Effect of pH on Antioxidant Activity of α-Tocopherol and Trolox in Oil-in-Water Emulsions

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The pH had a more significant effect on the antioxidant activity of Trolox than α-tocopherol in corn oil emulsified with Tween 20 at 60 °C. Although with increasing pH the oxidative stability of corn oil emulsions decreased, the antioxidant activity of α-tocopherol increased. Initially, Trolox and α-tocopherol were similar in inhibiting hydroperoxide formation with or without phosphate buffers at pH 3−7, but at later stages α-tocopherol was more effective than Trolox at pH 3, 4, 5, and 7 but not at pH 6. In contrast, α-tocopherol inhibited hexanal formation better than Trolox during the entire oxidation period. Trolox was more stable than α-tocopherol in bulk corn oil but less stable in Tween 20 solutions at different pH. Both antioxidants in Tween 20 solutions were most stable at pH 3 and least stable at pH 7. Although more Trolox partitioned into the oil phase and the oil−water interfaces at pH 3−4, its antioxidant activity was lower than at pH 5−6. The overall antioxidant activities of α-tocopherol and Trolox depend on their hydrogen-donating ability, relative stability, and distribution in emulsions.

Keywords: *Antioxidants; α-tocopherol; Trolox; corn oil triglycerides; emulsion; antioxidant mechanism; interfacial oxidation; hydroperoxides; hexanal; partition coefficient; oxidative stability*

INTRODUCTION

Previous studies from this laboratory showed that the physical states of lipid systems affected the antioxidant activities of α-tocopherol and of its water-soluble analogue, Trolox (Frankel et al., 1994; Huang et al., 1996). Trolox was more effective than α-tocopherol in bulk systems and in emulsified linoleic acid, while α-tocopherol was more active than Trolox in emulsified methyl linoleate and corn oil triglycerides (Huang et al., 1996). In bulk lipid systems, Trolox was suggested to be located at oil−air interfaces and could protect lipid more efficiently than α-tocopherol, which remained in the lipid phase. In emulsions, Trolox partitioned into the water phase and surfactant micelles, and thus, only a small amount of Trolox was present at oil−water interfaces and in the lipid phase to protect lipids, compared to α-tocopherol. Because linoleic acid forms unique mixed micelles with the surfactant Tween 20, Trolox easily diffused into these micelles and was able to better protect emulsified linoleic acid than did α-tocopherol. Thus, linoleic acid may not be a valid substrate for evaluating food antioxidants. These results showed that the effectiveness of antioxidants in heterogeneous lipid systems is very dependent on their locations and is thus not proportional to the total antioxidant concentration. However, these studies did not evaluate the effects of pH and buffers on lipid oxidation and on the antioxidant effectiveness of α-tocopherol and Trolox in emulsified lipid systems.

At pH 6.9, linoleic acid emulsified with Tween 20 oxidized much faster in 25 mM phosphate buffer than in acetate buffer, and the oxidation rate was intermediate in the absence of buffers (Cillard et al., 1980). Whether lipid oxidation was affected by pH or lipid substrate was not clear. However, at high concentrations of α-tocopherol, the oxidation of emulsified linoleic acid with or without buffers was accelerated at a similar rate at pH 6.9.

The oxidation of methyl linoleate emulsified with sodium dodecyl sulfate (SDS) was more rapid in acetate buffer at pH 6.5 than at pH 3.4 and slower in tris(hydroxymethyl)aminomethane−hydrochloric acid buffer at pH 8.8 (Saunders et al., 1962). The oxidation rate of methyl linoleate dispersed in 0.1 M phosphate buffer increased with pH from 6 to 8 (Mabrouk and Dugan, 1960). Lipid oxidation appeared to be accelerated at higher pH, but depended on the buffer system.

Trolox is either neutral or negatively charged, depending on the pH, and its pK_a value is 3.89 (Barclay and Vinqvist, 1994). In liposomes, the antioxidant activity of Trolox was reportedly affected by the surface charge of phospholipids, which is dependent on the pH (Horan et al., 1994). The affinities of ionizable antioxidants toward the membranes were affected by their surface charge. Dilinoleoylphosphatidylcholine (DLPC) is zwitterionic at pH 11 and 7 but positive at low pH. Trolox partitioned about 20% into the lipid phase of DLPC liposomes at pH 7 (Barclay and Vinqvist, 1994). The antioxidant activity of Trolox was slightly better at pH 4 than at pH 7, but Trolox had no antioxidant activity at pH 11 in DLPC liposomes oxidized with a lipid-soluble azo initiator. The partition of Trolox was not determined at pH 4 and 11. Because Trolox probably dissociates completely at pH 11, it would be located in the water phase, whereas at pH 4 Trolox is less dissociated and may partition more into the lipid phase. In contrast, Trolox did not inhibit the oxidation of a membrane of dilinoleoylphosphatidylglycerol when the membrane is negatively charged at pH 7−11. Little information is available in the literature on the effect of pH on the partition of Trolox and interfacial lipid oxidation in emulsions prepared with nonionic surfactants.

To determine the effect of pH on interfacial lipid oxidation and antioxidant activity, this study compared the effectiveness of α-tocopherol and Trolox in corn oil emulsified with nonionic Tween 20 at different pH at

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60 °C. The effectiveness of antioxidants was evaluated at different stages of oxidation by measuring both the formation of hydroperoxides (conjugated dienes) and the decomposition of hydroperoxides (hexanal). The partition coefficients of Trolox between different phases were also measured to define the relationship between the antioxidant activity and phase distribution. The oxidative stability of antioxidants in bulk oil and in Tween 20 micelle solution at different pH were determined by high-performance liquid chromatography (HPLC) to better understand the antioxidant mechanism for both antioxidants.

MATERIALS AND METHODS

Materials. The same corn oil triglycerides stripped of tocopherols were used as previously (Huang et al., 1995). α-Tocopherol was obtained from Fluka Chemical Co. (Ronkonkoma, NY), Tween 20 (poly(oxyethylene) sorbitan monolaurate) from Sigma Chemical Co. (St. Louis, MO), and Trolox C from Aldrich Chemical Co. (Milwaukee, WI). Monobasic potassium phosphate and dibasic potassium phosphate (certified grade) were purchased from Fisher Scientific (Fair Lawn, NJ).

Methods. *Preparation of Bulk Oil and Emulsion Samples.* Oil samples (10 g) were prepared as in previous studies (Frankel et al., 1994; Huang et al., 1996) using concentrations of antioxidants of 300 or 1161 μM, which are equivalent to 130 and 500 ppm of α-tocopherol and 76 and 294 ppm of Trolox, respectively. Antioxidants were added to corn oil directly and dissolved by heating to 60 °C for 10 min. Oil samples (6 g) were weighted into screw-capped 25-mL Erlenmeyer flasks. Oil-in-water emulsions (10%, 30 g) were prepared with or without added antioxidants (1161 μM) in distilled water or phosphate buffers (50 mM, pH 3−7) in 50-mL Erlenmeyer flasks, as described by Huang et al. (1996). Emulsification was carried out by sonicating for a total of 6 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 0.17−0.25 μm.

Oxidation. The oxidation of oil and emulsion samples was carried out at 60 °C in a shaker oven (Lab-Line Instrument, Inc., Melrose Park, IL). Oxidative stability of emulsion samples was determined by measuring conjugated diene hydroperoxides spectrophotometrically and hexanal by headspace gas chromatography (GC). All oxidations and analyses were done in duplicate and the results were calculated by one-way analysis of variance (Wagner, 1992).

Measurement of Conjugated Diene Hydroperoxides. Emulsion samples (0.1 g) were dispersed in 5 mL of methanol and then diluted with more methanol to a measurable absorbance. The absorbance was measured at 234 nm and calculated as hydroperoxides (in mmol/kg of oil) (Frankel et al., 1994).

Measurement of Hexanal by Static Headspace GC. Hexanal is one of many important volatile products that has proved to be a useful marker for the oxidative decomposition of *n*-6 polyunsaturated fatty acids (Frankel, 1982). The procedures used for hexanal measurements were described previously (Frankel et al., 1994), except for equilibrating emulsion samples at 60 °C for 15 min.

Partition Studies. The concentration of Trolox was measured in the aqueous phase separated from lipid−water mixtures, Tween 20 solution, and emulsions through Centri/Por centrifuge concentrators with cellulose ester membranes, molecular weight cutoff 1000 (Spectrum, Houston, TX), as described by Huang et al. (1996). Three different systems were prepared with or without 50 mM phosphate buffers containing Trolox (100 μg/g of solution) as described previously (Huang et al., 1996): (1) 1% (w/w) Tween 20 solution; (2) 10% (w/w) oil−water mixtures; and (3) 10% oil-in-water emulsified with 1% Tween 20. The pH of these samples ranged from 3.0 to 7.0. Trolox in the water phase was analyzed by HPLC (Huang et al., 1996).

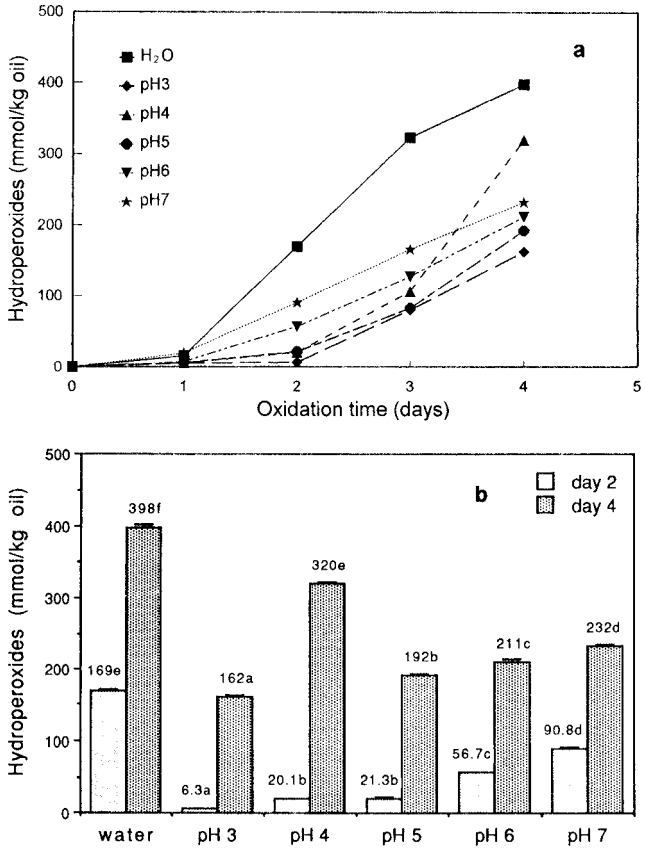

Figure 1. Effect of pH on oxidative stability of corn oil triglycerides emulsified with Tween 20 in 50 mM phosphate buffers at 60 °C by measuring hydroperoxide formation: (a) time curve; (b) on days 2 and 4. Values within each day followed by the same letter are not significantly different ($p < 0.05$).

Oxidative Stability of Antioxidants. The concentrations of antioxidants in bulk corn oil triglycerides during oxidation at 60 °C were determined by HPLC after methanol extraction. The oil samples (0.5 g) containing antioxidants were weighed into 10-mL test tubes and extracted five times with 1 mL of methanol containing citric acid (50 ppm) and erythorbic acid (50 ppm) by vortexing 20 s and centrifuging at 2000 rpm for 3 min. The methanol extracts were evaporated to dryness under nitrogen, then dissolved in 0.5 mL of methanol, vortexed, and centrifuged at 2000 rpm for 3 min. The clear methanol extracts were analyzed by HPLC. The oxidative stability of antioxidants in 1% Tween 20 micelle solutions at different pH was determined at 60 °C by directly measuring the concentrations of antioxidants in Tween 20 solutions by HPLC as described as below. Furthermore, the oxidative stability of Trolox in aqueous solutions at 60 °C was determined by the same HPLC method.

HPLC Analysis. The methanol extracts and Tween solutions containing antioxidants were 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 of methanol:water:2 M citric acid for Trolox (Huang et al., 1996), 100:1 of methanol:2 M citric acid for α-tocopherol. The flow rate was 0.3 mL/min^{-1}.

RESULTS

Effect of pH on Oxidative Stability of Emulsified Corn Oil Triglycerides. *Formation of Hydroperoxides.* The amount of hydroperoxides formed increased with increasing pH between 1 and 2 days (Figure 1a). At pH 4, the rate of hydroperoxide formation increased faster than at other pH after 3 days. Without buffers, the emulsion was initially pH 3.7, and after 4 days, it

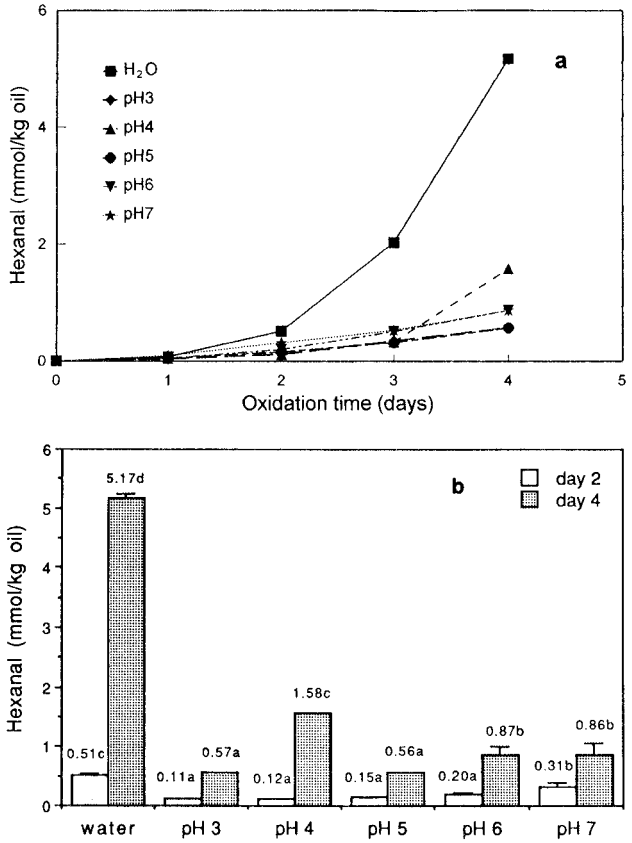

Figure 2. Effect of pH on oxidative stability of corn oil triglycerides emulsified with Tween 20 in 50 mM phosphate buffers at 60 °C by measuring hexanal formation: (a) time curve; (b) on days 2 and 4. Values within each day followed by the same letter are not significantly different ($p < 0.05$).

decreased to pH 2.6. The emulsion prepared without buffers oxidized more rapidly than those with phosphate buffer at different pH values after 1 day. The phosphate buffers may decrease the rate of oxidation by metal chelation. On day 2, the amount of hydroperoxides formed in corn oil emulsions increased in the following order: pH 3 < pH 4 = pH 5 < pH 6 < pH 7 (Figure 1b). On day 4, the amount of hydroperoxides increased with increasing pH (pH 3 < pH 5 < pH 6 < pH 7). However, at pH 4 the amount of hydroperoxides formed was highest.

Formation of Hexanal. The effect of pH on hexanal formation was similar to that of hydroperoxide formation, but the differences were less significant (compare Figures 1b and 2b). The amount of hexanal formed was greater in the absence of buffers than in the presence of buffers after 1 day (Figure 2a). On day 2, hexanal formation was higher at pH 7 than at pH 3−6 (Figure 2b). More hexanal was formed in the absence of buffers than in the presence of buffers on days 2 and 4. On day 4, the amount of hexanal formed was highest at pH 4, followed by pH 6 and 7.

Effect of pH on Antioxidant Activity of α-Tocopherol and Trolox. Without buffers, the emulsion was initially pH 3.5 with α-tocopherol and pH 3.2 with Trolox at 1161 μM and decreased to pH 2.8 and 2.6, respectively, after 4 days.

Formation of Hydroperoxides. In the presence of α-tocopherol, hydroperoxide formation increased at a constant low rate in most of the emulsions during the oxidation period (Figure 3). Conversely, in the presence of Trolox, hydroperoxide formation increased gradually and then increased sharply in most emulsions during

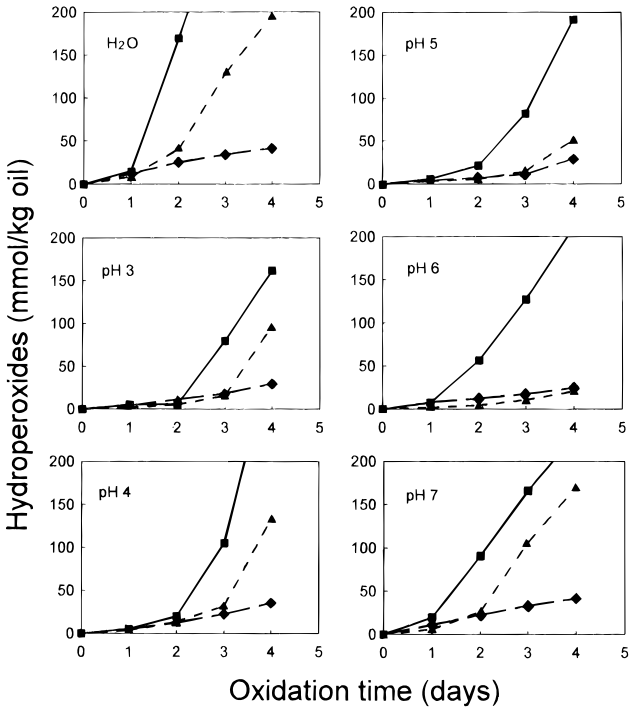

Figure 3. Effect of pH on the antioxidant activity of α-tocopherol and Trolox in corn oil triglycerides emulsified with Tween 20 in the absence and presence of 50 mM phosphate buffers at 60 °C by measuring hydroperoxide formation: ■, control; ▲, 1161 μM α-tocopherol; ◆, 1161 μM Trolox.

Table 1. Effect of pH on the Inhibition of Hydroperoxides and Hexanal Formation by α-Tocopherol and Trolox in Corn Oil Triglycerides Emulsified with Tween 20 at 60 °C (Percent Mean Inhibition ± SD)[a,b]

pH	hydroperoxides[c]	hexanal[d]
	α-Tocopherol (1161 μM or 500 ppm)	
water[e]	89.5 ± 0.2[a]	98.9 ± 0.1[a]
3	81.5 ± 0.5[d]	71.1 ± 0.2[e]
4	88.8 ± 0.2[b]	98.5 ± 0.1[a]
5	84.7 ± 0.1[c]	81.4 ± 0.1[d]
6	88.3 ± 0.1[b]	85.9 ± 0.2[b]
7	82.0 ± 0.1[d]	83.6 ± 0.6[c]
	Trolox (1161 μM)	
water[e]	51.0 ± 0.2[d]	87.0 ± 1.7[a]
3	40.1 ± 1.0[e]	22.6 ± 0.6[e]
4	58.4 ± 0.4[c]	74.3 ± 0.2[b]
5	73.2 ± 0.5[b]	49.7 ± 0.9[c]
6	90.1 ± 0.2[a]	76.2 ± 0.1[b]
7	26.7 ± 0.5[f]	41.8 ± 1.3[d]

[a] SD, standard deviation, $n = 2$. [b] Values for each antioxidant followed by the same letter are not significantly different ($p < 0.05$). [c] Figure 3, day 4. [d] Figure 4, day 4. [e] During oxidation pH decreased from 3.7 to 2.6.

the oxidation period. Initially, α-tocopherol and Trolox inhibited hydroperoxide formation similarly, but later, α-tocopherol inhibited hydroperoxide formation better than Trolox in all emulsions with the exception of the emulsion at pH 6 after 4 days of oxidation.

On day 4, α-tocopherol inhibited hydroperoxide formation between 81.5 and 89.5% in corn oil emulsions (Table 1). Its inhibition was highest in the absence of buffers, followed by pH 4 and pH 6 in the buffered emulsions. The lowest inhibition of hydroperoxide formation by α-tocopherol was observed at pH 3 and 7.

Trolox inhibited hydroperoxide formation between 26.7 and 90.1% and its inhibition was optimal at pH 6 on day 4 (Table 1). The inhibition by Trolox increased with increasing pH from 3 to 6. The least inhibition of hydroperoxide formation by Trolox occurred at pH

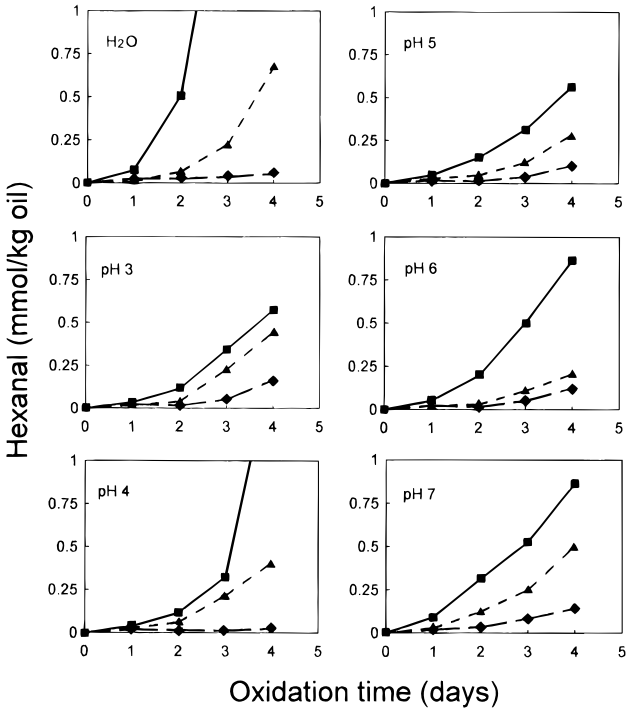

Figure 4. Effect of pH on the antioxidant activity of α-tocopherol and Trolox in corn oil triglycerides emulsified with Tween 20 in the absence and presence of 50 mM phosphate buffers at 60 °C by measuring hexanal formation: ■, control; ▲, 1161 μM α-tocopherol; ◆, 1161 μM Trolox.

7. In the absence of phosphate buffer, the inhibition of hydroperoxide formation by Trolox was intermediate between those of the emulsions at pH 3 and 4. The effect of pH on Trolox inhibition of hydroperoxide formation was much stronger than on α-tocopherol. α-Tocopherol inhibited hydroperoxide formation more effectively than Trolox in all emulsions except at pH 6 on day 4.

Formation of Hexanal. The results of hexanal formation were similar to those of hydroperoxide formation, but α-tocopherol was more effective than Trolox in all emulsions tested during the entire oxidation period (Figure 4). On day 4, α-tocopherol inhibited hexanal formation between 71.1 and 98.9% (Table 1). The inhibition by α-tocopherol was highest at pH 4 and in the absence of buffer and lowest at pH 3.

On day 4, Trolox inhibited hexanal formation between 22.6 and 87% in corn oil emulsions (Table 1). Trolox was more affected by pH than α-tocopherol. In the presence of phosphate buffer, Trolox inhibited hexanal formation better at pH 6 and pH 4 than at the other pH, and the inhibition was least at pH 3. In the absence of buffers, hexanal inhibition by Trolox was higher than those with buffers.

Partition Studies. The partitioning of Trolox (100 μg/g of water) between phases was determined to clarify the effect of pH on its antioxidant mechanism in corn oil emulsions. The amount of Trolox in the water phase decreased significantly with decreasing pH in corn oil−water mixtures, Tween 20 solutions, and corn oil emulsions (Table 2). Since the pK_a value of Trolox is 3.89 (Barclay and Vinqvist, 1994), this effect can be attributed to the dissociation of Trolox at higher pH values. The partition coefficient of Trolox between oil and water phases was lower than 1 at pH 5−7 because Trolox was mostly dissociated to form water-soluble anions. In contrast, at pH 3 and pH 4, its partition

Table 2. Partitioning of Trolox between Corn Oil Triglycerides and Water or Buffers and the Concentration of Trolox in Water Phase of Tween 20 Solution and Corn Oil Triglycerides Emulsified with 1% Tween 20 (Percent Mean ± SD)[a]

sample	% Trolox in water phase	calcd % Trolox[b]	partition coefficients[c]
10% corn oil−water			
water	68.3 ± 1.0[d]		3.83
pH 3	55.9 ± 0.5		6.50
pH 4	68.1 ± 1.9		3.86
pH 5	91.2 ± 2.1		0.80
pH 6	97.9 ± 1.0		0.18
pH 7	100 ± 0		0.00
1% Tween 20 solution			
water	43.3 ± 0.8[d]		
pH 3	31.0 ± 2.1[e]		
pH 4	51.2 ± 0.2		
pH 5	81.5 ± 0.4		
pH 6	95.1 ± 0.2		
pH 7	98.5 ± 2.2		
10% emulsified corn oil			
water	36.3 ± 1.5[d]	36.1	
pH 3	21.4 ± 0.8	24.9	
pH 4	41.5 ± 4.1	41.3	
pH 5	76.3 ± 0.1	75.6	
pH 6	93.2 ± 1.8[e]	93.2	
pH 7	98.4 ± 2.3	98.5	

[a] Buffers used were 0.05 M potassium phosphate buffers; SD, standard deviation, $n = 2$. [b] Calculated percent Trolox was determined on the basis of its partition ratios between lipid and water and between Tween 20 and water at a concentration of 100 μg/g. [c] Partition coefficient, $V_w/V_l(W_t/W_w - 1)$, where V_w is the volume of water or buffer; V_l is the volume of oil or Tween 20; W_t is the total amount of Trolox; and W_w is the amount of Trolox in water phase. The density (g/mL) used for calculating oil volume was 0.916 for corn oil. [d] Data from Huang et al. (1996). [e] $n = 3$.

coefficient was higher than 1 because the concentration of undissociated Trolox increased and more undissociated Trolox partitioned into the oil phase. The amount of Trolox in the water phase was less in 1% Tween 20 solutions than in 10% corn oil−water mixtures at the same pH. Therefore, Trolox was more soluble in Tween 20 micelles than in corn oil.

The amount of Trolox in the water phase of 10% corn oil emulsions with 1% Tween 20 was calculated on the basis of the partition coefficients between oil and water phases and between Tween 20 micelles and water phase. The experimental values were in excellent agreement with the calculated values. Without buffers, the pH ranged between 3.0 and 3.3 in all three systems (Huang et al., 1996). The partition coefficient of Trolox between oil and water phases in the oil−water mixture without buffers was similar to that with phosphate buffer at pH 4. In Tween 20 solutions and the corn oil emulsions, the amount of Trolox in the water phase without buffers ranged between those with buffers at pH 3 and pH 4. Although the partition of Trolox into the oil phase and at the oil−water interface increased from pH 7 to pH 3, the higher concentration of Trolox in the oil phase and at the oil−water interface at pH 3 and pH 4 than at pH 5 and pH 6 did not increase its inhibition of hydroperoxide formation.

Oxidative Stability of α-Tocopherol and Trolox at 60 °C. The effect of pH on the oxidative stability of α-tocopherol and Trolox was measured in different phases.

Bulk Corn Oil Triglycerides. Trolox was significantly more stable than α-tocopherol in bulk corn oil triglycerides oxidized at 60 °C (Figure 5). α-Tocopherol was completely depleted after 4 days at 300 μM and after 6 days at 1161 μM. Trolox was consumed at a similar

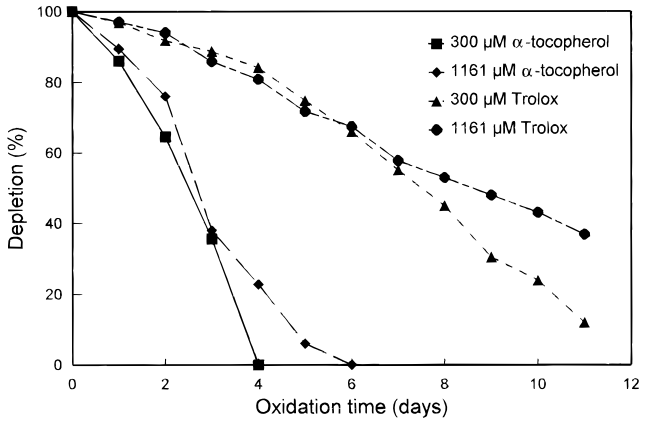

Figure 5. Oxidative stability of α-tocopherol and Trolox in bulk corn oil triglycerides at 60 °C.

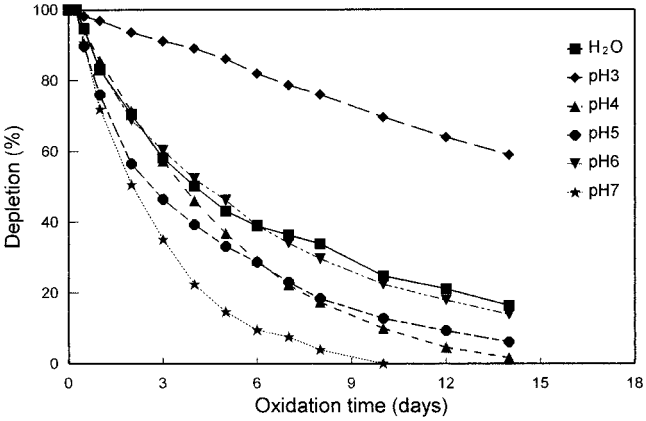

Figure 6. Effect of pH on oxidative stability of α-tocopherol (100 μg/mL solution) in 1% Tween 20 solutions in the absence or presence of 50 mM phosphate buffers at 60 °C.

rate at 300 and 1161 μM during the first 7 days. Fifty percent of Trolox was depleted after 7.6 days at both concentrations. After 11 days, about 10% of the original Trolox was left at 300 μM and 40% at 1161 μM.

Tween 20 Micelle Solution. To better understand the antioxidant mechanism of α-tocopherol and Trolox at the oil−water interfaces and in Tween 20 micelles, their oxidative stabilities were measured in Tween 20 micelles. The emulsion systems used contained Tween 20 micelles and the Tween 20 interfaces between oil and water phases (Huang et al., 1996). α-Tocopherol (100 μg/g) was most stable in Tween 20 micelles at pH 3 with 0.05 M phosphate buffer and least stable at pH 7 (Figure 6). The oxidative stability of α-tocopherol in Tween 20 micelles was higher at pH 6 than at pH 4 and pH 5. Without buffers, its stability was similar as in buffer at pH 6. The oxidative stability of α-tocopherol in Tween 20 micelles did not correspond to its antioxidant activity in inhibiting hydroperoxide and hexanal formation in corn oil emulsions during 4 days of oxidation (Table 1). At pH 3 the higher oxidative stability of α-tocopherol in the oil−water interfaces did not increase its antioxidant activity in emulsions, whereas at pH 7, its lower oxidative stability did not lower its antioxidant activity. Although the stability of α-tocopherol at pH 6 was intermediate between those at pH 3 and pH 7, it showed higher antioxidant activity at pH 6 than at pH 3 and pH 7.

The oxidative stability of Trolox in 1% Tween 20 solutions increased with decreasing pH between 0 and 1 day (Figure 7a). The stability of Trolox was similar at pH 4 and 5 and about 15% of Trolox was left after 4

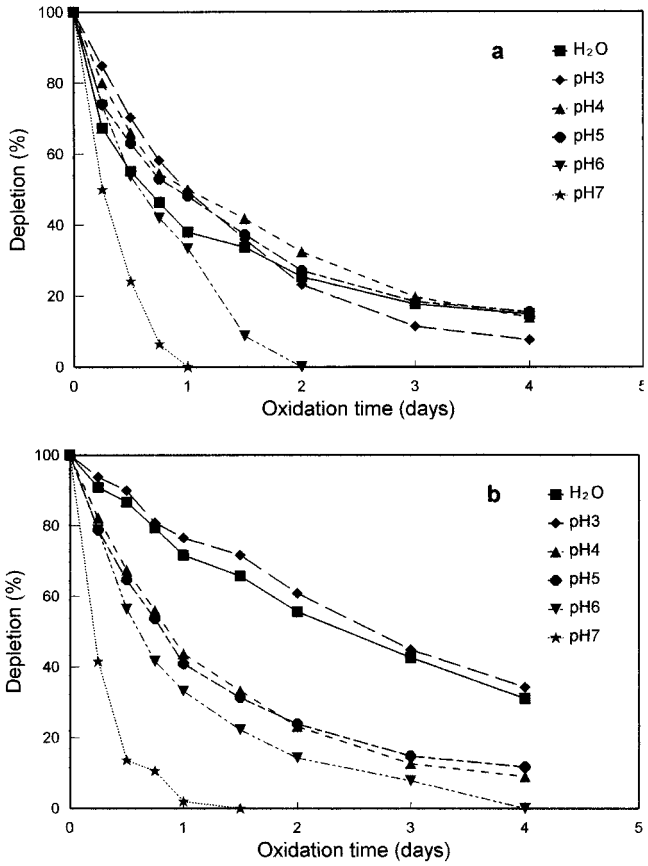

Figure 7. Effect of pH on oxidative stability of Trolox (100 μg/mL solution) at 60 °C in (a) 1% Tween 20 solutions and (b) water or 50 mM phosphate buffers.

days. After 1 day Trolox was depleted more rapidly at pH 3 than at pH 4 and 5. Trolox was completely depleted after 1 day at pH 7 and after 2 days at pH 6. In the absence of buffers, the depletion rate of Trolox was similar to that at pH 6, but after 1 day, this rate was much slower and about 15% of the original Trolox was left after 4 days. Trolox was much less stable in Tween 20 solutions than in bulk oils at 300 μM (Figures 5 and 7a). Although Trolox had low stability in Tween 20 micelles at pH 6, it had higher antioxidant activity at this pH (Table 1). Trolox was less stable than α-tocopherol in Tween 20 solutions (Figures 6 and 7a).

Aqueous Solutions. The oxidative stability of Trolox in buffers increased with decreasing pH (Figure 7b). The stability of Trolox in the control buffers was similar to that in Tween 20 solutions with the same buffers at pH 4, 5, and 7 (Figure 7a,b). In contrast, at pH 3 and 6, Trolox was more stable in phosphate buffers than in 1% Tween 20 solutions. Without buffers, the oxidative stability of Trolox decreased in the presence of Tween 20, apparently because Tween 20 emulsified and trapped air bubbles more readily than water or because of the presence of some impurities in Tween 20.

DISCUSSION

In the absence of antioxidants, lipid oxidation of corn oil emulsions with nonionic Tween 20 was accelerated by increasing pH in phosphate buffers as measured by formation of hydroperoxides and hexanal. The metal-catalyzed oxidation of methyl linoleate emulsions was accelerated by fructose as a reducing agent in phosphate buffer at pH 7 but not at pH 3 (Yamauchi et al., 1988). They suggested that the solubility of metal salts was

lower at pH 3 than at pH 7. Assuming trace metal ions were present in the emulsions used in the present study, if their solubilities were lower at lower pH, the concentrations of metal ions were reduced and the oxidative stability of the corn oil emulsions thus increased. Phosphoric acid may inactivate metal ions in catalyzing hydroperoxide decomposition, while dibasic phosphate may activate metal ions by decreasing the redox potentials of the metal ions. However, it is not clear why the oxidation of the emulsions was accelerated at pH 4 at the later stages of oxidation.

On the basis of the inhibition of hydroperoxide and hexanal formation, the antioxidant activity of α-tocopherol increased at pH 3, 5, and 6. In Tween 20 solution, the oxidative stability of α-tocopherol was highest at pH 3 and lowest at pH 7. Tween 20 is located at the oil−water interface and forms micelles above its critical micelle concentration in the water phase of the corn oil emulsions (Huang et al., 1996). The oxidative stability of α-tocopherol in Tween 20 micelles may be related to its oxidative stability at the oil−water interface, and to its hydrogen-donating ability. Thus, the hydrogen-donating ability of α-tocopherol at the oil−water interface can be reduced by protonization of its phenolic hydroxyl group at low pH. However, α-tocopherol at the interface or in Tween 20 micelles was depleted more slowly at pH 3 than at higher pH. The antioxidant activity of α-tocopherol may thus depend on both its hydrogen-donating activity and its depletion rate.

Initially, the antioxidant activity of α-tocopherol and Trolox was similar, but at later stages of oxidation, α-tocopherol was more effective than Trolox at the pH values tested except pH 6, on the basis of hydroperoxide formation. The higher antioxidant activity of Trolox than of α-tocopherol in bulk corn oil was explained by its being located at the oil-air interfaces and able to more efficiently protect the lipid against oxidation (Frankel et al., 1994; Huang et al., 1996). In the present study, α-tocopherol was less stable than Trolox in bulk corn oil. These results suggest that the lower stability of α-tocopherol decreases its antioxidant activity and the higher stability of Trolox prolongs its antioxidant activity in bulk oils.

In a homogeneous solution of styrene, Trolox was less reactive than α-tocopherol toward peroxyl radicals 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 (Burton et al., 1985). Although α-tocopherol is a stronger hydrogen donor, it reacts more easily with oxygen to form α-tocopheroxyl radicals (Cillard and Cillard, 1980; Gottstein and Grosch, 1990). Also, α-tocopheroxyl radicals (Loury et al., 1966) and α-tocoquinoperoxyl radicals (Gottstein and Grosch, 1990) may reinitiate chain reactions to promote lipid oxidation. However, in the present work, Trolox was shown to be much less stable than α-tocopherol in Tween 20 micelles and aqueous solutions than in bulk corn oil. When Trolox was located in the water phase and Tween 20 micelles, it was depleted more rapidly than in the oil phase. In emulsions, this difference between the depletion rates of Trolox in different phases becomes the driving force causing the diffusion of Trolox from the oil phase to the water phase and Tween 20 micelles until it is completely depleted. Initially, Trolox was partially located at the oil−water interface and partially in the oil phase, and protected the lipid better than α-tocopherol. At later stages of oxidation, Trolox would be expected to repartition out from these phases into the water phase and Tween 20 micelles. However, α-tocopherol was a better inhibitor of hexanal formation than Trolox in corn oil emulsions with or without buffers at different pH during oxidation because it reacts with alkoxyl radicals more effectively than Trolox in the oil phase and at the oil−water interfaces.

The hydrogen-donating ability of Trolox was increased in aqueous solutions by an inductive effect of the carboxylate ion of Trolox (Burton et al., 1985). In contrast, the hydrogen-donating ability of α-tocopherol at the oil−water interfaces may be reduced by hydrogen bonding between its phenolic hydroxyl group and water (Pryor et al., 1988; Roginsky, 1990). The higher hydrogen-donating ability of Trolox ions may also cause dehydrogenation of Trolox, resulting in more rapid oxidation and depletion of Trolox than α-tocopherol in Tween 20 solutions or water.

Although at pH 3 more Trolox was expected to be located at the interface and in the oil phase, its antioxidant activity was not higher than that at pH 4−6 on the basis of hydroperoxide formation. The lower antioxidant activity of Trolox at pH 3 than at pH 4−5 can be explained by its lower oxidative stability in Tween 20 solution. Trolox was a better antioxidant at pH 6, which was not consistent with the results of its partition and oxidative stability in Tween 20 solutions and aqueous solutions. These results suggest that the hydrogen-donating ability of Trolox was increased by an inductive effect of the carboxylate ion of Trolox after dissociation at higher pH (Burton et al., 1985). The higher hydrogen-donating ability of Trolox ions may also cause dehydrogenation and its more rapid depletion in Tween 20 solutions or aqueous solutions at higher pH. Therefore, the antioxidant activity of Trolox at a given pH is the result of a complex balance between its hydrogen-donating ability, location, and oxidative stability in different phases in emulsions at this pH.

To better understand the effect of pH on interfacial lipid oxidation, the oxidative stability of corn oil emulsions with or without added metal ions needs to be evaluated in nonchelating buffers. The effect of pH on the redox potentials of antioxidants needs to be determined to clarify the antioxidant mechanisms in emulsions. The effect of pH on the oxidative stabilities of antioxidants in corn oil emulsions also should to be measured to define the relationship between the oxidative stabilities and antioxidant activities of antioxidants in the emulsions. The partitioning of antioxidants between different phases during oxidation will complicate the study of antioxidant stability. Further studies are needed to develop methods to analyze antioxidants directly in different phases in emulsions to provide direct evidence for the stabilities of antioxidants in emulsions.

ABBREVIATIONS USED

Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Tween 20, poly(oxyethylene) sorbitan monolaurate; 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.

Burton, G. W.; Doba, T.; Dobe, 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.

Cillard, J.; Cillard, P.; Cormier, M. Effect of experimental factors on the prooxidant behavior of α-tocopherol. *J. Am. Oil Chem. Soc.* **1980**, *59*, 255−261.

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

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 properties of α- and γ-tocopherol in fats. *Fat Sci. Technol.* **1990**, *92*, 139−144.

Horan, K. L.; Lutzke, B. S.; Cazers, A. R.; McCall, J. M. Kinetic evaluation of lipophilic inhibitors of lipid peroxidation in DLPC liposomes. *Free Radical Biol. Med.* **1994**, *17*, 587−596.

Huang, S.-W.; Frankel, E. N.; German, J. B. Effects of individual tocopherols and tocopherol mixtures on the oxidative stability of corn oil triglyderides. *J. Agric. Food Chem.* **1995**, *43*, 2345−2350.

Huang, S.-W.; Hopia, A.; Schwarz, K.; Frankel, E. N.; German, J. B. Antioxidant activity of α-tocopherol and Trolox in different lipid substrates: bulk oils *vs* oil-in-water emulsions. *J. Agric. Food Chem.* **1996**, *44*, 444−452.

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

Mabrouk, A. F.; Dugan, L. R. A kinetic study of the autoxidation of methyl linoleate and linoleic acid emulsions in the presence of sodium chloride. *J. Am. Oil Chem. Soc.* **1960**, *37*, 484−490.

Saunders, D. H.; Coleman, J. E.; Hampson, J. W.; Wells, P. A.; Riemenschneider, R. W. Autoxidation of fatty materials in emulsions. I. Pro-oxidant effect of histidine and trace metals on the oxidation of linoleate esters. *J. Am. Oil Chem. Soc.* **1962**, *39*, 434−439.

Wagner, S. F. Analysis of variance. In *Introduction to Statistics*; Harper Perennial: New York, 1992; Chapter 11.

Yamauchi, R.; Tatsumi, Y.; Asano, M.; Kato, K.; Ueno, Y. Effect of metal salts and fructose on the autoxidation of methyl linoleate in emulsions. *Agric. Biol. Chem.* **1988**, *52*, 849−850.

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