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# Iron-catalyzed lipid oxidation in emulsion as affected by surfactant, pH and NaCl

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Corn oil-in-water emulsions stabilized by sodium dodecyl sulfate (SDS), Brij 35 or dodecyltrimethylammonium bromide (DTAB) were prepared to determine the influence of surface charge on iron-catalyzed lipid oxidation. Oxidation was measured using lipid peroxides, conjugated dienes, and thiobarbituric reactive substances. At pH 6.5, initial oxidation rates were in the order of SDS > Brij > DTAB. As pH was decreased from 8 to 3, oxidation of SDS-stabilized emulsions increased, while oxidation of Brij and DTAB emulsions were unaffected. NaCl (1.0%) decreased oxidation of the SDS-stabilized emulsion by 20% but had minimal influence on oxidation of Brij and DTAB emulsions. These results indicate that the surface charge of emulsion droplets plays an important role in their oxidative stability. © 1998 Elsevier Science Ltd. All rights reserved

## INTRODUCTION

Oxidation of lipids is often a determining factor in the quality and shelf-life of foods. Bulk lipids can oxidize when stored and transported and these oxidative processes have been well studied (Nawar, 1996; Frankel, 1982). However, in many processed foods, lipids are found in surfactant stabilized dispersions, where oxidation reactions are influenced by factors including water-soluble pro-oxidants and antioxidants, increased surface area and the presence of an interfacial membrane (Coupland & McClements, 1996). The lipids used in food emulsions normally contain small quantities of lipid peroxides (Huang *et al.*, 1996). The oxidative decomposition of these peroxides to form lipid radicals is accelerated by transition metals such as iron (Dunford, 1987). Since transition metals, especially iron, are common contaminants of water, it would be expected that water-soluble metal ions could catalyze the oxidation of dispersed lipids at the oil–water interface.

Properties of the lipid interface can be an important determinant in the rate of oxidation of lipids dispersed in water. Several authors have found that surface charge can influence the oxidation of lipids in micellar systems. Yoshida and Niki (1992) found that both iron and copper were capable of oxidizing methyl linoleate in negatively charged sodium dodecyl sulfate (SDS) micelles but not in positively charged tetradecyltrimethylammonium bromide (TTAB) micelles. Inhibition of linoleate oxidation by TTAB could be overcome by addition of tert-butyl hydroperoxide to the micelle system. Fukuzawa and Fujii (1992) reported that ferrous ions could catalyze the formation of alkoxyl radicals from linoleic acid hydroperoxides in SDS but not TTAB micelles. Oxidation of the TTAB micelles could be stimulated by iron in the presence of nitilotriacetic acid, a negatively charged iron chelator.

While research has been conducted on the influence of surface charge on the oxidation of lipids in micelles, most surfactant stabilized lipid dispersions in foods are found as emulsion droplets. Therefore, the objective of this research was to determine if the surface charge of emulsified corn oil would influence iron-catalyzed oxidation rates. The influence of pH and ionic strength on these reactions was also determined. By gaining a better understanding of how surface charge influences iron-catalyzed oxidation, we hope to identify techniques to increase the oxidative stability of emulsified oils.

## MATERIALS AND METHODS

Corn oil (tocopherol stripped) was purchased from ICN Biomedicals. Dodecyltrimethylammonium bromide (DTAB), Brij 35, sodium dodecyl sulfate (SDS) and 4,6-dihydroxy-2-mercaptopyrimidine (2-thiobarbituric acid; TBA) were obtained from Aldrich Chemical Company (Milwaukee, WI). L-Ascorbic acid and imidazole were

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obtained from Fisher Scientific (Fair Lawn, NJ). 1,1,3,3-Tetraethoxypropane, hydrogen peroxide (30%), cumene hydroperoxide, and potassium iodide were obtained from Sigma Chemical Company (St. Louis, MO). All other chemicals were reagent grade or purer.

To make the emulsions, 28.5 g of a 0.017 M solution of selected surfactants were mixed with 1.5 g corn oil. The solution was sonicated for 2.5 min using a Braun-Sonic 2000 U ultrasonic generator (Braun Biotech, Allentown, PA, USA) equipped with a 5T standard probe at a power setting of +200 and a 0.3 s repeating duty cycle. A static light scattering technique (Horiba LA-900, Horiba Instruments, Irvine, CA) was used to determine emulsion droplet sizes (Weiss *et al.*, 1996). Average droplet diameters were between 1.1 and 1.2 μm. Emulsion droplet sizes did not change during the course of the oxidation experiments.

Lipid oxidation in the emulsion system was catalyzed using an iron-ascorbic acid redox cycling system (Decker & Hultin, 1990). Buffer solution (acetate or imidazole) and ascorbic acid were added to the emulsion at a final concentration of 10 mM and 150 μM, respectively. The pH of the solution was adjusted to desired values, $FeCl_3$ (50 μM, final concentration) was immediately added and the emulsion was allowed to oxidize at 25°C. Lipid oxidation was measured by determining lipid peroxides (LOOH), thiobarbituric acid reactive substances (TBARS), and conjugated dienes (CD).

Lipid peroxides were quantitated using a modified method of Lovaas (1992). At various time intervals, emulsion (40 μl) was added to 2.7 ml of methanol/butanol (2:1, v/v), 120 μl of 25 mM HCl in methanol, 120 μl of 12.5 mM $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$, and 80 μl of saturated KI solution. This final assay mixture was centrifuged at 2000 *g* for 3 min and the solution absorbance was measured at 363 nm, exactly 15 min after addition of the saturated KI solution. Concentrations of peroxides were determined from standard curves prepared using cumene hydroperoxide.

TBARS were measured according to McDonald and Hultin (1987). A TBA solution was prepared by mixing 15 g trichloroacetic acid, 0.375 g TBA, 1.76 ml of 12 N HCl, and 82.9 ml $H_2O$. One hundred milliliters of TBA solution was mixed with 3 ml of 2% butylated hydroxytoluene in ethanol and 2 ml of this solution was mixed with 0.3 ml of emulsion and 0.7 ml $H_2O$. The mixture was heated in a boiling water bath for 15 min, cooled to room temperature using tap water, and centrifuged at 2000 *g* for 15 min. Absorbance was measured at 532 nm. Concentrations of TBARS were determined from standard curves prepared using 1,1,3,3-tetraethoxypropane.

Conjugated dienes were determined using a modified method of Huang *et al.* (1996). Emulsion samples (120 μl) were added to 2.7 ml of methanol/butanol (2:1, v:v) and absorbance was measured at 233 nm. Data were expressed as change in absorbance using unoxidized corn oil emulsions as the control.

All data represent the mean of four measurements of two different trials.

## RESULTS

Since it is possible that the charge of the buffer used could influence iron–surfactant interactions and therefore oxidation rates, the effect of cationic (imidazole) and anionic buffers on oxidation was determined (Table 1). Little changes in oxidation rates were observed for the different buffers over the concentration range of 0–20 mM. Since oxidation was not altered by buffer type or concentration, 10 mM buffer was used for all subsequent experiments.

Emulsions produced using three different surfactants showed different patterns of iron-catalyzed oxidation. Lipid oxidation markers were measured as a function of time (0–130 min) in order to monitor differences in oxidation kinetics. At pH 6.5 (Fig. 1), SDS emulsion oxidized rapidly during the first 20 min, after which time the oxidation rate became fairly constant as determined by LOOH, CD, and TBARS. The Brij emulsion initially had a slower oxidation rate than SDS, however, after 130 min, LOOH and CD concentration were similar to SDS emulsions. DTAB had the lowest rate of oxidation

**Table 1. Effect of imidazole and acetate buffers on lipid oxidation measured by thiobarbituric reactive substances (TBARS, mmol per kg oil) in SDS-, Brij 35- and DTAB-stabilized emulsions at pH 6.5 after 75 min of oxidation**

| Buffer (mM) | SDS | Brij 35 | DTAB |
|---|---|---|---|
| | | Imidazole | |
| 0 | 0.052 ± 0.001 | 0.028 ± 0.000 | 0.010 ± 0.000 |
| 5 | 0.048 ± 0.001 | 0.030 ± 0.000 | 0.009 ± 0.000 |
| 10 | 0.054 ± 0.001 | 0.028 ± 0.004 | 0.008 ± 0.001 |
| 20 | 0.042 ± 0.006 | 0.031 ± 0.001 | 0.008 ± 0.001 |
| 50 | 0.043 ± 0.001 | 0.032 ± 0.003 | 0.009 ± 0.001 |
| | | Acetate | |
| 0 | 0.041 ± 0.001 | 0.030 ± 0.000 | 0.009 ± 0.001 |
| 5 | 0.043 ± 0.003 | 0.034 ± 0.001 | 0.011 ± 0.001 |
| 10 | 0.045 ± 0.001 | 0.035 ± 0.000 | 0.011 ± 0.001 |
| 20 | 0.040 ± 0.002 | 0.035 ± 0.001 | 0.012 ± 0.000 |
| 50 | 0.041 ± 0.004 | 0.034 ± 0.001 | 0.014 ± 0.001 |

Data represent mean ± standard deviation ($n = 4$).

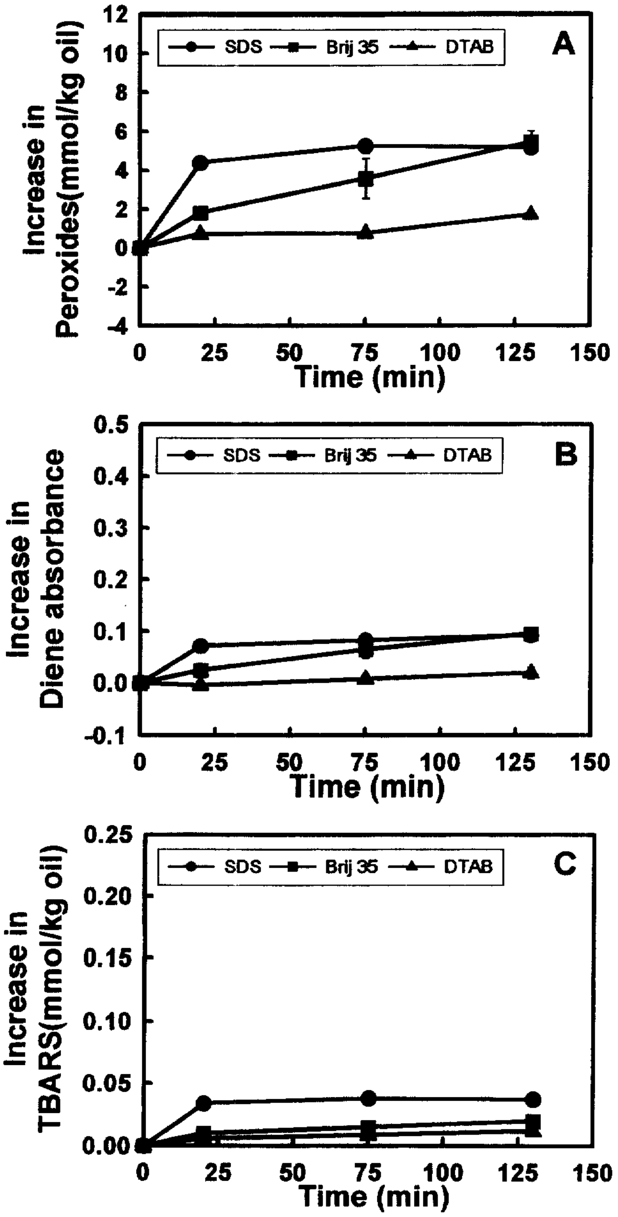

**Fig. 1.** Formation of lipid peroxides (A), conjugated dienes (B), and TBARS (C) in SDS-, Brij 35-, and DTAB-stabilized emulsions in imidazole buffer at pH 6.5.

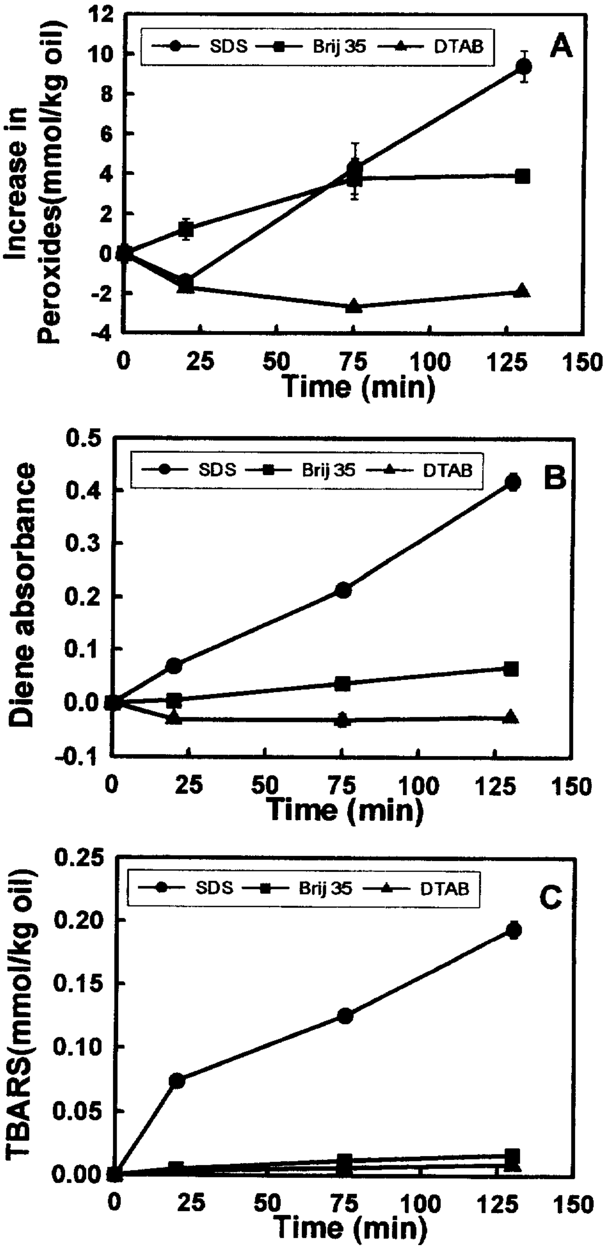

**Fig. 2.** Formation of lipid peroxides (A), conjugated dienes (B), and TBARS (C) in SDS-, Brij 35-, and DTAB-stabilized emulsions in acetate buffer at pH 4.

of all the emulsions with LOOH, CD, and TBARS being 37, 35 and 61% lower than SDS emulsions after 130 min of incubation.

At pH 4 (Fig. 2). LOOH of the SDS emulsions decreased during the first 20 min but rapidly increased thereafter, whereas CD and TBARS increased continuously from time zero. CD and TBARS formation in the SDS-stabilized emulsions were much higher than those of Brij and DTAB emulsions during the entire incubation. While much lower than SDS, Brij had a rate of oxidation higher than DTAB emulsions. DTAB again had the lowest oxidation rate with LOOH, CD, and TBARS being only 20, 16 and 7% of those of SDS emulsions after 130 min of incubation. The observed decrease in lipid peroxides during the early stages of oxidation in both SDS and DTAB emulsions may be due to the breakdown of pre-existing peroxides in the corn oil.

To better understand how pH and surface charge influenced iron-catalyzed oxidation of emulsified corn oil, LOOH and TBARS formation were determined at pH 3–8 after 75 min of incubation of the different emulsions in imidazole buffer (Fig. 3). Lipid peroxide and TBARS formation were not influenced by pH in the Brij and DTAB emulsions. For the SDS emulsion, LOOH and TBARS were lowest at pH 8 and increased slowly from pH 8 to 6, with both LOOH and TBARS being 1.14-fold higher at pH 6 compared with pH 8. Further decreasing pH from 6 to 3 increased TBARS formation 1.46-fold. LOOH increased 1.85-fold from pH 6 to 5, but decreased from pH 5 to 3, with a LOOH at pH 3 only 1.32-fold higher than at pH 6. Oxidation of the emulsions in acetate buffer (data not shown) showed similar pH effects as was observed with imidazole buffer.

Oxidation of the SDS emulsions was investigated in greater detail at pH 3 and 5 to determine why lower LOOH were observed at pH < 5. As shown in Fig. 4, LOOH was higher at pH 3 than pH 5 during the first 50 min of oxidation. As oxidation continued, the LOOH concentration at pH 5 surpassed that of pH 3. TBARS continued to increase during the entire 180 min of oxidation, with greater TBARS formation being observed at pH 3. The TBARS data indicate that oxidation rates were higher at pH 3. The low peroxide concentrations observed in the low pH (< 5) SDS emulsion after 75 min of incubation (Fig. 4) therefore seem to be due to an increase in LOOH decomposition rates, and not a decrease in lipid oxidation.

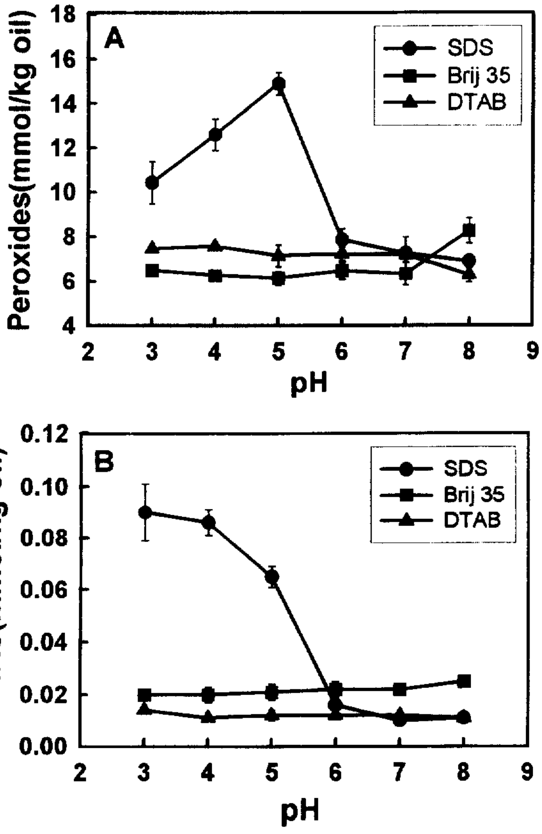

Fig. 3. Formation of LOOH (A) and TBARS (B) as a function of pH (3–8) in SDS-, Brij 35-, and DTAB-stabilized emulsions in imidazole buffer oxidized for 75 min.

NaCl decreased oxidation in the SDS emulsion at pH 6.5 with imidazole buffer (Table 2). Both LOOH and TBARS were approximately 20% lower in the presence of 0.173 M (1%) NaCl than in the absence of NaCl after 75 min of incubation. Addition of NaCl 0–0.173 M had no significant influence on LOOH and TBARS formation in Brij and DTAB emulsions at pH 6.5. The inability to observe NaCl effects on DTAB emulsions could be due to low oxidation rates.

## DISCUSSION

The activity of iron, a major lipid oxidation catalyst in foods, has been shown to be influenced by the surface charge of linoleic acid dispersed in a micelle system (Yoshida & Niki, 1992; Fukuzawa & Fujii, 1992). While micelles are often used experimentally, dispersed lipids in foods are more likely to exist as emulsion droplets. Interactions between iron and the surface of the dispersed lipid could be similar in both emulsions and micelles. However, several important differences exist between the micelle systems used by Yoshida and Niki (1992) and Fukuzawa and Fujii (1992) and emulsions including chemical structure of the lipid (triacylglycerols vs free and methylated fatty acids) and the fact that the majority of the lipid in emulsion droplets exist in the droplet interior (Coupland & McClements, 1996) thereby decreasing its accessibility at the interfacial surface. To better understand how surface charge of emulsion droplets can influence iron-catalyzed lipid oxidation, corn oil emulsion droplets were prepared using cationic [DTAB, $CH_3(CH_2)_{11}N(CH_3)_3Br$], anionic [SDS, $CH_3(CH_2)_{11}OSO_3Na$] and nonionic [Brij 35, $CH_3(CH_2)_{11}(OCH_2CH_2)_{23}OH$] surfactants. In order to

**Table 2. Effect of NaCl on formation of lipid peroxides (LOOH) and thiobarbituric reactive substances (TBARS) in SDS-, Brij 35-, and DTAB-stabilized emulsions in imidazole buffer at pH 6.5 after 75 min of oxidation**

| NaCl (mM) | SDS | Brij 35 | DTAB |
|---|---|---|---|
| | | LOOH (mmol per kg oil) | |
| 0.0 | 8.56 ± 0.29 | 7 ± 0.23 | 5.22 ± 0.27 |
| 8.6 | 8.07 ± 0.38 | 7 ± 0.02 | 5.30 ± 0.15 |
| 17.3 | 7.96 ± 0.29 | 7 ± 0.20 | 5.09 ± 0.15 |
| 86.5 | 6.93 ± 0.45 | 7 ± 0.13 | 5.30 ± 0.03 |
| 173.0 | 6.97 ± 0.51 | 7.10 ± 0.31 | 5.24 ± 0.06 |
| | | TBARS (mmol per kg oil) | |
| 0.0 | 0.030 ± 0.000 | 0.022 ± 0.001 | 0.011 ± 0.000 |
| 8.6 | 0.028 ± 0.002 | 0.022 ± 0.001 | 0.011 ± 0.001 |
| 17.3 | 0.028 ± 0.001 | 0.022 ± 0.001 | 0.011 ± 0.000 |
| 86.5 | 0.026 ± 0.002 | 0.023 ± 0.001 | 0.011 ± 0.000 |
| 173.0 | 0.025 ± 0.001 | 0.022 ± 0.001 | 0.012 ± 0.000 |

Data represent mean ± standard deviation ($n = 4$).

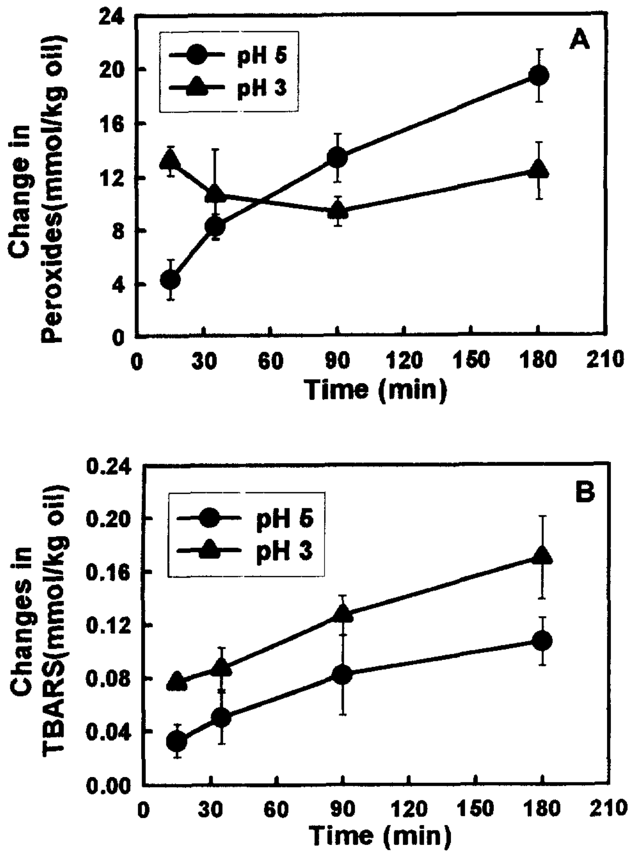

**Fig. 4.** Formation of LOOH (A) and TBARS (B) in SDS-stabilized emulsions in acetate buffer at pH 3 and 5.

minimize differences in surfactant properties, such as packing density, the surfactants chosen all contained 12 carbon saturated hydrocarbon tail groups. Since emulsion droplet size might also influence oxidation rates, all emulsions were produced under conditions that gave similar average droplet diameters (1.1–1.2 $\mu$m).

Iron-catalyzed oxidation was observed in both SDS and Brij emulsions at pH 6.5 and 4.0 but very little oxidation was detected with DTAB (Figs 1 and 2). Iron most likely catalyzes the oxidation of corn oil emulsions by promoting the breakdown of pre-existing lipid peroxides into radical species. Triacylglycerol peroxides are predominately hydrophobic molecules and would be expected to be contained within the oil phase. Nevertheless, these peroxides are more polar than unoxidized triacylglycerols and thus could accumulate near the inner surface of the emulsion droplets. Therefore, any factors which increase iron concentrations at the interface of lipid emulsions would be expected to increase oxidation rates. The low amount of iron-catalyzed oxidation observed in DTAB emulsions suggests that the positively charged emulsion droplets inhibited iron–lipid interactions, presumably by decreasing the ability of iron to interact with the emulsion interface through Coulombic repulsive forces. Conversely, the greater oxidation rates in SDS emulsions could be due to increased interfacial iron concentrations owing to attractive Coulombic forces. Inhibition of iron-catalyzed lipid oxidation by cationic surfactants and acceleration by anionic surfactants has also been observed in lipid micelle systems (Yoshida & Niki, 1992; Fukuzawa & Fujii, 1992).

Lipid oxidation was observed in Brij emulsions, suggesting that iron was capable of interacting with the lipid. Iron binding could occur through nonionic interactions with electrophilic oxygens in the Brij polar head groups. The lower oxidation rates observed in Brij emulsions compared with SDS suggests that the Brij polar head groups did not increase iron–lipid interactions to the same extent as SDS, probably because ion–dipole interactions are considerably weaker than ion–ion interactions.

Decreasing pH accelerated iron-catalyzed lipid oxidation in SDS emulsions but did not increase oxidation in the Brij and DTAB emulsions (Fig. 3). In addition to changes in the extent of oxidation, the oxidation pattern also changed. At pH 6.5 almost no oxidation was observed after 20 min of incubation. At pH 4.0, formation of oxidation products continued to increase for the entire 130 min of incubation. Increases in oxidation at low pH in the SDS emulsion could be due to increased iron solubility (Graf *et al.*, 1984) and/or ascorbate stability (Vanderslice & Higgs, 1993) or could be due to increased ascorbate interactions at the interface since decreasing pH would decrease ascorbate repulsion by SDS (ascorbate $pK_a = 4.04$; Dawson *et al.*, 1987).

The influence of NaCl on the iron-catalyzed oxidation of the different emulsions may also be explained from differences in iron–lipid interactions. Table 2 shows that NaCl decreased oxidation of the SDS emulsions but had little effect on Brij and DTAB. At its highest concentration (173 mM), NaCl was over 10 times greater than surfactant and over 3000 times greater than iron concentrations. Therefore, NaCl inhibition of oxidation could be due to decreased iron–lipid interactions possibly through the ability of sodium to decrease iron binding at the droplet surface or by formation of iron–chloride complexes which decreased iron binding. NaCl did not increase oxidation in the DTAB emulsion, indicating that neither sodium nor chloride was capable of altering the surface or iron charges in a manner that increased iron–lipid interactions. Even if NaCl decreased DTAB's iron repulsive forces, it is possible that no iron binding sites were available to increase iron interactions at the interface. Fukuzawa and Fujii (1992) found that the most effective way to overcome the iron repulsive forces of a cationic surfactant was to use a negatively charged iron chelator which allowed the binding of iron to the lipid interface.

## CONCLUSIONS

These results suggest that if iron is a lipid oxidation catalyst in a food emulsion, its activity would be influenced by the charge of the lipid droplets. Therefore, utilization of positively charged emulsifiers could be an effective way to control iron-catalyzed lipid oxidation. While cationic surfactants are not common in foods, it is possible that protein stabilized emulsions, at pHs below the pI could be used to decrease iron-catalyzed lipid oxidation. Reduction of iron-catalyzed oxidation by NaCl also suggests that ions which compete for iron binding on emulsion interfaces could be used to decrease oxidation rates.

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