Ability of Iron To Promote Surfactant Peroxide Decomposition and Oxidize α-Tocopherol

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Peroxides are an important factor in oxidative reactions in foods because their decomposition can result in formation of highly reactive free radicals. Emulsifiers such as the Brijs, Tweens, and lecithin were found to contain 4−35 μmol of peroxides/g of surfactant. Peroxide concentrations in Tween 20 micelles increased in the presence of low iron concentrations but decreased when iron concentrations were high, suggesting that iron was capable of promoting both peroxide formation and decomposition. Oxidation of α-tocopherol was observed in micelles high in peroxides (Tween 20) but not in micelles where peroxide concentrations were low (Brij). Transition metals accelerated the oxidation of α-tocopherol in Tween 20 micelles, whereas EDTA stabilized α-tocopherol in the presence of added Fe^{2+}. These results suggest that surfactant peroxides could decrease the oxidative stability of food emulsions by acting as a source of free radicals, especially in the presence of transition metals.

Keywords: *Lipid oxidation; peroxides; surfactants; emulsifiers; α-tocopherol; iron*

INTRODUCTION

Most food grade lipids contain preexisting peroxides that decompose under conditions such as heat and UV light or in the presence of transition metals. Decomposition of peroxides results in the formation of free radicals which in turn oxidize unsaturated fatty acids, leading to a deterioration of food quality (Nawar, 1996). Lipids in processed foods commonly exist as emulsions that are thermodynamically unstable and tend to separate into oil and water phases unless stabilized by emulsifiers (Dickinson and Stansby, 1992). Tween 20, a common food emulsifier, and other polyether surfactants often contain peroxides that can accumulate during storage (Jaeger et al., 1994; Lever, 1977). Peroxides originating from polyether surfactants are heterogeneous and unstable, one of which can be decomposed with catalase, suggesting it is H_2O_2 (Lever, 1977). In addition, BHT inhibits the formation of peroxides in Tween 20 during storage, suggesting that the reaction which forms peroxides involves free radicals (Jaeger et al., 1994).

Surfactants containing peroxides could serve as a source of free radicals, able to accelerate lipid oxidation in emulsions due to their close proximity to lipids at the emulsion droplet interface. The consequences of using a surfactant high in peroxides could be increased free radical formation, leading to the oxidation of unsaturated fatty acids, and/or a depletion of antioxidants especially in the presence of transition metals that promote peroxide breakdown (e.g., iron and copper). If peroxides originating from surfactants can participate in oxidative reactions, use of surfactants low in peroxides could help increase the oxidative stability of food emulsions.

The objective of this research was to determine whether surfactants represent a source of peroxides that could be involved in oxidative reactions in food emulsions. This was accomplished using a model system consisting of aqueous solutions of surfactant micelles containing iron or copper. Factors influencing the decomposition of peroxides originating from surfactant micelles were evaluated along with the impact of surfactant peroxide decomposition on the oxidation of α-tocopherol.

MATERIALS AND METHODS

Materials. Cumene hydroperoxide, polyoxyethylene sorbitan monolaurate (Tween 20), polyoxyethylene sorbitan palmitate (Tween 40), polyoxyethylene sorbitan monooleate (Tween 80), dodecyltrimethylammonium bromide (DTAB), polyoxyethylene 10 lauryl ether (Brij), polyoxyethylene 23 lauryl ether (Brij 35), imidazole, sodium acetate, cuprous chloride, ferrous sulfate, ferric chloride, lecithin (from soybean phosphotidylcholine type IV-S), and (±)-α-tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium dodecyl sulfate (SDS) was obtained from the Aldrich Chemical Co. (Milwaukee, WI). Potassium iodide was purchased from Fisher Scientific (Fair Lawn, NJ). Disodium ethylenediaminetetraacetic acid (EDTA) was purchased from Curtin Matheson (Cincinnati, OH). All other reagents were of analytical grade or purer.

Methods. *Micelle Preparation.* Micelles were prepared by mixing the various surfactants (0.1 M; 1224 was used for the average molecular weight of Tween 20) with an acetate−imidazole buffer solution (5 mM each) and stirring on a magnetic plate for 10 min. In some cases α-tocopherol (approximately 30 ppm, final concentration) was added directly to the surfactant before mixing with buffer. Ferrous sulfate (Fe^{2+}), ferric chloride (Fe^{3+}), and cuprous chloride (Cu^{+}) were added after formation of micelles. EDTA (0.25−2.0 mM) was added prior to metal addition. Micelles were incubated at 55 °C to increase reaction rates.

Peroxide Measurement. Peroxide concentrations were measured using the method of Lovass (1992) with slight modifications. A 0.1 mL aliquot of micelles was mixed into 2.7 mL of a 2:1 mixture of methanol/butanol containing 3% of an aqueous saturated KI solution by vortexing for 10 s. After 15 min, the absorbance was determined at 360 nm. Surfactants that contained low concentrations of peroxides (SDS and DTAB) and lecithin were dissolved directly into the methanol/butanol/

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Table 1. Peroxide Concentration of Various Surfactants (μM/g of Surfactant)

	μmol of peroxide/g of surfactant		μmol of peroxide/g of surfactant
Brij 10 lauryl ether	4.0 ± 0.2	SDS	0.6 ± 0.1
Brij 35	13.7 ± 0.9	DTAB	0.4 ± 0.2
Tween 20 (I)	34.9 ± 1.4	lecithin	13.0 ± 0.6
Tween 20 (II)	16.8 ± 0.6		
Tween 40	11.6 ± 0.5		
Tween 80	26.0 ± 0.2		

KI solution. Concentrations of peroxides were determined from standard curves prepared with cumene hydroperoxide.

Measurement of α-Tocopherol. α-Tocopherol was extracted from Tween 20 micelles by first diluting the micelle solution by half with imidazole−acetate buffer and then adding 0.4 mL of the diluted sample to 2.6 mL of hexane/isopropanal (3:2 v/v). The mixture was vortexed three times for 10 s and centrifuged at 2000*g* for 1 min before the top solvent layer was collected. Two subsequent wash steps were repeated by adding 1.0 mL of hexane to the aqueous phase, centrifuging at 2000*g* for 1 min, and collecting the solvent layer. Collected solvent was pooled and evaporated to dryness with nitrogen, and 1.0 mL of hexane was added to reconstitute the lipids. α-Tocopherol concentrations were determined by HPLC using a Waters 510 pump (Milford, MA) and a Waters 470 scanning fluorescence detector. An Alltech (Avondale, PA) hypersil C18 5 μm column (250 mm, 4.6 mm i.d.) was used with an acetontrile/methanol (3:1 v/v) mobile phase at 1.5 mL/min. Wavelengths for the fluorescence detector were 298 nm for excitation and 340 nm for emission (Hatam and Kayden, 1979). Concentrations were determined from peak areas using a standard curve made from authentic α-tocopherol.

Statistical Analysis. All data represent the mean ± standard deviation from three samples. Statistical analysis was performed using the Student's *t*-test (Snedecor and Cochran, 1989).

RESULTS

Peroxide Value of Various Emulsifiers. Different types of surfactants vary in peroxide concentration (Table 1). Nonionic, polyether surfactants such as those in the Tween and Brij families were found to be higher in peroxides than the ionic surfactants SDS and DTAB. Tween 20 (I), approximately 18 months old, had a higher peroxide content than Tween 20 (II), which was newly purchased. Lecithin originating from soybeans had intermediate levels of peroxides. Tween 20 (I) was used for this model due to its widespread usage in the food industry and its high peroxide concentrations.

Changes in Tween 20 Peroxides. A range of Fe^{2+} (0.01−0.25 mM) was studied for its effect on peroxide concentration in a 0.1 M Tween 20 micellar dispersion at pH 3.0 (Figure 1). After 4 h, 0.25 mM Fe^{2+} decreased peroxide concentration compared to a control (no iron). Decreasing Fe^{2+} to 0.10 mM resulted in no change in peroxide concentration, while further reduction to 0.05 and 0.01 mM Fe^{2+} resulted in a net increase in Tween 20 peroxides compared to the control after the first 4 h. Between 4 and 24 h, samples containing ≥0.05 mM Fe^{2+} had little change in peroxide concentrations. However, peroxide concentrations continued to increase in samples containing 0.01 mM Fe^{2+} and in the controls (which would contain low concentrations of contaminating transition metals), with peroxide concentrations at 24 h being over 7- and 2-fold higher, respectively, than zero time concentration.

Incubation of Tween 20 micelles (0.1 M) in the absence of added metals resulted in a significant ($p \leq 0.05$) increase in peroxide concentrations, being 1.3- and

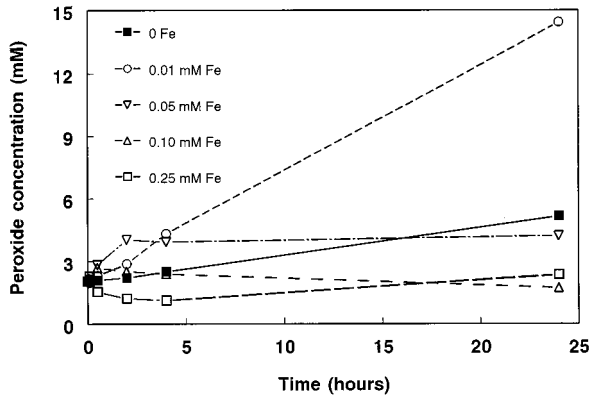

Figure 1. Effect of Fe^{2+} (0.01−0.25 mM) on peroxide concentration in 0.1 M Tween 20 micelles at pH 3.0 for 24 h at 55 °C. Standard deviation bars lie within the data points.

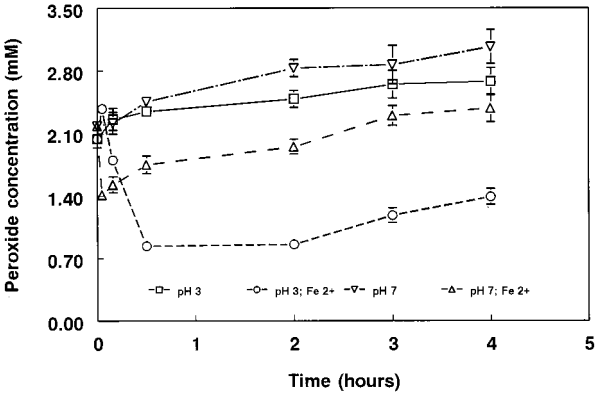

Figure 2. Effect of Fe^{2+} (0.25 mM) on peroxide concentration in 0.1 M Tween 20 micelles at pH 3.0 and 7.0 for 4 h at 55 °C. Some standard deviation bars lie within the data points.

1.4-fold higher than zero time concentrations after 4 h at pH 3.0 and 7.0, respectiveliy (Figure 2). Increases in peroxide concentrations tended to be greater at pH 7.0 than pH 3.0 ($p \leq 0.05$ at 2 and 4 h) in the absence of Fe^{2+}. At pH 3.0, Fe^{2+} (0.25 mM) decreased Tween 20 peroxides by 59% after 30 min of incubation, and after 2 h the peroxides began to increase (Figure 2). In the Tween 20 micelles at pH 7.0, a significant ($p < 0.05$) decrease of 35% was observed immediately after Fe^{2+} (0.25 mM) was added, followed by increasing peroxide concentration from 10 min to 4 h. The decrease in peroxides was greater at pH 3.0 than pH 7.0, possibly due to the increased solubility of Fe^{2+} at pH 3.0 and thus greater activity (Zumdahl, 1983; Mei et al., 1998).

EDTA (0.25−2.0 mM) was studied for its ability to alter Fe^{2+}−Tween 20 peroxide interactions (Figure 3). Tween 20 micelles (pH 3.0) containing 0.25−0.5 mM EDTA and Fe^{2+} (0.25 mM) had greater peroxide concentrations than Fe^{2+} alone after 0.5 h of incubation. The combination of EDTA (0.25−0.5 mM) and Fe^{2+} resulted in peroxide concentrations similar to no added Fe^{2+} controls for up to 2 h, after which the micelles containing EDTA (0.25−0.5 mM) and Fe^{2+} had greater peroxide concentrations (Figure 3). Micelles with 2 mM EDTA and Fe^{2+} (0.25 mM) resulted in a rapid 1.8-fold increase in peroxides after 0.5 h, after which concentration decreased slightly.

α-Tocopherol Oxidation. α-Tocopherol was incorporated into Tween 20 (high in peroxides) and Brij 10 lauryl ether (low in peroxides) micelles, to investigate the ability of surfactant peroxides to oxidize an antioxidant (Table 2). At pH 3.0 in the absence of added

Table 2. Relative Percent Decrease of α-Tocopherol in 0.1 M Brij and Tween 20 Micelles at pH 3.0 or 7.0 in the Presence or Absence of Fe^{2+} (0.25 mM) and EDTA (0.5 mM) (55 °C)

	relative decrease of α-tocopherol (%)						
time (h)	Brij[a] (pH 3.0)	Brij[a] + Fe^{2+} (pH 3.0)	Tween 20[b] (pH 3.0)	Tween 20[b] + Fe^{2+} (pH 3.0)	Tween 20[c] + Fe^{2+} + EDTA (pH 3.0)	Tween 20[d] (pH 7.0)	Tween 20[d] + Fe^{2+} (pH 7.0)
0	100 ± 24	100 ± 24	100 ± 9	100 ± 9	100 ± 16	100 ± 10	100 ± 10
0.5	106 ± 19	96 ± 12	103 ± 6	0[e]	107 ± 24	73 ± 3	75 ± 15
4	103 ± 30	91 ± 16	77 ± 4	0[e]	82 ± 5	52 ± 4	30 ± 0

[a] Initial α-tocopherol concentration 32.2 ppm. [b] Initial α-tocopherol concentration 26.4 ppm. [c] Initial α-tocopherol concentration 30.9 ppm. [d] Initial α-tocopherol concentration 35.4 ppm. [e] A zero value represents α-tocopherol at a concentration below the detection limits (8.5 ppm).

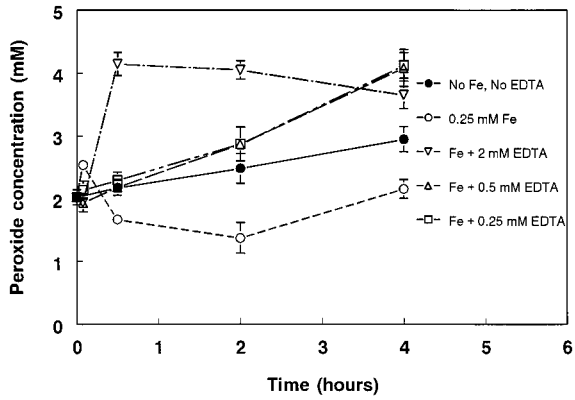

Figure 3. Effect of EDTA (0.25−2 mM) with Fe^{2+} (0.25 mM) on peroxide concentration in 0.1 M Tween 20 micelles at pH 3.0 for 4 h at 55 °C. Some standard deviation bars lie within the data points.

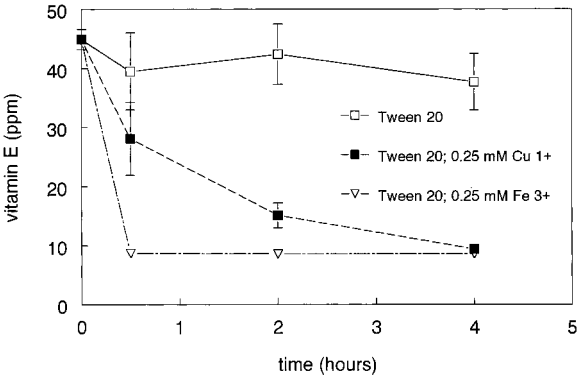

Figure 4. Effect of Fe^{3+} and Cu^{+} (0.25 mM) on the oxidation of α-tocopherol in 0.1 M Tween 20 micelles at pH 3.0 for 4 h at 55 °C. Some standard deviation bars lie within the data points.

iron, α-tocopherol concentration in the Brij micelles remained constant, while in the Tween micelles, α-tocopherol decreased by 23% after 4 h. Fe^{2+} (0.25 mM) did not result in a significant decrease in α-tocopherol in Brij micelles at pH 3.0, whereas all of the α-tocopherol was oxidized in the Tween 20 micelles by 0.5 h (Table 2). At pH 7.0, α-tocopherol in Tween 20 micelles decreased more rapidly than at pH 3.0 in the absence of iron, with a decrease of 48% after 4 h (Table 2). In the presence of Fe^{2+}, α-tocopherol in Tween 20 micelles was oxidized faster at pH 3.0 than pH 7.0. EDTA (0.5 mM) was able to significantly ($p \leq 0.05$) decrease the oxidation of α-tocopherol in the Tween 20 micelles containing Fe^{2+} at pH 3.0 (Table 2). At pH 3.0, Fe^{3+} (0.25 mM) decreased α-tocopherol concentration by 81% in 0.1 M Tween 20 micelles during the first 0.5 h, after which α-tocopherol levels remained constant (Figure 4). Cu^{+} resulted in a slower oxidation rate of α-tocopherol in the Tween 20 micelles than both Fe^{3+} and Fe^{2+} (Table 2) with a 37% decrease after 0.5 h and a total decrease of 79% after 4 h (Figure 4).

DISCUSSION

Free radicals arising from decomposition of fatty acid peroxides and hydrogen peroxide are known to accelerate lipid oxidation; however, little attention has been paid to the oxidation potential of peroxides originating from surfactants. Tween 20, noted for its susceptibility to peroxidation (Jaeger et al., 1994; Lever, 1977), may be able to promote lipid oxidation under conditions where its peroxides decompose into reactive free radicals. Transition metals are well known for their ability to decompose peroxides. Since transition metal−peroxide interactions will both decompose peroxides (resulting in a decrease in peroxide concentrations) and produce free radicals which promote oxidative reactions (resulting in an increase in peroxides), changes in peroxide concentrations in the presence of metals actually represent a balance between peroxide formation and decomposition.

Fe^{2+} (0.25 mM) initially caused a slight decrease in peroxide concentration in Tween 20 micelles, followed by a slight increase (Figure 1). The initial decrease in peroxides could represent the ability of Fe^{2+} to decompose preexisting Tween 20 peroxides, resulting in a burst in free radical formation which eventually causes peroxide formation during the later stages of oxidation. Lower concentrations of iron (0.01−0.10 mM) caused an increase instead of a decrease in peroxide concentrations (Figure 1). This could be due to the inability of the lower iron concentrations to substantially decrease peroxide concentrations while still producing enough free radicals to favor peroxide formation. Similar patterns have been observed for surfactant peroxides during exposure to low-intensity light (normal room lighting) favoring peroxide formation, resulting in an increase in peroxides, and high-intensity light (xenon arc lamp) favoring peroxide decomposition, resulting in a decrease in peroxide concentrations (Lever, 1977).

EDTA-containing samples had increased Tween 20 peroxide concentrations in the presence of added Fe^{2+} and equal or higher Tween-20 peroxide concentrations than the no added iron controls (Figure 3). The ability of EDTA to increase peroxide formation could therefore be due to its ability to inhibit peroxide decomposition by either added iron or endogenous transition metals.

Studying peroxide reactivity in systems containing unsaturated fatty acids is difficult because peroxide breakdown will product free radicals that produce additional fatty acid peroxides. Therefore, α-tocopherol was used as a marker to study the ability of free radicals originating from Tween 20 to oxidize lipids since the resulting low-energy tocopherol radicals do not rapidly autooxidize and form peroxide (Liebler, 1993). α-Toco-

pherol oxidized rapidly in Tween 20 micelles (high peroxide concentrations) while little α-tocopherol oxidation was observed in Brij 10 micelles (low peroxide concentrations) both in the presence and in the absence of added transition metals (Table 1 and Figure 4). The dependence on surfactant peroxides and the ability of EDTA to inhibit Fe^{2+}-promoted α-tocopherol oxidation suggests that free radicals originating from transition-metal-promoted decomposition was responsible for the oxidation of α-tocopherol.

Finally, in the absence of added iron, Tween 20 peroxide formation (Figure 2) and α-tocopherol oxidation (Table 2) were faster at pH 7.0 than pH 3.0. These reactions are likely due to the ability of contaminating transition metals to promote free radical formation from Tween 20 peroxides. Increased reactivity of peroxides originating from Tween 20 and pH 7.0 could help explain why oxidation of oil-in-water emulsions stabilized with Tween 20 increases with increasing pH (Mancuso et al., 1999; Huang et al., 1996).

In conclusion, peroxides originating from Tween 20 seem to be able to participate in oxidative reactions. If these surfactant peroxides are able to accelerate the oxidation of food emulsions, caution should be taken to monitor their concentration and prevent their formation during food-manufacturing operations.

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