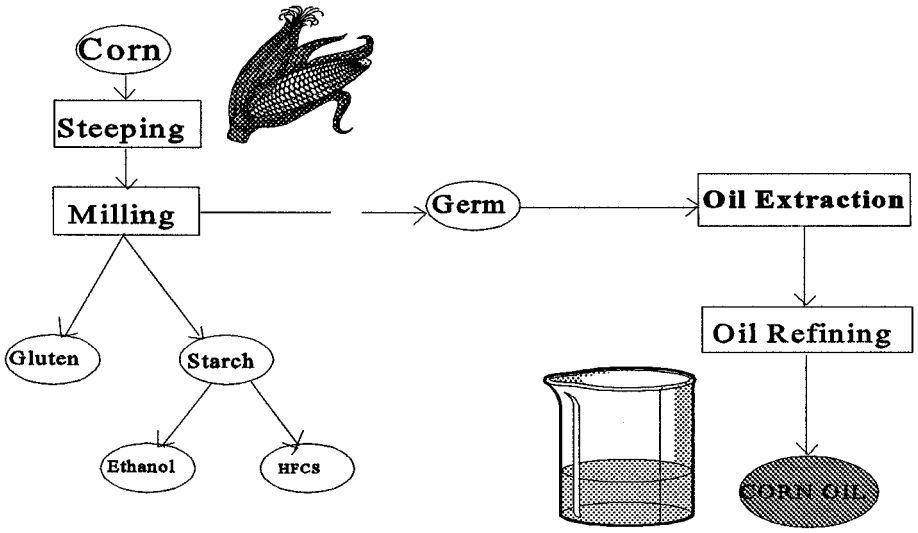

FIG. 2. Schematic chart of corn oil production. HFCS, high-fructose corn syrup.

HPLC system. The HPLC system consisted of a Beckman (Fullerton, CA) 110B pump, a Model 430 Turner spectrofluorometer (Sequoia-Turner Corporation, Mountain View, CA) with a flowcell (Hellma Cells, Inc., Forest Hills, NY) and a Kipp and Zonen recorder (Bohemia, NY). A 250 × 4 mm Lichrosorb Si 60 (5µm) column (Alltech Associates, Inc., Deerfield, IL) was used. The column was protected by a silica-packed guard column.

A mixture of hexane and isopropanol (99.5:0.5) was used as the mobile phase. The ratio was determined by trial and error. Samples were passed through a 0.45-µm nylon filter (Scientific Resources, Inc., Eatontown, NJ) and injected into the HPLC *via* a fully loaded 25-µL loop. The sample was separated on a silica column at ambient temperature at a flow rate of 1 mL/min. The excitation and emission wavelengths of the spectrofluorometer were set at 290 and 320 nm, respectively.

Peak identification and quantitation. Figure 3 shows a typical chromatogram. By comparing with standards, peak 1 and peak 3 of the chromatogram were identified as α- and γ-tocopherols, respectively. Because α- and γ-tocotrienols were not available, they were identified by their natural occurrence and comparison with published chromatograms (8).

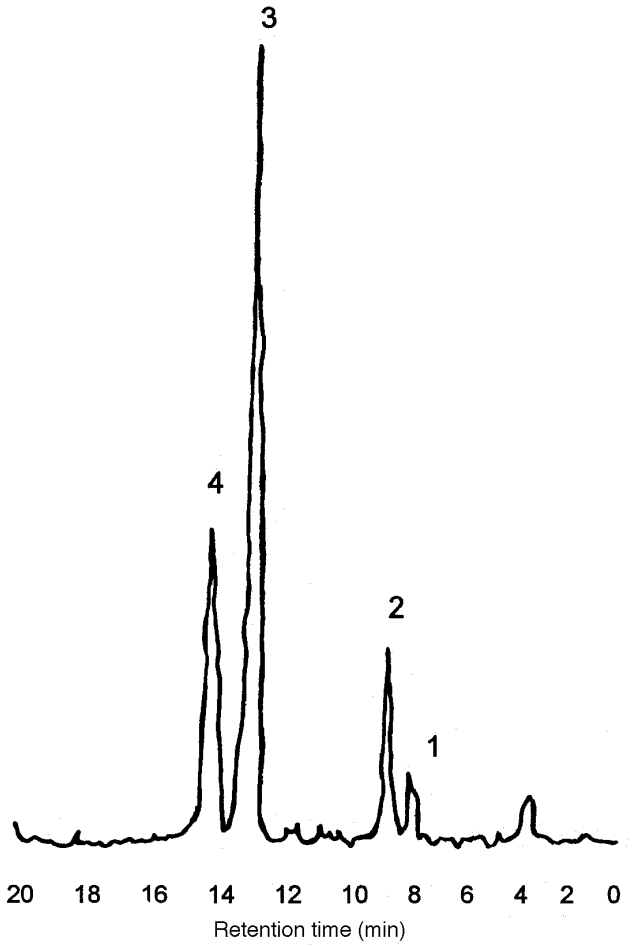

FIG. 3. Chromatogram of extract from corn. Peak identification: 1, α-tocopherol; 2, α-tocotrienol; 3, γ-tocopherol; 4, γ-tocotrienol.

TABLE 1
Gas Chromatograph–Mass Spectrometer Conditions for Peak Identification

Parameter	Value
Injector temperature	240°C
Detector temperature	280°C
Initial column temperature	80°C
Final column temperature	300°C
Rate of increase in temperature	10°C/min
Low mass	29
High mass	650
Threshold	500

The existence of the four tocols in the extracts from the corn was also verified by a Hewlett-Packard 5890 gas chromatograph (Avondale, PA) and 5971 mass spectrometer (GC–MS). The operating conditions of the GC–MS are listed in Table 1. The spectra were obtained by injection of the extract from the sample, and α- and γ-tocopherols were confirmed by comparing the spectra with the MS library. The spectra of α- and γ-tocotrienols were as described in the literature (9), and they were compatible with the calculated molecular weights.

Peak height was used for the quantitation of tocols. Because α- and γ-tocotrienols were not available as standards, α-tocopherol was used for quantitation for all four isomers, based on the assumption that they have the same fluorescence intensity (8).

Statistical analysis. The data were analyzed by the analysis of variance procedure of the Statistical Analysis System (10) program.

RESULTS AND DISCUSSION

Optimization of saponification conditions. Many saponification conditions have been used by different laboratories. Table 2 shows the effects of different saponification conditions on recoveries of tocols. Three saponification conditions

TABLE 2
Effects of Saponification Conditions on Recoveries of Tocol Isomers (µg/g)[a]

Temperature (°C)	Time (min)	α-T	α-T3	γ-T	γ-T3
26	5	2.6^{f}	6.3^{d}	39.0^{e}	4.6^{d}
	15	$3.2^{e,f}$	6.5^{d}	$43.7^{d,c}$	14.9^{d}
	40	$3.4^{c,d,e}$	7.7^{d}	$48.2^{a,b}$	15.5^{d}
50	5	$3.3^{d,e}$	7.5^{d}	$41.3^{d,e}$	$16.0^{c,d}$
	15	$4.1^{b,c}$	9.4^{c}	$47.9^{a,b}$	$17.7^{b,c}$
	40	$4.4^{a,b}$	$10.3^{a,b,c}$	$50.4^{a,b}$	$18.9^{a,b}$
70	5	$4.0^{b,c,d}$	$10.1^{b,c}$	$45.7^{b,c}$	$18.6^{a,b}$
	15	5.0^{a}	$11.2^{a,b}$	51.4^{a}	19.7^{a}
	40	4.9^{a}	11.7^{a}	50.1^{a}	20.0^{a}
90	5	$3.6^{c,d,e}$	$10.1^{b,c}$	$40.3^{d,e}$	$17.7^{b,c}$
	15	1.0^{g}	2.3^{e}	7.2^{f}	4.3^{e}
	40	$<0.5^{h}$	$<0.5^{f}$	2.7^{g}	1.7^{f}

[a]Values with the same letter in the same column are not significantly different ($P < 0.05$). α-T, α-tocopherol; α-T3, α-tocotrienol; γ-T, γ-tocopherol; γ-T3, γ-tocotrienol.

TABLE 3
Comparison of the Different Extraction Methods[a]

	α-T (μg/g)	α-T3 (μg/g)	γ-T (μg/g)	γ-T3 (μg/g)
Extract with saponification	4.8[a]	12.4[a]	53.2[a]	19.7[a]
Extract without saponification (70°C)	2.3[b]	5.3[b]	24.0[b]	9.7[b]
Extract without saponification (25°C)	1.7[c]	3.5[b]	17.2[c]	7.5[c]

[a]Values with the same letter in the same column are not significantly different ($P < 0.05$). For abbreviations see Table 2.

offer the highest recoveries for all tocols. They were 50°C for 40 min, 70°C for 15 min, and 70°C for 40 min. To shorten determination time, 70°C for 15 min was chosen as the optimal saponification condition. This condition was used in subsequent experiments. Saponification at 26°C offered low recovery due to low saponification and less tocols released. Saponifications at 90°C offered even lower recoveries. This is likely due to heat destruction of tocols.

Comparison of extraction with saponification and without saponification. Extraction methods with and without saponification have been used for tocol determination in the past by different laboratories (6,7). One of the objectives of this study was to determine whether saponification is necessary for tocol determination in steeped corn. Table 3 shows that extraction with saponification (70°C for 15 min) gave much higher recoveries for all tocols. Extractions without saponification were inefficient for steeped corn and gave erroneously low results. The low recoveries may be attributed to the following two factors: First, extraction of unsaponified samples was more difficult than of saponified samples owing to the formation of emulsion; and second, tocols may exist in ester forms without sufficient saponification. Therefore, we concluded that the steeped corn samples must be saponified before extraction to achieve high recoveries.

Effects of steeping conditions on the retention of tocols. The wet-milling process involves an initial water soak under carefully controlled conditions to soften the kernels. Commercially, solutions with 0.12–0.20% SO_2 are used for steeping. The SO_2 protects the corn from spoilage and assists in breaking down the protein–starch matrix, which is necessary for high starch yield and quality (11). Table 4 shows the effects of different steeping conditions on the retention of different tocol isomers. Tables 5 and 6 show the overall effects of steeping solution and time. Steeping with vitamin C (experimental control) clearly gave higher retention for all tocol isomers than steeping with SO_2 solutions. There is little overall difference among different SO_2 concentrations. However, higher concentrations of SO_2 seemed to give higher recoveries for γ-tocotrienol and lower recoveries for γ-tocopherol. The reason for this effect is not known. It may be due to isomerization of the two isomers during steeping. The recoveries for α-tocopherol and α-tocotrienol are not significantly different among the three different steeping concentrations of SO_2. At all SO_2 concentrations, the recoveries of γ-tocopherol increased with steeping time, whereas the recoveries of γ-tocotrienol decreased with steeping time. This could be due to the conversion of γ-tocotrienol to γ-tocopherol during the steeping process. The mechanism for this conversion is not understood. However, it is unlikely that the conversion is done by a hydrogenation process, which requires high pressure and metallic catalysts.

TABLE 4
Effect of Steeping Conditions on the Retention of Tocols[a] (μg/g)

Steeping solution	Time (h)	α-T	α-T3	γ-T	γ-T3
0.1% SO_2	18	5.2	12.8	53.5	19.2
	24	5.1	13.5	54.2	19.1
	48	5.6	13.1	55.4	18.4
0.2% SO_2	18	5.0	13.2	50.8	20.2
	24	5.1	13.7	49.7	19.8
	48	5.3	13.6	56.2	18.9
0.3% SO_2	18	5.1	12.9	49.3	20.3
	24	4.9	13.1	49.9	19.6
	48	5.5	14.8	54.7	19.9
1% vitamin C	18	7.1	17.1	60.0	20.8
	24	7.2	15.7	59.5	20.3
	48	6.8	16.3	60.2	20.8

[a]For abbreviations see Table 2.

This research has led to the following conclusions: (i) The corn variety contained α-tocopherol, γ-tocopherol, α-tocotrienol, and γ-tocotrienol. (ii) Steeped corn samples must be saponified prior to extraction of vitamin E isomers. (iii) The optimal conditions for saponification were 70°C for 15 min. Higher temperatures can destroy the vitamin E isomers. Shorter saponification time resulted in significantly lower recovery of the isomers. (iv) Steeping in SO_2 solutions significantly reduced the vitamin E content compared with steeping in 1% vitamin C. (v) Lower concentration of SO_2 and longer steeping time resulted in higher retention of γ-tocopherol and lower retention of γ-tocotrienols.

TABLE 5
Overall Steeping Solution Effects[a]

Concentration	α-T (μg/g)	α-T3 (μg/g)	γ-T (μg/g)	γ-T3 (μg/g)
0.1% SO_2	5.3[b]	13.1[b]	54.4[b]	18.9[c]
0.2% SO_2	5.1[b]	13.5[b]	52.1[c]	19.6[b]
0.3% SO_2	5.2[b]	13.6[b]	51.2[c]	19.9[b]
1% Vitamin C	7.0[a]	16.4[a]	59.9[a]	20.7[a]

[a]Values with the same letter in the same column are not significantly different ($P < 0.05$). For abbreviations see Table 2.

TABLE 6
Overall Steeping Time Effects[a]

Time (h)	α-T (μg/g)	α-T3 (μg/g)	γ-T (μg/g)	γ-T3 (μg/g)
18	5.6[a]	14.0[a]	53.4[b]	20.2[a]
24	5.6[a]	14.0[a]	53.3[b]	19.7[a,b]
48	5.8[a]	14.4[a]	56.5[a]	19.5[b]

[a]Values with the same letter in the same column are not significantly different ($P < 0.05$). For abbreviations see Table 2.

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