The Influence of Carotenoids and Tocopherols on the Stability of Safflower Seed Oil During Heat-Catalyzed Oxidation

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ABSTRACT: The stability and antioxidant effects of carotenoids and tocopherols in safflower seed oil were evaluated under thermal (75°C) and oxidative conditions and the oxidative stability index (OSI) determined. The antioxidant capability of butylated hydroxytoluene (BHT) was also compared with that of β-carotene in a model system. Lycopene and β-carotene (1 to 2000 ppm) were heated (75°C) and exposed to air (2.5 psi) in an oxidative stability instrument. β-Carotene had no antioxidant effect at concentrations below 500 ppm, because it did not alter the induction time. Lycopene increased the induction time only slightly at low concentrations. However, at concentrations greater than 500 ppm, both β-carotene and lycopene acted as prooxidants, significantly decreasing the induction period. At the highest concentration, 2000 ppm, lycopene was more prooxidative than β-carotene. α- and γ-Tocopherol (concentration, 1000 ppm) delayed the induction time by 16 and 26 h, respectively. There was no cooperative interaction between α-tocopherol and β-carotene in delaying the onset of oxidation. Furthermore, BHT was significantly more antioxidative than β-carotene. Thus, under thermal and oxidative conditions, β-carotene could not delay the onset of oxidation. The tocopherols and BHT were effective in suppressing the onset of oxidation, as determined by the oxidative stability measurement.
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KEY WORDS: Antioxidants, β-carotene, BHT, carotenoids, lycopene, oxidation stability, safflower seed oil, thermal stability, tocopherols.

Carotenoids have drawn considerable attention because of their role as natural colorants, provitamin A activity, and ability to prevent peroxidation processes. Many studies have focused on β-carotene's ability to retard potentially harmful oxidative reactions by trapping free radicals (1–3) and quenching singlet oxygen (4–6). Epidemiological studies indicate the carotenoids may function in a protective role against degenerative diseases that are influenced by radical reactions (7–9).

Tocopherols are important biological free-radical scavengers as well as a vitamin E source. Carotenoids may exert their radical-trapping function by acting cooperatively with tocopherols. It has been proposed that β-carotene and α-tocopherol cooperatively trap free radicals in microsomal membranes (10). A synergism between the two antioxidants was also detected in microsomal lipids (11). In addition soybean oil was found to be best protected against photooxidation by a mixture of β-carotene, tocopherols, and citric acid (12–14). The antioxidant effectiveness of β-carotene and α-tocopherol may be related to physical factors. It has been suggested that since β-carotene is more lipophilic, it is located in the interior of membranes and, therefore, is more effective at scavenging lipophilic radicals than α-tocopherol, which is concentrated at or near the surface of membranes (15,16).

The food industry is continuously exploring the use of natural compounds for food preservation. Carotenoids and tocopherols have antioxidant properties, provide vitamin activity, and are endogenously present in plant and animal tissues. Therefore, they are desirable ingredients for many food products. Much of the work to date on the antioxidant properties and stability of carotenoids and their cooperative activity with tocopherols has been studied in membrane- or solvent-based model systems, which may not translate to food systems. Information on their function in a model oil system under thermal and oxidative conditions would enhance our understanding of how these antioxidants might function in many food systems. The objectives of this study were to (i) evaluate the stability and antioxidant effects of lycopene and β-carotene on the heat-catalyzed oxidation of safflower seed oil; (ii) determine whether tocopherols can act cooperatively with β-carotene to delay the onset of oxidation; and (iii) compare the antioxidant capacity of β-carotene to that of butylated hydroxytoluene (BHT).

EXPERIMENTAL PROCEDURES

Materials. Safflower seed oil (raw oil without added preservatives), BHT, lycopene, and α- and δ-tocopherol were obtained from Sigma Chemical Co. (St. Louis, MO). β-Carotene was a generous gift from Hoffmann-La Roche, Inc. (Nutley, NJ). Structures of these compounds are shown in Scheme 1. The solvents, methanol and dichloromethane, were A.C.S.

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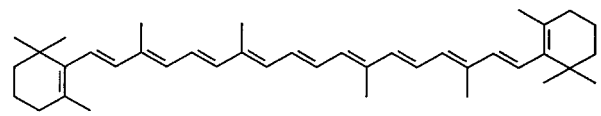
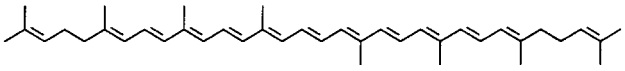
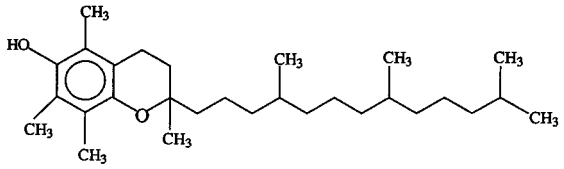


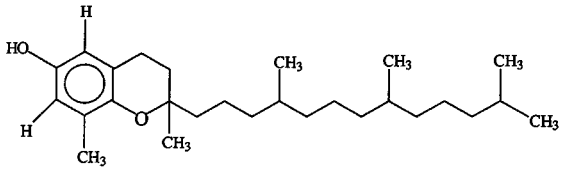
ALL-*TRANS* β-CAROTENE



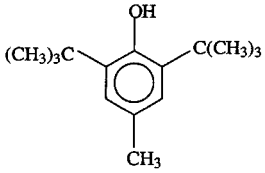
LYCOPENE



α-TOCOPHEROL



δ-TOCOPHEROL



BHT

SCHEME 1

certified grade (Fisher Scientific Co., Fairlawn, NJ). Methyl *tert*-butyl ether (MTBE) was high-performance liquid chromatography (HPLC) grade (Fisher).

Solvents used in the mobile phase for liquid chromatography were filtered through a 1.0 μm pore (47 mm) polytetrafluoroethylene (PTFE) filter (Fisher Scientific Co.).

Purification of standards. The all-*trans* fractions of lycopene and β-carotene were purified by using a semipreparatory column (10 mm i.d. × 250 mm) packed with C30 bonded to 3 μm silica particles (17,18). The HPLC system consisted of a Model 510 pump, a U6K injector, and a 990 photodiode array detector (Waters, Inc., Milford, MA) equipped with an NEC Powermate SX/20 computer (Boxborough, MA). Isocratic eluents of methanol/MTBE (89:11) and (62:38) were used to separate all-*trans* β-carotene and lycopene, respectively. The solvent conditions are similar to those reported by Emenhiser *et al.* (18). Chromatographic separations were monitored at 452 nm for β-carotene and 472 nm for lycopene. Sample injections of approximately 400 μL of carotenoid dissolved in 50:50 methanol/MTBE were used during the purification process. Following peak collection and evaporation of the solvent under a stream of nitrogen, the purified standards were stored at –20°C until further analysis.

Antioxidant/oil preparations. β-carotene and lycopene in oil. β-Carotene and lycopene were dissolved in dichloromethane and diluted as necessary to obtain concentrations of 2000, 1000, 500, 100, 10, and 1 ppm. The carotenoids in dichloromethane were added directly to disposable borosilicate glass reaction tubes containing 5 g of safflower oil. The solvent was evaporated under a stream of nitrogen. A blank sample was prepared with 5 g of safflower oil and dichloromethane, which was evaporated under nitrogen. Triplicate tubes were prepared for the blank and each mixture.

Tocopherols and β-carotene–oil mixtures. β-Carotene and α- and δ-tocopherol were each dissolved in dichloromethane. The β-carotene and α-tocopherol preparations were mixed to obtain the following ratios of α-tocopherol/β-carotene: 1:0, 0.9:0.1, 0.75:0.25, 0.5:0.5, and 0:1. The δ-tocopherol/β-carotene preparations had ratios of 1:0, 0.9:0.1, and 0:1. The total concentration in each system was 1.0 mM. The ratios and total concentration of tocopherols and β-carotene were selected based on the results of numerous preliminary studies in which various ratios and concentrations of the antioxidants were evaluated. Based on the oxidative conditions (75°C, air 2.5 psi; see below for details), a total concentration of 1 mM was selected, because at that level there were significant differences from the control (no β-carotene or tocopherol) that could be monitored in a reasonable time frame. The ratios of the compounds reflect levels and increments that had the most significant effects on the oxidative stability index from one increment to the next.

The β-carotene, α- and δ-tocopherol mixtures in dichloromethane were added directly to disposable borosilicate glass reaction tubes containing 5 g of safflower oil. Solvent evaporation, blank preparation procedures, and replications were identical to those outlined above.

BHT and β-carotene preparations. BHT and β-carotene were dissolved in dichloromethane and added directly to disposable borosilicate glass reaction tubes containing 5 g of safflower oil. The BHT and β-carotene were evaluated at the following percentages (wt%) of the sample: 0.003, 0.006, 0.013, and 0.020. An effective level of 0.020% BHT is commonly used commercially for antioxidant activity. Solvent evaporation, blank preparation procedures and replications were identical to those described in previous paragraphs.

Thermal and oxidative conditions. The oxidative stability index (OSI time) of the samples was measured with the Omnion Oxidative Stability Instrument (Archer Daniels Midland Co., Decatur, IL). The apparatus consists of a heating compartment, air inlet valves, and electrical conductivity probes. The heating compartment temperature was set at 75°C and the air at 2.5 psi. The OSI time was determined by a sharp increase in the total volatile secondary oxidation products present in the electrical conductivity cell; therefore, it allowed determination of the induction period. The OSI time is the point of maximal change in the oxidation rate and may be determined mathematically as the maximum of the second deriva-

tive with respect to time or by a graphical approximation in which manual tangents are drawn (19). The fully automated Oxidative Stability Instrument uses a microprocessor to determine the OSI time *via* a slope-change algorithm (19).

Statistical analysis. An analysis of variance was used to evaluate differences between treatments (20). There were two replicates for each treatment combination.

RESULTS AND DISCUSSION

Influence of β-carotene and lycopene on oil stability. Table 1 shows the effect of β-carotene and lycopene on the OSI time of safflower seed oil. The OSI times for the samples with carotenoids are compared to that of a control sample that contained only safflower seed oil. At concentrations up to 100 ppm, β-carotene had no effect on the induction period of the heated oil. Although significantly different from β-carotene, lycopene delayed the onset of oxidation only slightly at concentrations below 500 ppm. However, at concentrations above 500 ppm, both carotenoids acted as prooxidants, in that they shortened the induction period. The significant difference in OSI times for β-carotene and lycopene at concentrations of 1000 and 2000 ppm indicates that lycopene had a stronger prooxidative effect than β-carotene.

These results are in agreement with those of similar studies on the prooxidative potential of carotenoids reported in the literature (21,22). Burton and Ingold (23) studied the antioxidant potential of β-carotene and found that carotenoids are good radical-trapping antioxidants at low oxygen pressures (approximately 15 torr) which are prevalent in most tissues under physiological conditions. The authors also reported that at higher pressures (greater than 150 torr—the partial pressure of oxygen in air) β-carotene may act as a prooxidant. Kennedy and Liebler (24) also found that β-carotene more effectively scavenged radicals in lipid bilayers at low oxygen partial pressures.

Lycopene and β-carotene are potentially radical-forming compounds and therefore could propagate the reaction when the radicals are present at high concentrations. Therefore, the concentration of carotenoids in the system could play an important role in determining whether the carotenoid will act as a prooxidant or as an antioxidant.

TABLE 1
Effects of β-Carotene and Lycopene on the Oxidative Stability of Safflower Seed Oil[a]

Concentration (ppm)	β-Carotene OSI OSI time (h)	Lycopene OSI time (h)
0	21.27 ± 0.25[a]	21.30 ± 0.20[a]
1	21.43 ± 0.11[a]	22.29 ± 0.02[d]
10	21.58 ± 0.08[a]	22.43 ± 0.04[d]
100	22.04 ± 0.16[a]	22.64 ± 0.16[d]
500	20.96 ± 0.13[a]	21.13 ± 0.32[a]
1000	20.07 ± 0.16[b]	18.15 ± 0.14[e]
2000	15.90 ± 0.28[c]	14.19 ± 0.20[f]

[a]Oxidative stability index (OSI) times followed by the same superscript letter a–f are not significantly different ($P < 0.001$). The values reflect the means of two replicates for each treatment ± standard deviation. Duplicates of each treatment represent one replicate.

Cooperative activity of β-carotene and tocopherols. Figure 2 shows the effect of various ratios of tocopherols and δ-carotene on the OSI time. β-Tocopherol alone increased the induction period over that of the control by approximately 26 h and, therefore, was significantly more effective than all combined ratios of α-tocopherol and β-carotene. α-Tocopherol lengthened the induction time by 16 h. The $^1O_2$ quenching activity of δ-tocopherol was also found to be more effective than α-tocopherol during the oxidation of methyl linoleate photosensitized with methylene blue (25). Furthermore, Terao *et al.* (14) reported that δ-tocopherol had a more inhibitory effect on the production of peroxides than did α-tocopherol, by suppressing autooxidation and by quenching $^1O_2$.

In the current study, β-carotene did not delay the onset of oxidation (Table 1) and decreased the antioxidative capacity of α- and δ-tocopherol (Fig. 1). In addition, β-carotene acted as a prooxidant when used at a concentration equal to that of the tocopherols. Because β-carotene may react with oxygen to form β-carotene peroxyl radicals (15), these peroxyl radicals may enhance the propagation stage of the oxidation reaction because they supply the system with more oxidizable substrates. Therefore the antioxidant capacity or "sparing efficiency" of the tocopherols decreases. Although β-carotene and α-tocopherol cooperatively trap free radicals in microsomal membranes (10) and microsomal lipids (11), no synergism or cooperative effect existed between the carotenoid and tocopherols under the conditions of this study.

Comparison of β-carotene and BHT. At equal concentrations, BHT exhibited antioxidant activity whereas β-carotene did not (Fig. 2). A control that consisted of solely safflower seed oil had an OSI time of 22 h; at, for example, a concentration of 0.02%, the OSI times for β-carotene and BHT were 15 and 70 h, respectively. At this concentration, β-carotene significantly decreased the oil stability and caused the onset of rapid oxidation to occur approximately 7 h earlier than the control.

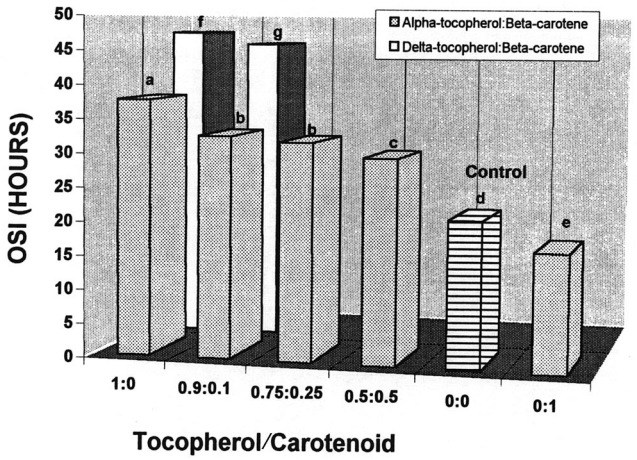


FIG. 1. The effect of β-carotene and tocopherol combinations on the stability of safflower seed oil. The control has a ratio of 0:0. Oxidative stability index (OSI) times followed by the same letter are not significantly different ($P < 0.001$). The values reflect the means of two replicates for each treatment. Duplicates of each treatment represent one replicate.

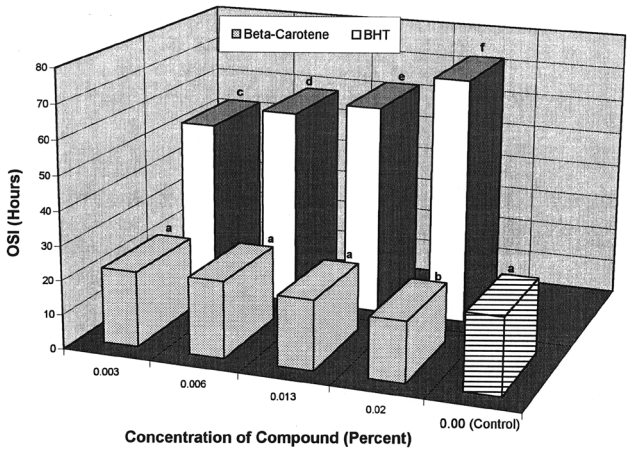


FIG. 2. Comparison of β-carotene and butylated hydroxytoluene (BHT) in the prevention of safflower seed oil oxidation. OSI times followed by the same letter are not significantly different ($P < 0.001$). The values reflect the means of two replicates for each treatment. Duplicates of each treatment represent one replicate. See Table 2 for abbreviation.

In conclusion, under the experimental conditions, β-carotene lacked the antioxidant capacity of other more commonly used antioxidants. BHT and α- and δ-tocopherols were better antioxidants than β-carotene and lycopene. Furthermore, unlike β-carotene and lycopene, BHT and α- and δ-tocopherols did not act as prooxidants at the levels tested. Food systems consist of many compounds that may alter the rate of oxidation; therefore, future work with antioxidants should involve evaluation of more complex food systems.

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