Impact of Tween 20 Hydroperoxides and Iron on the Oxidation of Methyl Linoleate and Salmon Oil Dispersions

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To determine the role of surfactant hydroperoxides on the oxidative stability of fatty acids, the oxidation of methyl linoleate micelles and salmon oil-in-water emulsions was measured as a function of varying Tween 20 hydroperoxide concentrations. Increasing Tween 20 hydroperoxide concentrations from 3.5 to 14.7 μmol hydroperoxide/g Tween 20 decreased the lag phase of headspace hexanal formation but did not increase the total amount of hexanal formed in methyl linoleate/Tween 20 micelles. In the micelle system, Fe^{2+} decreased the lag phase of hexanal formation but increased total hexanal concentrations only in micelles with the highest Tween 20 hydroperoxide concentrations (14.7 μmol hydroperoxide/g surfactant). Increasing Tween 20 surfactant hydroperoxide concentrations also increased the oxidation of salmon oil-in-water emulsions as determined by lipid hydroperoxides and headspace propanal. In both the micelle and emulsion systems, the prooxidant effect of Fe^{2+} decreased with increasing Tween 20 hydroperoxide concentrations. These data show that surfactant hydroperoxides such as those in Tween 20 could decrease the oxidative stability of lipids in food emulsions.

Keywords: *Lipid oxidation; fish oil; lipid hydroperoxides; Tween 20; emulsions; surfactants; propanal*

INTRODUCTION

Lipid oxidation is an important factor that influences the shelf life of various food products. The mechanism of lipid oxidation in bulk oils has been widely studied. However, lipids are commonly found as emulsions in processed foods (*1*). Oil-in-water emulsions consist of three distinct physical environments: the emulsion droplet's lipid core, the emulsion droplet interfacial membrane, and the continuous phase. The reactants involved in lipid oxidation can partition into these different environments, thus resulting in oxidation rates and mechanisms different from those of bulk oils (*2*−*4*). The interfacial membrane of the emulsion droplet is one of the main differences between oil-in-water emulsions and bulk oils that alters the chemistry of lipid oxidation.

There is an increasing interest in the incorporation of ω-3 polyunsaturated fatty acids into foods because of their many health benefits (*5*−*8*). However, these highly unsaturated fatty acids are extremely susceptible to oxidation, resulting in potential alterations in nutritional composition as well as in the sensory quality of the product. If ω-3 polyunsaturated fatty acids are to be added to foods it is likely to be in the form of lipid dispersions. For that reason it is necessary to develop methods that help control or slow oxidative reactions of emulsified ω-3 fatty acids if these bioactive lipids are to be successfully used as functional food ingredients.

Prooxidants, such as lipoxygenases, singlet oxygen, and transition metals, accelerate lipid oxidation. Transition metals, and in particular iron, are naturally present at levels high enough to promote lipid oxidation in many foods (*3*, *4*). These transition metals mainly promote lipid oxidation through their ability to decompose hydroperoxides into free radicals. Hydroperoxides are found in most lipid-containing foods, e.g., even high quality lipids contain hydroperoxides in the range of 10−100 nmol/g lipid, which is 400−1,000 times greater than the hydroperoxide concentrations found in biological sources such as plasma lipids (*9*). Another potential source of hydroperoxides in oil-in-water emulsions is surfactants. Commercial sources of Tween 20 and phospholipids have been found to contain 4−35 μmol hydroperoxides/g surfactant (*10*). Tween 20 hydroperoxides can be decomposed by Fe^{2+} resulting in the oxidation of lipids such as α-tocopherol. These results suggest that surfactant hydroperoxides could decrease the oxidative stability of emulsified lipids.

The overall objective of this work was to determine the role of surfactant hydroperoxides and iron on the oxidation of fatty acids in oil-in-water dispersions. The prooxidant activity of surfactant hydroperoxides was evaluated in micelles and lipid emulsions high in ω-3 fatty acids.

MATERIALS AND METHODS

Materials. Polyoxyethylene sorbitan monolaurate (Tween 20), imidazole, sodium acetate, ferrous sulfate, iminodiacetic acid (Chelex 100), and methyl linoleate were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade or purer. Glassware was acid-washed (concentrated HCl), rinsed with double-distilled water, and dried overnight before use.

Buffer Treatment with Chelex-100. Transition metals were removed from buffers by gentle mixing of 2 g of Chelex 100/l buffer for 24 h. The buffer was then separated from precipitated Chelex 100 by decantation (*11*).

Salmon Oil Isolation. Salmon oil was isolated and analyzed as described by Mei et al. (*12*). In short, fresh salmon was purchased from a local store, hand chopped, minced, and centrifuged at 10000*g* for 20 min at room temperature. The

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separated oil was decanted and stored at −80 °C until use. The isolated oil contained 99.5 ± 0.2% triacylglycerols, 0.048 μmol TBARS/g oil, and 0.36 μmol hydroperoxides/g oil (measured as described by Mei et al. (*12*)).

Methods. *Surfactant Hydroperoxides.* Polyoxyethylene sorbitan monolaurate (Tween 20) was used as purchased (3.5 μmol hydroperoxides/g) or exposed to UV light at 25 °C for 25 h to produce 14.7−15.6 μmol hydroperoxides/g Tween 20. Intermediate levels of hydroperoxides were produced by blending untreated and UV-light-treated Tween 20.

Micelle Preparation. Tween 20 (average molecular weight 1224 g/mol) and methyl linoleate were mixed together and then added to acetate−imidazole buffer (5 mM each) at final concentrations of 100 and 1 mM, respectively. This Tween 20/methyl linoleate ratio gave detectable levels of hexanal formation upon oxidation. The mixture was stirred on a magnetic plate for 10 min to form micelles, and then the pH was adjusted to 7.0.

Emulsion Preparation. Emulsions containing salmon oil (5%), Tween 20 (17 mM), and acetate−imidazole buffer (5 mM each) were prepared using a Braun-Sonic 2000 U ultrasonic generator (Braun Biotech, Allentown, PA) equipped with a 5T standard probe at a power setting of +250 and a 0.3 s repeating duty cycle for 90 s in an ice−water bath followed by adjustment of pH to 7.0. Particle size distributions were measured using a Horiba LA-900 laser scattering particle size analyzer (Horiba Instruments, Irvine CA) (*13*). The average droplet diameters ranged from 1.4 to 1.5 μm ± 0.05 μm and did not change during the course of the experiment.

Oxidation of Micelles and Emulsions. Ferrous sulfate solutions (1−50 μM, final concentrations) or water were added to the micelles or emulsions, and the samples (1 mL) were immediately placed in 10-mL glass vials, sealed with poly(tetrafluoroethylene) (PTFE)/butyl rubber septa using a crimper and aluminum seals, and incubated at 55 °C (micelles) or 32 °C (emulsions) in the dark. Oxidation was followed by measuring hydroperoxides and headspace aldehydes.

Lipid hydroperoxides were determined using a method adapted from Shanta and Decker (*14*). Emulsion (0.3 mL) was added to 1.5 mL of a mixture of isooctane/2-propanol (3:1), vortexed 3 times for 10 s each, followed by centrifuging for 2 min at 2000*g*. The organic phase (0.2 mL or less according to the oxidation state) was added to a mixture of methanol/butanol (2:1, v:v) followed by addition of 15 μL of 3.94 M thiocyanate and 15 μL of 0.072 M Fe^{2+}. The solution was vortexed, and after 20 min the absorbance was measured at 510 nm. The concentration of hydroperoxides was calculated from a cumene hydroperoxide standard curve.

Headspace aldehydes were determined with a method described by Mancuso et al. (*3*) using a Hewlett-Packard (HP) 5890 gas chromatograph (Avondale, PA) with a HP 19395A headspace sampler and coupled to a HP 3392A integrator. The headspace conditions were the following: sample temperature,40 °C for propanal and 55 °C for hexanal; sample loop and transfer line temperature, 110 °C; pressurization, 10 s; venting, 10 s; and injection, 1 min. The aldehydes were separated isothermally at 70 °C (propanal) or 65 °C (hexanal) on a HP methyl silicone (DB-1) fused silica capillary column (50 m, 0.31 mm i.d., 1.03 μm film thickness). The splitless injector temperature was 180 °C and the flame ionization detector temperature was 200 °C.

Statistics. All the experiments were done in triplicate. Statistical analyses were performed using the Student's *t*-test (*15*). Statistical differences were defined as $p \leq 0.05$.

RESULTS AND DISCUSSION

Tween 20 Hydroperoxide/Methyl Linoleate Interactions. The ability of surfactant hydroperoxides to oxidize a fatty acid was tested by incorporating methyl linoleate into Tween 20 micelles containing 3.5−14.7 μmol hydroperoxide/g Tween 20. This range of Tween 20 hydroperoxide concentrations is within the range commonly seen in commercial surfactants (*10*). Headspace hexanal (a secondary product of the oxidation of methyl linoleate) was used as an oxidation marker. Initial studies were conducted to determine the prooxidant role of transition metals from the buffer used to prepare the micelles. Hexanal formation was observed in methyl linoleate/Tween 20 micelles prepared with untreated and Chelex-treated buffer at both 3.5 and 14.7 μmol hydroperoxide/g Tween 20 (Figure 1a and b). Pretreatment of the buffer solutions with Chelex resin to decrease transition metal concentrations tended to increase the length of the lag phase at both hydroperoxide concentrations as compared to that with the untreated buffer; however, differences in hexanal concentrations were not statistically significant. Increasing Tween 20 hydroperoxide concentrations decreased the lag phases of hexanal formation for both untreated and Chelex-treated buffers.

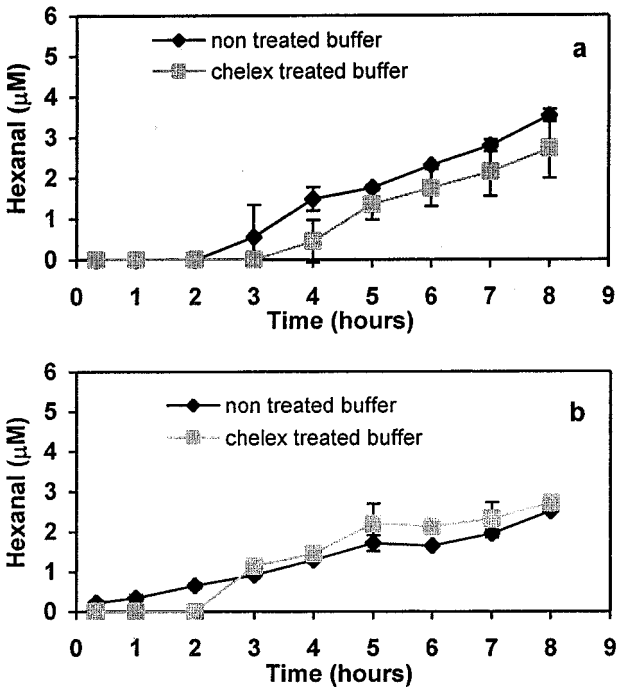

Figure 1. Effect of Chelex treatment of buffer solutions on hexanal formation in Tween 20/methyl linoleate micelles with different surfactant hydroperoxide concentrations: (a) 3.5 μmol hydroperoxide/g surfactant and (b) 14.7 μmol hydroperoxide/g surfactant. Data markers represent average ($n = 3$) ± standard deviation (some error bars lie within the markers).

Differences in oxidation of methyl linoleate in Tween 20 micelles (Chelex-treated buffer was used in all samples) were observed in the absence and presence of added Fe^{2+} (Figures 2−4). In the absence of added Fe^{2+}, increasing Tween 20 hydroperoxide concentrations decreased the lag phase of oxidation from 3 h for 3.5 μmol hydroperoxide/g Tween 20 to 2 h for 14.7 μmol hydroperoxide/g Tween 20. In the no-added-Fe^{2+} samples, increasing Tween 20 hydroperoxide concentration from 3.5 to 14.7 μmol hydroperoxide/g Tween 20 did not significantly increase the concentration of hexanal formed after 6 h of oxidation. Added Fe^{2+} resulted in acceleration of hexanal formation as was observed by the fact that oxidation proceeded rapidly, with no observable lag phases. In samples containing 3.5 and 6.0 μmol hydroperoxide/g Tween 20, no significant differences in total hexanal concentration between Fe^{2+} (10−35 μM) and no-added-Fe^{2+} samples were observed after 6 h of oxidation (Figures 2 and 3). Fe^{2+} did influence total hexanal concentrations in micelles con-

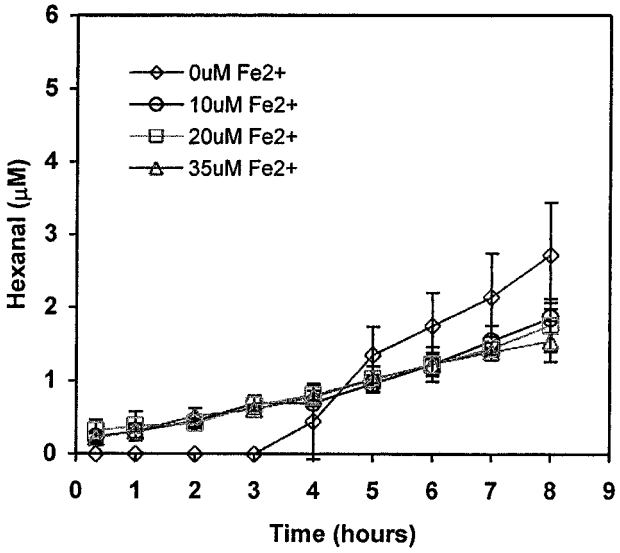

Figure 2. Effect of ferrous sulfate concentration (0, 10, 20, and 35 μM) on hexanal formation in Tween 20/methyl linoleate micelles containing 3.5 μmol hydroperoxide/g surfactant. Data markers represent average ($n = 3$) ± standard deviation (some error bars lie within the markers).

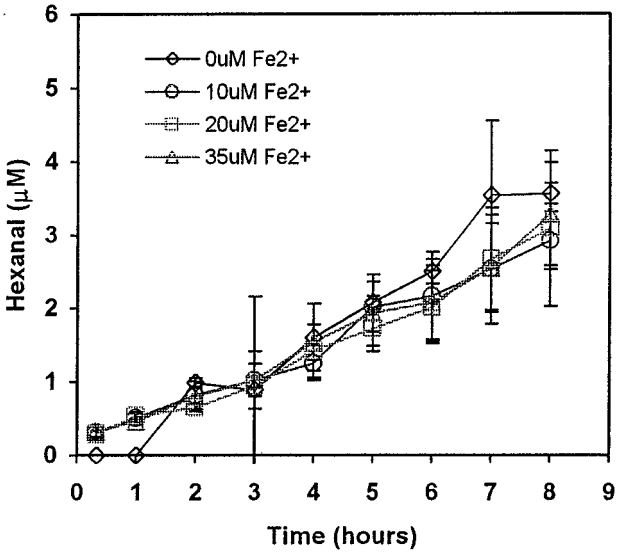

Figure 3. Effect of ferrous sulfate concentration (0, 10, 20, and 35 μM) on hexanal formation in Tween 20/methyl linoleate micelles containing 6.0 μmol hydroperoxide/g surfactant. Data markers represent average ($n = 3$) ± standard deviation (some error bars lie within the markers).

taining 14.7 μmol hydroperoxides/g Tween 20 with Fe^{2+}-containing samples containing 1.3 to 1.6-fold more hexanal than no-Fe^{2+} controls after 6 h of oxidation (Figure 4). No significant differences in hexanal concentrations in the micelles containing 14.7 μmol hydroperoxides/g Tween 20 were observed among the different iron concentrations (10−35 μM).

Tween 20 Hydroperoxide/Salmon Oil-in-Water Emulsions Interactions. To determine the influence of Tween 20 hydroperoxides on the stability of a food emulsion, salmon oil-in-water emulsions were prepared with Tween 20 that was either low (3.5 μmol hydroperoxide/g Tween 20) or high (15.6 μmol hydroperoxide/g Tween 20) in hydroperoxides. Headspace propanal (a breakdown product from the oxidation of ω-3 fatty acids) and lipid hydroperoxides were used as oxidation markers.

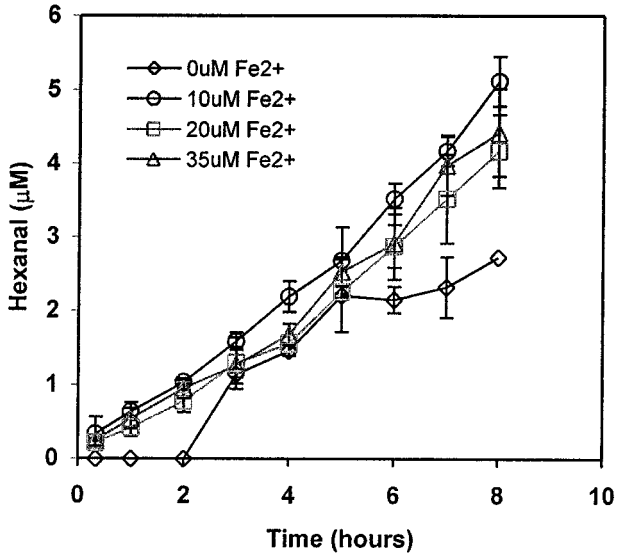

Figure 4. Effect of ferrous sulfate concentration (0, 10, 20, and 35 μM) on hexanal formation in Tween 20/methyl linoleate micelles containing 14.7 μmol hydroperoxide/g surfactant. Data markers represent average ($n = 3$) ± standard deviation (some error bars lie within the markers).

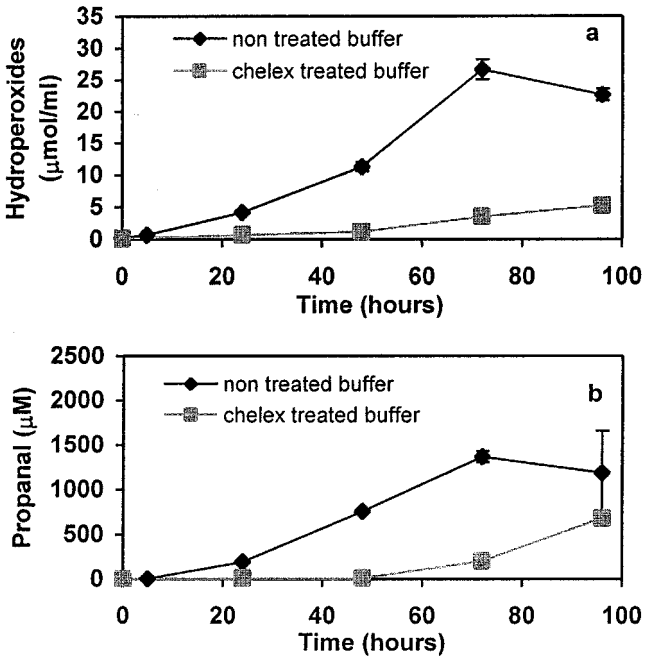

Figure 5. Effect of Chelex treatment of buffer solutions on the oxidation of salmon oil-in-water emulsions stabilized with Tween 20 (3.5 μmol hydroperoxide/g surfactant) as measured by hydroperoxide (a) and propanal (b) formation. Data markers represent average ($n = 3$) ± standard deviation (some error bars lie within the markers).

Unlike the Tween 20 micelle system, treatment of buffer with Chelex 100 had a dramatic effect on oxidation rates in the salmon oil-in-water emulsions. At low Tween 20 hydroperoxide concentration (3.5 μmol hydroperoxide/g Tween 20), Chelex treatment of the buffer decreased lipid hydroperoxide and propanal formation rates as observed by both an increase in the lag phase of oxidation and the total amount of lipid hydroperoxides and propanal produced (Figure 5a and b). At a Tween 20 hydroperoxide concentration of 15.6 μmol hydroperoxide/g surfactant, formation of lipid hydroperoxides was lower in the Chelex-treated buffer samples, yet no

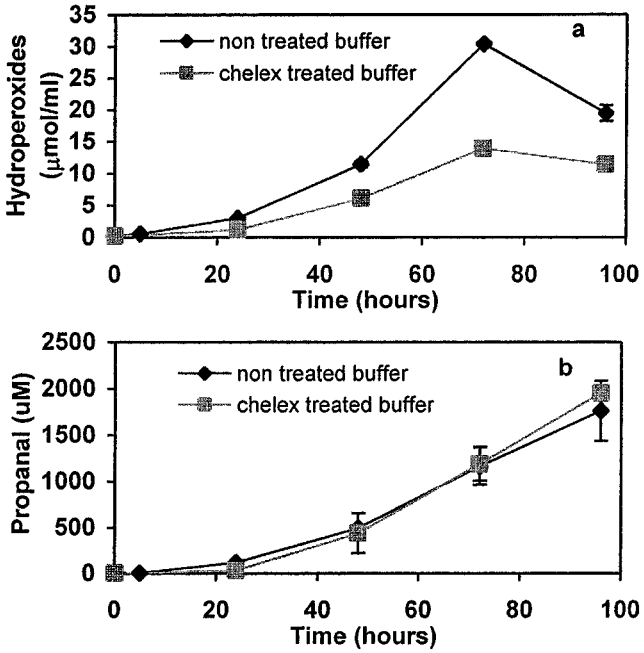

Figure 6. Effect of Chelex treatment of buffer solutions on the oxidation of salmon oil-in-water emulsions stabilized with Tween 20 (15.6 μmol hydroperoxide/g surfactant) as measured by hydroperoxide (a) and propanal (b) formation. Data markers represent average (n = 3) ± standard deviation (some error bars lie within the markers).

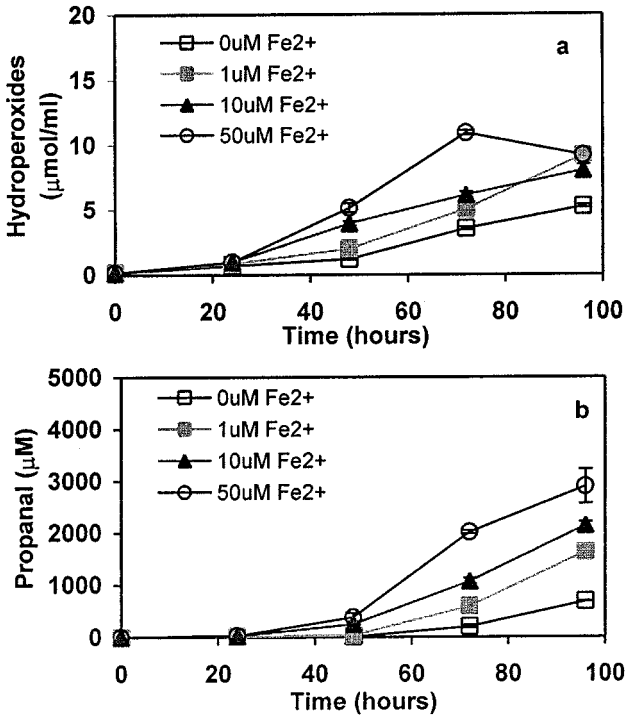

Figure 7. Effect of ferrous sulfate concentration (0 μM, 1 μM, 10 μM, and 50 μM) on the oxidation of salmon oil-in-water emulsions stabilized with Tween 20 (3.5 μmol hydroperoxide/g surfactant) as measured by hydroperoxide (a) and propanal (b) formation. Data markers represent average (n = 3) ± standard deviation (some error bars lie within the markers).

differences were observed in propanal formation rates (Figure 6a and b).

In emulsions containing 3.5 μmol hydroperoxide/g Tween 20, addition of Fe^{2+} increased both lipid hydroperoxide and propanal formation rates as compared to those of samples with no added Fe^{2+} (all samples contained Chelex-treated buffer). After 72 h of oxidation, lipid hydroperoxide concentrations were 1.4-, 1.7-, and 3.1-fold greater than those of no-added-Fe^{2+} controls for samples containing 1, 10, and 50 μM Fe^{2+}, respectively (Figure 7a). Propanal concentrations after 72 h of oxidation were 2.9-, 5.2-, and 9.7-fold higher than those of the no-Fe^{2+} controls (Figure 7b). In emulsions prepared with 15.6 μmol hydroperoxide/g Tween 20, lipid hydroperoxide concentrations after 72 h of oxidation did not show predictable differences as a function of added Fe^{2+} concentration (Figure 8a). This could be due to the ability of Fe^{2+} to promote both the breakdown and formation of hydroperoxides, with Tween 20 hydroperoxide concentrations potentially influencing the balance of these reactions. Previous work in our laboratory has shown that increasing Fe^{2+} concentrations and activity can cause a decrease in lipid hydroperoxide concentrations and an increase in secondary lipid oxidation products due to the ability of the iron to promote hydroperoxide decomposition (*12*). Alternately, the ability of iron to increase oxidation rates could also increase termination reactions which could result in decreased lipid hydroperoxides and propanal formation. Propanal formation did show a predictable pattern with propanal concentrations being 1.4-, 1.9-, and 1.9-fold greater in the presence of 1, 10, and 50 μM Fe^{2+}, respectively, compared to that of no-added-Fe^{2+} after 72 h of oxidation.

Salmon-oil-in-water emulsions prepared with Tween 20 high in hydroperoxide oxidized faster than emulsions low in Tween 20 hydroperoxides. In the absence of added Fe^{2+}, emulsions prepared with 15.6 μmol hydroperoxide/g Tween 20 had 1.7-, 5.0- and 3.9-fold higher lipid hydroperoxide concentrations than emulsions prepared with 3.5 μmol hydroperoxide/g Tween 20 after 24, 48 and 72 h of oxidation, respectively (Figure 7a and 8a). Propanal formation showed a similar pattern in samples without added Fe^{2+} with emulsions prepared

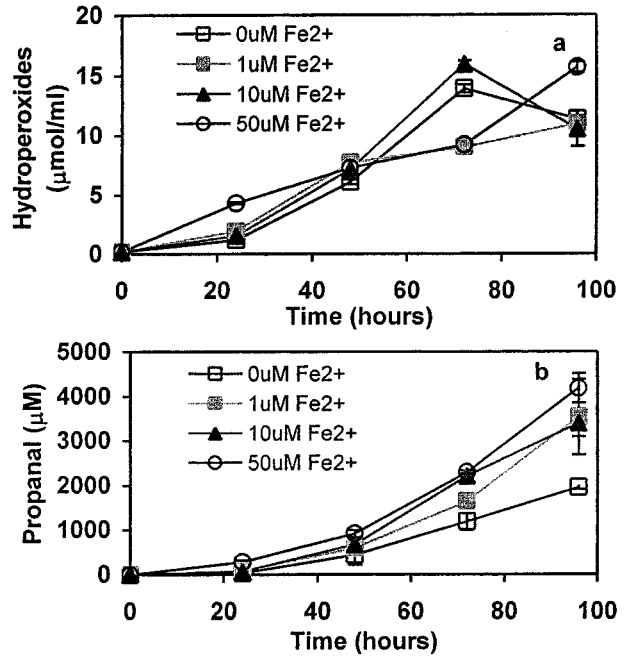

Figure 8. Effect of ferrous sulfate concentration (0 μM, 1 μM, 10 μM, and 50 μM) on the oxidation of salmon oil-in-water emulsions stabilized with Tween 20 (15.6 μmol hydroperoxide/g surfactant) as measured by hydroperoxide (a) and propanal (b) formation. Data markers represent average (n = 3) ± standard deviation (some error bars lie within the markers).

with 15.6 μmol hydroperoxide/g Tween 20 having 4.2-, 32- and 5.7-fold higher propanal concentrations than emulsions prepared with 3.5 μmol hydroperoxide/g Tween 20 after 24, 48 and 72 h of oxidation, respectively (Figure 7b and 8b). Increasing oxidation rates in the salmon-oil-in-water emulsions with increasing Tween 20 hydroperoxide concentrations was also seen in the presence of added Fe^{2+}. For instance, in the presence of 1 μM Fe^{2+}, lipid hydroperoxide concentrations were 2.3-, 3.9-, 1.8-fold greater and propanal concentrations were 6.0, 14.0, and 2.8-fold greater in the emulsions prepared with 15.6 μmol hydroperoxide/g Tween 20 compared to 3.5 μmol hydroperoxide/g Tween 20, after 24, 48 and 72 h of oxidation, respectively.

Discussion. Tweens are surfactants that contain polyether-based hydrophilic headgroups. These polyethers are easily oxidized to form hydroperoxides and various hydroperoxide breakdown products (*16, 17*). Factors such as elevated temperatures, light, and transition metals can cause both hydroperoxide formation and decomposition in Tweens (*10, 16, 18*). Because the hydrophilic headgroup is the site of hydroperoxide formation in Tweens, the surfactant hydroperoxides would be expected to be located at the interface of oil-in-water emulsion droplets where they could interact with both continuous-phase transition metals and lipid-phase unsaturated fatty acids. Previous work has shown that α-tocopherol is oxidized by iron and copper in the presence of Tween 20 hydroperoxides (*10*).

This research provides the first evidence that Tween 20 hydroperoxides can stimulate the oxidation of fatty acids in micelles and oil-in-water emulsions. Oxidation of methyl linoleate and salmon oil increased with both increasing surfactant hydroperoxide and Fe^{2+} concentrations. In both the micelle and emulsion systems, the prooxidant effect of Fe^{2+} decreased with increasing Tween 20 hydroperoxide concentrations. These data show that surfactant hydroperoxides such as those in Tween 20 could decrease the oxidative stability of food emulsions. In addition, if surfactant hydroperoxide concentrations are high, then less iron is needed to promote oxidation, suggesting that it may be more difficult to produce oxidatively stable food emulsions. The ability of Tween 20 hydroperoxide to accelerate lipid oxidation suggests that food manufacturers may wish to monitor hydroperoxide concentrations in their surfactants, as hydroperoxide concentrations can vary greatly in commercial sources and hydroperoxides can form during storage.

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