Antioxidant Activity of α-Tocopherol and Trolox in Different Lipid Substrates: Bulk Oils *vs* Oil-in-Water Emulsions

Shu-Wen Huang, Anu Hopia, Karin Schwarz, Edwin N. Frankel,* and J. Bruce German

Department of Food Science and Technology, University of California, Davis, California 95616

This study was aimed at evaluating the antioxidant effectiveness of α-tocopherol and its water-soluble analogue, Trolox, in different lipid systems. The oxidative stability of lipids in bulk and emulsion systems at 37 °C decreased in the order corn oil triglycerides > methyl linoleate > linoleic acid. In both bulk and emulsified linoleic acid, Trolox was a better inhibitor of hydroperoxide formation and decomposition than α-tocopherol. However, in bulk methyl linoleate and corn oil triglycerides, although Trolox was a better inhibitor of hydroperoxide formation, in emulsions, α-tocopherol was a better inhibitor of both the formation and decomposition of hydroperoxides. In emulsified methyl linoleate and corn oil, the partition coefficients of Trolox between lipids and water at high concentrations were not affected by Tween 20, and the antioxidant activity of Trolox decreased because it partitioned into the water phase and Tween 20 micelles. In contrast, in linoleic acid emulsified with Tween 20, the formation of mixed micelles of linoleic acid and Tween 20 increased the percentage of Trolox in the water phase and Trolox was more effective as an antioxidant than α-tocopherol because it diffused in the water phase and into mixed micelles. The physical states of lipid systems affect the distribution of antioxidants and thus significantly influence their antioxidant behavior. Because linoleic acid has unique physical properties in aqueous micelles, it may not be a valid substrate for evaluating food antioxidants.

Keywords: *Antioxidants; α-tocopherol; Trolox; partitioning; partition coefficient; linoleic acid; methyl linoleate; corn oil triglycerides; emulsion; mechanism; interfacial oxidation; hydroperoxides; hexanal*

INTRODUCTION

The effects of antioxidants have stimulated much interest in research aimed at preventing oxidation of lipids. Antioxidants have been evaluated using different methods to measure oxidation and within different lipid systems. The relative effectiveness of antioxidants is dependent on the lipid substrates, test system, concentration, oxidation time, and method used to determine lipid oxidation (Chipault et al., 1955; Lea and Ward, 1959; Porter, 1980; Pryor et al., 1988; Frankel et al., 1994; Huang et al., 1994). Antioxidant behavior is more complex when evaluated in emulsion systems than in bulk oil systems because more variables influence lipid oxidation, including emulsifier, pH, and buffer system (Cillard et al., 1980; Pryor et al., 1988; Barclay and Vinqvist, 1994). Hence, limited research has been done to systematically evaluate antioxidant activity with respect to the interaction between these system-dependent variables.

Some hydrophilic antioxidants were more effective than their lipophilic analogues in bulk oil but less active in emulsions (Chipault et al., 1956; Uri, 1961; Porter et al., 1989). Trolox, a water-soluble analogue of α-tocopherol, was more active than α-tocopherol in bulk corn oil triglycerides but less effective than α-tocopherol in corn oil-in-water emulsions (Frankel et al., 1994). A possible mechanism advanced to explain these differences in efficiency of antioxidants was related to their affinities toward the air−oil interfaces in bulk oil and the oil−water interfaces in emulsions. Although Trolox was more active than α-tocopherol in hemoglobin-catalyzed oxidation of a 10% safflower oil emulsion (Cort et al., 1975), α-tocopherol was more effective than Trolox in methyl linoleate emulsions with hemoglobin as catalyst (Taylor and Richardson, 1981). α-Tocopherol was a better scavenger of peroxyl radicals than Trolox in styrene systems (Burton et al., 1985) and in methyl linoleate with azo compound initiators (Roginsky, 1990). In the presence of an azo initiator, Trolox was more effective than α-tocopherol in linoleic acid emulsified in 0.5 M sodium dodecyl sulfate (SDS) solution (Barclay et al., 1985), while less effective than α-tocopherol in 0.015 M (Castle and Perkins, 1986) and 0.1 M SDS solution at pH 7.4 (Pryor et al., 1993). These results are difficult to interpret because of the various conditions and methods used to determine lipid oxidation.

Measurements of volatile oxidation products are important to estimate how much off-flavor is produced during autoxidation. Hexanal is one of many important volatile products that is a useful marker for the oxidative decomposition of oxidized *n*−6 PUFA (Frankel, 1982). Trolox inhibited both the formation of hydroperoxides and their decomposition to hexanal in bulk corn oil triglycerides but less efficiently in oil-in-water emulsions (Frankel et al., 1994). In contrast to Trolox, α-tocopherol promoted the formation of hydroperoxides at higher concentrations at the early oxidation stages but inhibited hexanal formation more strongly at higher concentrations in both bulk and emulsion systems (Frankel et al., 1994; Huang et al., 1994). Therefore, it is important to use more than one method to determine antioxidant activity to evaluate their effects on lipid oxidation at different stages (Frankel, 1993).

Although triglycerides are the major lipids in food systems, polyunsaturated fatty acids (PUFA) and their esters are commonly used as model lipid substrates to study lipid oxidation and antioxidant activity. These lipid substrates have different viscosities and polarities. Fatty acids are amphiphiles, and their pK_a values are

* Author to whom correspondence should be addressed [telephone (916) 752-4478; fax (916) 752-4759; e-mail enfrankel@ucdavis.edu].

0021-8561/96/1444-0444$12.00/0

about 5. Fatty acids dissociate in aqueous systems, depending on pH, to form fatty acid anions that are partially soluble in water and form micelles. In contrast to fatty acids, fatty acid esters and triglycerides are insoluble and are thus dispersed in water as emulsion particles. At pH 7, linoleic acid anion is homogeneously incorporated in the micellar structure, and its oxidizability is constant between 0 and 0.47 M in 0.5 M SDS with an azo compound initiator (Barclay et al., 1983). In contrast, methyl linoleate is solubilized in the micellar structure at lower concentrations and "dissolved" within the micelles at higher concentrations, and its oxidizability increases with increasing concentration. These differences, which affect antioxidant activity, have been largely overlooked in the literature.

In multiphase food systems, antioxidants partition into different phases according to their affinities toward and the amount of these phases. The relation between partition of antioxidants and their effectiveness in multiphase systems is not clear. Is the partition of antioxidants responsible for their different antioxidant activities in different physical states? Castle and Perkins (1986) found that α-tocopherol was more effective than Trolox because α-tocopherol was located in linoleic acid micellized in SDS at pH 7, compared to only 2% for Trolox. Barclay and Vinqvist (1994) also reported that Trolox partitioned partially into the lipid phase of liposomes at pH 7 and its antioxidant activity was influenced by the membrane surface charge that varied with pH. Cornell et al. (1970) only determined the partition coefficients of some antioxidants between butter oil and water, and the partition toward the water phase increased with increasing temperature. Yamamura et al. (1991) used a three-phase model to investigate the distribution of a diterpenoid in lipid emulsions by determining its partition coefficients between oil and water, between oil and water with emulsifiers, and between lipid emulsions and water. However, there is apparently no information in the literature on the distribution of highly lipophilic antioxidants in lipid emulsions.

This study compared the effectiveness of α-tocopherol and Trolox in different lipid substrates by systematically studying the interactive effects of three variables, concentration, physical state, and oxidation stage, on antioxidant activities in bulk and emulsions at 37 or 60 °C. The effectiveness of α-tocopherol and Trolox was evaluated at different stages of oxidation by measuring both the formation of hydroperoxides (conjugated dienes) and the decomposition of hydroperoxides (hexanal) in these lipid systems. To define the relationship between the antioxidant activity and phase distribution in different emulsified lipids, partitioning of Trolox between different phases and water was investigated according to classical methods.

MATERIALS AND METHODS

Materials. Linoleic acid and its methyl ester were obtained from Nu-Chek Prep Inc., Elysian, MN. Corn oil triglycerides stripped of tocopherols were purchased from Eastman Kodak Co., Rochester, NY. Corn oil was found to be free of tocopherols by high-performance liquid chromatography (HPLC) using a fluorescence detector (Handelman et al., 1985), and its peroxide value was less than 5. The fatty acid composition determined by gas chromatography (GC) of the methyl esters was as follows: 10.5% 16:0; 2.1% 18:0; 25.5% 18:1; 60.8% 18:2; and 1.1% 18:3. α-Tocopherol and Tween 20 (polyoxyethylene sorbitan monolaurate) were obtained from Sigma Chemical Co., St. Louis, MO. Tween 20 (molecular weight, 1226) has a density of 1.095 g mL^{-1} and a critical micelle concentration of approximately 3.5×10^{-5} M (Courthaudon et al., 1991). Trolox C was obtained from Aldrich Chemical Co., Milwaukee, WI.

Preparation of Bulk Oil and Emulsion Samples. Lipid samples (5.0 g) were prepared with or without added 150 or 300 μM antioxidants. These concentrations are equivalent to 65 and 130 ppm of α-tocopherol and 38 and 76 ppm of Trolox, respectively. Antioxidants were added in methanol solutions, and samples were purged of methanol with nitrogen. Ten percent oil-in-water emulsions (20 g) were prepared in 50-mL Erlenmeyer flasks. The emulsions were prepared by mixing 2.0 g of oils, 1.8 g of deionized water, and 0.2 g of Tween 20 as emulsifier, sonicating this mixture for 1 min. Sonication was continued in an ice bath for another 4.5 min during which 16 mL of deionized water was added in four 4-mL portions every 30 s. This emulsification was carried out by sonicating for a total of 5.5 min at high power (sonicator, cell disruptor, Model W-10, Heat Systems, Ultrasonics, Inc., New York). The particle sizes of emulsions were determined with a Microtrac Ultrafine particle analyzer (Leeds & Northrup, North Wales, PA). The average particle size in fresh samples of emulsions was 0.1−0.25 μm. The pH of emulsified linoleic acid and corn oil triglyceride samples with and without antioxidants ranged from 4.8 to 5.0. The pH of emulsified methyl linoleate samples was approximately 6.1.

Methods. *Oxidation.* Bulk oil samples (2.5 g in 11.1-mL screw-capped vial) and emulsion samples were oxidized at 37 °C in a shaker water bath (Model M76D, New Brunswick Scientific Co., Inc., Edison, NJ) or at 60 °C in a shaker oven (Lab-Line Instrument, Inc., Melrose Park, IL). Oxidative stability was determined by measuring conjugated diene hydroperoxides spectrophotometrically and hexanal by headspace GC. All oxidation and analyses were done in duplicate, and the results were calculated by one-way analysis of variance (Wagner, 1992).

Measurement of Conjugated Diene Hydroperoxides. Measurements in oil samples were carried out according to the same procedures described previously (Frankel et al., 1994). For emulsion samples, samples (0.1 g) were dispersed in 5 mL of methanol and then diluted to a measurable absorbance with methanol. The absorbance was measured at 234 nm and calculated on the same unit as the oil samples.

Measurement of Hexanal by Static Headspace GC. The procedures used for hexanal measurements were those described previously (Frankel et al., 1994), except that all oil and emulsion samples were equilibrated at 60 °C for 15 min.

Partition Studies. Two methods were used to determine the partition coefficients of Trolox between different lipids and water.

Method A. Lipids (0.50 g) and deionized water (4.5 g) containing different concentrations of Trolox were weighed into 10-mL screw-capped test tubes and vortexed twice for 15 s after purging with nitrogen. These samples were then centrifuged at 2000 rpm for 3 min. The water layer was transferred into a test tube and centrifuged again to remove lipid residue. The water layer containing Trolox was analyzed by HPLC (method given below) to obtain the partition coefficients of Trolox between different lipids and water. The calculation for the partition coefficient of Trolox between lipid and water was as follows: partition coefficient $= V_w/V_l(W_t/W_w - 1)$, where V_w = volume of water; V_l = volume of lipid or Tween 20; W_t = total amount of Trolox; and W_w = amount of Trolox in water phase; the densities (g mL^{-1}) used for calculating lipid volume were 0.904 for linoleic acid, 0.889 for methyl linoleate, and 0.916 for corn oil.

Method B. The concentration of Trolox was measured in the aqueous phase separated from lipid−water mixtures, Tween 20 solution, and emulsions. Three different systems containing 30 −100 μg/g Trolox (25 g) were prepared as follows: (1) 1% (w/w) Tween 20 solution; (2) 10% (w/w) lipid−water mixtures sonicated for 20 s at power level 4, about 70−80 W (Sonifier cell disruptor, Model W185D, Heat Systems-Ultrasonics, Inc., Plainview, NY) and vortexed for 10 s; (3) 10% lipids emulsified with 1% Tween 20 prepared as described previously. The pH of these samples ranged from 3.0 to 3.3. These samples were purged with nitrogen and kept at 5 °C

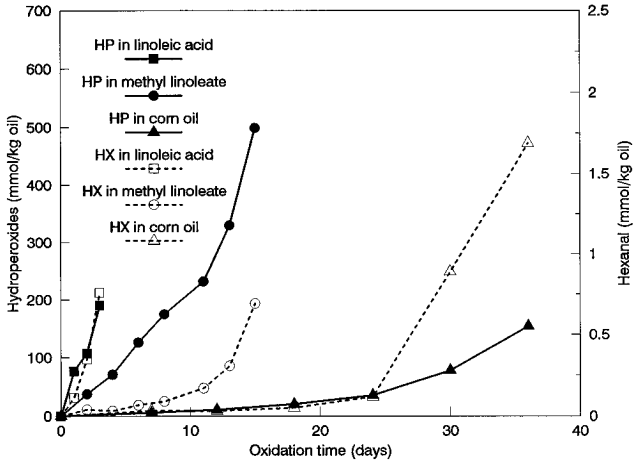

Figure 1. Oxidative stability of bulk linoleic acid, methyl linoleate, and corn oil triglycerides at 37 °C. HP, hydroperoxides; HX, hexanal.

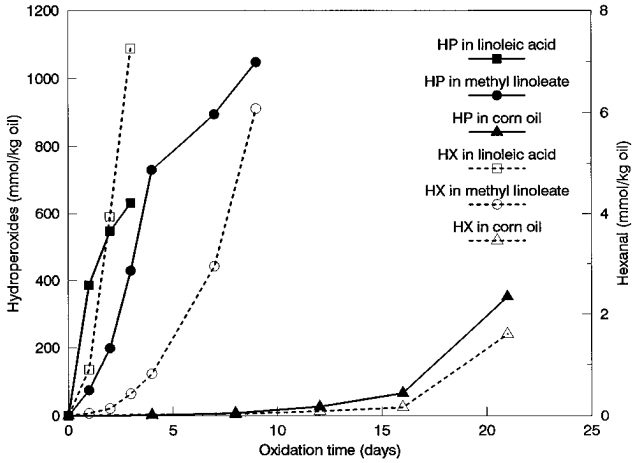

Figure 2. Oxidative stability of 10% emulsions of linoleic acid, methyl linoleate, and corn oil triglycerides at 37 °C. HP, hydroperoxides; HX, hexanal.

during the experiment. These samples (2 mL) were placed in Centri/Por centrifuge concentrators with cellulose ester membranes, molecular weight cutoff 1000 (Spectrum, Houston, TX), and centrifuged at 1500*g* for 20 min at 20 °C. This procedure was repeated eight times, and the last four filtrates were analyzed by HPLC. Each centrifuge concentrator was rinsed three times with 3 mL of deionized water and centrifuged at 1500*g* for 30 min each time before use; the recovery of each concentrator was determined with different concentrations of Trolox solution. The percentage of Trolox in the water phase was determined by analyzing the filtrates of each sample. This analysis was normalized on the basis of the composition of the lipid sample and was corrected by multiplying the recovery of Trolox in the Centri/Por centrifuge concentrator, which ranged from 69 to 89%.

HPLC Analysis. Trolox in the water phase was analyzed on a Hewlett−Packard 1090 HPLC system using a Supelcosil LC-18-DB column (particle size, 5 μm; 2.1 mm i.d. × 25 cm, Supelco, Inc., Bellefonte, PA) and peak detection at 290 nm. The elution solvent was 60:40:1 methanol−water−2 M citric acid, and the flow rate was 0.3 mL min^{-1}.

RESULTS

Oxidative Stability of Bulk Linoleic Acid, Methyl Linoleate, and Corn Oil Triglycerides. *Formation of Hydroperoxides.* At 37 °C, linoleic acid oxidized at the highest rate, followed by methyl linoleate and then corn oil triglycerides (Figure 1). Linoleic acid had no induction period compared to an induction period of about 1 day for methyl linoleate. The accumulation of hydroperoxides formed in methyl linoleate was about one-third of that in linoleic acid between 1 day and 3 days. The rate of hydroperoxide formation (the ratio of hydroperoxide formation to oxidation time) in corn oil increased after 24 days. The lower oxidative stability of linoleic acid and methyl linoleate may be attributed to the lower viscosity resulting in increasing rates of collision and reaction and greater circulation to the surface. The lower linoleate content (60%) of corn oil may account for its higher oxidative stability.

Decomposition of Hydroperoxides. Hexanal formation was also faster in linoleic acid samples than in those composed of methyl linoleate and corn oil (Figure 1). If linoleic acid hydroperoxides decompose more quickly to initiate free radical chain reactions, linoleic acid would be expected to have lower oxidative stability in the presence of trace amount of hydroperoxides acting as initiators. The rate of hexanal formation (the ratio of hexanal formation to oxidation time) in all of these lipids increased when the rate of hydroperoxide formation increased or changed. When the amount of hydroperoxides reached 100 mmol/kg of oil, the ratio of hexanal formation to hydroperoxide formation decreased in the order corn oil > linoleic acid > methyl linoleate. Although corn oil triglyceride hydroperoxides appeared to decompose more readily than the hydroperoxides of methyl linoleate and linoleic acid, the differences were small but exaggerated in the hexanal scale (Figure 1, right *y*-axis). At 60 °C, the rate of hydroperoxide formation increased more rapidly than the rate of hexanal formation (data not shown).

Oxidative Stability of Emulsified Linoleic Acid, Methyl Linoleate, and Corn Oil Triglycerides. *Formation of Hydroperoxides.* The different emulsified lipid substrates showed the same order of oxidative stability as bulk systems (Figures 1 and 2). However, these lipids when emulsified exhibited lower oxidative stability than the bulk lipids. In corn oil emulsions, the induction period was 16 days compared to 24 days in bulk corn oil.

Decomposition of Hydroperoxides. In emulsified linoleic acid and methyl linoleate, the rate of hexanal formation increased largely when the speed of the accumulation of hydroperoxides decreased (Figure 2). When the amount of hydroperoxides reached 350 mmol/kg of oil, the ratio of hexanal formation to hydroperoxide formation was highest in corn oil, followed by linoleic acid and methyl linoleate. This ratio increased after an induction period in linoleic acid and methyl linoleate in both bulk and emulsified systems. When their rates of hydroperoxide formation decreased, the amount of accumulated hydroperoxides was smaller in emulsified linoleic acid than in emulsified methyl linoleate. In contrast, hydroperoxides decomposed to a greater extent in emulsified linoleic acid than in emulsified methyl linoleate. In emulsion systems as in bulk systems, linoleic acid hydroperoxides decomposed more quickly than methyl linoleate hydroperoxides and were accumulated to a lesser extent.

Effects of α-Tocopherol and Trolox in Bulk Linoleic Acid. *Formation of Hydroperoxides.* The formation of hydroperoxides in bulk linoleic acid was decreased by α-tocopherol and Trolox at 37 °C (Figure 3a). The rate of hydroperoxide formation increased in the presence of 150 μM α-tocopherol after 1 day. At 300 μM, the rate increased more slowly than at 150 μM, and it was higher between 2 and 3 days than between 1 and 2 days. The formation of hydroperoxides was increased

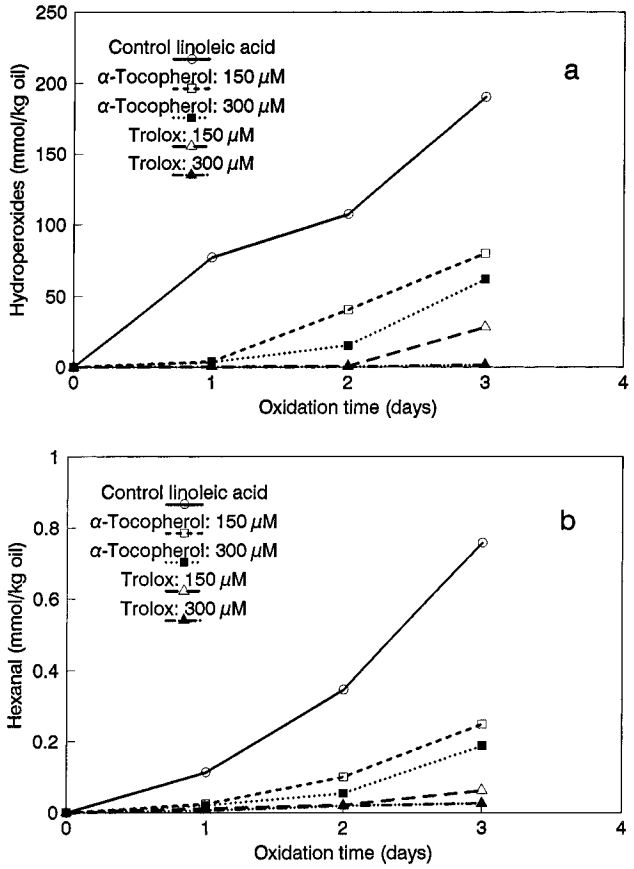

Figure 3. Effect of α-tocopherol and Trolox on oxidative stability of bulk linoleic acid at 37 °C: (a) hydroperoxide formation; (b) hexanal formation.

at 150 μM Trolox after 2 days but inhibited at 300 μM Trolox between 1 and 3 days. The inhibition by both antioxidants decreased after 3 days (Table 1). On day 3, Trolox was a more effective inhibitor of hydroperoxide formation than α-tocopherol at 150 and 300 μM.

Decomposition of Hydroperoxides. Hydroperoxide decomposition was strongly inhibited by α-tocopherol and Trolox (Figure 3b). Hexanal was produced after the formation of hydroperoxides. The rate of hexanal formation in the presence of α-tocopherol increased between 1 and 3 days. Hexanal formed more rapidly at 150 μM α-tocopherol than at 300 μM. Hexanal formation increased in the presence of 150 μM Trolox after 2 days but did not increase at 300 μM during 3 days of oxidation (Figure 3b). The extent of inhibition of hydroperoxide decomposition by α-tocopherol decreased between 1 and 3 days, whereas the inhibition by Trolox actually increased during this period (Table 1). On day 3, the inhibition by both antioxidants was higher at 300 μM than at 150 μM, but with Trolox the difference between the two levels was not significant. Trolox was more effective than α-tocopherol.

Effects of α-Tocopherol and Trolox in Emulsified Linoleic Acid. *Formation of Hydroperoxides.* The formation of hydroperoxides was also retarded by added α-tocopherol and Trolox (Figure 4a). With 150 μM α-tocopherol, the rate of hydroperoxide formation increased sharply between 1 and 2 days, as in the control the apparent rate of hydroperoxide formation decreased between 2 and 3 days. With 300 μM α-tocopherol, the increase after 1 day was less than with 150 μM. Hydroperoxides were formed significantly in the presence of 150 μM Trolox only after 2 days, whereas with 300 μM, only traces of hydroperoxides were detected even after 3 days. The inhibition by both antioxidants decreased with oxidation time (Table 1). On day 3, the order of inhibition by both antioxidants was the same as that in bulk linoleic acid.

Decomposition of Hydroperoxides. The effects of α-tocopherol and Trolox on hexanal formation were similar in emulsified and in bulk linoleic acid (Figures 4b and 3b). On day 3, α-tocopherol and Trolox inhibited hydroperoxide decomposition more effectively at 300 μM than at 150 μM (Table 1). Also, Trolox was a better inhibitor of hydroperoxide decomposition in emulsified linoleic acid than α-tocopherol.

Effects of α-Tocopherol and Trolox in Bulk Methyl Linoleate. *Formation of Hydroperoxides.* Hydroperoxide formation was strongly inhibited by α-tocopherol and Trolox in bulk methyl linoleate compared

Table 1. Inhibition of Hydroperoxide and Hexanal Formation by α-Tocopherol and Trolox C in Bulk and in Emulsified Linoleic Acid and Methyl Linoleate (Percent Mean Inhibition ± SD)[a,b]

	bulk				emulsified			
	hydroperoxides		hexanal		hydroperoxides		hexanal	
sample	day 1	day 3	day 1	day 3	day 1	day 3	day 1	day 3
linoleic acid	0.0 ± 0.8[c]	0.0 ± 4.9[e]	0.0 ± 3.6[d]	0.0 ± 1.6[d]	0.0 ± 3.0[c]	0.0 ± 1.8[e]	0.0 ± 1.1[c]	0.0 ± 0.5[e]
+ α-tocopherol								
150 μM	94.7 ± 0.1[b]	58.1 ± 0.2[d]	79.0 ± 3.6[c]	67.1 ± 3.6[c]	93.3 ± 0.2[b]	14.1 ± 0.6[d]	92.8 ± 0.8[b]	57.0 ± 0.2[d]
300 μM	95.3 ± 0.1[b]	67.5 ± 0.7[c]	82.4 ± 1.3[bc]	77.5 ± 1.8[b]	94.7 ± 0.1[b]	40.9 ± 2.0[c]	91.1 ± 0.9[b]	79.2 ± 0.3[c]
+ Trolox								
150 μM	99.5 ± 0.2[a]	85.0 ± 0.1[b]	89.1 ± 0.4[ab]	91.8 ± 0.3[a]	99.9 ± 0.2[a]	69.2 ± 1.0[b]	96.2 ± 0.1[a]	93.8 ± 0.3[d]
300 μM	99.5 ± 0.2[a]	99.1 ± 0.3[a]	94.2 ± 1.1[a]	96.6 ± 0.1[a]	100.0 ± 1.8[a]	99.4 ± 0.1[a]	98.3 ± 0.3[a]	99.1 ± 0.1[a]
	bulk				emulsified			
	hydroperoxides		hexanal		hydroperoxides		hexanal	
sample	day 11	day 15	day 11	day 15	day 7	day 9	day 7	day 9
methyl linoleate	0.0 ± 2.0[b]	0.0 ± 0.7[b]	0.0 ± 2.3[b]	0.0 ± 3.0[c]	0.0 ± 0.5[c]	0.0 ± 0.1[c]	0.0 ± 1.3[c]	0.0 ± 2.4[c]
+ α-tocopherol								
150 μM	98.0 ± 0.1[a]	98.5 ± 0.1[a]	95.1 ± 0.4[a]	97.1 ± 0.1[a]	99.4 ± 0.1[a]	99.4 ± 0.1[a]	98.8 ± 0.3[a]	98.6 ± 0.1[a]
300 μM	97.2 ± 0.1[a]	97.9 ± 0.1[a]	97.2 ± 0.4[a]	98.5 ± 0.3[a]	99.6 ± 0.1[a]	99.5 ± 0.1[a]	99.3 ± 0.1[a]	99.6 ± 0.1[a]
+ Trolox								
150 μM	99.1 ± 0.1[a]	99.0 ± 0.1[a]	−18.6 ± 2.0[c]	−66.4 ± 2.3[d]	85.3 ± 0.1[b]	64.9 ± 0.5[b]	96.2 ± 0.2[b]	92.2 ± 0.1[b]
300 μM	99.1 ± 0.1[a]	99.4 ± 0.1[a]	92.8 ± 1.8[a]	63.2 ± 1.8[b]	99.4 ± 0.1[a]	99.3 ± 0.1[a]	98.9 ± 0.1[a]	99.2 ± 0.1[a]

[a] % inhibition = $[(C - S)/C] \times 100$, where C = hydroperoxide or hexanal formed in control and S = hydroperoxides or hexanal formed in sample. Negative values represent prooxidant activity. SD, standard deviation, $n = 2$. [b] Values within each column followed by the same letter are not significantly different ($p < 0.05$).

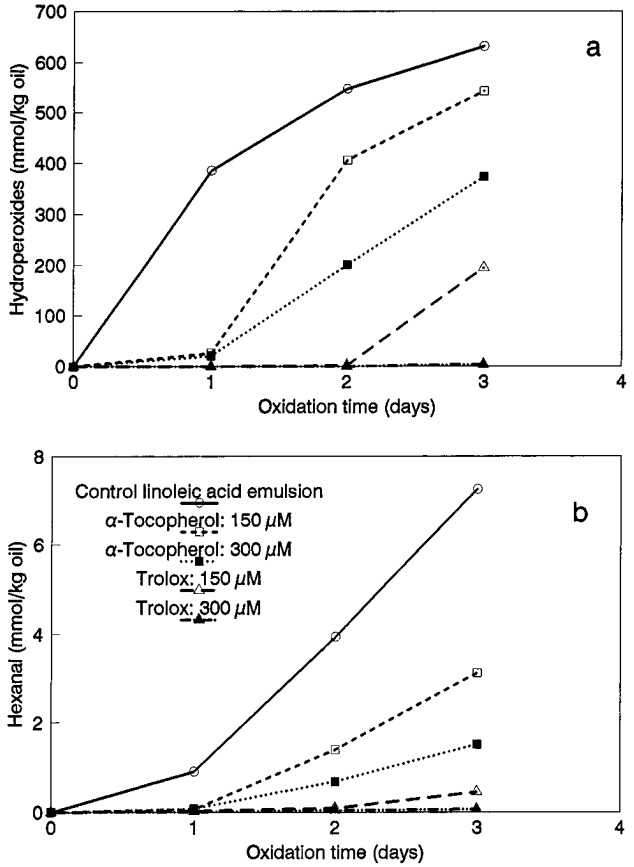

Figure 4. Effect of α-tocopherol and Trolox on oxidative stability of 10% emulsified linoleic acid at 37 °C: (a) hydroperoxide formation (same symbols as in panel b); (b) hexanal formation.

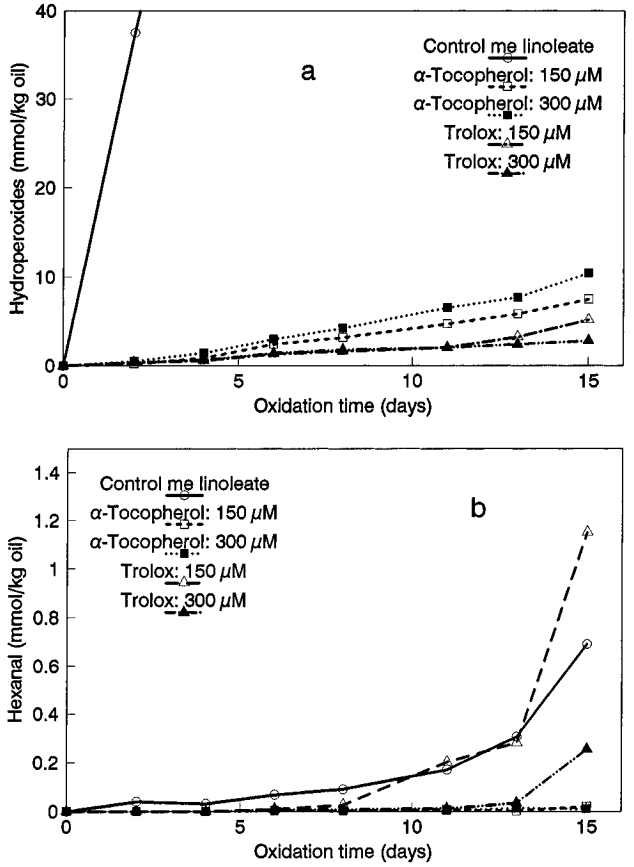

Figure 5. Effect of α-tocopherol and Trolox on oxidative stability of bulk methyl linoleate at 37 °C: (a) hydroperoxide formation; (b) hexanal formation.

to linoleic acid at 37 °C (Figures 3a and 5a). Trolox was again a more effective inhibitor of hydroperoxide formation than α-tocopherol. However, the inhibition by both antioxidants at the two levels used was between 97 and 99% over the 25 days tested and was not significantly different (Table 1).

Decomposition of Hydroperoxides. Hexanal formation in methyl linoleate was inhibited by α-tocopherol and Trolox between 1 and 8 days (Figure 5b). After 8 days, 150 μM Trolox did not inhibit hexanal formation and showed a prooxidant effect after 11 and 15 days of oxidation (Table 1). In contrast to their effects on hydroperoxide formation, α-tocopherol was more effective than Trolox in inhibiting hydroperoxide decomposition.

Effects of α-Tocopherol and Trolox in Emulsified Methyl Linoleate. *Formation of Hydroperoxides.* In contrast to bulk methyl linoleate, in emulsions α-tocopherol was a better inhibitor of hydroperoxide formation than Trolox (Figures 5a and 6a). During 14 days of oxidation, α-tocopherol strongly inhibited hydroperoxide formation and was not different between 150 and 300 μM. The rate of hydroperoxide formation increased after 4 days at 150 μM Trolox and after 11 days at 300 μM. On day 9, the inhibition was 65% with 150 μM of Trolox and exceeded 99% with all other samples (Table 1).

Decomposition of Hydroperoxides. Hydroperoxides decomposed extensively in the control emulsion after 2 days (Figure 6b). The rate of hexanal formation in the presence of 150 μM Trolox increased between 7 and 11 days. In the presence of 150 and 300 μM α-tocopherol and 300 μM Trolox, hexanal formation was strongly inhibited during 14 days of oxidation. On day 9, the inhibition of hexanal formation was 92% with 150 μM Trolox and 99% with 300 μM Trolox and 150 and 300 μM α-tocopherol (Table 1).

Effects of α-Tocopherol and Trolox in Bulk Corn Oil Triglycerides. *Formation of Hydroperoxides.* At 37 °C, in the presence of both antioxidants, hydroperoxide formation was inhibited and its initial rate did not increase during 36 days of oxidation (Figure 7a). Trolox was a more effective inhibitor of hydroperoxide formation than α-tocopherol (Table 2). Neither antioxidant showed significant differences in activity between 150 and 300 μM.

Because the oxidation of corn oil triglycerides was slow at 37 °C, the antioxidants were also evaluated at 60 °C. The activities of both antioxidants in inhibiting hydroperoxide formation in bulk corn oil were less at 60 °C than at 37 °C (Table 2), yet Trolox was still a better inhibitor of hydroperoxide formation than α-tocopherol, especially at 300 μM. At 60 °C, α-tocopherol was more active at 300 μM than at 150 μM on day 4 and day 5, but this trend was lost by day 6.

Decomposition of Hydroperoxides. Hexanal formation did not increase in the control until after 18 days, but its rate increased sharply after 24 days (Figure 7b). The antioxidant activity of 150 and 300 μM α-tocopherol was not significantly different (Table 2). α-Tocopherol was a more effective inhibitor of hexanal formation than Trolox at 150 μM but less active than Trolox at 300 μM. The same trends were observed at 60 °C.

Effects of α-Tocopherol and Trolox in Emulsified Corn Oil Triglycerides. *Formation of Hydroperoxides.* At 37 °C, both antioxidants inhibited hydroperoxide formation in emulsions (Figure 8a). The rate

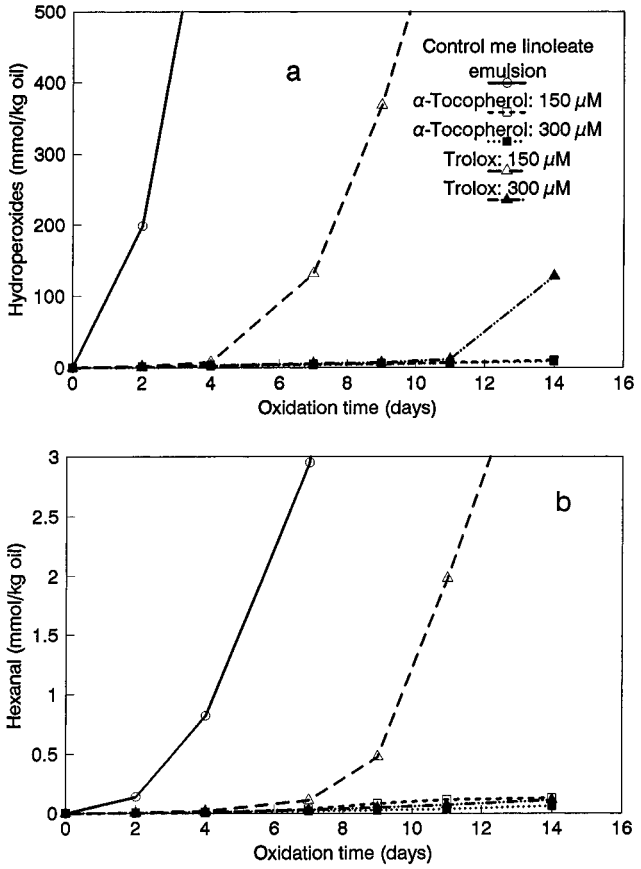

Figure 6. Effect of α-tocopherol and Trolox on oxidative stability of 10% methyl linoleate emulsions at 37 °C: (a) hydroperoxide formation; (b) hexanal formation (same symbols as in panel a).

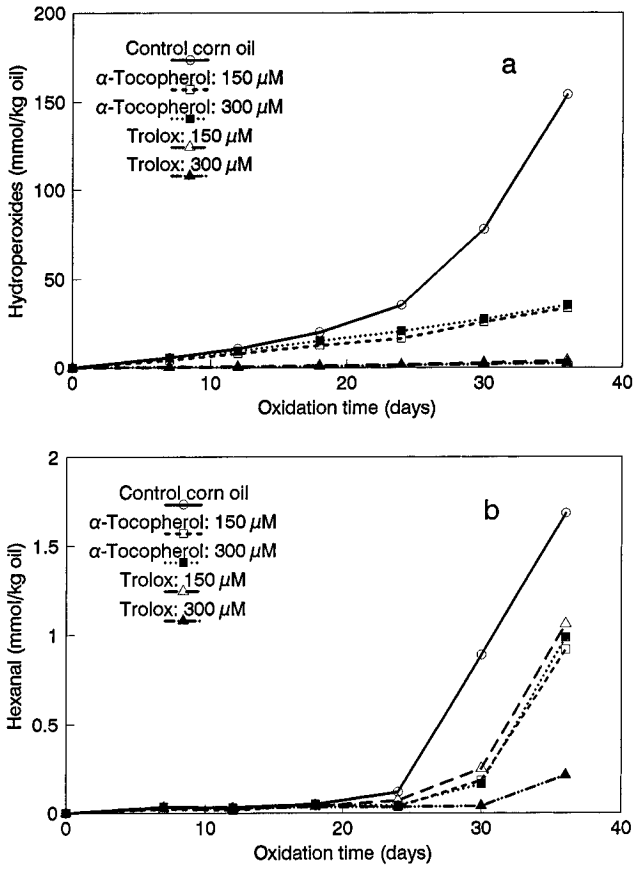

Figure 7. Effect of α-tocopherol and Trolox on oxidative stability of bulk corn oil triglycerides at 37 °C: (a) hydroperoxide formation; (b) hexanal formation.

of hydroperoxide formation in the presence of Trolox increased significantly after 16 days (Table 2). In contrast to bulk corn oil but similar to methyl linoleate emulsions, α-tocopherol was a more effective inhibitor of hydroperoxide formation than Trolox.

At 60 °C, α-tocopherol inhibited hydroperoxide formation to a greater extent than an equal concentration of Trolox. At 300 μM, α-tocopherol strongly inhibited hydroperoxide formation after 4 days, whereas Trolox inhibited hydroperoxide formation only slightly (Table 2). In contrast to results obtained at 37 °C, the activities of Trolox at 150 and 300 μM were not significantly different after 4 days of oxidation.

Decomposition of Hydroperoxides. At 37 °C, in the control emulsion, large amounts of hexanal were formed after 16 days (Figure 8b). Hexanal formation was inhibited by both antioxidants (Table 2). On day 21, α-tocopherol was more active than Trolox at 150 μM but not at 300 μM.

At 60 °C, the same trend in antioxidant activity was observed (Table 2). α-Tocopherol was more active at 150 μM between 1 day and 3 days but less active than at 300 μM after 3 days. In contrast, Trolox inhibited hexanal formation only between 1 and 2 days but promoted hexanal formation between 2 and 4 days at 150 μM and after 3 days at 300 μM.

Partition Studies. The partitioning of Trolox between different phases was determined to clarify its mechanism of antioxidant activity in different emulsified lipid systems. The partition coefficients of Trolox between different lipids and water increased with increasing Trolox concentration as measured with two different methods (Table 3). At 100 μg/g, the partition coefficients measured according to these two methods between corn oil and water were similar. The apparent partition coefficients of Trolox between different lipids and water depended on its concentration due to surface adsorption and to the change of degree of dissociation of Trolox with concentration. At the low pH values of these samples (pH 3.0−3.3) linoleic acid was mainly in the undissociated form ($pK_a \sim 5$). Although Trolox was in both dissociated and undissociated forms ($pK_a = 3.89$) (Barclay and Vinqvist, 1994), the partition coefficient of Trolox between linoleic acid and water was similar to that between methyl linoleate and water determined by either method and was higher than that between corn oil and water. Trolox had similar high affinity toward linoleic acid and methyl linoleate but lower affinity toward corn oil.

The proportion of Trolox in the micelles of 1% Tween 20 solutions also increased with increasing Trolox concentration (Table 3). When the ratio of lipid to water was 1:9 w/w, 55.5−68.3% of 100 μg/g Trolox partitioned into the water phase as determined by method B. With 1% Tween 20, the concentration of Trolox in the water phase decreased from 55.8 to 42.2% in emulsified linoleic acid, from 55.5 to 30.7% in methyl linoleate emulsions, and from 68.3 to 37.8% in corn oil emulsions.

Although the partition of Trolox was complicated in the presence of Tween 20 at the oil−water interface and in the micelles, the concentrations of Trolox in the water phase of different emulsified lipids were estimated on the basis of its partition ratios between lipids and water and between Tween 20 and water at a concentration of 100 μg/g. For emulsified corn oil and methyl linoleate, the calculated concentrations of Trolox agreed with the experimental values much better (0 and 1.4%) than for

Table 2. Inhibition of Hydroperoxide and Hexanal Formation by α-Tocopherol and Trolox C in Bulk Corn Oil Triglyceride and in Corn Oil-in-Water Emulsions at 37 and 60 °C (Percent Mean Inhibition ± SD)[a,b]

	bulk oil at 37 °C				emulsions at 37 °C			
	hydroperoxides		hexanal		hydroperoxides		hexanal	
sample	day 24	day 36	day 24	day 36	day 16	day 21	day 16	day 21
corn oil	0.0 ± 0.3[d]	0.0 ± 4.2[c]	0.0 ± 2.9[c]	0.0 ± 0.7[d]	0.0 ± 1.4[e]	0.0 ± 0.9[d]	0.0 ± 5.0[d]	0.0 ± 9.4[c]
+ α-tocopherol								
150 μM	53.1 ± 1.0[b]	78.0 ± 0.3[b]	69.6 ± 1.7[a]	45.6 ± 1.4[b]	75.4 ± 0.9[b]	93.1 ± 0.2[a]	86.8 ± 1.1[b]	97.1 ± 0.1[d]
300 μM	41.5 ± 1.7[c]	76.9 ± 0.6[b]	63.9 ± 0.1[a]	41.5 ± 0.3[b]	79.0 ± 0.7[a]	91.7 ± 0.4[a]	100.0 ± 0.3[a]	99.6 ± 0.1[a]
+ Trolox								
150 μM	95.0 ± 1.0[a]	97.4 ± 0.3[a]	39.6 ± 2.7[b]	37.1 ± 1.8[c]	51.9 ± 0.2[d]	54.3 ± 1.6[c]	74.1 ± 0.5[c]	81.7 ± 1.2[b]
300 μM	95.4 ± 0.6[a]	98.3 ± 0.4[a]	69.6 ± 0.9[a]	87.5 ± 0.1[a]	65.1 ± 1.8[c]	85.2 ± 0.8[b]	74.8 ± 2.7[c]	92.9 ± 0.4[ab]
	bulk oil at 60 °C				emulsions at 60 °C			
	hydroperoxides		hexanal		hydroperoxides		hexanal	
sample	day 4	day 6	day 4	day 6	day 3	day 4	day 3	day 4
corn oil	0.0 ± 0.5[e]	0.0 ± 2.2[d]	0.0 ± 14.8[a]	0.0 ± 11.8[c]	0.0 ± 4.5[c]	0.0 ± 1.5[d]	0.0 ± 5.8[c]	0.0 ± 7.7[c]
+ α-tocopherol								
150 μM	19.3 ± 0.8[d]	15.3 ± 2.7[c]	−33.7 ± 22.6[a]	35.0 ± 0.8[b]	87.4 ± 0.2[a]	26.0 ± 0.9[b]	83.1 ± 3.5[a]	54.3 ± 1.9[b]
300 μM	36.6 ± 0.8[c]	8.7 ± 1.4[d]	13.3 ± 15.0[a]	39.6 ± 0.4[b]	93.3 ± 0.1[a]	87.6 ± 0.8[a]	70.6 ± 2.0[b]	75.4 ± 1.2[a]
+ Trolox								
150 μM	49.0 ± 3.1[b]	30.3 ± 4.3[b]	−34.5 ± 95.8[a]	5.5 ± 2.0[c]	3.6 ± 0.2[c]	14.7 ± 5.6[c]	−19.9 ± 2.0[d]	2.0 ± 1.7[c]
300 μM	96.0 ± 0.9[a]	83.8 ± 0.6[a]	35.5 ± 4.9[a]	87.9 ± 0.4[a]	12.7 ± 4.5[b]	12.5 ± 3.0[c]	−6.1 ± 2.0[c]	−27.1 ± 2.9[d]

[a] % inhibition = $[(C - S)/C] \times 100$, where C = hydroperoxide or hexanal formed in control and S = hydroperoxides or hexanal formed in sample. Negative values represent prooxidant activity. SD, standard deviation, $n = 2$. [b] Values within each column followed by the same letter are not significantly different ($p < 0.05$).

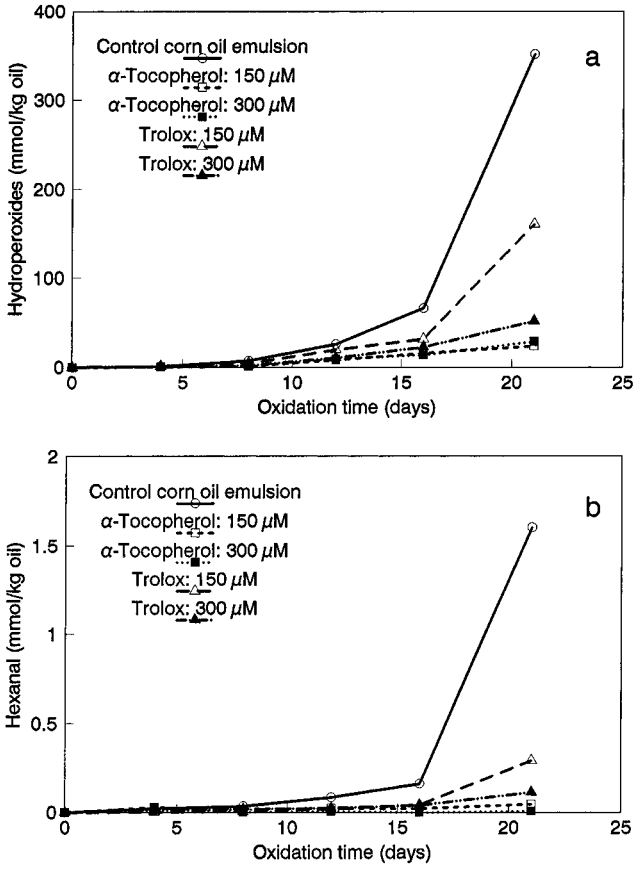

Figure 8. Effect of α-tocopherol and Trolox on oxidative stability of 10% corn oil triglyceride emulsions at 37 °C: (a) hydroperoxide formation; (b) hexanal formation.

emulsified linoleic acid (10%). These results indicate that in emulsified lipids the concentration of Trolox in the water phase decreased because of its affinity toward Tween 20. Methyl linoleate and corn oil form oil-in-water emulsions with Tween 20 at the interface, whereas linoleic acid not only forms oil-in-water emulsions but also remains partly in the water phase and partly in mixed micelles with Tween 20.

Table 3. Partitioning of Trolox between Different Lipids and Water and the Percentages of Trolox in Water Phase of Tween 20 Solution and Different Emulsified Lipids with 1% Tween 20 (Percent Mean ± SD)[a]

sample	method[b]	total Trolox (μg/g)	% Trolox in water phase	calcd % Trolox[c]	partition coeff
10% linoleic	A	50	60.3 ± 2.3		5.47
acid−water	B	100	55.8 ± 0.2		6.44
10% methyl	A	50	60.8 ± 0.7		5.16
linoleate−water	B	100	55.5 ± 0.9		6.41
10% corn	A	33	78.5 ± 0.5		2.25
oil−water	A	50	76.5 ± 0.7		2.53
	A	65	74.5 ± 1.2		2.83
	A	100	66.6 ± 0.7		4.13
	B	100	68.3 ± 1.0		3.83
1% Tween 20	B	40	59.7 ± 1.9		
solution	B	65	50.9 ± 1.6		
	B	100	43.3 ± 0.8[d]		
10% emulsified linoleic acid	B	100	42.2 ± 1.3	32.2	
10% emulsified methyl linoleate	B	100	30.7 ± 2.2	32.1	
10% emulsified corn oil	B	100	36.3 ± 1.5[d]	36.1	

[a] SD, standard deviation, $n = 3$. [b] Methods A and B are described under Materials and Methods. [c] Percent Trolox in the water phase was calculated on the basis of its partition ratios between lipid and water and between Tween 20 and water at a concentration of 100 μg/g as determined by method B. [d] $n = 4$.

DISCUSSION

The present study demonstrates that the effectiveness of α-tocopherol and Trolox is very dependent on their physical properties and the physical states of lipid systems used as substrates. Polarity and solubility of antioxidants determine their concentrations in different locations in various multiphase systems. Their antioxidant activity is affected by their diffusion rates, stability, and degree of dissociation, which may change with their location in these systems. If the relationship between activities and phase distribution of antioxidants can be defined, the antioxidant activity could be predicted on the basis of phase distribution.

The results of this study suggest that the relative amounts of hydroperoxides formed were higher in the presence of α-tocopherol than of Trolox in bulk methyl linoleate. The reverse trend was observed with hexanal formation. Although antioxidants compete with unoxidized lipid as hydrogen donors, their ability to donate hydrogen is different for peroxyl radicals from that for alkoxyl radicals. In addition, the effectiveness of antioxidants may also depend on their location, mobility, stability, reactivity toward lipid radicals, and the tendency of their peroxyl and alkoxyl radicals to form secondary products.

Burton et al. (1985) suggested that Trolox was less reactive than α-tocopherol toward peroxyl radicals in a homogeneous solution of styrene because of a deactivating effect induced by hydrogen bonding between the close proximity of the carboxylic group and the oxygen atom in the heterocyclic ring. The present study with heterogeneous systems indicated that Trolox might also be less reactive than α-tocopherol toward alkoxyl radicals. The lower hexanal formation in the presence of α-tocopherol than Trolox in bulk methyl linoleate may thus be explained by the higher reactivity of α-tocopherol toward alkoxyl radicals. Although α-tocopherol is considered to be a strong hydrogen donor, α-tocopheroxyl radicals (Labuza, 1971; Loury et al., 1966; Terao and Matsushita, 1986) and α-tocoquinoperoxyl radicals (Gottstein and Grosch, 1990) may act as chain initiators. Also, α-tocopherol may react with oxygen (Cillard and Cillard, 1980; Gottstein and Grosch, 1990) to form α-tocopheroxyl radical. Previously, we found that α-tocopherol at higher concentrations was less effective in inhibiting hydroperoxide formation but more active in inhibiting hexanal formation (Frankel et al., 1994; Huang et al., 1994). However, there is no information in the literature on the fate of Trolox phenoxyl radicals in bulk lipids. Trolox quinone and lactone were identified as potential oxidation products of Trolox in alkaline aqueous solutions with potassium ferricyanide and potassium bromide, and neither oxidation product of Trolox had antioxidant activity (Cort et al., 1975). No dimers or trimers were found. Porter (1980) suggested that the high activity of Trolox in oils might stem from the high surface energy produced by the hydrophilic strong acid group and the lipophilic nucleus or the tendency to form a lactone product. On the other hand, Frankel et al. (1994) suggested an interfacial effect for Trolox that might better protect lipid against oxidation by being located at oil−air interfaces or the oil surface.

In contrast to bulk lipids, α-tocopherol was a better inhibitor of hydroperoxide formation and decomposition than Trolox in emulsified methyl linoleate and corn oil triglycerides. To clarify this interfacial phenomenon, partition studies were carried out with Trolox in different lipid systems according to classical methods. As emulsifier, Tween 20 was expected partly to be located at the interface and partly to form micelles because its concentration was above its critical micelle concentration (3.5×10^{-5} M). Therefore, the emulsified lipid systems contained at least four phases including water, lipid, interface, and Tween 20 micelles. For oil-in-water emulsions, the amount of Tween 20 distributed at the oil−water interface was calculated from the particle size of lipid droplets. For 10 mL of these lipid emulsions, the physical dimensions were assumed as follows: if the volume of lipid was about 1.1 mL and the diameter of lipid droplet was in the range of 0.1−0.25 μm, then

surface area of each droplet = 3.1×10^{-10} cm^2 (0.1 μm), 2.0×10^{-9} cm^2 (0.25 μm)

volume of each droplet = 5.2×10^{-16} cm^3 (0.1 μm), 8.2×10^{-15} cm^3 (0.25 μm)

number of lipid droplet = 2.1×10^{15} (0.1 μm), 1.3×10^{14} (0.25 μm)

total surface area = 6.6×10^5 cm^2 (0.1 μm), 2.6×10^5 cm^2 (0.25 μm)

If the radius of equivalent spheres for Tween 20 micelles is 3.57 nm and the aggregation number is 80 at 25 °C (Mandal et al., 1985), the cross-section area per molecule of Tween 20 can be estimated to be about 2×10^{-14} cm^2 and the number of moles at the oil−water interface can be estimated to be about 2.2×10^{-5}−5.5×10^{-5}. The emulsified lipids used in this study contained about 8×10^{-5} mol of Tween 20/10 mL of emulsion. Therefore, about 26−68% of Tween 20 was at the oil−water interface and 32−74% formed micelles, which meant at least four phases existed in emulsified lipid systems.

According to Wedzicha (1988), Trolox is surface-active because it is soluble both in water and in the organic phase and concentrates at the lipid−water interface. The present study showed that Trolox moved more freely into the lipid phase and its partition coefficients between lipids and water increased at high concentrations. The concentration effect of Trolox on its partition between different phases may be explained by the degree of its dissociation, which depends on its concentration in water. Although the affinity of Trolox toward linoleic acid was similar to that toward methyl linoleate, the concentration of Trolox in the water phase was much higher in emulsified linoleic acid than in emulsified methyl linoleate. Linoleic acid appeared to compete with Trolox for Tween 20 in the polar region of the micelles and at the oil−water interface, resulting in an increase of Trolox concentration in the water phase in emulsified linoleic acid. These results suggest that the physical state of emulsified linoleic acid is different from those of emulsified methyl linoleate and corn oil, in agreement with the finding of Barclay et al. (1983). Apparently, Trolox partitions into the water phase and into Tween 20 micelles and protects methyl linoleate and corn oil triglycerides less effectively than α-tocopherol, which may be oriented at the oil−water interfaces and in the lipid phase. In contrast, Trolox was a better antioxidant than α-tocopherol in emulsified linoleic acid, which could be better protected by Trolox in the water phase and in mixed micelles with Tween 20. Castle and Perkins (1986) found that Trolox diffused more quickly than α-tocopherol to scavenge linoleic acid radicals between SDS micelles at pH 7, based on their concentrations in the micelles. However, Trolox was less effective than α-tocopherol due to its greater partitioning into the water phase.

The partitioning of the lipophilic α-tocopherol between lipids and Tween 20 is difficult to determine with classical methods because lipids cannot be separated from miscible Tween 20. The stability of Trolox in different phases needs to be evaluated to determine if it affects antioxidant activity in different phases in emulsions.

Overall, the behavior of α-tocopherol and Trolox in methyl linoleate was more similar to that in corn oil triglycerides in both bulk and emulsion systems. However, in linoleic acid α-tocopherol and Trolox had the same order of activity in inhibiting hydroperoxide formation but not in inhibiting hydroperoxide decomposition. In emulsified linoleic acid, α-tocopherol and Trolox had the opposite trend of activity in inhibiting both formation and decomposition of hydroperoxides, compared to emulsified methyl linoleate and corn oil triglycerides. These results suggest that linoleic acid cannot be used as a representative model food system since antioxidant behavior will be significantly different from that in foods composed mainly of triglycerides. This study revealed that antioxidant activity is markedly affected by the physical state of different lipid systems and by the concentration and location of different antioxidants.

ABBREVIATIONS USED

PUFA, polyunsaturated fatty acid; Tween 20, polyoxyethlene sorbitan monolaurate; GC, gas chromatography; HPLC, high-performance liquid chromatography.

LITERATURE CITED

Barclay, L. R. C.; Vinqvist, M. R. Membrane peroxidation: inhibiting effects of water-soluble antioxidants on phospholipids of different charge types. *Free Radical Biol. Med.* **1994**, *16*, 779−788.

Barclay, L. R. C.; Locke, S. J.; MacNeil, J. M. The autoxidation of unsaturated lipids in micelles. Synergism of inhibitors vitamin C and E. *Can. J. Chem.* **1983**, *61*, 1288−1290.

Barclay, L. R. C.; Locke, S. J.; MacNeil, J. M. Autoxidation in micelles. Synergism of vitamin C with lipid-soluble vitamin E and water-soluble Trolox. *Can. J. Chem.* **1985**, *63*, 366−374.

Burton, G. W.; Doba, T.; Gabe, E. J.; Lughes, L.; Lee, F. L.; Prasad, L.; Ingold, K. U. Autoxidation of biological molecules. 4. Maximizing the antioxidant activity of phenols. *J. Am. Chem. Soc.* **1985**, *107*, 7053−7065.

Castle, L.; Perkins, M. J. Inhibition kinetics of chain-breaking phenolic antioxidants in SDS micelles. Evidence that intermicellar diffusion rates may be rate-limiting for hydrophobic inhibitors such as α-tocopherol. *J. Am. Chem. Soc.* **1986**, *108*, 6381−6382.

Chipault, J. R.; Mizuno, G. R.; Lundberg, W. O. Antioxidant properties of spices in oil-in-water emulsions. *Food Res.* **1955**, *20*, 443−448.

Chipault, J. R.; Mizuno, G. R.; Lundberg, W. O. The antioxidant properties of spices in foods. *Food Technol.* **1956**, *10*, 209−212.

Cillard, J.; Cillard, P. Behavior of alpha, gamma, and delta tocopherols with linoleic acid in aqueous media. *J. Am. Oil Chem. Soc.* **1980**, *57*, 39−42.

Cillard, J.; Cillard, P.; Cormier, M. Effect of experimental factors on the prooxidant behavior of α-tocopherol. *J. Am. Oil Chem. Soc.* **1980**, *57*, 255−261.

Cornell, D. G.; DeVilbiss, E. D.; Pallansch, M. J. Partition coefficients of some antioxidants in butteroil-water model systems. *J. Dairy Sci.* **1970**, *53*, 529−532.

Cort, W. M.; Araujo, M.; Mergens, W. J.; Cannalonga, M. A.; Osadca, M.; Harley, H.; Parrish, D. R.; Pool, W. R. Antioxidant activity and stability of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid. *J. Am. Oil Chem. Soc.* **1975**, *52*, 174−178.

Courthaudon, J.-L.; Dickinson, E.; Dalgleish, D. G. Competitive adsorption of β-casein and nonionic surfactants in oil-in-water emulsions. *J. Colloid Interface Sci.* **1991**, *145*, 390−395.

Frankel, E. N. Volatile lipid oxidation products. *Prog. Lipid Res.* **1982**, *22*, 1−33.

Frankel, E. N. In search of better methods to evaluate natural antioxidants and oxidative stability in food Lipids. *Trends Food Sci. Technol.* **1993**, *4*, 220−225.

Frankel, E. N.; Huang, S.-W.; Kanner, J.; German, J. B. Interfacial phenomena in the evaluation of antioxidants : bulk oils *versus* emulsions. *J. Agric. Food Chem.* **1994**, *42*, 1054−1059.

Gottstein, T.; Grosch, W. Model study of different antioxidant properites of α- and γ-tocopherol in fats. *Fat Sci. Technol.* **1990**, *92*, 139−144.

Handelman, G. J.; Machlin, L. J.; Fitch, K.; Weiter, J. J.; Dratz, E. A. Oral α-tocopherol supplements decrease plasma γ-tocopherol levels in humans. *J. Nutr.* **1985**, *115*, 807−813.

Huang, S.-W.; Frankel, E. N.; German, J. B. Antioxidant activity of α- and γ-tocopherols in bulk oils and in oil-in-water emulsions. *J. Agric. Food Chem.* **1994**, *42*, 2108−2114.

Labuza, T. P. Kinetics of lipid oxidation in food. *CRC Crit. Rev. Food Technol.* **1971**, *2*, 355−405.

Lee, C. H.; Ward, R. J. Relative activities of the seven tocopherols. *J. Sci. Food Agric.* **1959**, *10*, 537−548.

Loury, M.; Bloch, C.; Francois, R. Use of tocopherol as an antioxidant in fats. *Rev. Fr. Corps Gras* **1966**, *13*, 747−754.

Mandal, A. B.; Gupta, S.; Moulik, S. P. Characterisation of Tween 20 and Tween 80 micelles in aqueous medium from transport studies. *Ind. J. Chem.* **1985**, *24*, 670−673.

Porter, W. L. Recent trends in food applications of antioxidants. In *Autoxidation in Food and Biological Systems*; Simic, M. G., Karel, M., Eds.; Plenum Press: New York, 1980; pp 295−365.

Porter, W. L.; Black, E. D.; Drolet, A. M. Use of polyamide oxidative fluorescence test on lipid emulsions: contrast in relative effectiveness of antioxidants in bulk versus dispersed systems. *J. Agric. Food Chem.* **1989**, *37*, 615−623.

Pryor, W. A.; Strickland, T.; Church, D. F. Comparison of the efficiencies of several natural and synthetic antioxidants in aqueous sodium dodecyl sulfate micelle solutions. *J. Am. Chem. Soc.* **1988**, *10*, 2224−2229.

Pryor, W. A.; Cornicelli, J. A.; Devall, L. J.; Tait, B.; Trivedi, B. K.; Witiak, D. T.; Wu, M. A rapid screening test to determine the antioxidant potencies of natural and synthetic antioxidants. *J. Org. Chem.* **1993**, *58*, 3521−3532.

Roginsky, V. A. Effectiveness of lipid- and water-soluble phenolic antioxidants in the autoxidation of polyunsaturated fatty acid esters in microheterogeneous solutions. *Biol. Membr.* **1990**, *4*, 437−451.

Taylor, M. J.; Richardson, T. Antioxidant activity of amino acids bound to Trolox-C. *J. Am. Oil Chem. Soc.* **1981**, *58*, 622−626.

Terao, J.; Matsushita, S. The peroxidizing effect of α-tocopherol on autooxidation of methyl linoleate in bulk phase. *Lipids* **1986**, *21*, 255−260.

Uri, N. Mechanism of antioxidation. In *Autoxidation and Antioxidants*; Lundgerg, W. O., Ed.; Interscience: New York, 1961; Vol. 1, Chapter 4.

Wagner, S. F. Analysis of variance. In *Introduction to Statistics*; Harper Perennial: New York, 1992; pp 239−273.

Wedzicha, B. L. Distribution of low-molecular-weight food additives in dispersed systems. In *Advances in Food Emulsions and Foams*; Dickinson, E., Stainsby, G., Eds; Elsevier Applied Science: New York, 1988; pp 329−371.

Yamamura, K.; Nakao, M.; Yano, K.; Miyamoto, K.-I.; Yotsuyanagi, T. Stability of forskolin in lipid emulsions and oil/water partition coefficients. *Chem. Pharm. Bull.* **1991**, *39*, 1032−1034.

Received for review August 22, 1995. Accepted November 13, 1995.[⊗]

JF9505685

[⊗] Abstract published in *Advance ACS Abstracts*, January 15, 1996.