Optimal Tocopherol Concentrations to Inhibit Soybean Oil Oxidation

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ABSTRACT: The optimal concentration for tocopherols to inhibit soybean oil oxidation was determined for individual tocopherols (α-, γ-, and δ-tocopherol) and for the natural soybean oil tocopherol mixture (tocopherol ratio of 1:13:5 for α-, γ-, and δ-tocopherol, respectively). The concentration of the individual tocopherols influenced oil oxidation rates, and the optimal concentrations were unique for each tocopherol. For example, the optimal concentrations for α-tocopherol and γ-tocopherol were ~100 and ~300 ppm, respectively, whereas δ-tocopherol did not exhibit a distinct concentration optimum at the levels studied ($P < 0.05$). The optimal concentration for the natural tocopherol mixture ranged between 340 and 660 ppm tocopherols ($P < 0.05$). The antioxidant activity of the tocopherols diminished when the tocopherol levels exceeded their optimal concentrations. Above their optimal concentrations, the individual tocopherols and the tocopherol mixture exhibited prooxidation behavior that was more pronounced with increasing temperature from 40 to 60°C ($P < 0.05$). A comparison of the antioxidant activity of the individual tocopherols at their optimal concentrations revealed that α-tocopherol (~100 ppm) was 3–5 times more potent than γ-tocopherol (~300 ppm) and 16–32 times more potent than δ-tocopherol (~1900 ppm).

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Tocopherols are phenolic antioxidants that occur naturally in vegetable oils and function to maintain oil quality by terminating free radicals. Soybean oil contains a mixture of four tocopherol homologues (α-, β-, γ-, and δ-tocopherols) whose relative concentrations are: *d*-α-tocopherol (4–10%), *d*-β-tocopherol (1–3%), *d*-γ-tocopherol (60–66%), and *d*-δ-tocopherol (24–29%) (1–3). The total concentration of tocopherols in commercial oils depends on the processing steps and the conditions used to convert crude oils into edible products. For example, crude soybean oil contains 1300 to 1600 ppm tocopherol before processing, whereas deodorized oil contains between 800 and 1100 ppm (3). However, the relative amounts of the individual tocopherols do not change significantly with processing (3).

The tocopherol concentration is an important factor that influences tocopherol antioxidant activity in bulk oils. Generally, antioxidant activity is greatest at lower concentrations and decreases or may become prooxidant at higher concentrations. For example, α-tocopherol exhibits optimal antioxidant activity at concentrations between 100 and 250 ppm (4–6). The optimal concentration for γ-tocopherol is between 250 and 500 ppm (4–7), and the optimal concentration for δ-tocopherol is between 500 and 1000 ppm (5,7–10). The optimal concentration for the mixture of tocopherol homologues present in soybean oil is between 500 and 750 ppm (9,11).

The apparent relationship between tocopherol concentration and its antioxidant activity may be influenced by the conditions used to accelerate oxidation in lab studies and by the end points selected to measure oxidation. These factors have contributed to the contradictory opinions in the published literature regarding the concentrations necessary to stabilize soybean oil. Understanding the origin of these ambiguities may enable soybean oil processors to optimize tocopherol antioxidant activity, and oil stability, by managing the final concentrations remaining in edible products. Therefore, the purpose of this research was to further define the optimal concentration limits for the individual tocopherols and for the mixture of tocopherols existing in soybean oil.

MATERIALS AND METHODS

Materials. Refined, bleached, and deodorized soybean oil was obtained from Cargill, Inc. (Des Moines, IA). Individual tocopherols including *d*-α-tocopherol (99.0%) were obtained from Acros Organics (Pittsburgh, PA), and *d*-γ-tocopherol (99.9%) and *d*-δ-tocopherol (92.4%) were obtained from Sigma-Aldrich, Inc. (St. Louis, MO). A mixture of tocopherols in corn oil containing *d*-α-tocopherol (10.8%), *d*-β-tocopherol and *d*-γ-tocopherol (34.2%), and *d*-δ-tocopherol (14.9%) was obtained from Universal Oil Products (Des Plaines, IL).

Preparation of tocopherol-reduced soybean oil. The tocopherols were removed by short-path vacuum distillation (SPVD) using a model KDL-4 distillation unit (UIC, Inc., Joliet, IL). The distillation conditions were 0.004 mm Hg pressure, 225°C evaporator temperature, 70°C condenser temperature, 60°C oil feed temperature, and 3.2 mL/min oil feed rate. The composition of the original and tocopherol-reduced oil was analyzed for free fatty acid content (12), conjugated diene (13), refractive index (14), fatty acid distribution (15), β-carotene (16), and sterols (17). The tocopherol-reduced oil was then blanketed with argon and stored at 4°C prior to use.

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Sample preparation. Tocopherol concentrates were prepared by adding individual tocopherols to tocopherol-reduced oil. The concentration of tocopherols in each concentrate was then quantified (18) and diluted to the tocopherol concentration of interest using tocopherol-reduced oil. Similarly, mixed tocopherol concentrates were produced using individual tocopherols and tocopherol mixture to produce oil with a tocopherol profile that was similar to soybean oil (6.5, 70.6, and 22.9% α-tocopherol, β-tocopherol/γ-tocopherol, and δ-tocopherol, respectively). Each concentrate was then diluted to the final tocopherol concentration using tocopherol-reduced oil.

Soybean oil oxidation. Independent oil samples (1 g each) were oxidized in open glass vials (20 mL). Oxidation was accelerated under dark conditions by storing the oil at 40, 50, and 60°C inside a Blue M (Asheville, NC) or a Lab-Line Instruments, Inc. (Melrose, IL) gravity convection oven. Each vial was mixed prior to analysis, and then the oil (0.05 to 0.1 g) was diluted with 100 mL high-performance liquid chromatography grade isooctane (Fisher Scientific, Fairlawn, NJ). The solution absorbance was measured at 233 nm and conjugated diene concentration calculated as described in the AOCS method (13). Oils were analyzed in duplicate vials, and then the average value was calculated to create each data point. The oils were oxidized until the conjugated diene concentration increased to at least 0.5%. This level of oxidation corresponded to a peroxide value of ~27 meq/kg and was chosen as the cutoff because soybean oil develops objectionable organoleptic properties when the peroxide value exceeds 10 meq/kg (19).

Determination of oxidation rates. The oxidation data were analyzed as described by Labuza (20). Conjugated diene formation rate constants were determined as first-order for each treatment after oils reached similar oxidation levels (~0.5% conjugated diene). Oxidation rate constants were calculated by plotting $\log_{10}$[% conjugated diene] vs. time using linear coordinates. The total number of data points plotted ranged from 6 to 8 (for 60°C data), from 8 to 12 (for 50°C data), and from 10 to 18 (for 40°C data). Coefficient of determination (r^2) values were typically greater than 0.9. The rate constants (k_1) were calculated from the slope ($k_1 = 2.3 \times \text{slope}$) of the curves generated by least squares regression analysis.

Statistical analysis of data. The oxidation rates were analyzed statistically by the analysis of variance method.

Antioxidant potency. The antioxidant potency of each tocopherol was calculated at the optimal concentration for each homologue. Antioxidant potency was defined as the amount of time required for the conjugated diene concentration to increase from 0.2 (initial value) to 0.5% per mole of tocopherol.

RESULTS AND DISCUSSION

Preparation of tocopherol-reduced soybean oil. SPVD achieves much higher vacuum conditions than conventional positive displacement vacuum pumps, thus permitting the removal of tocopherols at lower temperatures. In the present study, ~99% of the tocopherols were removed without significantly altering the free fatty acid content, conjugated diene concentration, refractive index, fatty acid distribution, or the amount of *trans* fatty acids (Table 1). SPVD also reduced the β-carotene and total sterols concentrations. β-Carotene has been shown to interact synergistically with tocopherols in protecting oil oxidative stability (23,24); thus, SPVD processing may also remove other minor constituents that contribute to oil stability.

Effect of individual tocopherols on oxidation. The addition of *d*-α-tocopherol inhibited soybean oil oxidation; however, the antioxidant activity was dependent on both its initial concentration and the oxidation temperature (Fig. 1). For example, oxidation was lowest when oil contained 50 to 250 ppm α-tocopherol at 40°C, 50 to 100 ppm at 50°C, and 100 ppm at 60°C ($P < 0.05$). These results agreed well with earlier reports where the optimal concentration was between 100 and 250 ppm when oils were oxidized between 37 and 60°C (4–6). In the present study, the antioxidant activity diminished and the oxidation rates increased when α-tocopherol concentrations were increased further from 150 to 500 ppm. The extent that the antioxidant activity diminished, at concentrations above 100 ppm, was dependent on the oxidation temperature and was more distinct when the temperature was raised from 40 to 60°C ($P < 0.05$).

The addition of *d*-γ-tocopherol reduced oil oxidation, and its antioxidant activity was also dependent on its initial concentration and the oxidation temperature (Fig. 2). For γ-tocopherol, oxidation was lowest when oil contained 164 to 780 ppm at 40°C, 164 to 318 ppm at 50°C, and 318 ppm at 60°C ($P < 0.05$). These results agreed with earlier work where the optimal concentration for γ-tocopherol was between 250 and 500 ppm (4–7). The antioxidant activity of γ-tocopherol decreased as the concentration increased from 369 to 780 ppm. Similar to α-tocopherol, a significant ($P < 0.05$) reduction of antioxi-

TABLE 1
Composition of Soybean Oil Before and After Tocopherol Removal by Short-Path Vacuum Distillation

Oil composition	Original oil[a]	Tocopherol-reduced oil
Total tocopherol (ppm)	1030	10
α-Tocopherol (ppm)	50	None detected
β- and γ-Tocopherol (ppm)	710	10
δ-Tocopherol (ppm)	270	None detected
Free fatty acids (%)	0.02	0.02
Conjugated diene (%)	0.2	0.2
Refractive index @ 40°C	1.4660	1.4658
Palmitic acid, C16:0 (%)	10.2	10.3
Stearic acid, C18:0 (%)	4.1	4.1
Oleic acid, C18:1 (%)	19.2	19.2
Linoleic acid, C18:2 (%)	53.0	52.6
Linolenic acid, C18:3 (%)	8.2	8.1
trans Fatty acids (%)	2.1	2.2
β-Carotene (ppm)	3.7	2.2
Total sterol (ppm)	2140	100

[a] The original oil was also found to contain iron (0.1 ppm) and copper (<0.02 ppm) (Ref. 21) and citric acid (<5 ppm, Ref. 22).

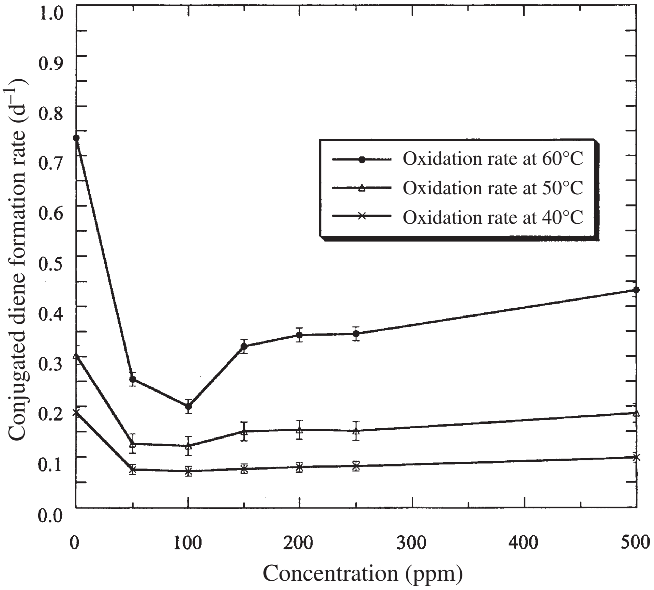

FIG. 1. Effect of α-tocopherol concentration on conjugated diene formation rates. The rates were determined based on the time required for the conjugated diene concentration to increase from 0.2 to 0.5%.

dant activity was found as the temperature increased and γ-tocopherol concentrations increased above 318 ppm.

The concentration and the oxidation temperature also influenced the antioxidant activity of *d*-δ-tocopherol (Fig. 3). The oxidation rates declined steadily as δ-tocopherol concentrations increased from 284 to 1890 ppm, when the oils were oxidized at 40 and 50°C. But oxidation increased sharply when oils contained 284 to 473 ppm δ-tocopherol at 60°C ($P < 0.05$). We speculated that the optimal concentration for δ-tocopherol may exist below 500 ppm, but this disagrees with earlier reports (5,7–10). The earlier studies showed that the antioxidant activity of δ-tocopherol increased with concentration (from 100 to 500 ppm) and exhibited no optimal concentration over this concentration range.

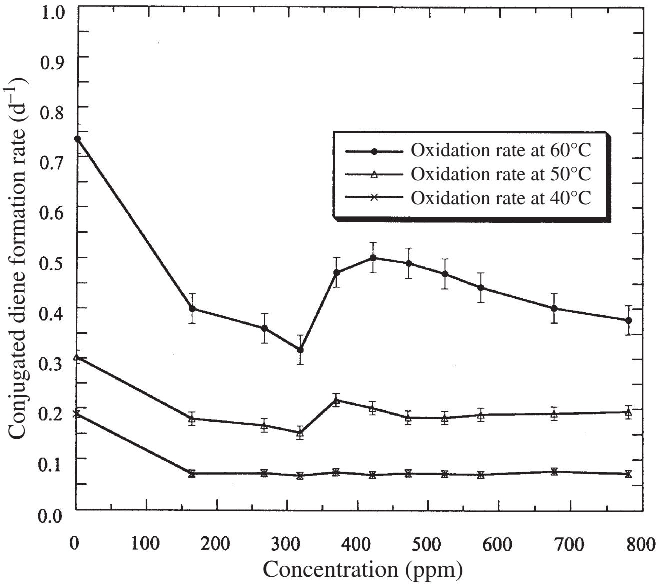

FIG. 2. Effect of γ-tocopherol concentration on conjugated diene formation rates. The rates were determined based on the time required for the conjugated diene concentration to increase from 0.2 to 0.5%.

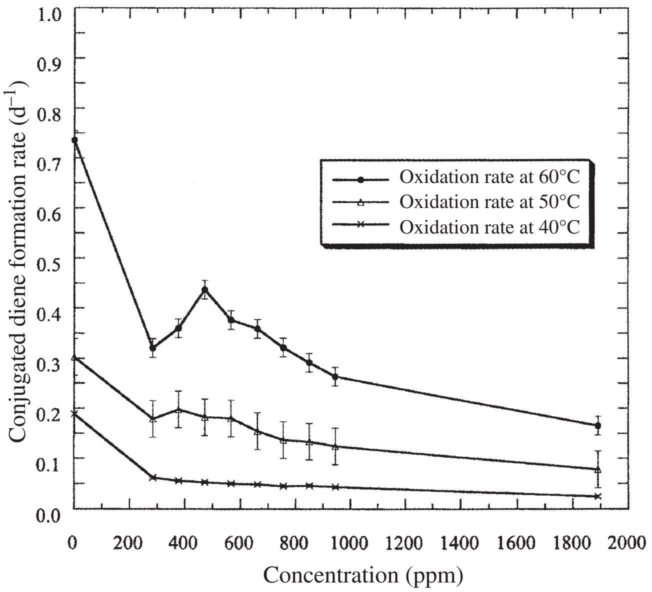

FIG. 3. Effect of δ-tocopherol concentration on conjugated diene formation rates. The rates were determined based on the time required for the conjugated diene concentration to increase from 0.2 to 0.5%.

The earlier reports suggested that the optimal concentration for δ-tocopherol existed between 500 and 1000 ppm so the present research focused on this concentration range. However, no clear optimal concentration was observed because oxidation decreased steadily when oils contained 473 to 1890 ppm δ-tocopherol. These results agreed with a much earlier report (8) but contradicted more recent work where the antioxidant activity remained constant (9) or diminished (5) when δ-tocopherol was increased from 500 to 1000 ppm. These latter reports evaluated δ-tocopherol at 500 and 1000 ppm (5) or at 500, 750, and 1000 ppm (9), perhaps an insufficient number of observations to establish a clear trend over this concentration range. Overall, the antioxidant activity of δ-tocopherol was unique among the tocopherols because it was the only tocopherol homologue whose antioxidant activity improved in the >500 ppm concentration range.

Typically, the concentration of *d*-β-tocopherol accounts for only about 2% of the soybean oil tocopherol composition. Also, it is structurally similar to *d*-γ-tocopherol, so the two tocopherols have similar reactivity with peroxyl radicals (25). For these reasons, the antioxidant activity of β-tocopherol was not studied in this research.

Tocopherol antioxidant activity inversion. Kamal-Eldin and Appelqvist (26) referred to the reduction of antioxidant activity at high tocopherol concentrations as antioxidant activity inversion. The reactions that are responsible may consume tocopherol phenolic hydrogen atoms ($H^{\bullet}$) without effective peroxide chain-breaking. The tocopherols with lower reduction potentials exhibit antioxidant activity inversion at lower concentrations than tocopherols with higher reduction potentials (26). Burton and Ingold (25) quantified the rates that tocopherols were oxidized by peroxyl radicals and showed that tocopherol reactivity decreased in the order α-tocopherol > β-tocopherol ≈ γ-tocopherol > δ-tocopherol. The relative reactivity suggests that less energy is needed to oxidize α-tocopherol than δ-tocopherol and may explain why α-tocopherol antioxidant activity inverts at lower concentrations than δ-tocopherol. Similarly, the intermediate energy levels needed to oxidize γ-tocopherol may explain why its optimal concentration (~300 ppm) lies between α- and δ-tocopherols.

Relative potency of the individual tocopherols. The antioxidant potency of the tocopherol homologues may be related to the rates that they terminate peroxyl radicals. For example, the termination rates reported for the tocopherols were 23.5×10^5, 15.9×10^5, and 6.5×10^5 $M^{-1}s^{-1}$ for α-, γ-, and δ-tocopherol, respectively (25), suggesting that α-tocopherol may be a more potent antioxidant than γ-tocopherol or δ-tocopherol. However, reactivity with lipid radicals is not the only criterion that determines antioxidant potency because the tocopherol concentration and the oxidation temperature also influence its antioxidant activity. To account for these variables, the potencies of α-, γ-, and δ-tocopherol were calculated at their optimal concentrations (Table 2). Since an optimal concentration for δ-tocopherol was not clearly established, the oxidation rates for the oil containing ~1900 ppm δ-tocopherol were used. The antioxidant potency was defined as the amount of time required to increase the conjugated diene concentration from 0.2 to 0.5% per mole of tocopherol. The analysis revealed that α-tocopherol was 3–5 times more potent than γ-tocopherol and 16–32 times more potent than δ-tocopherol. Therefore, the antioxidant potency coincided with the tocopherol reactivity when tocopherols were compared at their optimal concentrations.

Effect of tocopherol mixture on oxidation. Earlier reports showed that the optimal concentration for total tocopherols was between 500 and 750 ppm (9,11). However, these reports did not evaluate tocopherol concentrations between 500 and 750 ppm so the present work sought to further define these concentration limits (Fig. 4). Oxidation was minimized when the oil initially contained 340 to 660 ppm total tocopherols at 40, 50, and 60°C ($P < 0.05$). The antioxidant activity diminished when the tocopherol concentrations were increased further from 720 to 1090 ppm ($P < 0.05$). The oxidation temperature influenced antioxidant activity at concentrations above 660 ppm since the activity inversion was more pronounced as temperatures increased from 40 to 60°C ($P < 0.05$).

TABLE 2
Tocopherol Antioxidant Potency at Their Optimal Concentrations

Oxidation temperature (°C)	[a]Potency (d/mole tocopherol) × 10^{-3}			Relative potency (α:γ:δ)
	α-Tocopherol (~100 ppm)	γ-Tocopherol (~300 ppm)	δ-Tocopherol ~1900 ppm)	
40	54.6	17.6	3.3	16.5:5.3:1.0
50	32.3	7.9	1.2	26.9:6.6:1.0
60	19.7	3.8	0.6	32.8:6.3:1.0

[a]Potency is defined as the oxidation time required for the conjugated diene concentration to increase from 0.2 to 0.5% per mole of tocopherol added initially to the oil.

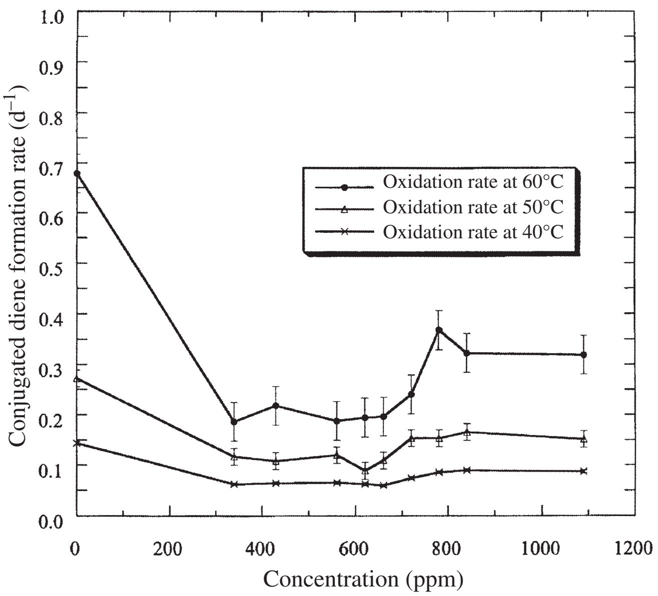

FIG. 4. Effect of mixed tocopherol concentration on conjugated diene formation rates. The rates were determined based on the time required for the conjugated diene concentration to increase from 0.2 to 0.5%.

The lower antioxidant activity of mixed tocopherols at concentrations above 660 ppm may be related to the levels of individual tocopherols. For example, oil containing 720 ppm mixed tocopherols contained ~50 ppm α-tocopherol and ~160 ppm δ-tocopherol. These concentrations were lower than the levels shown earlier to cause activity inversion. However, γ-tocopherol (~510 ppm) was present at a sufficiently high concentration (>300 ppm) to cause activity inversion. Therefore, the concentration of γ-tocopherol may have a significant impact on the antioxidant activity of mixed tocopherols in soybean oil. In conclusion, the optimal concentrations for α-tocopherol (~100 ppm), γ-tocopherol (~300 ppm), and soybean oil tocopherols (300-600 ppm) were further defined. For δ-tocopherol, no clear optimal concentration was identified as its antioxidant activity increased with increasing concentration (up to ~1900 ppm).

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