# The Effects of Surfactant Type, pH, and Chelators on the Oxidation of Salmon Oil-in-Water Emulsions

Jennifer R. Mancuso,† D. Julian McClements, and Eric A. Decker*

Department of Food Science, Chenoweth Lab, University of Massachusetts, Amherst, Massachusetts 01003

Lipid oxidation in emulsions is influenced by the ability of transition metals to associate with emulsion droplets. The oxidative stability of 5% salmon oil-in-water emulsion was influenced by surfactant type, with oxidation rates being greatest in emulsions stabilized by anionic sodium dodecyl sulfate (SDS) followed by nonionic Tween 20 and cationic dodecyltrimethylammonium bromide (DTAB). EDTA inhibited lipid oxidation in all the emulsions, and apo-transferrin inhibited oxidation in the Tween 20-stabilized emulsions at pH 7.0, suggesting that continuous-phase iron was an active prooxidant. Iron associated with Tween-20 stabilized hexadecane emulsion droplets could be partitioned into the continuous phase by lowering the pH to ≤4.0 or by the presence of EDTA, which could help explain why low pH and EDTA decrease lipid oxidation rates. These data suggest that iron is an important lipid oxidation catalyst in salmon oil emulsions, and factors that increase iron-emulsion droplet interactions will increase oxidation rates.

**Keywords:** *Lipid oxidation, emulsions, surfactants, emulsifiers, iron*

## INTRODUCTION

In many foods, lipids exist as emulsifier-stabilized dispersions. These emulsions can be considered to contain three regions: the interior of a droplet, the continuous phase, and the interfacial membrane. The interfacial membrane consists of a narrow region surrounding each emulsion droplet. This region is potentially very important in lipid oxidation since it represents the region where lipid- and water-soluble components interact and it is where surface-active compounds such as lipid peroxides and chain breaking antioxidants concentrate (Coupland and McClements, 1996; Buettner, 1993).

Recent studies in our laboratory on emulsified corn (Mei et al., 1998a), salmon (Mei et al., 1998b) and menhaden (Donnelly et al., 1998) oil found that iron-promoted lipid oxidation was affected by the repulsion or attraction of iron to the lipid droplet interface. This suggested that if the interfacial region of emulsion droplets could be altered to decrease lipid−iron interactions, this could provide an additional technique to decrease oxidative rancidity. However, these studies used iron concentrations that are much greater than those found in typical food emulsions.

The objective of this research was to determine the effect of surface charge, pH, and chelators on lipid oxidation in emulsions without added prooxidative metals. The surface of the emulsion droplets was varied by using different types of surfactants. Dodecyltrimethylammonium bromide (DTAB), sodium dodecyl sulfate (SDS), and Tween 20 were used as surfactants because they are cationic, anionic, and nonionic, respectively. If endogenous transition metals are active prooxidants in the emulsions, we would expect that lipid oxidation rates will depend on surfactant type. Studies with chelators were also designed to verify the possible prooxidative role of endogenous transition metals. Our

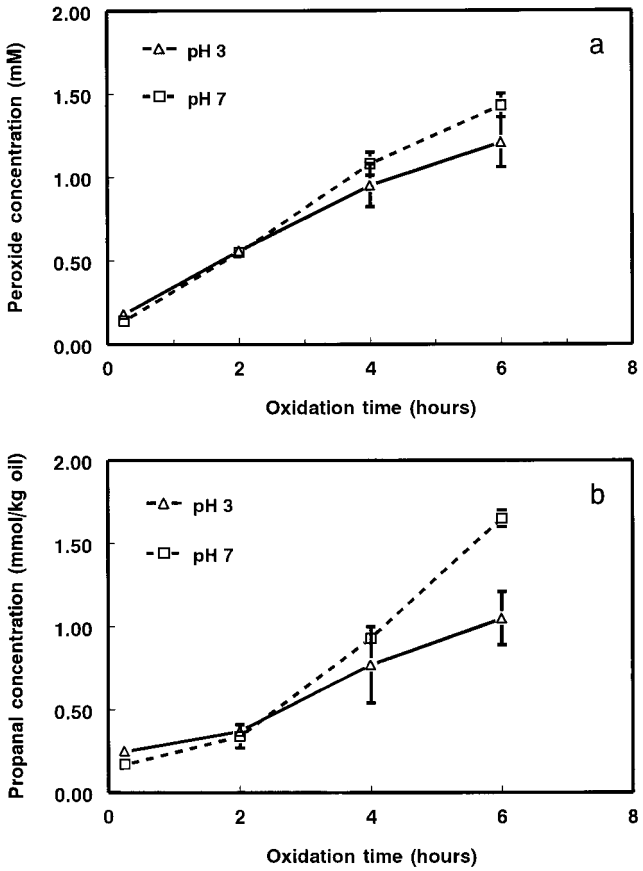


**Figure 1.** Effect of pH on SDS-stabilized salmon oil-in-water emulsions as measured by (a) formation of lipid peroxides and (b) formation of headspace propanal for the first 6 h of incubation.

* To whom correspondence should be addressed. Tel: 413-545-1026. Fax: 413-545-1262. E-mail: edecker@foodsci.umass.edu.

† Current affiliation: General Mills, Minneapolis, MN.

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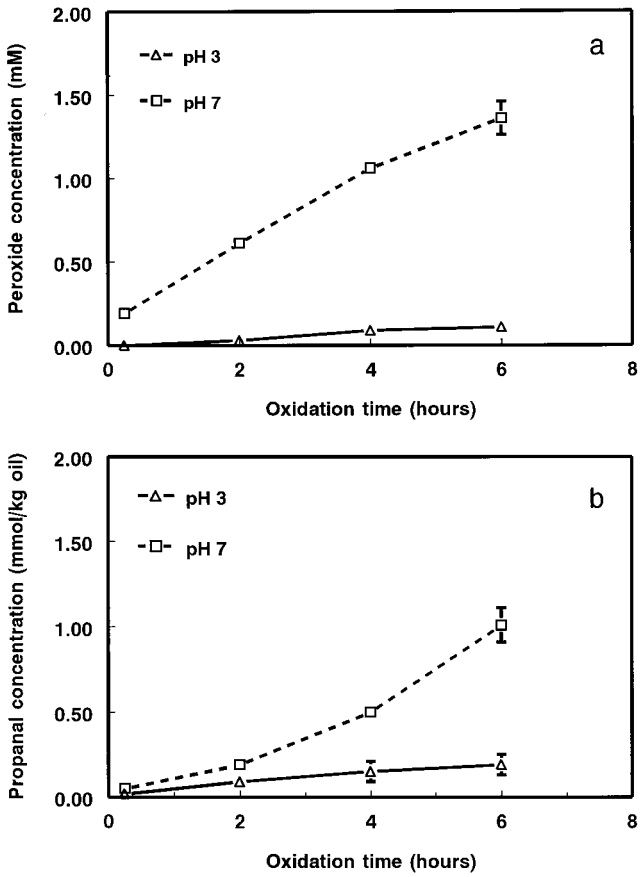


**Figure 2.** Effect of pH on Tween 20-stabilized salmon oil-in-water emulsions as measured by (a) formation of lipid peroxides and (b) formation of headspace propanal for the first 6 h of incubation.

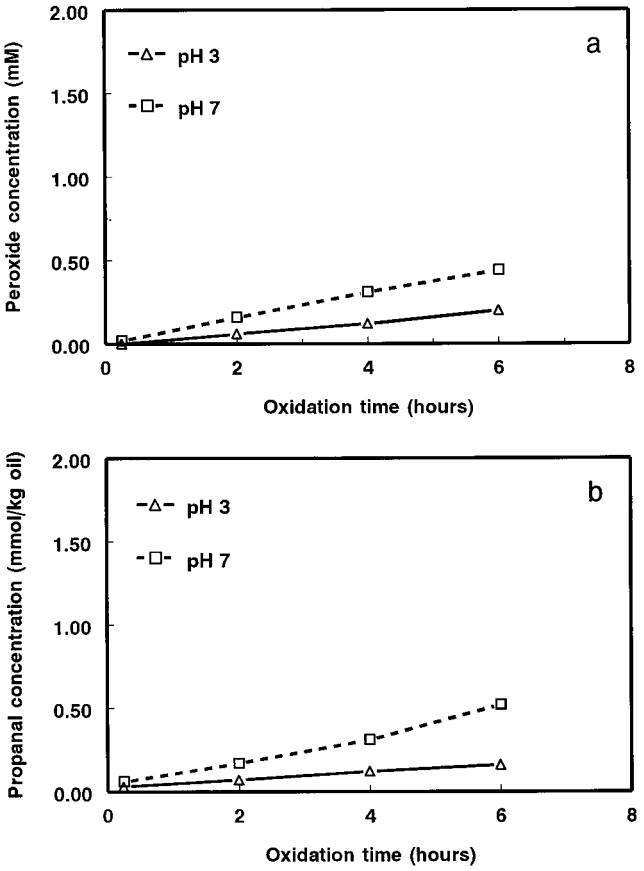


**Figure 3.** Effect of pH on DTAB-stabilized salmon oil-in-water emulsions as measured by (a) formation of lipid peroxides and (b) formation of headspace propanal for the first 6 h of incubation.

results are compared to studies on emulsions to which iron was added to accelerate lipid oxidation.

## MATERIALS AND METHODS

**Materials.** Salmon oil was isolated by centrifuging minced salmon muscle (Mei et al., 1998b). Dodecyltrimethylammonium bromide (DTAB) and sodium dodecyl sulfate (SDS) were obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). Imidizole, apo-transferrin, cumene hydroperoxide, and polyoxyethylene sorbitan monolaurate (Tween 20) were purchased from the Sigma Chemical Co. (St. Louis, MO). Disodium ethylenetetraacetic acid (EDTA) was obtained from Curtin Matheson Scientific, Inc. (Houston, TX). Sodium acetate was purchased from Fisher Scientific (Fair Lawn, NJ). All other chemicals were reagent grade or better.

**Methods. Preparation and Characterization of Emulsions.** A coarse emulsion premix was prepared by homogenizing 5% salmon oil and 95% aqueous phase or 10% hexadecane and 90% aqueous phase using a Model PT 10/35 Brinkman Polytron (Westbury, NY) at a setting of 7 for 1 min. The aqueous phase consisted of 17.0 mM of emulsifier dispersed in a buffer solution containing 5.0 mM imidazole and 5.0 mM sodium acetate in distilled water. The coarse emulsion was then recirculated through a high-pressure laboratory valve homogenizer (Rannie Pro APV-Gaulin, Copenhagen, Denmark) at approximately 45 MPa. Between each pass, the emulsion was collected in a beaker submerged in an ice−water bath so as to keep the temperature at ≃25 °C. All emulsions were made up at pH 3.0, and if needed, they were adjusted to pH 7.0 by the addition of NaOH after homogenization. EDTA (50−200 $\mu$M) and apo-transferrin (31 $\mu$M) were added to some of the emulsions after homogenization.

Particle size distributions were measured using a Horiba LA-900 laser scattering particle size distribution analyzer (Horiba Instruments, Irvine CA) (Weiss et al., 1996). Since the droplet size and thus surface area could influence the oxidation rates, the mean droplet diameter of every emulsion was measured. The mean droplet diameters of Tween 20-, DTAB-, and SDS-stabilized emulsions immediately after homogenization were 0.29−0.30 $\mu$m. To obtain this droplet size, SDS-stabilized emulsions required four passes through the homogenizer, while Tween 20- and DTAB-stabilized emulsions required six passes. Preliminary studies showed that lipid oxidation rates were not influenced by these homogenization conditions for up to 10 passes (data not shown). Droplet sizes were checked periodically to monitor emulsions stability.

**Measurement of Lipid Oxidation.** Oxidation was carried out at 32 °C in the dark with constant shaking (Gyrotory Shaker-model G2, New Brunswick Scientific Co., Inc, New Brunswick, NJ). Oxidative stability was evaluated by measuring lipid peroxides and headspace propanal. Peroxides were measured by a modified method of Shantha and Decker (1994) after an extraction step in which 0.3 mL of emulsion was added to 1.5 mL of isooctane−2-propanal followed by vortexing three times for 10 s each and centrifuging for 2 min at 2000*g*. Next, the organic phase (0.2 mL total volume containing 0.015 to 0.2 mL of lipid extract) was added to 2.8 mL of methanol−butanol (2:1 v:v), followed by 15 $\mu$L of thiocyanate solution (3.94 M) and 15 $\mu$L ferrous iron (0.072 M acidic solution). The solution was vortexed, and the absorbance at 510 nm was measured after 20 min. Lipid peroxide concentrations were determined using a cumene hydroperoxide standard curve.

For headspace analysis, emulsion samples (1 mL) were placed into 10-mL headspace vials and sealed with poly(tetrafluoroethylene) (PTFE)/butyl rubber septa using a crimper and aluminum seals. Emulsion samples were heated at 40 °C for 15 min immediately prior to analysis. Headspace propanal was determined using a Hewlett-Packard (HP) 5890 gas chromatograph (Avondale, PA) with a HP 19395A headspace

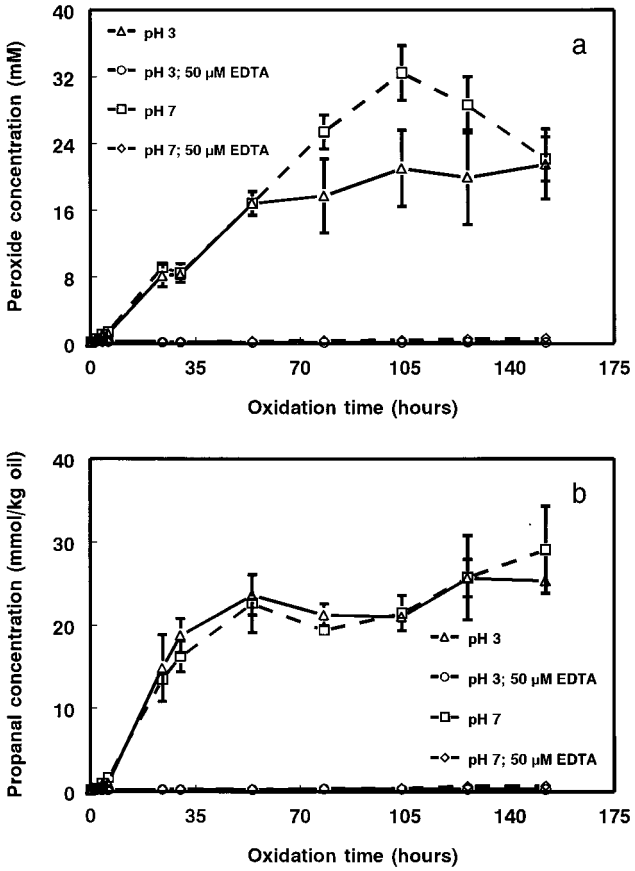


**Figure 4.** Effect of pH and EDTA on SDS-stabilized salmon oil-in-water emulsions as measured by (a) formation of lipid peroxides and (b) formation of headspace propanal.

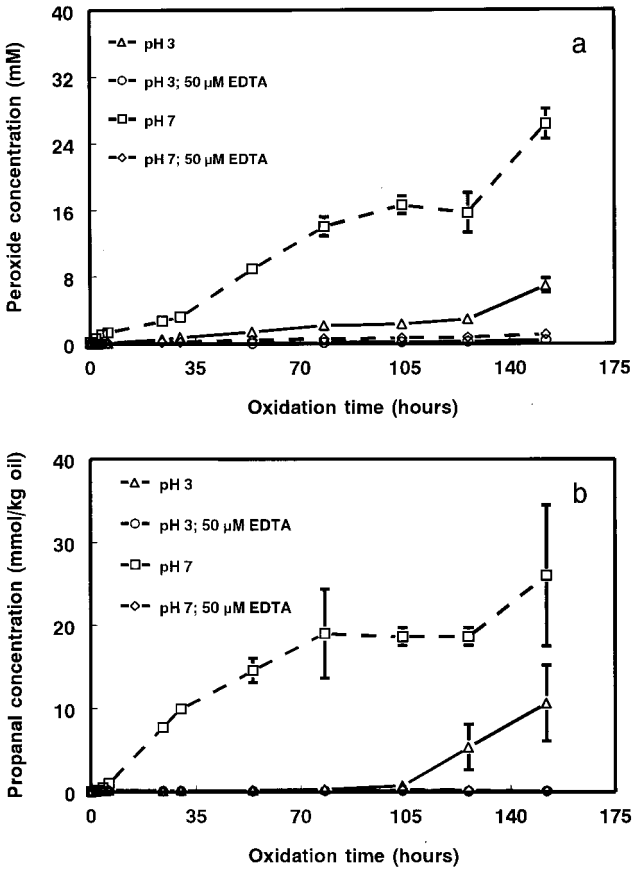


**Figure 5.** Effect of pH and EDTA on Tween 20-stabilized salmon oil-in-water emulsions as measured by (a) formation of lipid peroxides and (b) formation of headspace propanal.

sampler and coupled to a HP 3392A integrator. The headspace conditions were as follows: sample loop and transfer line temperature, 100 °C; pressurization, 10 s; venting, 10 s; injection, 1 min. The aldehydes were separated isothermally at 70 °C on a HP methyl silicone (DB-1) fused silica capillary column (50 m, 0.31 mm i.d., 1.03 $\mu$m film thickness). The splitless injector temperature was 180 °C, and the eluted compounds were detected with a flame ionization detector at 200 °C. Concentrations were determined from peak areas using a standard curve made from authentic propanal.

**Effect of pH and EDTA on Iron Association with Tween 20-Stabilized Emulsions.** In an attempt to understand why lipid oxidation is higher at pH 7.0 than at pH 3.0 in Tween 20 emulsions, a model was developed to observe whether iron associates with oil droplets. For this experiment, 1.0 mM ferric chloride was added to a Tween 20 (1%)-stabilized hexadecane (10%) emulsion at pH 7.0. Hexadecane was used so that lipid oxidation would not occur during the experiment. To favor iron−emulsion droplet interactions, the emulsion and iron were allowed to equilibrate at room temperature at pH 7.0 for 24 h, during which time the iron precipitated out of solution. Emulsion droplets were then separated from insoluble iron by centrifugation at $(1.4 \times 10^4)g$ for 20 min (Sorvall Superspeed RC2-B, Dupont Company, Wilmington, DE). The creamed emulsion layer was collected and reconstituted to its original concentration using imidizole−acetate buffer. To determine the influence of pH on iron distribution, the emulsion was divided into five aliquots that were adjusted to pH 3, 4, 5, 6, and 7, respectively. To determine the influence of chelators on iron distribution, EDTA (0.5−4.0 mM) was added to the reconstituted emulsions. Three hours after pH adjustment or EDTA addition, the emulsion samples were centrifuged at $(1.4 \times 10^4)g$ for 15 min (18 °C). The creamed layer was once again removed, and iron was measured in the continuous phase of the emulsion using a Perkin-Elmer 703 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, CT) at 248.3 nm.

**Statistical Analysis.** Assays were measured in triplicate. Statistical analysis was performed using the Student *t*-test (Snedecor and Cochran, 1989).

## RESULTS AND DISCUSSION

**Influence of Surfactant Type on Oxidation of Salmon Oil Emulsions.** A comparison of oxidation rates in salmon oil emulsions stabilized by different surfactants was made by incubating the emulsions at 32 °C for up to 152 h. When comparisons were made over the first 6 h of incubation, oxidation rates were faster at pH 7.0 than 3.0, with the effect being most pronounced in the nonionic, Tween 20-stabilized emulsion. Initial (first 6 h) oxidation rates at pH 3.0 were in the following order: SDS > Tween 20 ≅ DTAB, while at pH 7.0 they were SDS ≅ Tween 20 > DTAB as determined by both lipid peroxides and headspace propanal (Figures 1−3). Mei et al. (1998a) found that in corn oil-in-water emulsions with iron added oxidation rates at pH 4.0 were SDS > Brij 35 (nonionic) ≅ DTAB and at pH 6.5 were SDS ≥ Brij 35 > DTAB.

In the SDS-stabilized salmon oil emulsions, peroxide (Figure 4a) and propanal (Figure 4b) concentrations increased over time and formation rates at pH 3.0 and 7.0 were similar up to 54 and 152 h, respectively. At pH 7.0, the peroxides increased over time until concentrations reached a peak at 104 h followed by a period of decline. At pH 3.0, there was no significant change ($p \leq 0.05$) in peroxides after 54 h. This trend in peroxide formation is in agreement with Mei et al. (1998a), who

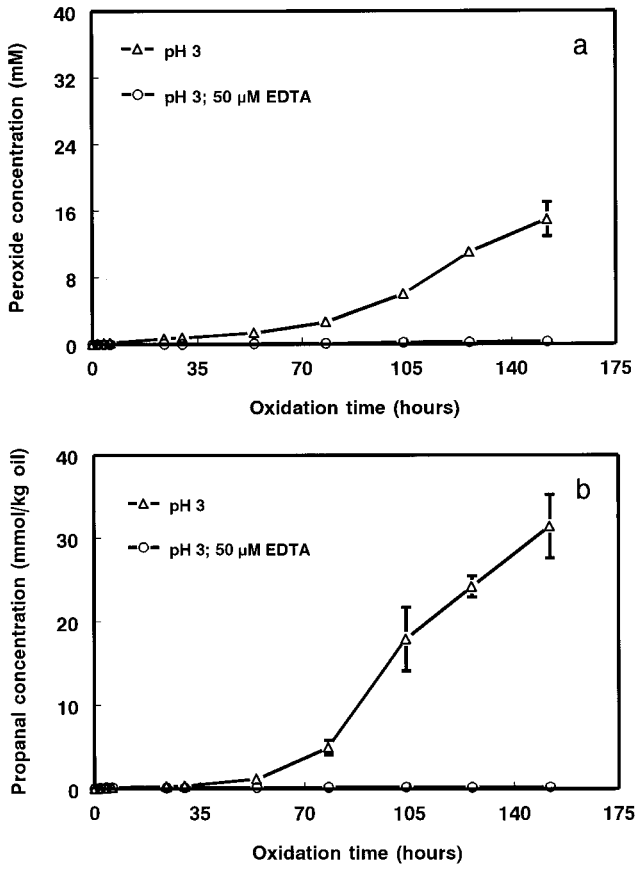


**Figure 6.** Effect of EDTA on DTAB-stabilized salmon oil-in-water emulsions at pH 3.0 as measured by (a) formation of lipid peroxides and (b) formation of headspace propanal.

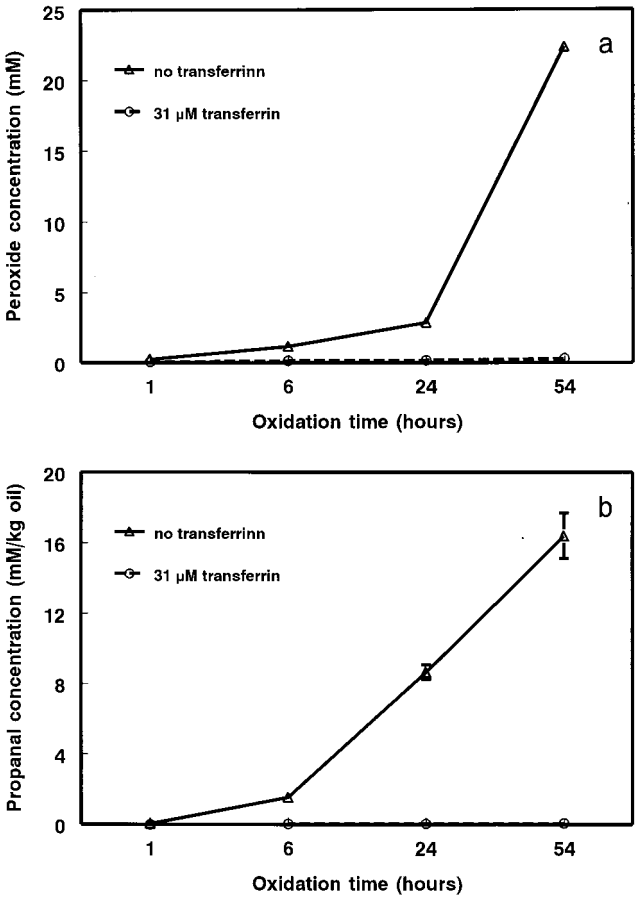


**Figure 7.** Oxidative stability of Tween 20-stabilized salmon oil-in-water emulsion (pH 7) in the presence and absence of apo-transferrin. Lipid oxidation was measured using (a) lipid peroxides and (b) headspace propanal.

observed lower peroxide concentrations at pH 3.0 than 7.0 in a SDS-stabilized corn oil emulsion containing added iron. We propose that the lower peroxide concentrations at pH 3.0 are not due to lower lipid oxidation rates since propanal concentrations were similar at pH 3.0 and 7.0 but instead are due to iron's increased ability to decompose lipid peroxides, thus preventing peroxide accumulation.

Figure 5 shows that, for Tween 20-stabilized emulsions, lipid oxidation was greater and more rapid at pH 7.0 than at pH 3.0. At pH 7, peroxide formation increased throughout the entire incubation period (Figure 5a) while propanal formation increased rapidly for the first 78 h and thereafter remained fairly constant (Figure 5b). At pH 3.0, peroxides increased slowly over time and propanal concentrations only became significant after 105 h. Increasing oxidation rates with increasing pH have also been observed in Tween-20 stabilized tocopherol-stripped corn oil emulsions as determined by conjugated dienes and headspace analysis (Huang et al., 1996).

Peroxide concentration (Figure 6a) and headspace propanal (Figure 6b) increased throughout the oxidation period in the DTAB-stabilized emulsion at pH 3.0. The DTAB-stabilized emulsions at pH 7.0 separated after 30 h of incubation; therefore, further comparisons of oxidation rates as a function of pH could not be made. Separation of the DTAB-stabilized emulsion suggested that the concentration of DTAB was too low, anions were present that caused destabilization, and/or the increase in pH caused a decrease in droplet charge.

EDTA (50 $\mu$M) significantly ($p \leq 0.05$) inhibited oxidation in Tween 20- and SDS-stabilized salmon oil emulsions at both pH 3.0 and pH 7.0 and in DTAB-stabilized emulsions at pH 3.0 for the entire incubation period as indicated by both lipid peroxides and headspace propanal (Figures 4−6). The results suggest that lipid oxidation of the emulsion is promoted by endogenous transition metals that are naturally present in the oil, surfactant, and/or water. Transferrin, an iron-binding transport protein found in biological systems, was compared to EDTA in the Tween 20-stabilized emulsion at pH 7.0 in order to verify that EDTA was acting specifically as an iron chelator and also to evaluate if iron chelation was occurring in the lipid or aqueous phase (EDTA has been observed to solubilize in oil; Schaich, 1988). Transferrin (31 $\mu$M) was compared at approximately one-half the concentration of EDTA, since every transferrin molecule binds two iron molecules (Halliwell and Gutteridge, 1989). Transferrin inhibited oxidation for 54 h of incubation as determined by both peroxide (Figure 7a) and headspace propanal (Figure 7b) formation. Transferrin's ability to inhibit lipid oxidation supports the hypothesis that iron is a major promoter of lipid oxidation in the oil-in-water emulsions and that iron is active in the continuous phase. However, transferrin could also be inhibiting oxidation by strong chelation of iron, which would favor partitioning of lipid-soluble iron out of the oil phase of the emulsion droplets where it can be chelated by the transferrin.

**Factors Influencing Iron−Tween 20 Emulsion Droplet Interactions.** There is some uncertainty about the role that pH plays on lipid oxidation rates in

**Table 1. Continuous Phase Iron Concentrations in Tween 20-Stabilized Hexadecane Emulsions Ranging from pH 3 to 7**

| | pH | Fe (mM) |
|---|---|---|
| emulsion with added iron | 3.0 | 0.107 ± 0.003 |
| | 4.0 | 0.016 ± 0.001 |
| | 5.0 | nd[a] |
| | 6.0 | nd[a] |
| | 7.0 | nd[a] |
| emulsion without added iron | 3.0 | nd[a] |
| | 7.0 | nd[a] |
| no emulsion | 7.0 | nd[a] |

[a] nd = not detected, detection limit ≥ 0.01 μM.

**Table 2. Continuous-Phase Iron Concentrations for Tween 20-Stabilized Hexadecane Emulsions at pH 7.0 with EDTA (0.5−4 mM)**

| | EDTA (mM) | Fe (mM) |
|---|---|---|
| emulsion with added iron | 0 | nd[a] |
| | 0.5 | 0.099 ± 0.001 |
| | 1.0 | 0.166 ± 0.000 |
| | 2.0 | 0.194 ± 0.001 |
| | 4.0 | 0.198 ± 0.002 |
| emulsion without added iron | 4.0 | nd[a] |
| no emulsion | 0 | nd[a] |

[a] nd = not detected, detection limit ≥ 0.01 μM.

emulsions. Iron is known to be more soluble at low pH's (Zumdahl, 1989). Therefore, we would have expected lipid oxidation rates to be higher at pH 3.0 than 7.0. However, our data showed the opposite to be true, which appears to contradict the inhibitory effects observed with the metal chelators EDTA and apo-transferrin. To explain this apparent contradiction, we hypothesized that at pH 7.0 low iron solubility results in precipitation of metal onto the lipid droplet surface, thereby bringing iron in closer contact with the lipid compared to pH 3.0, where the water solubility of iron is dramatically ($10^{8-12}$ M; Zumdahl, 1989) higher. To test this hypothesis, we prepared nonionic, Tween 20-stabilized emulsions at pH 7.0, let them stand for 24 h to allow iron to precipitate out of solution, and centrifuged them to remove insoluble iron from the continuous phase of the emulsion. The emulsion droplets were then resuspended in buffer followed by pH adjustment or addition of EDTA. The emulsions were allowed to equilibrate for 3 h, and the iron concentration of the continuous phase was determined by atomic absorption spectroscopy after the droplets had been removed by centrifugation. Iron could not be detected in the buffer (data not shown), the continuous phase of the emulsion where iron was not added, or the continuous phase of iron-added emulsions at pH 5.0−7.0 (Table 1). Continuous-phase iron concentrations increased when the pH of the emulsions was decreased to ≤4.0 (Table 1). The ability of low pH's to increase continuous phase iron concentrations suggests that iron precipitated to the emulsion droplet surface was being solublized. Presumably at pH 5.0−7.0, the majority of insoluble iron remained attached to the droplet surface. At pH 7.0, EDTA (0.5−4.0 mM) was also able partition iron precipitated to the surface of emulsion droplets into the continuous phase (Table 2). These observations support the hypothesis that, at pH 7.0, insoluble iron precipitates onto the emulsion droplet surface, which may increase lipid oxidation rates because of the close proximity of the iron and lipid substrate.

## CONCLUSIONS

Overall, salmon oil emulsions stabilized by anionic (SDS) surfactants exhibited increased lipid oxidation compared to emulsions stabilized by nonionic (Tween 20) and cationic (DTAB) surfactants. Although anionic surfactants such as SDS are not used in the food industry, other emulsifiers such as proteins at pH's greater than their pI's will produce negatively charged emulsion droplets that may be more susceptible to metal-promoted oxidation. Oxidation in salmon oil-in-water emulsions seems to be promoted by iron since oxidation can be effectively inhibited by EDTA and the iron-specific chelator apo-transferrin. Iron associates with Tween 20-stabilized hexadecane emulsions more at pH 7.0 than 3.0. If the iron that associated with Tween 20 stabilized-droplets is capable of promoting lipid oxidation, this could explain why Tween 20-stabilized emulsions are less stable oxidatively at pH 7.0, where iron-emulsion droplet interactions are greater. This work supports the hypothesis that techniques that decrease iron-emulsion droplet interactions will increase the oxidative stability of oil-in-water emulsions.

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