Antioxidant Activity of α- and γ-Tocopherols in Bulk Oils and in Oil-in-Water Emulsions

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This study was aimed at evaluating the effectiveness of α- and γ-tocopherols in inhibiting the formation and decomposition of hydroperoxides in bulk corn oil stripped of natural tocopherols and in 10% oil-in-water emulsion systems. Whether α-tocopherol acted as an antioxidant or as a prooxidant depended on the test system, the concentration, the oxidation time, and the method used to determine lipid oxidation. On the basis of hydroperoxide formation, α-tocopherol showed maximum antioxidant activity at 100 ppm in bulk oil and at 250–500 ppm in emulsions, while γ-tocopherol showed maximum activity at 250–500 ppm in bulk oil but showed no significant difference in antioxidant activity between 250 and 1000 ppm in emulsions. α-Tocopherol had a slight, initial prooxidant effect at 250 ppm or higher concentrations in bulk oil and at 500 ppm or higher concentrations in emulsions, whereas γ-tocopherol showed no prooxidant activity in either system. However, γ-tocopherol showed less antioxidant activity than α-tocopherol at 100 ppm but higher antioxidant activity at higher concentrations in both systems. In contrast to hydroperoxide formation, the ability of both α- and γ-tocopherols in inhibiting hexanal formation improved with increased concentration and oxidation time.

Keywords: *Antioxidants; tocopherols; corn oil; emulsion; antioxidation; mechanism; interfacial oxidation; hydroperoxides; hexanal*

INTRODUCTION

The antioxidant activities of α- and γ-tocopherols have been investigated in different test systems, including vegetable oils (Lea and Ward, 1959; Jung and Min, 1990), animal fats (Olcott and van der Veen, 1968), polyunsaturated fatty acids (PUFAs) and their methyl esters (Lea and Ward, 1959; Gottstein and Grosch, 1990), emulsions of PUFAs (Pryor et al., 1988, 1993) and esters (Cillard and Cillard, 1980, 1986; Cillard et al., 1980a,b), and styrene in chlorobenzene solution initiated with an azo compound (Burton and Ingold, 1981, 1986). The relative antioxidant activity of different tocopherols depended on temperature, lipid composition, physical state (bulk phase, emulsion), and tocopherol concentrations (Lea and Ward, 1959). On the basis of hydroperoxide formation, α-tocopherol was reported to have antioxidant activity at low concentrations but prooxidant activity at high concentrations (Loury et al., 1966; Cillard and Cillard, 1980; Koskas et al., 1984). γ-Tocopherol, on the other hand, had antioxidant activity at higher concentrations than α-tocopherol (Cillard and Cillard, 1980; Koskas et al., 1984). However, the effectiveness of γ-tocopherol was not improved with increased concentration in purified soybean oil (Jung and Min, 1990). For tocopherol-free soybean oil, the reported optimum concentrations of α- and γ-tocopherols were 100 and 250 ppm, respectively (Jung and Min, 1990). In aqueous sodium dodecyl sulfate micelle solutions of linoleic acid with an azo initiator, α-tocopherol was more effective than γ-tocopherol (Pryor et al., 1993). Similarly, in a simple styrene solution in the presence of an azo initiator, α-tocopherol was a better scavenger of peroxyl radicals than γ-tocopherol (Burton and Ingold, 1981). However, the effects of tocopherol concentrations on oxidative stability in different lipid emulsion systems have not yet been established. Much of the confusion in the literature is probably due to the influence of the oil/water interface on oxidation events. This important variable has been largely overlooked in previous investigations.

Antioxidants have been difficult to evaluate in oils and in emulsions due to the different interfacial affinities between air/oil and oil/water interfaces involved (Porter, 1980). In addition, emulsifiers have been reported to affect lipid oxidation and antioxidant activity (Pryor et al., 1993; Cillard et al., 1980b). We previously showed that α-tocopherol was more effective in an oil-in-water emulsion system than in bulk oil, whereas the opposite trend was found for Trolox C, a hydrophilic carboxylic acid derivative of α-tocopherol (Frankel et al., 1994). The interfacial properties affecting the antioxidant or prooxidant activity of tocopherols in emulsion systems have not been well understood.

Previous work comparing the effectiveness of various antioxidants is difficult to interpret because of the use of different methodologies and end points and because of the variation in interfacial properties between oils and food emulsions (Frankel, 1993). Although α-tocopherol had a prooxidant effect based on the formation of hydroperoxides, it inhibited malondialdehyde formation determined by high-performance liquid chromatography (HPLC) (Husain et al., 1987). Also, α-tocopherol was reported to inhibit the volatile thermal decomposition products of methyl linoleate hydroperoxides (Frankel and Gardner, 1989). However, only limited work has been reported on the effects of tocopherols on both hydroperoxide formation and hydroperoxide decomposition. In the literature, many researchers used emulsions of PUFA or PUFA esters as model systems, but results from these model systems may not be simply related to food emulsion systems in which triglycerides are the major lipids.

The study of antioxidant activity in lipid systems is a complex subject in which much of the complexity is due to the interaction between discrete variables, and

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Table 1. Inhibition of Formation of Hydroperoxides and Hexanal by α- and γ-Tocopherols in Bulk Corn Oils and Corn Oil-in-Water Emulsions (Percent Mean Inhibition ± SD)[a,b]

	bulk oils			oil-in-water emulsions			
	hydroperoxides		hexanal[c]	hydroperoxides		hexanal	
sample	day 3	day 7	day 7	day 2	day 4	day 2	day 4
control	0.0 ± 2.9 a	0.0 ± 1.5 e	0.0 ± 4.5 c	0.0 ± 1.8 a	0.0 ± 5.8 d	0.0 ± 2.8 d	0.0 ± 1.1 e
+ α-tocopherol							
100 ppm	9.4 ± 4.6 a	87.8 ± 0.5 a	87.6 ± 0.3 b	5.7 ± 9.8 a	70.4 ± 2.6 b	9.4 ± 1.4 c	30.2 ± 0.3 d
250 ppm	−25.8 ± 6.4 b	84.8 ± 0.0 b	98.8 ± 0.0 a	−5.3 ± 4.2 a	80.7 ± 1.2 a	23.6 ± 0.2 b	63.4 ± 0.7 c
500 ppm	−39.1 ± 5.2 b	81.5 ± 0.4 bc	99.0 ± 0.0 a	−23.6 ± 1.1 b	75.4 ± 1.8 ab	25.4 ± 0.6 b	71.9 ± 3.0 b
750 ppm	−84.7 ± 4.8 c	79.1 ± 0.1 cd	99.5 ± 0.0 a	−101.0 ± 8.1 c	57.2 ± 0.8 c	35.8 ± 1.3 a	72.7 ± 0.4 b
1000 ppm	−97.3 ± 2.6 d	76.9 ± 0.3 d	99.3 ± 0.3 a	−99.6 ± 1.6 c	69.3 ± 3.7 b	37.3 ± 1.7 a	85.1 ± 0.6 a
control	0.0 ± 1.6 b	0.0 ± 1.5 d	0.0 ± 1.4 d	0.0 ± 1.8 c	0.0 ± 5.8 c	0.0 ± 2.8 c	0.0 ± 1.1 e
+ γ-tocopherol							
100 ppm	25.8 ± 0.9 a	72.5 ± 0.2 c	56.3 ± 0.1 c	−5.1 ± 9.8 c	66.0 ± 8.2 b	−1.7 ± 3.6 c	46.8 ± 1.4 d
250 ppm	27.0 ± 2.9 a	82.9 ± 0.6 a	84.9 ± 0.8 b	26.2 ± 3.0 ab	85.9 ± 0.0 a	23.1 ± 0.3 b	68.6 ± 0.7 c
500 ppm	24.0 ± 1.9 a	81.6 ± 0.5 ab	99.0 ± 0.3 a	45.1 ± 0.7 a	88.0 ± 3.6 a	20.3 ± 1.2 b	82.3 ± 0.1 b
750 ppm	22.5 ± 5.6 a	79.7 ± 0.3 b	98.8 ± 0.0 a	28.5 ± 9.0 ab	85.7 ± 0.4 a	24.0 ± 2.8 b	83.7 ± 0.6 ab
1000 ppm	9.7 ± 3.9 a	78.5 ± 0.6 b	98.9 ± 1.1 a	6.9 ± 2.8 bc	81.8 ± 1.1 a	31.1 ± 0.4 a	86.4 ± 0.2 a

[a] % inhibition = $[(C - S)/C] \times 100$; C = hydroperoxide or hexanal formed in control and S = hydroperoxide or hexanal formed in sample. Negative values represent prooxidant activity; SD, standard deviation. [b] Values within each column followed by the same letter are not significantly different ($p < 0.05$). [c] The initial inhibition of hexanal formation was not calculated because hexanal formation did not increase until after 5 days of oxidation.

yet much of the literature deals with these variables independently. This paper presents a systematic study of the interactive effects of three variables, including concentration, physical state, and oxidation stage, on the activities of α- and γ-tocopherols in stripped corn oil and in corn oil-in-water emulsions oxidized at 60 °C. The effectiveness of α- and γ-tocopherols was evaluated at different stages of oxidation by measuring both the formation of hydroperoxides (conjugated dienes) and the decomposition of hydroperoxides (hexanal) in corn oil.

MATERIALS AND METHODS

Materials. Corn oil, stripped of tocopherols, and γ-tocopherol were obtained commercially (Eastman Kodak Co., Rochester, NY). Corn oil was found to be free of tocopherols by HPLC, and its peroxide value was less than 5. The fatty acid composition determined by gas chromatography (GC) of the methyl esters was 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 purchased from Sigma Chemical Co. (St. Louis, MO).

Preparation of Oil and Emulsion Samples. Stripped corn oil containing different amounts of α- or γ-tocopherols was prepared by direct addition. Ten percent oil-in-water emulsions (30 mL) were made with 3.0 g of oil, made up with deionized water in a 50-mL Erlenmeyer flask and emulsified with 0.3 g of Tween 20. The same procedure of emulsification was used as described previously (Frankel et al., 1994). The particle sizes of emulsions were determined using a Microtrac ultrafine particle analyzer (Leeds & Northrup, North Wales, PA).

Methods. Oxidations at 60 °C, spectrophotometric measurements of hydroperoxides, and headspace GC determinations of hexanal were carried out by the same procedures described previously (Frankel et al., 1994). However, for the measurements of hexanal, conditions for the static headspace GC were changed by heating the emulsions at 60 °C for 15 min instead of 80 °C for 10 min. All oxidations and determinations were done in duplicate. Results of measurements taken after each day of oxidation were calculated by one-way analysis of variance (Steel and Torrie, 1980).

RESULTS

Effect of α-Tocopherol in Bulk Oils. The effect of α-tocopherol concentration on the oxidative stability of tocopherol-stripped corn oil was evaluated by determining formation of hydroperoxides and hexanal due to decomposition of linoleate hydroperoxides at 60 °C.

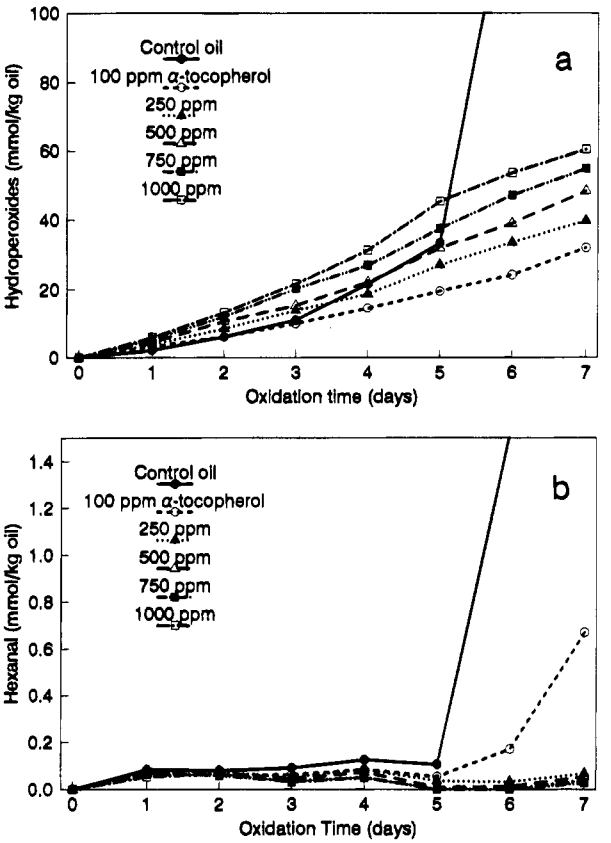

Figure 1. Effect of α-tocopherol on oxidative stability of tocopherol-stripped corn oil at 60 °C: (a) hydroperoxide formation; (b) hexanal formation.

Hexanal is one of many important volatile products that has proved to be a useful marker for the oxidative decomposition of $n-6$ PUFAs (Frankel, 1982).

Formation of Hydroperoxides. In the control oil, the initial rate of hydroperoxide formation increased steeply after an induction period of 5 days. In the presence of α-tocopherol, the rate of hydroperoxide formation increased with concentration of α-tocopherol during 7 days of oxidation (Figure 1a). The inhibition of hydroperoxide formation by α-tocopherol between the control and a sample containing α-tocopherol increased with oxidation time (Table 1). After 3 days, α-tocopherol inhibited

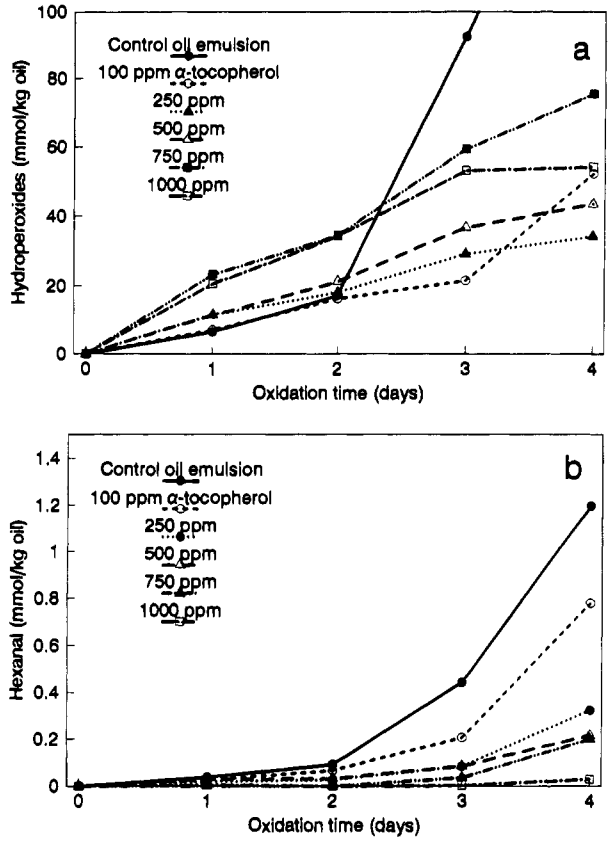

Figure 2. Effect of α-tocopherol on oxidative stability of 10% tocopherol-stripped corn oil emulsion at 60 °C: (a) hydroperoxide formation; (b) hexanal formation.

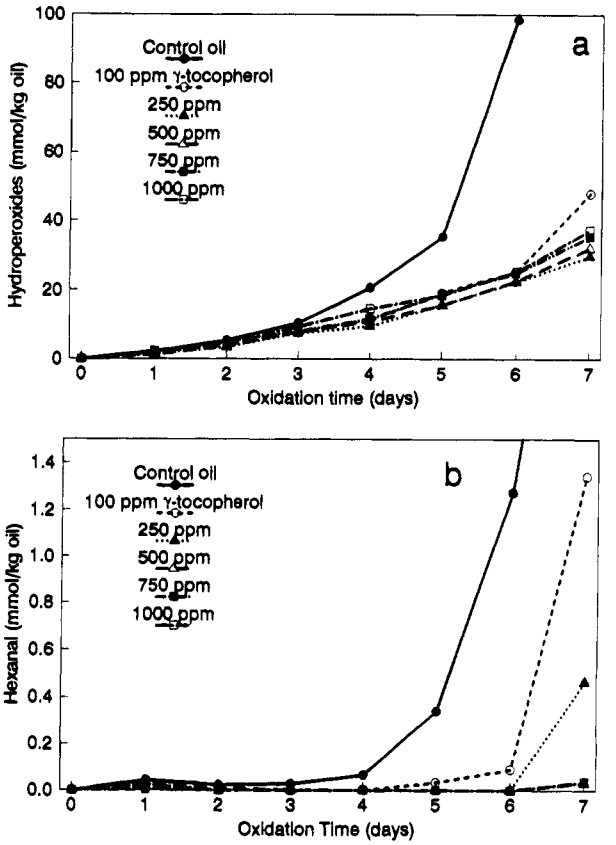

Figure 3. Effect of γ-tocopherol on oxidative stability of tocopherol-stripped corn oil at 60 °C: (a) hydroperoxide formation; (b) hexanal formation.

hydroperoxide formation at 100 ppm but promoted hydroperoxide formation at higher concentrations. After 5 days, hydroperoxide formation was inhibited by α-tocopherol at all concentrations tested because the rate of hydroperoxide formation increased significantly in the control (Figure 1a). After 7 days, α-tocopherol showed the highest inhibition of hydroperoxide formation at 100 ppm.

Decomposition of Hydroperoxides. Hexanal was detected after the appearance of hydroperoxides. Hexanal was formed initially in the control corn oil to only slight levels and increased steeply after 5 days of oxidation (Figure 1b). In contrast to hydroperoxide formation, α-tocopherol inhibited hexanal formation at all levels tested. With 100 ppm of α-tocopherol, hexanal increased after 6 days. At higher levels of α-tocopherol, no hexanal formation was observed during oxidation. This inhibition of hexanal formation increased at higher concentrations of α-tocopherol (Table 1) and oxidation time (Figure 1b).

Effect of α-Tocopherol in Oil-in-Water Emulsions. These oil-in-water emulsions were physically stable during 5 days of oxidation at 60 °C, and the particle sizes averaged between 0.8 and 1.1 μm.

Formation of Hydroperoxides. Oxidation was more rapid in corn oil-in-water emulsions than in bulk oils. In the control emulsion, the initial rate of hydroperoxide formation increased sharply after an induction period of 2 days of oxidation (Figure 2a). In the presence of 100 ppm of α-tocopherol, the rate of hydroperoxide formation increased after 3 days of oxidation, but at higher concentrations of α-tocopherol, the rate did not change significantly during 4 days of oxidation. The initial rate of hydroperoxide formation increased with the concentration of α-tocopherol, but the rate was smaller with 1000 ppm than with 750 ppm of α-tocopherol. On day 2 α-tocopherol significantly promoted the formation of hydroperoxide at 500 ppm or higher (Table 1). After 2 days, α-tocopherol significantly inhibited hydroperoxide formation. As in bulk oil, α-tocopherol showed an inhibitive effect at higher concentrations when the rate of hydroperoxide formation increased greatly in the control. α-Tocopherol showed the highest inhibition of hydroperoxide formation at 100 ppm after 3 days and at 250 ppm after 4 days (Figure 2a). However, on day 4 the inhibition was not significantly different between 250 and 500 ppm (Table 1).

Decomposition of Hydroperoxides. Hexanal formation was detected earlier in emulsions than in bulk oils. In the control emulsion, the rate of hexanal formation increased sharply after 2 and 3 days (Figure 2b). In the presence of α-tocopherol, hexanal formation was inhibited at all levels used in emulsions (Table 1). After 3 days, the rate of hexanal formation decreased with the concentration of α-tocopherol (Figure 2b). Therefore, the inhibition of hexanal formation was improved by increasing concentrations of α-tocopherol and oxidation time.

Effect of γ-Tocopherol in Bulk Oils. *Formation of Hydroperoxides.* At all levels tested γ-tocopherol inhibited hydroperoxide formation, and this inhibition increased with oxidation time (Figure 3a; Table 1). In the presence of 100 ppm of γ-tocopherol, the rate of hydroperoxide formation increased sharply after 6 days. Initially, the inhibition of γ-tocopherol was not significantly different at all levels tested, but this inhibition was the least at 1000 ppm (Table 1). On day 7 the inhibition of γ-tocopherol was significantly higher at 250–500 ppm and lower at 100 ppm. At 100 ppm, the

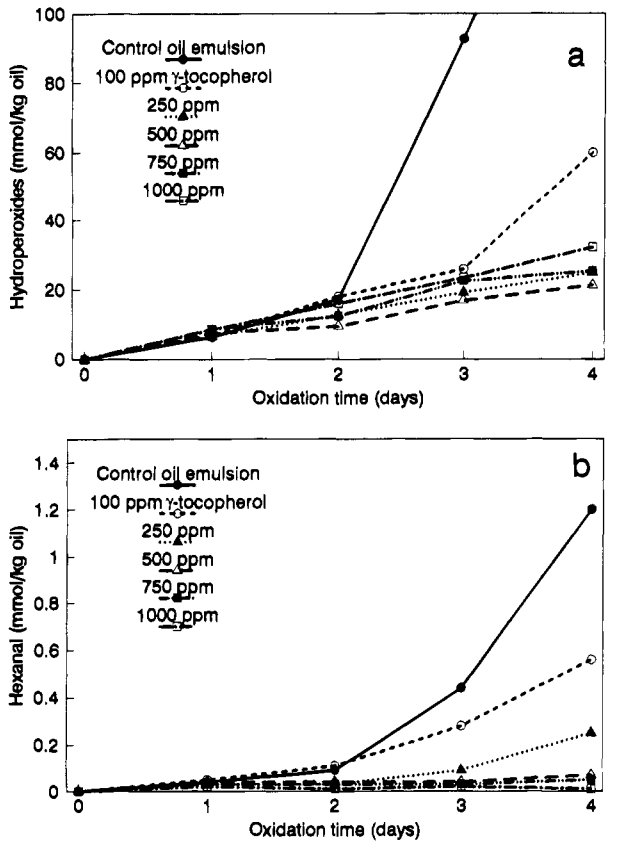

Figure 4. Effect of γ-tocopherol on oxidative stability of 10% tocopherol-stripped corn oil emulsion at 60 °C: (a) hydroperoxide formation; (b) hexanal formation. The same control was used as in Figure 2 because the oxidation was run together in one experiment.

inhibition of hydroperoxide formation by γ-tocopherol was less than that of α-tocopherol.

Decomposition of Hydroperoxides. Inhibition of hexanal formation increased significantly at higher concentrations of γ-tocopherol (Figure 3b; Table 1). In the presence of 100–250 ppm of γ-tocopherol, the rate of hexanal formation increased after 6 days of oxidation, and the change in the rate was greater at 100 ppm of γ-tocopherol than at 250 ppm. At higher levels, hexanal formation did not increase during 7 days of oxidation. After 7 days, the inhibition of hexanal by γ-tocopherol was significantly higher at 500–1000 ppm than at 100–250 ppm (Table 1). At 100–250 ppm, the activity of γ-tocopherol was less than that of α-tocopherol in inhibiting hexanal formation (Figures 3a and 1a; Table 1).

Effect of γ-Tocopherol in Oil-in-Water Emulsions. *Formation of Hydroperoxides.* Stripped corn oil containing added γ-tocopherol, as with α-tocopherol, also oxidized more rapidly in emulsions than in bulk oils (Figure 4a). In the presence of 100 ppm of γ-tocopherol, the rate of hydroperoxide formation increased after 3 days of oxidation, while at higher levels the rate of hydroperoxide formation was constant during 4 days of oxidation. The inhibition of hydroperoxide by γ-tocopherol was evident after 1 day of oxidation. After 2 days, the highest inhibition was observed at 500 ppm of γ-tocopherol in emulsions (Table 1). However, this inhibition was not significantly different between 250 and 750 ppm. After 4 days, a significantly higher inhibition was observed between 250 and 1000 ppm (Table 1). The inhibition of γ-tocopherol was higher than that of α-tocopherol at 250 ppm or higher (Figures 4a and 2a; Table 1).

Decomposition of Hydroperoxides. The inhibition of hexanal formation increased with increasing concentration of γ-tocopherol and with oxidation time (Figure 4b; Table 1). At 100 ppm, this inhibition was evident after 2 days of oxidation. At higher concentrations, γ-tocopherol inhibited hexanal formation after 1 day of oxidation. After 2 days, the highest inhibition of hexanal was observed at 1000 ppm of γ-tocopherol (Table 1). On day 4, γ-tocopherol showed significantly higher inhibition at 750–1000 ppm. The inhibition of hexanal formation by γ-tocopherol was also higher than that of α-tocopherol at 100–750 ppm after 4 days of oxidation (Figures 4b and 2b; Table 1).

DISCUSSION

This study showed that α-tocopherol had either antioxidant or prooxidant activity depending on concentration, oxidation time, method to determine oxidation, and physical state. On the basis of hydroperoxide formation, α-tocopherol had an antioxidant effect at 100 ppm in tocopherol-stripped corn oil and a prooxidant effect at higher concentrations at the early stage of oxidation. However, on the basis of hexanal formation, α-tocopherol showed only antioxiant activity and was more active at higher concentrations. Therefore, using hexanal as a marker of volatile products α-tocopherol was an effective inhibitor of the decomposition of hydroperoxides. These results are consistent with the report that malondialdehyde formation decreased at higher concentrations of α-tocopherol (Husain et al., 1987). α-Tocopherol was also found to act as a potent hydrogen donor in inhibiting the formation of total volatile thermal decomposition products of methyl linoleate hydroperoxides (Frankel and Gardner, 1989).

In contrast to α-tocopherol, γ-tocopherol showed an antioxidant effect toward tocopherol-stripped corn oil, on the basis of both hydroperoxide and hexanal formations. On the basis of hydroperoxide formation, the concentration of γ-tocopherol for maximum antioxidant activity was around 250–500 ppm. This result is in agreement with those of Jung and Min (1990), who reported similar concentrations of α- and γ-tocopherols for optimum antioxidant activity in purified soybean oil, based on peroxide value and headspace oxygen consumption. Moreover, Labuza concluded (1971) that for nonhindered phenolic antioxidants, the protective factor or induction time increased up to a point and then decreased with increasing antioxidant concentration. However, on the basis of inhibition of hexanal formation, as with α-tocopherol, γ-tocopherol was more effective at higher concentrations. At 100 ppm, γ-tocopherol was less effective in inhibiting formation of both hydroperoxides and hexanal than α-tocopherol.

The mechanism of lipid autoxidation in the presence of antioxidants was described by the steps found in Scheme 1 (Bolland and ten Have, 1947; Uri, 1961; Mahoney, 1969; Labuza, 1971; Frankel, 1982).

Several mechanisms may be considered to explain the prooxidant activity of phenolic antioxidants. Bolland and ten Have (1947) and Bailey (1962) proposed that if the resonance energy of the phenoxyl radical (A•) was not much greater than that of the parent phenol (AH), a hydrogen chain transfer reaction with lipid substrate (LH) (reaction 14) could occur to reinitiate the chains. Mahoney (1969) also suggested that for nonhindered phenols hydrogen transfer from a phenol to a peroxyl radical (LOO•) could be reversible (reaction −6). Therefore, the antioxidant activity of phenolic antioxidant can

Scheme 1

initiation

$$\text{LOOH} \xrightarrow{\text{(metal ions)}} \text{LOO}^{\bullet} \quad \text{(reaction 1)}$$

propagation

$$\text{LOO}^{\bullet} + \text{LH} \rightarrow \text{LOOH} + \text{L}^{\bullet} \quad \text{(reaction 2)}$$

$$\text{L}^{\bullet} + \text{O}_2 \rightarrow \text{LOO}^{\bullet} \quad \text{(reaction 3)}$$

termination

$$\text{LOO}^{\bullet} + \text{LOO}^{\bullet} \rightarrow \text{product} \quad \text{(reaction 4)}$$

$$\text{A}^{\bullet} + \text{A}^{\bullet} \rightarrow \text{A}_2 \quad \text{(reaction 5)}$$

inhibition

$$\text{LOO}^{\bullet} + \text{AH} \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} \text{LOOH} + \text{A}^{\bullet} \quad \text{(reaction 6)} \quad \text{(reaction } -6\text{)}$$

$$\text{LOO}^{\bullet} + \text{A}^{\bullet} \rightarrow \text{LOOA} \quad \text{(reaction 7)}$$

$$\text{LO}^{\bullet} + \text{AH} \rightarrow \text{LOH} + \text{A}^{\bullet} \quad \text{(reaction 8)}$$

$$\text{LO}^{\bullet} + \text{A}^{\bullet} \rightarrow \text{LOA} \quad \text{(reaction 9)}$$

decomposition

$$\text{LOOH} \xrightarrow{\text{(metal ions)}} \text{LO}^{\bullet} \quad \text{(reaction 10)}$$

$$\text{LO}^{\bullet} + \text{LH} \rightarrow \text{LOH} + \text{L}^{\bullet} \quad \text{(reaction 11)}$$

$$\text{LO}^{\bullet} \rightarrow \text{aldehydes or hydrocarbons and shorter fatty esters} \quad \text{(reaction 12a)}$$

$$\text{LO}^{\bullet} \rightarrow \text{secondary products (epoxy, keto, and hydroxy derivatives)} \quad \text{(reaction 12b)}$$

oxidation of antioxidants

$$\text{AH} + \text{O}_2 \rightarrow \text{HOO}^{\bullet} + \text{A}^{\bullet} \quad \text{(reaction 13)}$$

$$\text{A}^{\bullet} + \text{LH} \xrightarrow{k_{14}} \text{AH} + \text{L}^{\bullet} \quad \text{(reaction 14)}$$

$$\text{A}^{\bullet} \rightarrow \text{Q}^{\bullet} \quad \text{(reaction 15)}$$

$$\text{Q}^{\bullet} + \text{O}_2 \rightarrow \text{QOO}^{\bullet} \quad \text{(reaction 16)}$$

$$\text{QOO}^{\bullet} + \text{LH} \rightarrow \text{QOOH} + \text{L}^{\bullet} \quad \text{(reaction 17)}$$

$$\text{QOOH} + \text{LH} \rightarrow \text{QOH} + \text{L}^{\bullet} + {}^{\bullet}\text{OH} \quad \text{(reaction 18)}$$

be reversed to a prooxidant effect when its concentration increases (Labuza, 1971; Loury et al., 1966; Terao and Matsushita, 1986). Mukai et al. (1993a,b) measured reaction rates k_6 and k_{-6} (reactions 6 and −6) and k_{14} (reaction 14) for the reactions of α-tocopherol with methyl linoleate peroxyl radical, α-tocopheroxyl radical with methyl linoleate hydroperoxide, and α-tocopheroxyl radical with methyl linoleate, respectively. The k_{-6} value (5.0×10^{-1} M^{-1} s^{-1}) was found to be about 7 orders of magnitude lower than the k_6 value (3.2×10^6 M^{-1} s^{-1}). The value for k_{14} was difficult to determine because of the instability of the α-tocopheroxyl radical and was estimated to be about 5.0×10^{-2} M^{-1} s^{-1}. Mukai et al. also suggested that reactions −6 and 14 may be related to the prooxidant effect of α-tocopherol at higher concentrations in oils.

α-Tocopherol is more readily oxidized by air than γ-tocopherol to form α-tocopheroxyl radical, which acts as chain initiator (reaction 13). Cillard and Cillard (1980) and Gottstein and Grosch (1990) suggested that this oxidizability of α-tocopherol is related to its prooxidant activity. Therefore, the prooxidant effect of α-tocopherol increases with increasing concentration due to the formation of α-tocopheroxyl radical. However, according to Bolland and ten Have (1947), when the difference between the resonance energies of the antioxidant radical and its parent phenol becomes sufficiently great, resulting in ease of dehydrogenation, the oxidation of antioxidant by oxygen (reaction 13) may occur. In contrast to this oxidation, the reaction of an antioxidant radical with a lipid substrate (reaction 14) may occur when the difference of the resonance energies is sufficiently small. α-Tocopherol is well-known not to be stable when exposed to air, so the difference between the resonance energies of α-tocopheroxyl radical and α-tocopherol is sufficiently great. Thus, for α-tocopherol, reaction 13 may be more important than reaction 14 during lipid oxidation. Nevertheless, reaction 14 may occur at high temperatures and antioxidant concentrations (Ragnarsson and Labuza, 1977). In addition, Uri (1961) suggested that alkyl substitution in a phenolic compound may have a dual effect. On the one hand, antioxidant efficiency is increased by weakening an O−H bond; on the other hand, this effect is counteracted by the splitting of some C−H bonds, which would then readily act as oxygen carriers. Also, Gottstein and Grosch (1990) hypothesized that α-tocoquinoperoxyl radical (QOO•) formed by oxidation of α-tocopherol could abstract hydrogen atoms from lipid substrates to promote lipid oxidation (reactions 15−18).

Cillard and Cillard (1986) showed that the prooxidant effect of α-tocopherol in linoleic acid dispersed with Tween 20 can be decreased by metal chelators such as EDTA and phosphoric, malonic, and citric acids. Obviously, metal chelation will affect the mechanism in Scheme 1 since trace metals are involved in the initiation reaction 1 and the decomposition reaction 10. The effect of metal chelators on oxidative stability of our corn oil-in-water emulsion systems will be the subject of another publication.

γ-Tocopherol was considered a weaker hydrogen donor (Porter et al., 1980) and was found to be more resistant to oxygen than α-tocopherol (Cillard and Cillard, 1980; Gottstein and Grosch, 1990). Thus, because the difference between the resonance energies of γ-tocopheroxyl radical and γ-tocopherol is smaller than that of α-tocopherol, reaction 13 may be less important for γ-tocopherol during oxidation. Nevertheless, this energy difference is not sufficiently small to promote prooxidant effect by reaction 14. The weaker hydrogen-donating ability and higher oxidative stability of γ-tocopherol can explain its higher antioxidant effectiveness compared to that of α-tocopherol (Cillard and Cillard, 1980; Gottstein and Grosch, 1990).

In addition, the oxidation products of γ-tocopherol were identified as diphenyl ether dimer and biphenyl dimers (reaction 5) (Ishikawa, 1982). Gottstein and Grosch (1990) found the dimers of 2,2,7,8-tetramethyl-6-hydroxychroman, an analog of γ-tocopherol, to be still effective as antioxidants. On the other hand, the oxidation products of α-tocopherol were identified mainly

as α-tocopherylquinone and small amounts of a dimer of α-tocopherol (Cillard and Cillard, 1980). In contrast, α-tocopherylquinone did not show antioxidant activity (Gottstein and Grosch, 1990). These differences in mechanisms can thus explain the greater antioxidant activity of γ-tocopherol compared to that of α-tocopherol.

Hydroperoxides could be cleaved either to form peroxyl radicals (reaction 1) or to produce the highly reactive, alkoxyl radicals (reaction 10). The alkoxyl radicals can undergo a number of reactions including (a) β-cleavage to produce aldehydes, short-chain hydrocarbons, and shorter fatty acids (reaction 12a) (Frankel, 1982); (b) hydrogen abstraction from the lipid substrate to form an alcohol (reaction 11) (Labuza, 1971; Frankel, 1982); (c) cyclization to form epoxy derivatives (reaction 12b) (Hamberg and Gotthammar, 1973; Gardner et al., 1974; Frankel, 1985, 1987; Dix and Marnett, 1985); and (d) formation of ketone and hydroxy derivatives (reaction 12b) (Frankel, 1987). Tocopherols may compete with lipid substrates by donating their hydrogen atoms to alkoxyl radicals to form more stable alcohols and tocopheroxyl radicals (reaction 8). Also, alkoxyl radicals can be trapped by tocopheroxyl radicals (reaction 9). This mechanism may explain the inhibition by tocopherols of secondary cleavage products such as hexanal formed by reaction 12a. Therefore, *in the evaluation of antioxidant activity of tocopherols and of other antioxidants, it is just as important to determine their effect in inhibiting the decomposition of hydroperoxides as in their formation.*

The more rapid lipid oxidation in oil-in-water emulsions than in bulk oil can be explained by the high surface/volume ratio suggested by Porter (1980). In emulsions, α-tocopherol showed the same tendency to inhibit hydroperoxide formation at lower concentrations as in oils and to increase at higher concentrations at the early stage of oxidation. Although the amounts of hydroperoxides formed initially were higher in emulsions with 250 ppm of α-tocopherol than in the control emulsion, α-tocopherol exhibited a significant prooxidant effect at 500 ppm. After 3 days, the concentration of α-tocopherol to achieve maximum antioxidant activity increased from 100 to 250–500 ppm.

Pryor et al. (1988) reported that the effectiveness of tocopherols was greater in a lipophilic environment than in sodium dodecyl sulfate micelles. They suggested that water was likely to penetrate into the neighborhood of inhibitors and reduce their effectiveness by hydrogen bonding in micelles. In emulsions, because tocopherols contain both hydrophilic (O–H group) and hydrophobic groups, they would be expected to accumulate in the interface. In phospholipid bilayers ^{13}C NMR experiments with shift reagents support the assumption that α-tocopherol is oriented with the chromanol group toward the surface and with the phytyl group within the hydrocarbon layer (Perly et al., 1985; Burton and Ingold,1986).

The change in effective antioxidant activity of tocopherols may be related to their interfacial property and hydrogen bonding with water near or in the interface. To clarify the mechanism of interfacial lipid oxidation, the influence of emulsifiers on initiation of oxidation and the distribution of both hydroperoxides and tocopherols need to be determined. The effects of tocopherols on hydroperoxide decomposition need to be further researched to better understand how they inhibit the formation of secondary oxidation products.

ABBREVIATIONS USED

PUFA, polyunsaturated fatty acid; Tween 20, polyoxyethlene sorbitan monolaurate; GC, gas chromatography; LH, lipid substrate; L•, alkyl radical; LOOH, lipid hydroperoxide; LOO•, peroxyl radical; AH, antioxidant or phenol; A•, antioxidant radical; Q•, quinone radical; QOO•, quinoperoxyl radical.

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