Ability of Surfactant Headgroup Size To Alter Lipid and Antioxidant Oxidation in Oil-in-Water Emulsions

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Oxidation of oil-in-water emulsion droplets is influenced by the properties of the interfacial membrane surrounding the lipid core. To evaluate how surfactant headgroup size influences lipid oxidation rates, emulsions were prepared with polyoxyethylene 10 stearyl ether (Brij 76) or polyoxyethylene 100 stearyl ether (Brij 700), which are structurally identical except for their hydrophilic headgroups, with Brij 700 containing 10 times more polyoxyethylene groups than Brij 76. Fe^{2+}-promoted decomposition of cumene hydroperoxide was lower in Brij 700-stabilized than in Brij 76-stabilized hexadecane emulsions. Fe^{2+}-promoted α-tocopherol oxidation rates were similar in hexadecane emulsion regardless of surfactant type. Brij 700 decreased production of hexanal from methyl linoleate and the formation of lipid peroxides and propanal from salmon oil compared to emulsions stabilized by Brij 76. These results indicate that emulsion droplet interfacial thickness could be an important determinant in the oxidative stability of food emulsions.

Keywords: *Lipid oxidation; emulsions; iron; surfactants; fish oil; antioxidants*

INTRODUCTION

Numerous foods contain lipids dispersed in water as membrane bilayers or emulsion droplets. The fact that oxidative reactions in dispersed lipids are mechanistically different from bulk oils has been the subject of several recent publications (Frankel et al., 1994; Huang et al., 1996a,b; Heinonen et al., 1998; Mei et al., 1998a,b; Mancusco et al., 1999, 2000; Pekkarinen et al., 1999). One of the potential mechanisms that differs in the bulk and emulsified oils is the large surface area of the emulsified lipids, which presents a situation in which water-soluble prooxidants can readily interact with lipids.

Oil-in-water emulsions contain three distinct regions: a lipid core, droplet interfacial membrane, and continuous phase. Recent work in our laboratory has shown that the oxidation of oil-in-water emulsions involves the decomposition of lipid peroxides by transition metals, with iron being the most important prooxidant metal (Mancuso et al., 1999a). The interaction of continuous phase iron with lipid-containing emulsion droplets is influenced by the properties of the emulsion droplet interfacial membrane. Oil-in-water emulsions prepared with anionic, cationic, and nonionic surfactants have various susceptibilities to oxidation. Lipid peroxides are more rapidly broken down by Fe^{2+} in anionic emulsion droplets prepared with sodium dodecyl sulfate (SDS) than in emulsions stabilized with nonionic (polyoxyethylene 10 lauryl ether; Brij) or cationic (dodecyltrimethylammonium bromide; DTAB) surfactants (Mei et al., 1998a; Mancuso et al., 1999a,b). Although Fe^{2+} can promote decomposition of lipid peroxides in anionic, cationic, and nonionic emulsion droplets, Fe^{3+} promotes peroxide decomposition only in anionic emulsion droplets (Mancuso et al., 1999). This increased reactivity of iron is believed to be because of the electrostatic attraction between the positively charged metal and the negatively charged emulsion droplet membrane. Oxidation of emulsified corn and salmon oil behaves very similarly to the stability of lipid peroxides with oxidation rates being greatest in emulsion droplets stabilized with anionic SDS compared to cationic DTAB and nonionic Brij or Tween 20 (Mei et al., 1998b; Mancuso et al., 1999). The impact of surface charge on the oxidative stability of oil-in-water emulsions is also observed in whey protein stabilized emulsions, with oxidation rates being greatest when the pH is less than the p*I* of the protein, and thus the emulsion droplet is negatively charged (Donnelly et al., 1998).

Although several studies have shown that emulsion droplet charge is an important factor in the oxidative stability of emulsified oil, very little is known about how other emulsion droplet interfacial membrane properties impact oxidation rates. Therefore, the purpose of this research was to use two different surfactants that varied in hydrophilic headgroup size to evaluate their impact on the stability of lipid peroxides and antioxidants as well as the oxidation of salmon oil. This was accomplished by preparing emulsions with polyoxyethylene 10 stearyl ether (Brij 76) or polyoxyethylene 100 stearyl ether (Brij 700), which are structurally identical except for hydrophilic headgroups, with Brij 700 containing 10 times more polyoxyethylene groups than Brij 76.

EXPERIMENTAL PROCEDURES

Materials. Salmon oil was isolated by centrifuging minced salmon muscle (Mei et al., 1998b). Hexadecane, cumene hydroperoxide, polyoxyethylene 10 stearyl ether (Brij 76),

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10.1021/jf991162l CCC: $19.00
Published on Web 05/26/2000

polyoxyethylene 100 stearyl ether (Brij 700), α-tocopherol, ferrous sulfate, imidazole, sodium acetate, and methyl linoleate were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade or purer.

Methods. *Preparation of Emulsions.* Emulsions were prepared by mixing either hexadecane or salmon oil with aqueous solutions of 17 mM polyoxyethylene 10 stearyl ether (Brij 76) or polyoxyethylene 100 stearyl ether (Brij 700) in an acetate/imidizole buffer solution (5 mM each; pH 7.0) to give a final concentration of 5% lipid. Cumene hydroperoxide (22 mmol/L of emulsion), cumene hydroperoxide (5.7 mmol/L of emulsion) plus methyl linoleate (0.1 mmol/L of emulsion), or cumene hydroperoxide (5.7 mmol/L of emulsion) plus α-tocopherol (80 μmol/L of emulsion) were added to hexadecane prior to sonication. Salmon oil emulsions were prepared with and without 140 μM α-tocopherol. Solutions were sonicated 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 cycle (Mei et al., 1998a). Emulsions were sonicated for different times (hexadecane, Brij 76 = 2.5 min and Brij 700 = 1.5 min; salmon oil, Brij 76 = 3.5 min and Brij 700 = 2.5 min) to obtain mean emulsion droplet diameters of 0.2 ± 0.03 μm. Particle size distributions were measured using a Horiba LA-900 laser scattering particle size distribution analyzer (Horiba Instruments, Irvine, CA) (Weiss et al., 1996). Particle size distributions were measured periodically and did not change over the course of the experiments, indicating that coalescence or Oswald ripening did not occur. The sonication conditions used did not alter initial concentrations of peroxides, tocopherol, hexanal, or propanal. Hexadecane emulsions were oxidized at 55 °C in the presence or absence of 500 μM ferrous sulfate. Salmon oil emulsions were oxidized at 32 °C in the dark.

Determination of Cumene Hydroperoxide Concentrations in Hexadecane Emulsions. Cumene hydroperoxide concentrations in the hexadecane emulsions were determined by adding 0.3 mL of the emulsions to 1.5 mL of isooctane/isopropanal (3:2, v/v), followed by vortexing three times for 10 s each. After centrifugation for 2 min at 2000*g*, 0.2 mL of the clear upper layer was collected and peroxides were quantitated using a modified method of Shantha and Decker (1994). The sample extract (0.2 mL) was mixed with 2.8 mL of methanol/1-butanol (2:1, v/v) and 30 μL of thiocyanate/Fe^{2+} solution and then vortexed. The thiocyanate/Fe^{2+} solution was made by mixing one part of 3.94 M thiocyanate solution with one part of 0.072 M Fe^{2+} solution (obtained from the supernatant of a mixture of one part of 0.144 M $FeSO_4$ and one part of 0.132 M $BaCl_2$ in 0.4 M HCl). After 20 min of incubation at room temperature, absorbance was measured at 510 nm. Peroxide concentrations were determined using a cumene hydroperoxide standard curve.

Determination of α-Tocopherol Concentrations in Hexadecane Emulsions. α-Tocopherol concentrations in the hexadecane emulsions were determined by vortexing 0.1 mL of emulsions with 1.0 mL of hexane three times for 10 s each. The mixture was then centrifuged at 2000*g* for 1 min, and the solvent layer was collected. Two subsequent wash steps were repeated with 1.0 mL of hexane followed by centrifugation and collection of the solvent layer. Pooled solvent was evaporated under nitrogen to dryness and reconstituted with 0.1 mL of hexane. α-Tocopherol concentrations were determined by HPLC using a Waters 510 pump and a Waters 470 fluorescence detector (Milford, MA). A Hypersil C18, 5 μm column (250 × 4.6 mm; Alltech, Avondale, PA) was used with an acetonitrile/methanol (3:1, v/v) mobile phase at 1.5 mL/min. Wavelengths for detection were 298 for excitation and 340 for emission (Hatam and Kayden, 1979). Concentrations were determined from peak areas using a standard curve made from authentic α-tocopherol.

Determination of Hexanal Concentrations in Hexadecane Emulsions. Hexanal concentrations in the hexadecane emulsions containing methyl linoleate were determined by sealing 1.0 mL of the emulsion samples in 10-mL headspace vials that were then sealed with poly(tetrafluoroethylene) (PTFE)/butyl rubber septa using a crimper and aluminum seals. Headspace hexanal was determined using a Hewlett-Packard (HP) 5890 gas chromatograph (Avondale, PA) with an HP 19395A headspace sampler and coupled to an HP 3392A integrator. The headspace conditions were as follows: sample temperature, 55 °C; sample loop and transfer line temperature, 110 °C; pressurization, 10 s; venting, 10 s; injection, 1 min. The aldehydes were separated isothermally at 65 °C on an 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 eluted compounds were detected with a flame ionization detector at 250 °C. Concentrations were determined from peak areas using a standard curve made from authentic hexanal in solutions containing Brij 76 or 700 micelles (17.0 mM).

Determination of Lipid Peroxides and Propanal in Salmon Oil Emulsions. Lipid oxidation in the salmon oil-in-water emulsions was determined by measuring headspace propanal using the same conditions as described for headspace hexanal above with the exception of the following: sample loop and transfer line temperature, 100 °C; oven temperature, 70 °C; detector temperature, 200 °C. Concentrations were determined from peak areas using a standard curve made from authentic propanal. Lipid peroxides in the salmon oil emulsions were measured according to 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/isopropanal followed by vortexing three times for 10 s each and centrifuging for 2 min at 2000*g*. Next, organic phase (0.2 mL) was added to 2.8 mL of methanol/butanol (2:1, v/v), followed by 15 μL of thiocyanate solution (3.94 M) and 15 μL of 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.

Statistical Analysis. Assays were measured in triplicate. Statistical analysis was performed using analysis of variance. Means separations were achieved using Duncan's multiple-range test (Snedecor and Cochran, 1989).

RESULTS

The impact of emulsion droplet interfacial thickness on the ability of Fe^{2+} to break down cumene hydroperoxide was studied in emulsions prepared with hexadecane. Hexadecane was used as a nonoxidizable lipid to prevent the formation of additional peroxides that would have occurred in emulsions containing food oils. The stability of cumene hydroperoxide in hexadecane emulsions has previously been shown to be very similar to that of trilaurin, suggesting that it represents a good model lipid system to study lipid peroxide−iron interactions (Mancuso et al., 1999b). Cumene hydroperoxide concentrations in Brij 76- and Brij 700-stabilized emulsions decreased during storage in the absence of added Fe^{2+} (Figure 1) with no significant differences occurring between different surfactants. Addition of Fe^{2+} significantly ($p \leq 0.05$) increased cumene hydroperoxide decomposition (compared to no added Fe^{2+} controls) in both the Brij 76- and Brij 700-stabilized emulsions (Figure 1). Fe^{2+} promoted cumene hydroperoxide decomposition more quickly in the Brij 76-stabilized (22−29% more decomposition than no iron controls) than in the Brij 700-stabilized (3−10% more than no iron controls) emulsions.

Upon decomposition of lipid peroxides, the subsequent free radicals can attack unsaturated fatty acids or antioxidants. To evaluate how emulsion droplet interfacial thickness could impact the ability of free radicals originating from Fe^{2+}−cumene hydroperoxide interactions to oxidize antioxidants, α-tocopherol oxidation was monitored by measuring concentration changes by

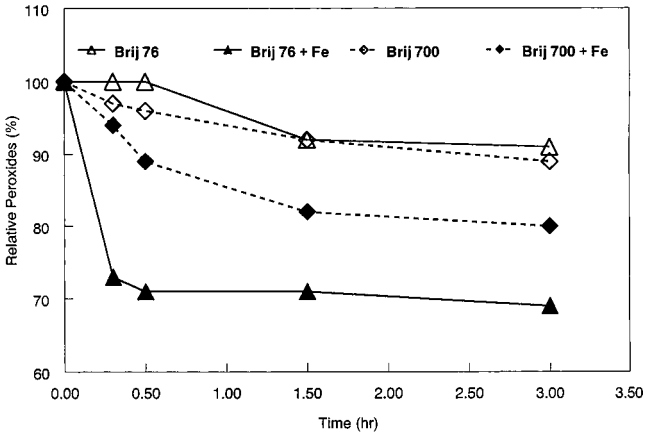

Figure 1. Decomposition of cumene hydroperoxide (initial concentrations ≈ 22 mmol/L of emulsion) in hexadecane emulsions stabilized by Brij 76 or Brij 700 at pH 7.0 and 55 °C in the presence or absence of ferrous sulfate (500 μM). Data points represent means ($n = 3$) ± standard deviations.

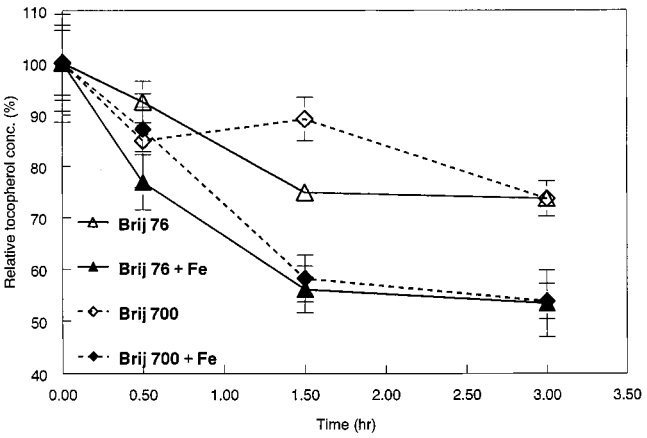

Figure 2. Changes in α-tocopherol concentrations (initial concentrations ≈ 80 μmol/L of emulsion) in hexadecane emulsions stabilized by Brij 76 or Brij 700 at pH 7.0 and 55 °C in the presence or absence of ferrous sulfate (500 μM). Emulsions contained 5.7 mmol of cumene hydroperoxide/L of emulsion. Data points represent means ($n = 3$) ± standard deviations.

HPLC. α-Tocopherol concentrations were observed to decrease in the absence of added Fe^{2+} (Figure 2), with no significant differences being observed between the surfactants. Fe^{2+} significantly ($p \leq 0.05$) increased α-tocopherol oxidation, compared to no added iron controls, after 0.5 h of incubation in the Brij 76-stabilzed emulsions. No significant differences ($p \leq 0.05$) in α-tocopherol oxidation were observed between the surfactants in the presence of Fe^{2+}.

To assess how emulsion droplet interfacial thickness impacts the ability of free radicals originating from cumene hydroperoxide−Fe^{2+} interactions to oxidize fatty acids, emulsions were prepared with cumene hydroperoxide and methyl linoleate. Oxidation of methyl linoleate was monitored by measuring headspace hexanal, a common breakdown product of the oxidation of linoleic acid. For both surfactants, hexanal concentrations increased ($p \leq 0.05$) in the absence of added Fe^{2+} (Figure 3**),** with hexanal formation being lower in the Brij 700-stabilized than in the Brij 76-stabilized emulsions ($p \leq 0.05$). In both surfactant systems, Fe^{2+} significantly increased hexanal concentrations, with Brij 700-stabilized emulsions again having less hexanal formation than Brij 76-stabilized emulsions.

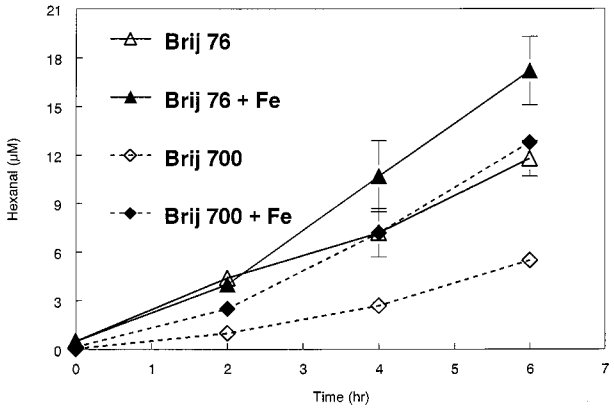

Figure 3. Formation of hexanal in hexadecane and methyl linoleate (0.1 mmol/L of emulsion) emulsions stabilized by Brij 76 or Brij 700 at pH 7.0 and 55 °C in the presence or absence of ferrous sulfate (500 μM). Emulsions contained 5.7 mmol of cumene hydroperoxide/L of emulsion. Data points represent means ($n = 3$) ± standard deviations.

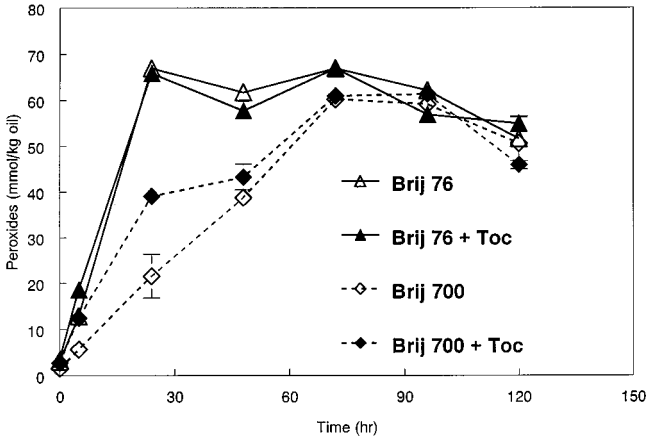

Figure 4. Formation of lipid peroxide in salmon oil emulsions stabilized by Brij 76 or Brij 700 at pH 7.0 and 32 °C. Data points represent means ($n = 3$) ± standard deviations.

The impact of emulsion droplet interfacial thickness on lipid oxidation in salmon oil emulsions stabilized with Brij 76 or Brij 700 at pH 7.0 in the absence of added iron was determined by monitoring the formation of lipid peroxides and headspace propanal (a common lipid oxidation product of $n-3$ fatty acids). Formation of lipid peroxides was significantly ($p \leq 0.05$) lower in the Brij 700-stabilized than in the Brij 76-stabilized emulsions during the first 96 h of oxidation, after which time peroxide concentrations were the same (Figure 4). α-Tocopherol did not decrease lipid peroxide formation in either the Brij 76- or Brij 700-stabilized emulsions. Propanal formation was significantly lower in the Brij 700-stabilized than in the Brij 76-stabilized emulsions during the entire incubation period (Figure 5). α-Tocopherol inhibited propanal formation but was less effective in the Brij 76-stabilized emulsion (significant decrease in propanal at 5, 25, and 72 h of incubation with decrease ranging from 2 to 32%; $p \leq 0.05$) than in the Brij 700-stabilized emulsion (significant decrease in propanal at 5, 24, 48, and 72 h with decrease ranging from 38 to 60%; $p \leq 0.05$). The observation that α-tocopherol had no effect on lipid peroxide formation but inhibited propanal formation in the salmon oil emulsions is likely due to the antioxidant mechanism of α-tocopherol that involves the donation of hydrogen to peroxyl radicals resulting in the formation of lipid

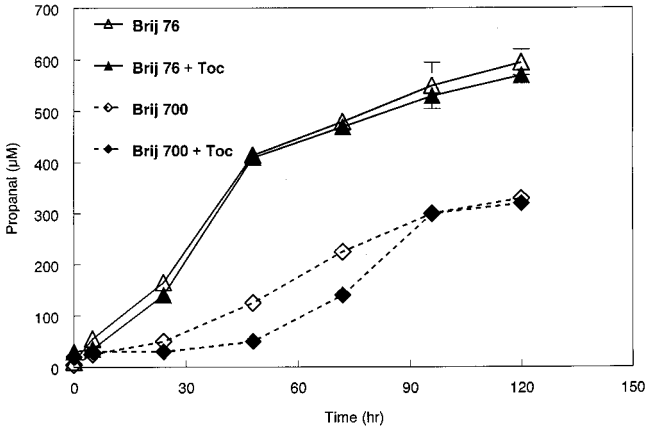

Figure 5. Formation of headspace propanal in salmon oil emulsions stabilized by Brij 76 or Brij 700 at pH 7.0 and 32 °C. Data points represent means ($n = 3$) ± standard deviations.

peroxides (thus, no net decrease in peroxide formation) and inhibition of the formation of secondary lipid oxidation products (thus decreasing propanal formation; Frankel, 1998).

DISCUSSION

Oxidation of cumene hydroperoxide, α-tocopherol, methyl linoleate, and salmon oil occurred in the absence of added iron in both the Brij 76- and Brij 700-stabilized emulsions (Figures 1−5). The observed oxidation in the absence of iron in these experiments is similar to earlier work in our laboratory in which oxidation in salmon oil emulsions in the absence of added iron was inhibited by EDTA and transferrin, suggesting that "autoxidation" was promoted by endogenous iron (Mancuso et al., 1999a).

Addition of Fe^{2+} resulted in an acceleration of cumene hydroperoxide, α-tocopherol, and methyl linoleate oxidation. Fe^{2+} primarily decreased cumene hydroperoxide concentrations during the first 30 min of incubation with smaller changes in peroxide concentrations occurring over the subsequent 2.5 h of incubation. This trend of rapid decrease in cumene hydroperoxide concentrations followed by little to no subsequent change during prolonged storage has also been observed in emulsions stabilized by Tween 20 and is likely due to the rapid conversion of Fe^{2+} to Fe^{3+}, with the latter being unable to promote peroxide breakdown (Mei et al., 1998b; Mancuso et al., 1999b). Fe^{2+} did not decrease α-tocopherol concentrations (Figure 2) and increase hexanal formation (Figures 3) as quickly as was observed for changes in cumene hydroperoxide concentrations. This could be due to the necessary sequence of the chemical reactions involved because cumene hydroperoxide would first need to break down into free radicals (Figure 6; reaction 1) that in turn would oxidize α-tocopherol or methyl linoleate (Figure 6; reactions 2a and 2b, respectively).

Fe^{2+} promoted the decomposition of cumene hydroperoxide and methyl linoleate in the Brij 76-stabilized emulsions more rapidly than in the Brij 700-stabilized emulsions; differences between surfactants were less evident during α-tocopherol oxidation (Figures 1−3). Oxidation of salmon oil was faster in the Brij 76- than in the Brij 700-stabilized emulsions as determined by both lipid peroxide and headspace propanal formation (Figures 4 and 5). Lower cumene hydroperoxide, methyl linoleate, and salmon oil oxidation rates in the Brij 700-

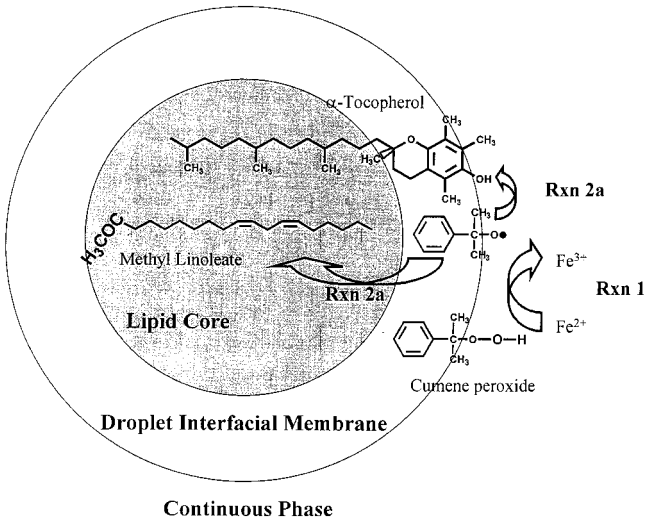

Figure 6. Schematic showing potential reaction pathways 1 and 2 and partition of the different lipids in oil-in-water emulsions. Components are not drawn to scale.

stabilized emulsions indicate that the larger surfactant headgroup was able to decrease lipid oxidation reactions. Many compounds can inactivate high-energy radicals such as those produced from the decomposition of lipid peroxides. Therefore, it is possible that the large polar headgroups of Brij 700 were decreasing methyl linoleate and salmon oil oxidation by being more effective at inactivating cumene hydroperoxide-derived free radicals than Brij 76. However, if inactivation of free radicals by the surfactant headgroups was occurring, then Brij 700 should also have been able to protect α-tocopherol from oxidation. In addition, there should not have been any differences in cumeme hydroperoxide decomposition rates between the surfactants (Figure 1) because this step is not influenced by free radical inactivation.

The polarity of the lipids used in these experiments could help to explain the differences observed in their oxidation rates in emulsions stabilized by the different surfactants. An oil-in-water emulsion contains three distinct regions: the lipid core, droplet interfacial membrane (surrounding the lipid core), and the continuous phase (surrounding the interfacial membrane). Lipid peroxides and α-tocopherol are more polar than methyl linoleate and salmon oil triacylglycerols. These polarity differences would result in greater partitioning of cumene hydroperoxide and α-tocopherol near or in the emulsion droplet interfacial membrane, with methyl linoleate and salmon oil triacylglycerols partitioning more in the lipid core (Figure 6).

The free radicals produced in the emulsion system used in this research would originate from the interaction of cumene hydroperoxide with Fe^{2+} (Figure 6; reaction 1). The slower decomposition of cumene hydroperoxide in the Brij 700-stabilized than in the Brij 76-stabilized emulsion suggests that greater concentrations of cumene hydroperoxide were found near the continuous phase−interfacial membrane boundary in the Brij 76-stabilized emulsions, where it could more readily react with continuous phase iron. When lipid peroxides are decomposed, the resulting radicals will migrate to the interfacial membrane boundary, where they are strongly solvated by water (Barclay, 1992). The inability of the large hydrophilic headgroup of Brij 700 to protect α-tocopherol from oxidation could be due to the parti-

tioning of α-tocopherol in the same region as the cumene hydroperoxide-derived radicals, thus exposing the α-tocopherol to similar levels of free radical attack in both surfactant systems (Figure 6; reaction 2a). No differences in α-tocopherol oxidation rates between the different surfactant systems were observed despite the fact that cumene hydroperoxide was being decomposed more rapidly (and presumably free radicals were being produced more rapidly). This suggests that free radical concentrations were high enough in both surfactant systems to not limit the rate of free radical−α-tocopherol interactions.

Methyl linoleate and salmon oil, which are more nonpolar than α-tocopherol, would have greater partitioning into the lipid core or the inner regions of the interfacial membrane. For cumene hydroperoxide-derived free radicals to react with methyl linoleate, these radicals would have to migrate through the interfacial membrane to the lipid core (Figure 6; reaction 2b). This would also occur in the salmon oil emulsions, in which free radicals originating from salmon oil peroxides (0.12 μmol of peroxide/g of oil) in the emulsion droplet interfacial membrane would not react as readily with the triacylglycerols in the lipid core in the Brij 700-stabilized emulsion. Although the physical location of lipids provides a potential hypothesis to help explain why increased interfacial thickness can decrease lipid oxidation, more detailed information of the partitioning behavior of oxidizable lipids in emulsions is needed.

In conclusion, the characteristics of surfactant polar headgroups can be an important factor in the oxidative stability of oil-in-water emulsions. This research suggests that the thickness of the emulsion droplet interfacial membrane could be an important determinant in the ability of lipid peroxides to oxidize fatty acids. Although nonionic surfactants with large polar headgroups (such as the Brij 700 used in this study) are not available as food additives, protein-stabilized oil-in-water emulsions can exhibit large differences in droplet interfacial membrane thickness. Research is currently underway to determine if protein-stabilized emulsions with thick interfacial membranes can be utilized to decrease lipid oxidation in food emulsions.

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Received for review October 25, 1999. Revised manuscript received March 15, 2000. Accepted March 22, 2000. This research was supported in part by Grant 9901521 from the NRI Competitive Grants Program/USDA. M.P.C.S. was supported by CNPq-Brazil.

JF991162L