

Impact of Lipid Physical State on the Oxidation of Methyl Linolenate in Oil-in-Water Emulsions

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The purpose of this research was to examine the influence of the physical state of lipids on iron-promoted oxidation of methyl linolenate in octadecane oil-in-water emulsions. Octadecane and methyl linolenate oil-in-water emulsions were prepared that contained droplets having the octadecane as either liquid or solid. The physical state of the octadecane was confirmed by a differential scanning calorimeter (DSC). The effect of the physical state of the lipid on oxidation rates was determined as a function of iron concentration (80 and 160 μM), pH (3.0 or 7.0), emulsifier type, and cooling rate. Oxidation of methyl linolenate was determined by lipid hydroperoxides and thiobarbituric acid reactive substances (TBARS). Emulsions containing solid octadecane had higher rates of lipid hydroperoxide and TBARS formation than those containing liquid octadecane. The rate at which the emulsions were cooled had no influence on oxidation rates. Oxidation rates in both emulsions increased with increasing iron concentration and decreasing pH. Oxidation rates were lowest in emulsions with cationic droplet membranes (dodecyl trimethylammonium bromide-stabilized), presumably due to the repulsion of iron from the oxidizable methyl linolenate in the emulsion droplet core. These results suggest that upon crystallization of octadecane, the liquid methyl linolenate migrated to the emulsion droplet surface, where it was more prone to oxidation because it was in closer contact with the iron ions in the aqueous phase.

KEYWORDS: Crystallization; lipids; lipid oxidation; methyl linolenate; emulsions; solid fat content

INTRODUCTION

The lipids in many food products contain small oil droplets that are dispersed in an aqueous continuous phase, for example, milk, cream, dressings, mayonnaise, beverages, soups, sauces, desserts, dips, and ice cream (1, 2). These lipids are often susceptible to oxidation, which leads to the formation of off-flavors and toxic compounds. Many factors such as lipid type, prooxidant and antioxidant concentrations, pH, and interfacial membrane properties can influence lipid oxidation rates in oil-in-water emulsions. One of the most common mechanisms of lipid oxidation in emulsions is the interaction between lipid hydroperoxides located at the droplet surface and transition metals originating in the aqueous phase (3), which accelerate the decomposition of hydroperoxides into highly reactive peroxy and alkoxy radicals. Most food emulsions contain ample concentrations of transition metals that can oxidize lipids in emulsions. Therefore, a better understanding of lipid oxidation by transition metals is needed to improve the oxidative stability of food emulsions.

The physical state of lipids in oil-in-water emulsions might be expected to influence lipid oxidation because it alters the relative location of different types of lipids within the droplets, the partitioning of oil soluble components between the oil and

water phases, and the diffusion rate of molecules through the droplets. The crystallization of lipids in emulsions may be either deleterious or desirable to food product quality. For example, lipid crystallization is an essential step in the production of butter, margarine, and whipped cream because it leads to a desired texture and flavor, but it is undesirable in dressings and creams (4, 5). Many factors can affect lipid crystallization in emulsions such as the type of emulsifier, droplet size, the composition and purity of the fat, and temperature variation (6–10). Currently, many researchers have studied the influence of fat crystals on the stability of emulsions (11–16). However, the influence of the lipid crystalline state on lipid oxidation has not been systematically investigated. It could be of great interest to the food industry if the physical state of lipids could be altered in a manner that would improve oxidation stability.

The objective of this study was to produce oil-in-water emulsions with either solid or liquid lipids to determine how the physical state of the lipid affects lipid oxidation kinetics. The influence of iron concentration, pH, emulsifier type, and cooling rate on the oxidation of methyl linolenate was also investigated.

MATERIALS AND METHODS

Materials. Octadecane and methyl linolenate were acquired from Aldrich Chemical Co., Inc. (Milwaukee, WI). Sodium dodecyl sulfate (SDS), polyoxyethylene sorbitan monolaurate (Tween 20), dodecyl

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trimethylammonium bromide (DTAB), ethylenediaminetetraacetic acid (EDTA), 2-thiobarbituric acid (TBA), ferrous sulfate, butylated hydroxytoluene, barium chloride, and imidazole were obtained from Sigma Chemical Co. (St. Louis, MO). Trichloroacetic acid was purchased from Acros Organics (Morris Plains, NJ). All other chemicals and solvents were of reagent or HPLC grade and were obtained from Fisher Scientific (Suwanee, GA). Distilled and deionized water from a water purification system (Barnstead NANO pure infinity ultrapure, Dubuque, IA) was used in all experiments.

Methods. Emulsion Preparation. Octadecane was heated to 35 °C to ensure that it was completely liquid. A coarse oil-in-water emulsion consisting of 4.9 wt % octadecane, 0.1 wt % methyl linolenate, and 0.5 wt % emulsifier (SDS, Tween 20, or DTAB) in 10 mM sodium acetate/imidazole buffer (pH 3.0 or 7.0) was made by homogenizing the lipid and aqueous phases for 2 min using a two-speed hand-held homogenizer (Biospec Products, Inc., Bartlesville, OK) at the highest speed setting. The size of the droplets in the coarse emulsion was then reduced by sonicating it for 1 min at a frequency of 20 kHz, an amplitude of 50%, and a duty cycle of 0.5 s (model 500, sonic dismembrator, Fisher Scientific, Pittsburgh, PA). Care was taken to ensure that all samples were maintained at 35 °C to prevent lipid crystallization during emulsion preparation.

The emulsions were cooled to produce droplets containing either liquid or solid octadecane. To produce emulsions with liquid octadecane, samples were cooled from 35 to 20 °C. To produce emulsions with solid octadecane, the samples were cooled from 35 to 5 °C and then heated to 20 °C. Slow cooling was performed in a 2 °C incubator (−0.5 °C/min), whereas rapid cooling was performed in an ice–water bath (−8.8 °C/min). The emulsions were then kept in a temperature-controlled room (20 °C) for the duration of the experiments. At this temperature, octadecane in droplets could either be solid or liquid depending on their thermal history. The particle size distribution of the emulsions was determined using a laser diffraction particle size analyzer (Mastersizer X., Malvern Instruments, Ltd., Worcestershire, U.K.). The mean particle size was reported as the volume-surface mean diameter, d_{32} ($=\sum n_i d_i^3 / \sum n_i d_i^2$), where n_i is the number of particles with diameter d_i . The mean particle diameter in all emulsions ranged from 0.28 to 0.30 μm and did not change during the course of the studies.

Verification of whether the emulsified octadecane was solid or liquid was determined by a differential scanning calorimeter (DSC; Q1000, TA Instruments, New Castle, DE). Emulsions (8–10 mg) were placed in aluminum pans (ME-00026763, Mettler Toledo, Columbus, OH) and sealed. Samples were cycled at the scan rate of 0.35 °C/min. An empty aluminum pan was used as a reference.

Lipid Oxidation Measurement. Oxidation of emulsions was carried out in capped glass test tubes (13 × 100 mm) in the presence of ferrous sulfate (80 or 160 μM) at 20 °C for up to 7 days. EDTA (300 μM) was added to the emulsions before measurement of oxidation products to stop further oxidation. Oxidation of the methyl linolenate was determined by measuring lipid hydroperoxide and thiobarbituric acid-reactive substances (TBARS). Lipid hydroperoxide was measured according to a modified method of Shanta and Decker (17). Lipids in the emulsions (0.3 mL) were extracted by vortexing (10 s, three times) with 1.5 mL of iso-octane/2-propanol (3:1, v/v) followed by centrifugation for 2 min at 3400g (Centrifuge, Fisher Scientific, Fair Lawn, NJ) to collect the organic phase. The organic phase (0.2 mL) was added to 2.8 mL of methanol/1-butanol (2:1, v/v) followed by 15 μL of 3.94 M ammonium thiocyanate and 15 μL of ferrous iron solution (prepared by adding equal amounts of 0.132 M BaCl_2 and 0.144 M FeSO_4 in acidic solution). The solution was vortexed, and the absorbance was measured after 20 min at 510 nm using a UV–vis scanning spectrophotometer (Shimadzu UV-2101PC, Kyoto, Japan). Hydroperoxide concentrations were determined using a standard curve made from cumene hydroperoxide.

TBARS were determined by using the method of McDonald and Hultin (18). Emulsions (0.05–0.5 mL, depending on state of oxidation) were combined with water (0.5–0.95 mL) to a total of 1.0 or 2.0 mL of TBA reagent (100 mL of 15% w/v trichloroacetic acid and 0.375% w/v thiobarbituric acid in 0.25 M HCl) was mixed with 3.0 mL of 2% BHT in ethanol) in test tubes. The mixtures were vortexed and heated in a boiling water bath for 15 min. After cooling to room temperature,

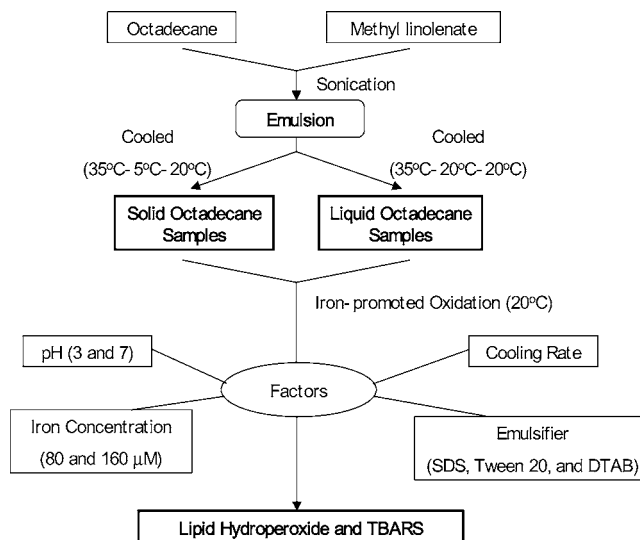


Figure 1. Preparation of emulsions and experimental design.

samples were centrifuged at 1000g for 15 min and the absorbance of the supernatant was measured at 532 nm. Concentrations of TBARS were determined from a standard curve prepared using 1,1,3,3-tetraethoxypropane. A flow diagram of the experimental protocol is shown in Figure 1.

Statistics. All experiments, except DSC, were conducted twice, and measurements were performed on triplicate samples. The results are reported as the means and standard deviations. Statistical analyses were performed using Student's *t* test (19). Significance was set at the 5% level.

RESULT AND DISCUSSION

Verification of Physical State of Octadecane in Oil-in-Water Emulsions. A differential scanning calorimeter was used to determine the physical state of the octadecane in the emulsions. In the bulk state, the melting point of pure octadecane is around 28 °C. Initially, we examined the change in enthalpy when the emulsions were cooled from a temperature at which the emulsified octadecane should be completely liquid (35 °C) to a temperature at which it should be completely solid (5 °C) and then heated back to a temperature at which the octadecane should be completely liquid (35 °C) (Figure 2A). Upon cooling from 35 to 5 °C, a minor exothermic enthalpy change was detected at 24.9 °C and a major exothermic enthalpy change was detected at 11.8 °C. The minor peak at 24.9 °C can be attributed to the crystallization of a small fraction of nonemulsified lipid or large lipid droplets, because this temperature is close to the melting point of pure octadecane (12). The major peak at 11.8 °C can be attributed to crystallization of emulsified octadecane, because a high degree of supercooling is usually observed when hydrocarbons are dispersed as small isolated emulsion droplets (12). Upon heating from 5 to 35 °C, a large endothermic enthalpy change was detected at 26.5 °C, which can be attributed to melting of both the emulsified and nonemulsified oil.

"Liquid Droplet" Emulsions. Figure 2B (1) shows the DSC thermograph of the emulsions that were prepared using conditions under which the octadecane should remain completely liquid, for example, cooling from 35 to 20 °C. When these emulsions were heated in the DSC from 20 to 35 °C, only a minor endothermic enthalpy change was detected at 26.5 °C, which can be attributed to the presence of the small fraction of nonemulsified octadecane mentioned above. Hence, we confirmed that the majority of octadecane was in a liquid form in the emulsions prepared using this method.

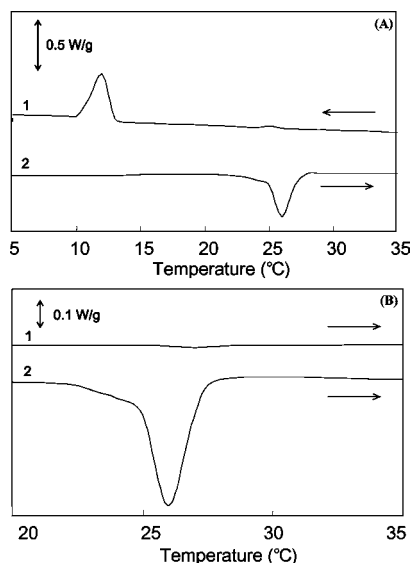


Figure 2. Example of DSC thermograms of octadecane and methyl linolenate oil-in-water liquid octadecane emulsion (A, 1; B, 1) and solid octadecane emulsion (A, 2; B, 2) stabilized by sodium dodecyl sulfate.

“Solid Droplet” Emulsions. **Figure 2B** (2) shows the DSC thermograph of the emulsions that were prepared using conditions under which the octadecane should end up completely solid, for example, cooling from 35 °C to 5 °C and then heating to 20 °C. Upon heating in the DSC from 20 to 35 °C, a large endothermic enthalpy change was detected at 26.5 °C, which is associated with the melting of the emulsified and nonemulsified octadecane. Hence, we confirmed that the majority of the octadecane was in a solid form in the emulsions prepared using this method.

Influence of the Physical State of the Lipid on the Oxidation of Methyl Linolenate in Oil-in-Water Emulsions. **Figure 3** shows how two different concentrations of iron (80 and 160 μM) promoted the oxidation of methyl linolenate in SDS-stabilized oil-in-water emulsions containing solid and liquid octadecane at pH 7.0. As expected, oxidation rates of methyl linolenate were faster in the presence of 160 than 80 μM iron as determined by both lipid hydroperoxides (**Figure 3A**) and TBARS (**Figure 3B**). It is interesting to note that at both iron concentrations the oxidation rate of methyl linolenate in emulsions containing solid octadecane was significantly greater ($p < 0.05$) than in emulsions containing liquid octadecane.

Lipid oxidation in oil-in-water emulsions can be promoted by the interaction of iron in the aqueous phase or near the surface of emulsion droplets with lipid hydroperoxides present at the emulsion droplet interface. Fats used in foods commonly contain a mixture of liquid and solid triacylglycerols. In products such as butter and margarines, the liquid triacylglycerols are entrapped in a solid fat matrix. If a similar arrangement was found in the emulsions used in these experiments, the liquid methyl linolenate would be entrapped in the solid octadecane. In this arrangement, oxidation rates would be expected to be slower because the iron in the aqueous phase of the emulsion or at the emulsion droplet surface would have limited access to the methyl linolenate. However, the observation that oxidation rates were faster in emulsions containing solid octadecane suggests that the methyl linolenate was not entrapped in the solid but instead had separated from the solid octadecane, possibly by accumulating in microenvironments at or near the emulsion droplet interface, where it could interact with iron. Two possible distributions of the methyl linolenate and octadecane within the

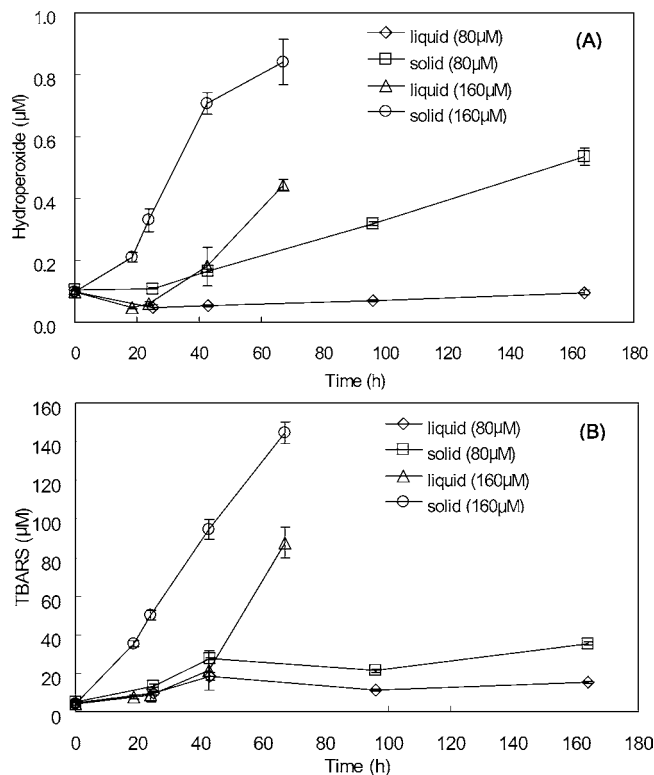


Figure 3. Formation of lipid hydroperoxides (A) and thiobarbituric acid reactive substances (TBARS) (B) in octadecane and methyl linolenate oil-in-water emulsions stabilized by sodium dodecyl sulfate at pH 7.0 and stored at 20 °C. The concentrations of ferrous iron in the emulsions were 80 and 160 μM . Solid represents emulsions with crystallized octadecane. Liquid represents emulsions with noncrystallized octadecane. Data points represent mean ($n = 3$) \pm standard deviation.

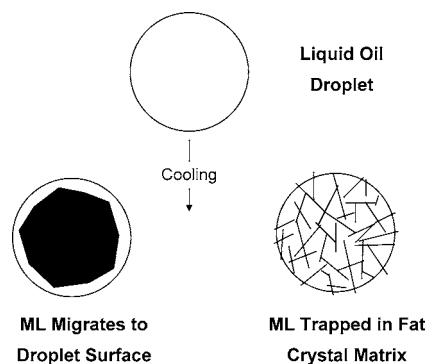


Figure 4. Schematic diagram of the possible physical locations of methyl linolenate (ML) in oil-in-water emulsions containing solid or liquid octadecane.

oil droplets upon crystallization of the octadecane are shown in **Figure 4**. Our results suggest that the methyl linolenate accumulates at the droplet surfaces, where it can interact with the iron.

The physical location of the liquid methyl linolenate in the solid octadecane could be influenced by different cooling conditions because cooling rate affects lipid nucleation and crystal growth. For example, we might expect the formation of a crystal network that would trap the methyl linolenate within the droplets if a fast cooling rate was used (**Figure 4**). Therefore, the oxidation rates of methyl linolenate in oil-in-water emulsions, where octadecane was crystallized by slow ($-0.5^\circ\text{C}/\text{min}$) or rapid ($-8.8^\circ\text{C}/\text{min}$) cooling, were determined. **Figure 5** shows that there are no differences in methyl linolenate oxidation

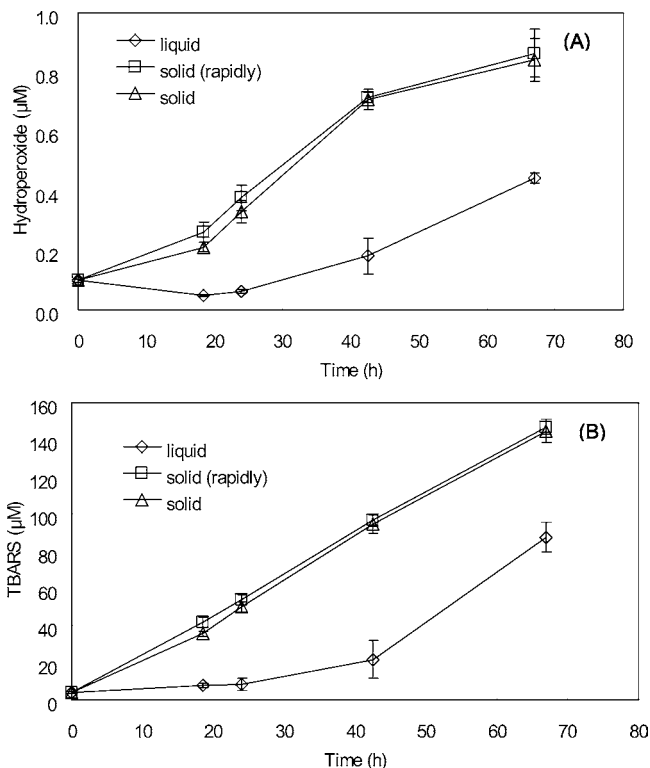


Figure 5. Formation of lipid hydroperoxides (A) and thiobarbituric acid reactive substances (TBARS) (B) in sodium dodecyl sulfate-stabilized octadecane and methyl linolenate oil-in-water emulsions (pH 7.0, 20 °C in the presence of 160 μM ferrous ions) exposed to slow (−0.5 °C/min) or rapid (−8.8 °C/min) cooling treatments. Solid represents emulsions with crystallized octadecane. Liquid represents emulsions with noncrystallized octadecane. Data points represent mean ($n = 3$) ± standard deviation.

rates in rapidly or slowly cooled emulsions as determined by both lipid hydroperoxides and TBARS, suggesting that the physical location of the methyl linolenate was not influenced by the cooling rates used in this study.

Influence of pH and Surfactant Type on the Oxidation of Methyl Linolenate in Oil-in-Water Emulsions Containing Solid or Liquid Octadecane. The reactivity, solubility, and physical location of iron is strongly influenced by pH. In addition, surfactant type also influences the ability of iron to interact with lipids to promote oxidation. Therefore, the influence of these factors on the oxidation of methyl linolenate in oil-in-water emulsion droplets containing either solid or liquid octadecane was investigated to determine if conditions could be altered to help stabilize the polyunsaturated fatty acids.

Figure 6 shows a comparison of iron-promoted oxidation of methyl linolenate in SDS-stabilized oil-in-water emulsions containing either solid or liquid octadecane at pH 3.0 and 7.0. At both pH values, methyl linolenate was again observed to oxidize more quickly when the octadecane was in the solid state as determined by both lipid hydroperoxides and TBARS. At pH 3.0, lipid hydroperoxides values were lower than at pH 7.0, whereas TBARS were higher at pH 3.0 than at pH 7.0. Iron promotes oxidation in oil-in-water emulsions by accelerating the decomposition of lipid hydroperoxides into free radicals. The ability of iron to decompose hydroperoxides is dependent on its physical location and solubility. The observed low lipid hydroperoxides concentrations at pH 3.0 are not due to low oxidation rates. Instead, at pH 3.0, iron has a higher water solubility and reactivity, which causes a rapid decomposition

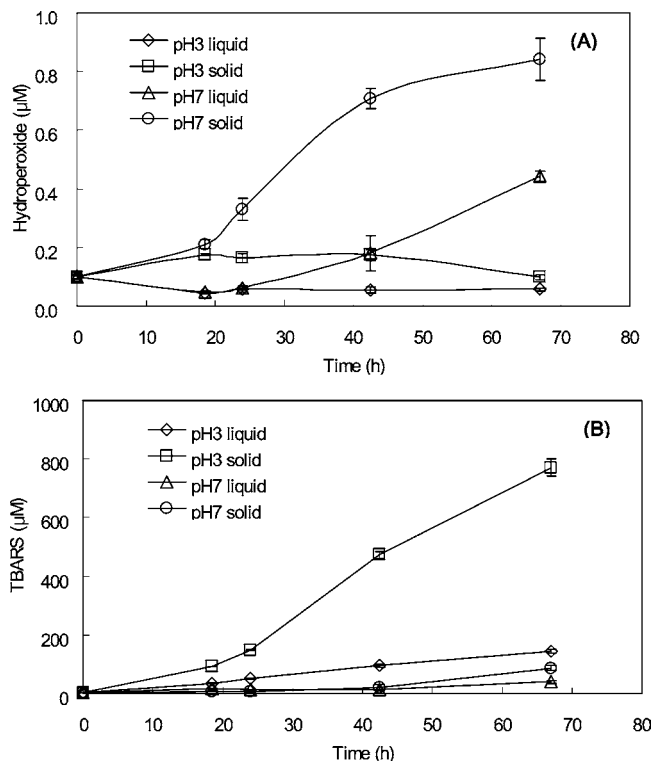


Figure 6. Formation of lipid hydroperoxides (A) and thiobarbituric acid reactive substances (TBARS) (B) in octadecane and methyl linolenate oil-in-water emulsions stabilized by sodium dodecyl sulfate at pH 3.0 and 7.0 stored at 20 °C in the presence of 160 μM ferrous ions. Solid represents emulsions with crystallized octadecane. Liquid represents emulsions with noncrystallized octadecane. Data points represent mean ($n = 3$) ± standard deviation.

of lipid hydroperoxides, thus preventing hydroperoxide accumulation. This rapid decomposition of the hydroperoxides at pH 3.0 can be seen by a low hydroperoxide concentration simultaneous with a high concentration of secondary lipid oxidation products (TBARS). Therefore, oxidation rates were higher at pH 3.0 than at pH 7.0 as can be seen by the differences in TBARS. A similar trend of formation of lipid hydroperoxides and TBARS has also been reported in oil-in-water emulsions at pH 3.0 and 7.0 by Mei and co-workers (20) and by Mancuso and co-workers (21).

Figure 7 shows a comparison of iron-promoted oxidation of methyl linolenate oil-in-water emulsions stabilized by Tween 20, SDS, or DTAB (pH 7.0) containing solid and liquid octadecane. These three surfactants were chosen because they produce emulsion droplets with various surface charges, which can influence the physical location and thus reactivity of iron. Little to no hydroperoxide or TBARS formation was observed in the DTAB emulsions, presumably due to the ability of the cationic emulsion droplets to repel iron and minimize its interactions with methyl linolenate in the droplet core. The faster rate of oxidation in the Tween 20- and SDS-stabilized emulsion droplets is likely due to the fact that they produce anionic emulsion droplets that could attract iron to the emulsion droplet surface. In the SDS- and Tween 20-stabilized emulsions, oxidation was again higher in droplets containing the solid octadecane. TBARS formation was higher in the SDS-stabilized than in Tween 20-stabilized emulsion droplets containing crystallized lipid, suggesting that the SDS-stabilized oil-in-water emulsion was oxidizing more rapidly. The low amounts of lipid hydroperoxides in the SDS- and Tween 20-stabilized emulsions

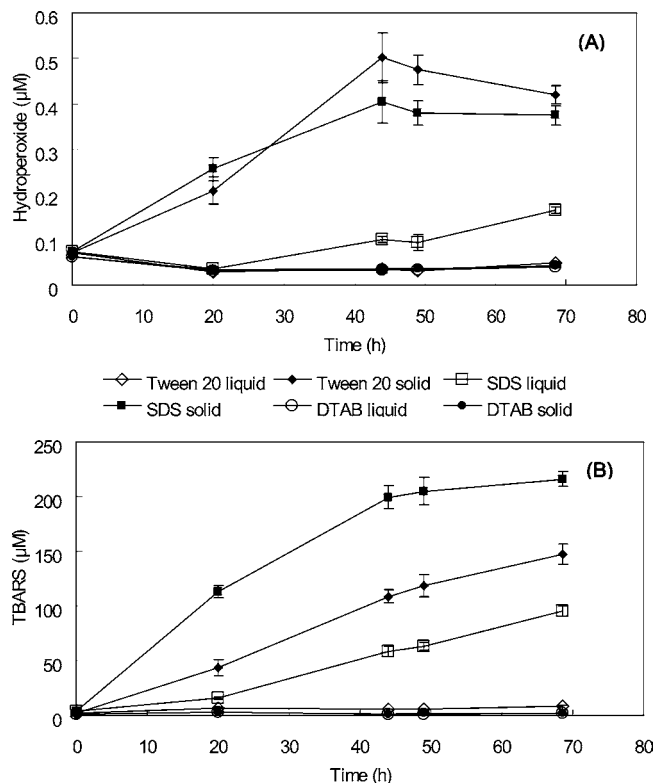


Figure 7. Formation of lipid hydroperoxides (A) and thiobarbituric acid reactive substances (TBARS) (B) in octadecane and methyl linolenate oil-in-water emulsions stabilized by Tween 20, sodium dodecyl sulfate (SDS), and dodecyl trimethylammonium bromide (DTAB) at pH 7.0 and stored at 20 °C in the presence of 160 μ M ferrous ions. Solid represents emulsions with crystallized octadecane. Liquid represents emulsions with noncrystallized octadecane. Data points represent mean ($n = 3$) \pm standard deviation.

containing solid octadecane are again likely due to the rapid decomposition of hydroperoxides. The lower rate of oxidation in the Tween 20-stabilized compared to the SDS-stabilized emulsions could be due to differences in surface charge (-27.2 mV for Tween 20 compared to -100.7 mV for SDS), with the greater surface charge attracting more iron to the emulsion droplet surface.

Conclusions. The oxidation rate of methyl linolenate in emulsions containing solid octadecane was significantly faster than that in emulsions containing liquid octadecane. These results suggest that the liquid methyl linolenate could be concentrating in physical domains near the surface of the emulsion droplet, where it would be more susceptible to oxidation by aqueous phase or interfacial iron. The rate at which the emulsions were cooled had no influence on oxidation rate, suggesting that the physical location of the methyl linolenate was not influenced by cooling rate. Oxidation rates in both emulsions decreased with decreasing iron concentration, increasing pH, and increasing surface charge at the emulsion droplet interface, suggesting that these conditions could be used to increase oxidative stability. These results suggest that oxidation rates in food emulsions could be altered by controlling the physical state of the lipid.

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