JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Ability of Surfactant Micelles To Alter the Physical Location and Reactivity of Iron in Oil-in-Water Emulsion

YOUNG-JE CHO,† D. JULIAN MCCLEMENTS,‡ AND ERIC A. DECKER*,‡

Food Engineering, Sangju National University, Sangju, Gyeongbuk 742-711, Korea, and
Food Science, Chenoweth Laboratory, University of Massachusetts, Amherst, Massachusetts 01003

The purpose of this research was to determine how surfactant micelles influence iron partitioning and iron-promoted lipid oxidation in oil-in-water emulsions. Lipids containing ferric ions were used to produce oil-in-water emulsions, and continuous-phase iron concentrations in emulsions were measured as a function of varying continuous-phase polyoxyethylene 10-lauryl ether (Brij) concentrations. Continuous-phase iron concentrations increased with increasing surfactant micelle concentrations (0.1−2.0%) and storage time (1−7 days). At pH 3.0, the concentration of continuous-phase iron was higher than at pH 7.0. Similar trends in iron solubilization by Brij micelles were observed when either hexadecane or corn oil was used as the lipid phase. Lipid oxidation rates, as determined by the formation of lipid hydroperoxides and headspace hexanal, in corn oil-in-water emulsions containing iron decreased with increasing surfactant concentrations (0.5−2.0%). These results indicate that surfactant micelles could alter the physical location and prooxidant activity of iron in oil-in-water emulsions.

KEYWORDS: Emulsions; lipid oxidation; iron; micelles; solubilization; hexanal; hydroperoxides; surfactants; emulsifiers

INTRODUCTION

Emulsified lipids are one of the most common forms of lipids found in foods (*1*). In foods, these emulsified lipids are susceptible to oxidative deterioration leading to the formation of volatile off-flavors and deterioration of quality. Oxidation of emulsified lipids is dependent on many factors including the properties of the emulsion droplet interface and the physical location and concentration of prooxidants, antioxidants, and oxidizable substrates (e.g., lipid hydroperoxides) (for review see *2*). One of the major mechanisms of oxidation of emulsified lipids is the iron-promoted degradation of lipid hydroperoxides into free radicals that can oxidize unsaturated fatty acids. Food emulsions typically contain ample endogenous concentrations of both iron and lipid hydroperoxides for this reaction to cause quality degradation (*3*). Thus, factors that impact iron−hydroperoxide interaction can have a dramatic effect on lipid oxidation rates. Iron is soluble in both water and oil. However, the water solubility of iron and in particular, ferric iron, is typically low except at acidic pH levels (*4*). Thus, the physical location of iron in food systems is often not well understood.

Oil-in-water emulsions commonly contain more surfactant than is required to saturate the emulsion droplet surface. Excess surfactants not associated with the emulsion droplet will form surfactant micelles in the continuous phase (*1*). The presence of surfactant micelles may alter the partitioning of lipids, prooxidants, antioxidants, and lipid hydroperoxides between the dispersed phase, continuous phase, and interfacial region (*5*−*7*). Surfactant micelles have been shown to decrease lipid oxidation rates in oil-in-water emulsions (*6*, *7*). The ability of surfactant micelles to inhibit lipid oxidation does not seem to be due to direct antioxidant activity because surfactants such as the Brijs have minimal free radical scavenging activity. Surfactant micelle solubilization of antioxidants also does not seem to be responsible for the observed inhibition of lipid oxidation by surfactant micelles in oil-in-water emulsions because the micelles result in removal of antioxidants away from the lipid (*6*). Therefore, a potential mechanism by which surfactant micelles could inhibit lipid oxidation in oil-in-water emulsion is through mechanisms that decrease the activity of prooxidants. Surfactant micelles will increase the partitioning of lipid hydroperoxides out of emulsion droplets (*7*), a factor that could decrease lipid oxidation rates by removing a source of free radicals from the lipid environment. If surfactant micelles can also influence the physical location of prooxidant transition metals, this could be an additional mechanism that would decrease oxidation rates.

The objective of this research was to determine the ability of surfactant micelles to alter the partitioning of iron in oil-in-water emulsions. The ability of surfactant micelles to solubilize iron from lipid droplets and the effects of iron solubilization on lipid oxidation rates was determined.

* To whom correspondence should be addressed. Phone: 413-545-1026. Fax: 413-545-1262. E-mail: edecker@foodsci.umass.edu.
† Sangju National University.
‡ University of Massachusetts.

10.1021/jf020433g CCC: $22.00
Published on Web 08/30/2002

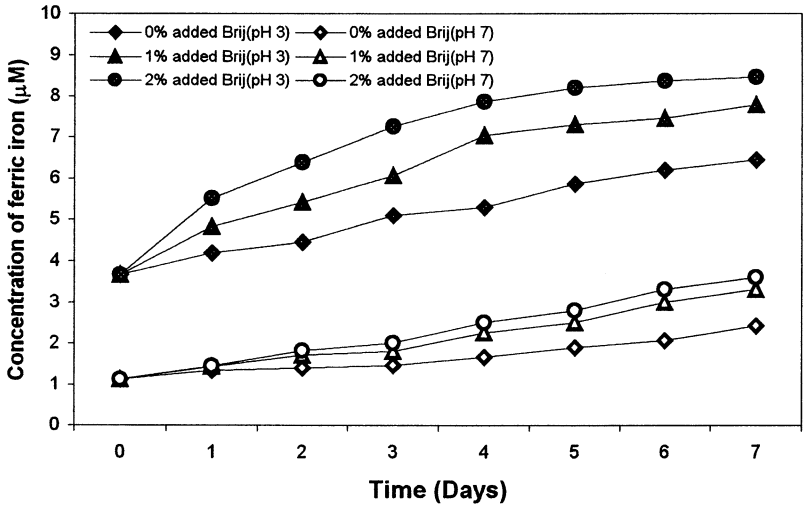

Figure 1. Ability of Brij micelles to increase continuous-phase iron concentrations in hexadecane oil-in-water emulsions at pH 3.0 and pH 7.0. Ferric ions were added to the hexadecane prior to formation of the emulsion. Data points represent means ($n = 3$) ± standard deviation (some error bars may lie within the data points).

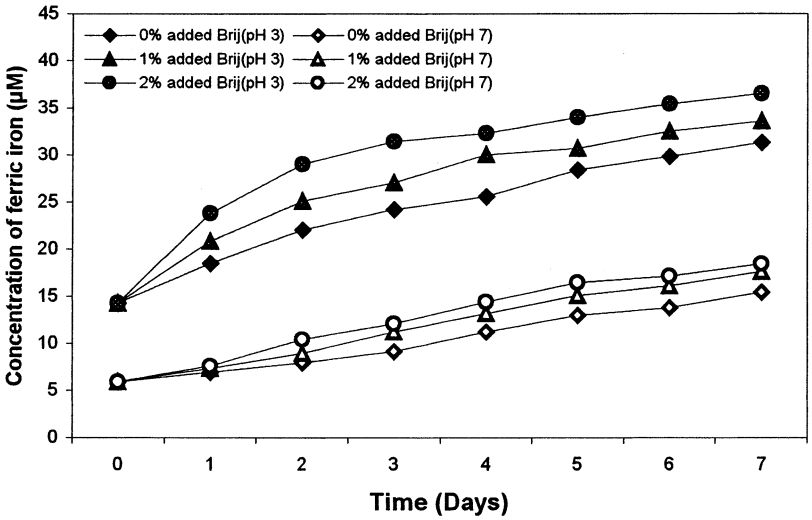

Figure 2. Ability of Brij micelles to increase continuous-phase iron concentrations in corn oil-in-water emulsions at pH 3.0 and pH 7.0. Ferric ions were added to the corn oil prior to formation of the emulsion. Data points represent means ($n = 3$) ± standard deviation (some error bars may lie within the data points).

MATERIALS AND METHODS

Chemicals. Polyoxyethylene 10-lauryl ether (Brij), imidazole, ferric chloride, ferrous sulfate, citric acid, ferrozine, hydroxylamine-hydrochloride, barium chloride, ammonium thiosulfate, sodium acetate, and *n*-hexadecane were purchased from Sigma Chemical Co. (St. Louis, MO). Methanol, butanol, isooctane, and 2-propanol were purchased from Fisher Scientific (Fair Lawn, NJ). All other reagents were of analytical grade or purer. Corn oil was purchased from a local retail outlet in the United States and was used without further purification. Glassware was acid-washed with concentrated HCl, rinsed with double-distilled water, and dried overnight before use.

Preparation of Hexadecane and Corn Oil with Ferric Ions. Ferric chloride (Fe III, 500 μM) was mixed with hexadecane or corn oil and stirred for 12 h at 22 °C. After the mixture was stirred, the lipids were filtered through Whatman No. 40 filter paper to remove insoluble iron. The filtered lipids were then used to prepare the emulsions. The iron concentrations in the lipids were determined by the difference of the total iron added to the lipids minus the iron that was not dissolved in the lipids. The iron that was not dissolved in the lipids was collected by solubilization of the iron associated with the filter paper. This was achieved by grinding the filter paper in 0.2 N HCl with a Texmar homogenizer for 1 min, followed by centrifugation at 2000*g* for 10 min and collection of the supernatant. Under these acidic conditions, the iron associated with the filter paper dissolves, allowing iron concentrations in the supernatant to be measured as described below.

Preparation of Emulsions. Emulsions were prepared by mixing the iron-containing corn oil or hexadecane with aqueous solutions of either 34 mM (used for 10% lipid emulsions) or 128 mM (used for 40% lipid emulsions) Brij with 10 mM acetate-imidazole buffer (pH 3.0 or 7.0). 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 duty cycle for 90 s. Particle size distributions of emulsion droplets were measured using a Horiba LA-900 laser scattering particle size analyzer (Horiba Instruments, Irvine CA) (*8*). The average droplet diameters ranged from 1.4 to 1.6 μm and did not change during the course of the experiment.

Brij micelles (0−2%) were added by mixing equal volumes of Brij solutions (0−4%) to the emulsions and stirring for 10 min to produce emulsions with final lipid concentrations of 5 or 20%. To isolate the continuous phase, emulsions were centrifuged at 24 000*g* (Serv-all model RC2-B, Newtown, CT) for 30 min at 10 °C. A 5-mL disposable syringe with an 18-gauge needle was pressed against the wall of the centrifuge tube and gently pushed down to the bottom of the tube where approximately 5 mL of the continuous phase was removed.

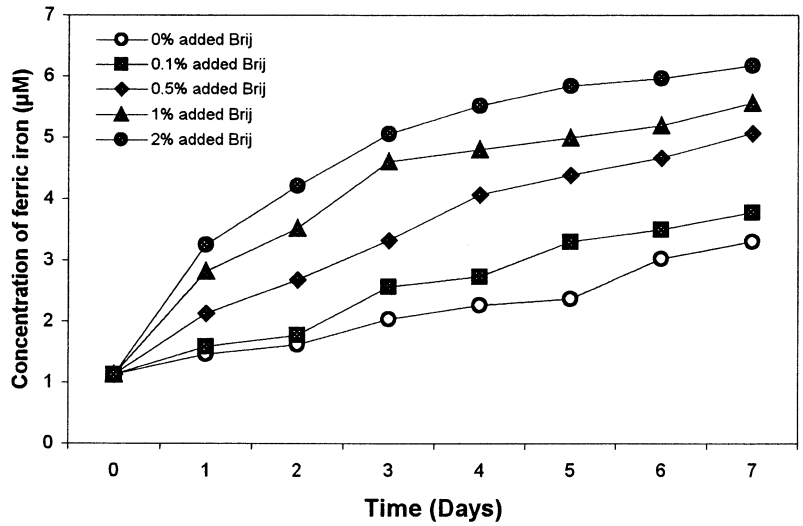

Figure 3. Ability of low concentrations of Brij micelles to increase continuous-phase iron concentrations in hexadecane-in-water emulsions at pH 3.0. Ferric ions were added to the hexadecane prior to formation of the emulsion. Data points represent means ($n = 3$) ± standard deviation (some error bars may lie within the data points).

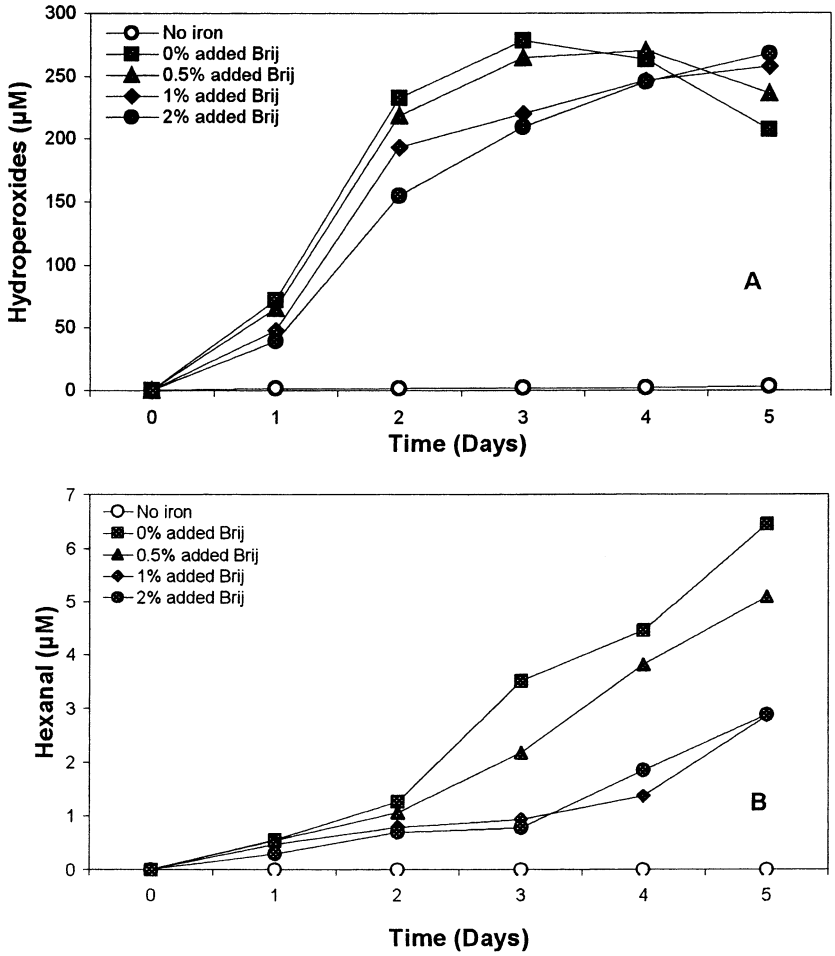

Figure 4. Formation of lipid hydroperoxides (A) and headspace hexanal (B) in corn oil-in-water emulsions containing Brij micelles at pH 7.0. Ferric ions were added to the corn oil prior to formation of the emulsion. Data points represent means ($n = 3$) ± standard deviation (some error bars may lie within the data points).

Measurement of Iron Concentrations. Quantification of iron not dissolved in the lipids or iron in the continuous phase of the oil-in-water emulsions was determined spectrophotometrically using a method adapted from Fukuzawa and Fujii (9). A 1-mL aliquot of the iron-containing sample was reduced with 1.0 mL of 10% hydroxylamine-hydrochloride in 2 N HCl for 15 min at room temperature followed by the addition of 1.0 mL of 9.0 mM ferrozine. After 10 min, the absorbance was measured at 562 nm, and concentrations were determined from a standard curve constructed using ferric chloride. In a separate experiment we found that Brij micelles did not interfere with iron quantitation.

Lipid Oxidation Studies. The emulsions were immediately placed in 10-mL glass vials. The vials were sealed with poly(tetrafluoroethylene) (PTFE)/butyl rubber septa using a crimper and aluminum seals,

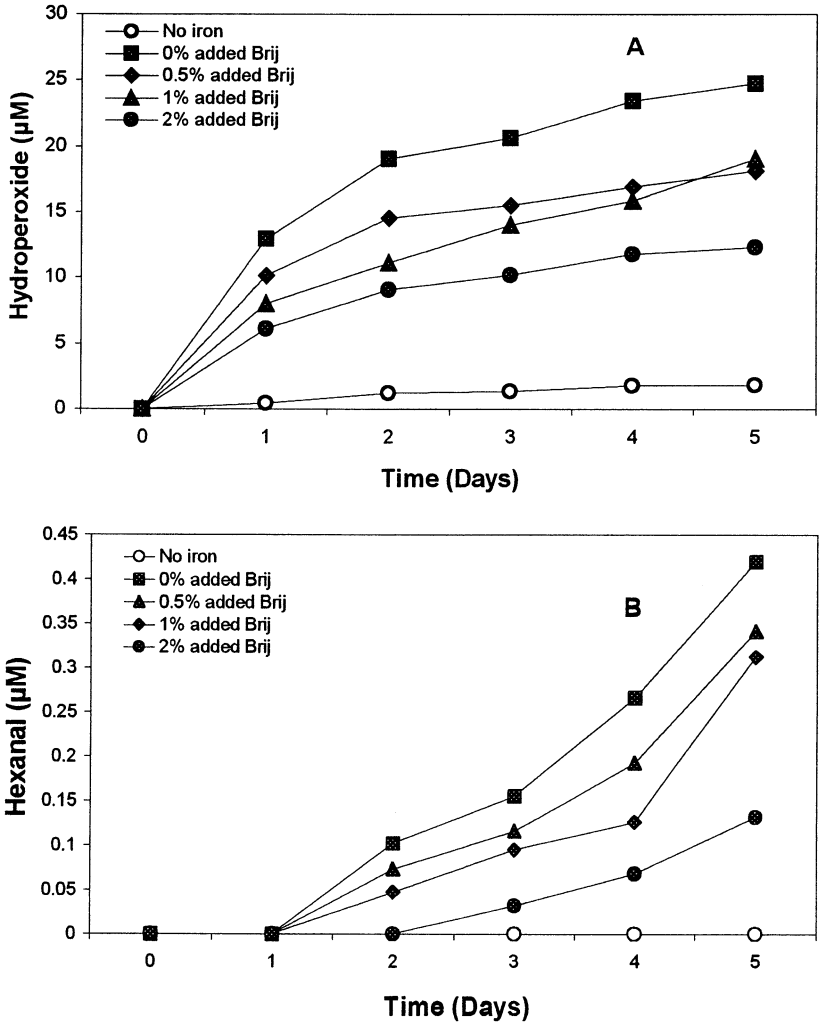

Figure 5. Formation of lipid hydroperoxides (A) and headspace hexanal (B) in corn oil-in-water emulsions containing Brij micelles at pH 3.0. Ferric ions were added to the corn oil prior to formation of the emulsion. Data points represent means ($n = 3$) ± standard deviation (some error bars may lie within the data points).

and incubated at 22 °C in the dark. Lipid hydroperoxides were determined using a method adapted from Shanta and Decker (*10*). Emulsions (0.3 mL) were added to 1.5 mL of isooctane/2-propanol (3:1), vortexed 3 times for 10 s, and centrifuged for 2 min at 2000*g*. The organic phase (0.2 mL or less according to the oxidation state) was added to 2.8 mL of methanol/butanol (2:1, v:v) followed by addition of 15 μL of 3.94 M ammonium thiocyanate and 15 μL of 72 mM ferrous (Fe II) solution. After 20 min, the absorbance was measured at 510 nm. The concentration of hydroperoxides was calculated from a hydroperoxide standard curve.

Oxidation was also followed by measuring headspace hexanal using a modified method of Mancuso et al. (*11*). Separations were performed on a Shimadzu GC-17A gas chromatograph (Columbia, MD) attached to a Hewlett-Packard 19395A headspace sampler (Avondale, PA). The chromatograms were integrated using a Shimadzu CLASS-VP chromatography data system software. The headspace conditions were as follows: sample temperature 55 °C, sample loop and transfer line temperature 110 °C, pressurization 10 s, venting 10 s, and injection 1 min. The aldehydes were separated isothermally at 65 °C 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 250 °C. Concentrations were determined from peak areas using a standard curve made from authentic hexanal in 10 mM acetate-imidazole buffer because it was determined that the surfactant micelles did not influence the amount of hexanal partitioning into the headspace.

Statistics. All the experiments were done in triplicate. Statistical analyses were performed using the Student's *t* test (*12*). Significance was set at the 5% level.

RESULTS

Iron-saturated hexadecane or corn oil was produced by mixing 500 μM of ferric chloride with the lipid for 12 h followed by removal of undissolved iron by filtration. Elevation of lipid hydroperoxide and headspace hexanal concentrations was not detected in the corn oil after mixing for 12 h, indicating that minimal lipid oxidation had occurred. Iron concentrations in hexadecane and corn oil after ferric chloride incorporation were 48.5 ± 0.9 μmol/kg lipid and 297.4 ± 2.4 μmol/kg lipid, respectively. The higher concentration of iron in corn oil than in hexadecane could be due to differences in polarity as hexadecane is less polar than corn oil. In addition, unlike hexadecane, corn oil would be expected to contain compounds such as free fatty acids, phospholipids, sterols, tocopherols, and mono and diacylglycerols. These surface active compounds could form reverse micelles in the oil which could aid in the solubilization of iron. Additionally, these compounds could directly chelate iron (e.g., free fatty acids) thus increasing its oil solubility.

Iron-partitioning experiments were conducted with 20% oil-in-water emulsions in order to increase the ability to detect

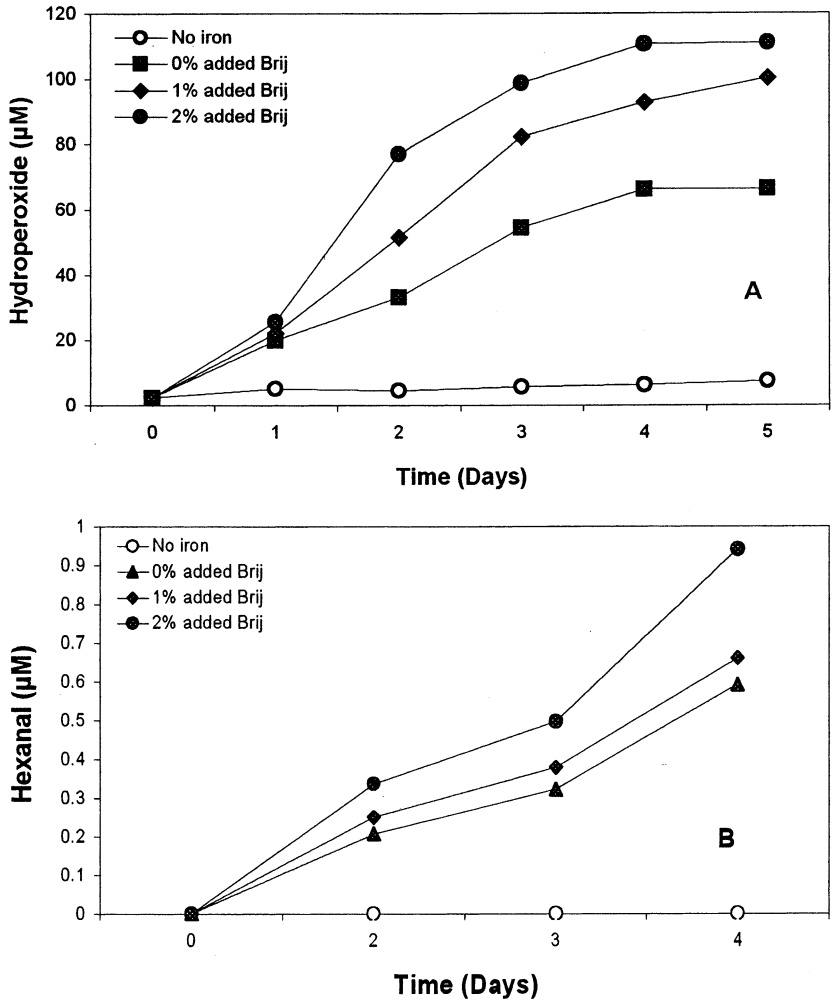

Figure 6. Formation of lipid hydroperoxides (A) and headspace hexanal (B) in corn oil-in-water emulsions containing Brij micelles that were high in hydroperoxide. Ferric ions were added to the corn oil prior to formation of the emulsion. Data points represent means ($n = 3$) ± standard deviation (some error bars may lie within the data points).

continuous-phase iron. Upon formation of the oil-in-water emulsions, initial continuous-phase iron concentrations were 3.7 ± 0.2 μM and 1.1 ± 0.2 μM at pH 3.0 and 7.0, respectively, in hexadecane-in-water emulsions and 14.3 ± 0.8 μM and 5.9 ± 0.2 at pH 3.0 and 7.0, respectively, in corn oil-in-water emulsions (**Figures 1** and **2**). Continuous-phase iron concentrations in emulsions containing no added Brij increased during incubation, suggesting that the iron partitioned into the continuous phase without the aid of surfactant micelles. However, previous work in similar oil-in-water emulsion systems has found that 40−60% of the surfactant used to prepare the emulsion remains in the continuous phase (*6*). The critical micelle concentration (CMC) of Brij surfactants is low (<1 mM; *13*) which means that the surfactant in the emulsion that is not associated with the emulsion droplet interface would likely form surfactant micelles in the continuous phase. Therefore, aqueous-phase Brij would likely form micelles, and these may be responsible for the observed partitioning of iron into the continuous phase in the absence of added Brij.

Higher continuous-phase iron concentrations (in the absence of added Brij micelles) were observed at pH 3.0 compared to those at pH 7.0 in both the hexadecane and corn oil emulsions. The water solubility of ferric iron increases with decreasing pH (*4*), suggesting that the higher continuous-phase iron concentration at pH 3.0 could be partially due to micelle-independent solubilization of iron into the water. However, increases in continuous-phase iron concentrations at pH 7.0 in the absence of added Brij after 7 days of storage were 2.4 and 15.4 μM for the hexadecane and corn oil emulsions, respectively (**Figures 1** and **2**), despite the fact that water solubility of iron at pH 7.0 is reported to be 4×10^{-17} M (*13*). Addition of Brij micelles further increased continuous-phase iron concentrations at both pH 3.0 and 7.0. For example, in hexadecane-in-water emulsions stored for 7 days, 2.0% added Brij micelles increased continuous-phase iron concentrations 1.3- and 1.5-fold at pH 3.0 and 7.0, respectively (**Figure 1**). Likewise, in corn oil-in-water emulsions stored for 7 days, 2.0% added Brij micelles increased continuous-phase iron concentrations 1.2-fold at both pH 3.0 and 7.0 (**Figure 2**).

The solubilization of iron out of the lipid droplets increased gradually over the 7 days of incubation (**Figures 1**−**3**). This is in contrast to the ability of Brij micelles to solubilize phenolic antioxidants and lipid hydroperoxides where the majority of solubilization into the continuous phase occurred within the first hour with little additional solubilization occurring with prolonged storage (*6*, *7*). Increasing Brij concentrations increased iron partitioning into the continuous phase, with added Brij concentrations as low as 0.1% causing significant ($p \leq 0.05$) iron solubilization in hexadecane oil-in-water emulsions at pH 3.0 (**Figure 3**). The fraction of iron solubilized out of the emulsion droplets after 7 days of storage ranged from 5.4 to

17.4% for hexadecane- and 5.2−12.4% corn oil-containing emulsions (**Figures 1**−**3**).

Ability of Brij Micelles To Alter Iron-Promoted Lipid Oxidation in Oil-in-Water Emulsions. To determine if iron solubilization by Brij impacted the oxidative stability of 5% corn oil-in-water emulsions, emulsions (pH 3.0 and 7.0) were prepared with iron-containing oil followed by the addition of 0−2% Brij. Lipid hydroperoxides and headspace hexanal formation was used to monitor lipid oxidation during 5 days of storage. In emulsions made with corn oil without added iron, low levels of lipid hydroperoxides were detected at both pH 3.0 (1.3−2.9 μM) and pH 7.0 (0.43−1.8 μM), whereas headspace hexanal was not detected at either pH (**Figures 4** and **5**). As expected, addition of ferric ions to the corn oil resulted in a dramatic increase in both lipid hydroperoxide and headspace hexanal formation at both pHs with oxidation proceeding faster at pH 3.0 than at pH 7.0 (**Figures 4** and **5**). A similar trend was observed in Tween 20 stabilized menhaden oil-in-water emulsions where iron-promoted oxidation increased with decreasing pH (*14*). Addition of Brij micelles to the emulsions decreased lipid hydroperoxide and headspace hexanal formation at both pH 3.0 and 7.0. Even though Brij micelles were found to solubilize more iron out of corn oil emulsion at pH 3.0 than at pH 7.0, the ability of the Brij micelles to inhibit lipid oxidation was not dramatically better at pH 3.0 than at 7.0. For example, after 3 days of storage, lipid hydroperoxide and headspace hexanal concentrations were 51 and 81% lower, respectively, for emulsions containing 2.0% added Brij compared to no-added-Brij controls at pH 7.0 (**Figure 4A**,**B**). At pH 3.0, 2.0% added Brij decreased lipid hydroperoxide and headspace hexanal concentrations 25 and 78%, respectively, compared to no-added-Brij controls after 3 days of storage (**Figure 5A**,**B**). The inability of the Brij micelles to inhibit oxidation more effectively at pH 3.0 where they were most efficient at partitioning iron into the continuous phase could be due to the higher rates of lipid oxidation at pH 3.0. If iron was more active at pH 3.0, the greater proportion of the lipid-phase iron that was solubilized into the continuous phase might not have been great enough to inhibit lipid oxidation more effectively than at pH 7.0.

The impact of Brij micelles on lipid oxidation rates in iron-containing oil-in-water emulsion was also tested using Brij that was high in hydroperoxides (73.1 $\pm$ 1.5 μmol hydroperoxide/g Brij vs 5.1 $\pm$ μmol hydroperoxide/g Brij). The Brij that was high in hydroperoxides originated from an old bottle of Brij that was stored in our laboratory at room temperature for approximately 2 years compared to the Brij low in hydroperoxides used in the studies described above that was newly purchased. Storage time, transition metal concentrations, and light are factors that accelerate hydroperoxide formation in nonionic surfactants such as Brij and Tween (*15*, *16*). When Brij high in hydroperoxides was added to iron-containing corn oil-in-water emulsions at pH 3.0, the formation of lipid hydroperoxide and headspace hexanal increased with increasing Brij concentrations (**Figure 6A**,**B**), which is opposite to the effect that was observed with Brij low in hydroperoxides (**Figure 5**). Brij micelles that were high in hydroperoxides were found to solubilize iron into the continuous phase in a manner similar to Brij micelles low in hydroperoxides (data not shown). The acceleration of lipid oxidation caused by Brij micelles high in hydroperoxides, therefore, occurred despite the fact that less iron would be present in the lipid droplets due to micelle solubilization. This result is likely due to the ability of iron to decompose Brij hydroperoxides into free radicals that in turn can promote lipid oxidation. Hydroperoxides associated with Tweens and Brij have previously been shown to promote the oxidation of tocopherol and fatty acids in oil-in water emulsions (*15*, *16*).

CONCLUSIONS

Surfactant micelles have previously been shown to inhibit lipid oxidation in oil-in-water emulsions. The results of this research suggest that one potential mechanism by which surfactant micelles could inhibit lipid oxidation is through their ability to solubilize iron out of the lipid droplets into the continuous phase where the iron would be less likely to interact with oxidizable lipids. Solubilization of iron from the lipid droplets increased with increasing Brij micelle concentrations, increasing storage time, and decreasing pH. Addition of Brij micelles low in hydroperoxides to emulsion containing high concentrations of lipid-phase iron was antioxidative, i.e., it decreased the formation of lipid hydroperoxides and headspace hexanal in corn oil-in-water emulsions. However, if the Brij micelles added to the emulsion contained high concentrations of preexisting hydroperoxide, the micelles were prooxidative. This research suggests that surfactant micelles could impact the physical location of iron in oil-in-water emulsions, and through their ability to partition iron, they could impact lipid oxidation rates.

LITERATURE CITED

(1) McClements, D. J. *Food Emulsions: Principles, Practice, and Techniques*; CRC Press: Boca Raton, FL, 1999.
(2) McClements, D. J.; Decker, E. A. Lipid oxidation in oil-in-water emulsions: impact of molecular environment on chemical reactions in heterogeneous food system. *J. Food Sci.* **2000**, *65*, 1270−1282.
(3) Decker, E. A.; McClements, D. J. Transition Metal and Hydroperoxide Interactions: An Important Determinant in the Oxidative Stability of Lipid Dispersions. *Inform* **2001**, *12*, 251−255.
(4) Dunford, H. B. Free radicals in ion-containing systems. *Free Radical Biol. Med.* **1987**, 3, 405−421.
(5) Coupland, J. N.; Weiss, J.; Lovy, A.; McClements, D. J. Comparison of the solubilization kinetics of triacylglycerol and hydrocarbon emulsion droplets in a micellular solution. *J. Food Sci.* **1996**, *61*, 1114−1117.
(6) Richards, M. P.; Chaiyasit, W.; McClements, D. J.; Decker, E. A. Ability of surfactant micelles to alter the partitioning of phenolic antioxidants in oil-in-water emulsions. *J. Agric. Food Chem.* **2002**, *50*, 1254−1259.
(7) Nuchi, C. D.; Hernandez, P.; McClements, D. J.; Decker, E. A. Ability of lipid hydroperoxides to partition into surfactant micelles and alter lipid oxidation rates in emulsions. *J. Agric. Food Chem.* **2002**, in press.
(8) Weiss, J.; Coupland, J. N.; McClements, D. J. Solubilization of hydrocarbon droplets suspended in a nonionic surfactant solution. *J. Phys. Chem.* **1996**, *100*, 1066−1071.
(9) Fukuzawa, K; Fujii, T. Peroxide dependent and independent lipid peroxidation site-specific mechanisms of initiation by chelated iron and inhibition by α-tocopherol. *Lipids* **1992**, *27*, 227−233.
(10) Shanta, N. C.; Decker, E. A. Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxides values of food lipids. *J. AOAC* **1994**, *77*, 421−424.
(11) Mancuso, J. R.; McClements, D. J.; Decker, E. A. The effects of surfactant type, pH, and chelators on the oxidation of salmon oil-in-water emulsions. *J. Agric. Food Chem.* **1999**, *47*, 4112−4116.
(12) Snedecor, G. W.; Cochran, G. W. *Statistical Methods*; Iowa State University Press: Ames, IA, 1989.
(13) Zumdahl, S. S. *Chemistry*, 2nd ed.; Heath: Lexington, MA, 1989; p A26.

(14) Donnelly, J. L.; Decker, E. A.; McClements, D. J. Iron-catalyzed oxidation of menhaden oil as affected by emulsifiers. *J. Food Sci*. **1998**, *63*, 997−1000.

(15) Mancuso, J. R.; McClements, D. J.; Decker, E. A. Ability of iron to promote surfactant peroxide decomposition and oxidize alpha-tocopherol. *J. Agric. Food Chem.* **1999**, *47*, 4146−4149.

(16) Nuchi, C. D., McClements, D. J.; Decker, E. A. Impact of Tween 20 hydroperoxides and iron on the oxidation of methyl linoleate and salmon oil dispersions. *J. Agric. Food Chem.* **2001**, *49*, 4912−4916.

Received for review April 12, 2002. Revised manuscript received July 11, 2002. Accepted July 24, 2002. This research was partially funded by Grant 2001-4526 from Nutritional Impacts of Functional Food Program, IFAFS, CSREES, USDA.

JF020433G