DOI:10.1021/jf901270m

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Prooxidant Mechanisms of Free Fatty Acids in Stripped Soybean Oil-in-Water Emulsions

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The prooxidant role of free fatty acids was studied in soybean oil-in-water emulsions. Addition of oleic acid (0−5.0% of oil) to the emulsions increased lipid hydroperoxides and headspace hexanal formation and increased the negative charge of the emulsion droplet with increasing oleic acid concentration. Methyl oleate (1.0% of oil) did not increase oxidation rates. The ability of oleic acid to promote lipid oxidation in oil-in-water emulsions decreased with decreasing pH with dramatic reduction in oxidation observed when the pH was low enough so that the oleic acid was not able to increase the negative charge of the emulsion droplet. Ethylenediaminetetraacetic acid (EDTA, 200 μm) strongly inhibited lipid oxidation in emulsions with oleic acid, indicating that transition metals were responsible for accelerating oxidation. Oleic acid hydroperoxides did not increase oxidation rates, suggesting that hydroperoxides on free fatty acids are not strong prooxidants in oil-in-water emulsion. These results suggest that the prooxidant activity of free fatty acids in oil-in-water emulsions is due to their ability to attact prooxidant metals to the emulsion droplet surface.

KEYWORDS: Lipid oxidation; oil-in-water emulsion; free fatty acid; fatty acid methyl ester; emulsion droplet surface charge; pH; EDTA

INTRODUCTION

Lipid oxidation is a common cause of quality deterioration in lipid-containing food products resulting in changes in quality attributes such as taste, appearance, texture, and shelf life as well as the loss of important nutrients and formation of potentially toxic reaction products (*1*−*4*). Many lipid-containing food products are in the form of oil-in-water emulsions such as milk, fruit, and nutritional beverages, salad dressings, soups, and sauces. There are many factors that affect lipid oxidation rates in oil-in-water emulsions, including fatty acid composition, oxygen concentration, type and concentration of antioxidants, interfacial characteristics of emulsion droplet such as electrical charge, and the ability of aqueous phase prooxidants such as transition metals to interact with oxidizable lipids (*3*).

Even though edible oils are refined to remove undesirable components, commercial oils still contain small amounts of minor components including free fatty acids, monoacylglycerols, diacylglycerols, phospholipids, and sterols. These minor components are surface active compounds that could affect lipid oxidation by altering the chemical and physical properties of oils. Free fatty acids are formed during lipid extraction and refining by hydrolysis of triacylglycerols by lipases and high temperature in the presence of water. Free fatty acids are removed from crude oils by neutralization and deodorization. However, these refining steps are not 100% efficient, with commercial oils typically containing 0.05−0.70% of free fatty acids (*5*−*7*). Besides negatively affecting oil quality by causing foaming and reducing the smoke point of the oils, free fatty acids can also act as prooxidants in bulk oils. Several researchers have reported that the prooxidant effect of free fatty acids in bulk oils is the result of the carboxylic acid group because methyl esters of free fatty acids are not prooxidative (*8*−*11*). The current hypothesis for the prooxidant activity of free fatty acids is to directly promote the acid-catalyzed decomposition of lipid hydroperoxides and/or form prooxidative complexes with trace metals.

Free fatty acids are surface active compounds because they are more polar than triacylglycerols due to the presence of unesterified carboxylic acid groups. The surface activity of free fatty acids allows them to diffuse and concentrate at the water−lipid interface of the oil-in-water emulsions (*12*). Thus, free fatty acids could potentially make the emulsion droplet more negatively charged when pH values are above their pK_a values [4.8−5.0 for medium- and long-chain (C ≥10) fatty acids in aqueous solution (*13*−*15*)]. Previous research has shown that negatively charged colloidal lipid systems dispersed in water can attract prooxidant transition metals that can increase metal−lipid interactions, thus accelerating oxidation (*16*−*19*).

Although there are several studies on the prooxidant effects of free fatty acids in bulk oils, there are almost no studies on the impact of free fatty acids on lipid oxidation of oil-in-water emulsions. Because the mechanisms of lipid oxidation in oil-in-water emulsions can be very different from those in bulk oils (*3*), this study was conducted to investigate the role free fatty acids on

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 Published on Web 07/02/2009

oxidation in emulsions as a function of free fatty acid concentration and pH as well as in the presence of free fatty acid hydroperoxides and metal chelators. Understanding how free fatty acids affect lipid oxidation in oil-in-water emulsions could provide fundamental knowledge that could be used to improve the oxidative stability of oils in emulsions and other food dispersions.

MATERIALS AND METHODS

Materials. Soybean oil was purchased from a local retail store. Oleic acid and methyl oleate were purchased from Nu-Chek Prep, Inc. (Elysian, MN). Ethylenediaminetetraacetic acid (EDTA), potassium phosphate monobasic, potassium phosphate dibasic heptahydrate, silicic acid (100−200 mesh, 75−150 μm, acid washed), activated charcoal (100−400 mesh), polyoxyethylene (*20*) sorbitan monolaurate (Tween 20), ammonium thiocyanate, and iron(II) sulfate heptahydrate were obtained from Sigma Chemical Co. (St. Louis, MO). Iso-octanol, *n*-hexane, 2-propanol, methanol, and 1-butanol were purchased from Fisher Scientific (Fair Lawn, NJ). All of the chemicals used in this experiments were of analytical grade or purer. Glassware was incubated in 3 mM HCl overnight to remove metals followed by rinsing with double-distilled water before use. Double-distilled water was used throughout the study.

Methods. *Preparation of Stripped Soybean Oil*. Stripped soybean oil as prepared according to the method of Boon et al. (*20*) was used in all experiments. In short, silicic acid (100 g) was washed three times with a total of 3 L of distilled water followed by filtering with Whatman filter paper in a Buchner funnel and drying at 110 °C for 20 h. The washed silicic acid (22.5 g) and activated charcoal (5.625 g) were suspended in 100 and 70 mL of *n*-hexane, respectively. A chromatographic column (3.0 cm internal diameter × 35 cm height) was then packed sequentially with 22.5 g of silicic acid followed by 5.625 g of activated charcoal and then another 22.5 g of silicic acid. Thirty grams of soybean oil was dissolved in 30 mL of *n*-hexane and passed through the column by eluting with 270 mL of *n*-hexane. To retard lipid oxidation during stripping, the collected triacylglycerols were held in an ice bath, which was covered with aluminum foil. The solvent in the stripped oils was removed with a vacuum rotary evaporator (RE 111 Buchi, Flawil, Switzerland) at 37 °C, and traces of the remaining solvent were removed by flushing with nitrogen. Then 3 g of the stripped oil was transferred into 3 mL vials, flushed with nitrogen, and kept at −80 °C for subsequent studies.

Preparation of Free Fatty Acids. Hydroperoxides are primary products from lipid oxidation and are substrates for decomposition of secondary products such as aldehydes and ketones. Therefore, in this study, the initial hydroperoxides in commercial oleic acid were removed to ensure that the effects of added free fatty acids on oxidation in emulsions were not due to the addition of lipid hydroperoxides. The hydroperoxide reduction process was adapted from that of Miyashita and Takagi (*8*) using silicic acid. Column chromatography was set up using a glass syringe (2.0 cm internal diameter × 10.5 cm height), the outlet of which was covered with three layers of nylon membrane filters (Nylaflo Nylon membrane filters, 47 mm 0.45 μm, Gelman Sciences, Ann Arbor, MI). Silicic acid was pretreated as described for the preparation of stripped soybean oil. Silicic acid (5.0 g) was suspended in 22.5 mL of *n*-hexane and then poured into the column. Three grams of oleic acids diluted in 3 mL of *n*-hexane was loaded onto the column and followed by elution with 40.0 mL of *n*-hexane. The eluent was collected in an ice bath covered with aluminum foil to retard lipid oxidation. The solvent with oleic acid was kept in a glass tube with a seal cap at −80 °C until use. Solvent was removed by flushing with nitrogen prior to use. Hydroperoxide residues in oleic acids after the treatment were reduced from 6.8 to < 0.05 mmol of hydroperoxides/kg of fatty acids. For experiments on the effect of oleic acid hydroperoxides on oxidation rates, purified oleic acid was incubated at 55 °C in the dark to allow for the formation of hydroperoxides (5.0 mmol/kg of oleic acid final concentration) and was then added to the 1.0% stripped soybean oil-in-water emulsions, keeping total oleic acid concentration at 1.0% of the oil.

Emulsion Preparation and Storage Conditions. Oil-in-water emulsions were prepared using 1.0% (wt) stripped soybean oil in a 10 mM phosphate buffer solution (pH 7.0). Tween 20 was used as an emulsifier at a 1:10 emulsifier/oil ratio. The emulsion was prepared by adding purified oleic acid in *n*-hexane into a beaker and flushing with nitrogen gas to remove the solvent. Then stripped soybean oil, Tween 20, and phosphate buffer were added to the beaker, and a coarse emulsion was made by blending with a hand-held homogenizer (M133/1281-0, Biospec Products, Inc., Bartlesville, OK) for 2 min. The coarse emulsion was then homogenized with a microfluidizer (Microfluidics, Newton, MA) at a pressure of 9 kbar for three passes. During homogenization, ice was used to cover the homogenizer chamber and coil to maintain the emulsion temperature at ≤25 °C. One milliliter of each emulsion was transferred into 10 mL GC vials (Supelco, Bellefonte, PA), capped with aluminum caps with PTFE/silicone septa and stored in the dark at 15 °C.

Measurement of Particle Size Distributions and Zeta Potential (ζ). Samples for droplet size distribution and zeta potential measurements were diluted into 10 mM phosphate buffer at the same pH as the emulsions at an emulsion/buffer ratio of 1:50. Both particle size distributions and zeta potential of the emulsions were analyzed in a ZetaSizer Nano-ZS (Malvern Instruments, Worcestershire, U.K.). Each measurement was repeated twice at room temperature.

Measurement of Lipid Oxidation. Lipid hydroperoxides, which are primary products of lipid oxidation, were measured using the method adapted from Shantha and Decker (*21*). Each sample (0.3 mL) was vortexed three times (10 s each) with 1.5 mL of an isooctanol+isopropanol (3:1 v/v) solution. The samples were then centrifuged for 2 min at 3400*g* (Centrific TM Centrifuge, Fisher Scientific), and 0.2 mL of the upper organic layer or diluted with methanol/butanol (depending on the extent of lipid oxidation) was mixed with 2.80 mL of methanol+butanol solution (2:1 v/v), 15 μL of 3.94 M ammonium thiocyanate, and 15 μL of ferrous iron solution (prepared by mixing 0.132 M $BaCl_2$ and 0.144 M $FeSO_4$). The absorbance of the samples was measured at 510 nm using a Genesys 20 spectrophotometer (ThermoSpectronic, Waltham, MA) 20 min after the addition of the iron. Hydroperoxide concentrations were quantitated on the basis of a cumene hydroperoxide standard curve.

Hexanal was measured as a secondary lipid oxidation product as described by Boon et al. (*20*) using a GC-17A Shimadzu gas chromatograph equipped with an AOC-5000 autosampler (Shimadzu, Kyoto, Japan). Emulsions (1 mL) in 10 mL glass vials capped with aluminum caps with PTFE/silicone septa were shaken and heated at 55 °C for 13 min in an autosampler heating block before measurement. A 50/30 μm DVB/Carboxen/PDMS solid-phase microextraction (SPME) fiber needle from Supelco was injected into the vial for 1 min to absorb volatiles and then was transferred to the injector port (250 °C) for 3 min. The injection port was operated in split mode, and the split ratio was set at 1:5. Volatiles were separated on a Supleco 30 m × 0.32 mm Equity DB-1 column with a 1 μm film thickness at 65 °C for 10 min. The carrier gas was helium at 15.0 mL/min. A flame ionization detector was used at a temperature of 250 °C. Hexanal concentrations were determined from peak areas using a standard curve prepared from authentic hexanal.

Statistical Analysis. All experiments were conducted in triplicate samples and were repeated at least two times. Data were presented as mean ± standard deviation. Data results were analyzed by analysis of variance (ANOVA) using SPSS (SPSS Inc., Chicago, IL). The differences between mean values were compared using Duncan's multiple-range test with significance defined as $p < 0.05$.

RESULTS AND DISCUSSION

Physical Stability of Emulsions. The droplet size of the emulsions was measured immediately after emulsion preparation and every 24 h throughout storage. Emulsion droplets size ranged from 165 to 185 nm and did not change significantly during the course of the experiments (data not shown). The stability of the emulsions was also confirmed by no visual observation of creaming during storage (data not shown). These indicated that the emulsions were stable to droplet aggregation, flocculation, or coalescence (*22*).

Effect of Oleic Acid Concentrations on the Physical and Chemical Properties of Oil-in-Water Emulsions. Previous studies by Miyashita and Takagi (*8*) and Mistry and Min (*9*) showed that free fatty acids are prooxidative in bulk soybean oil. To determine how free fatty acids influence the oxidation of oil-in-water

Table 1. Droplet Surface Charge or Zeta Potential (ζ) of 1.0% Stripped Soybean Oil-in-Water Emulsions without (Control) and with Addition of 1.0, 2.5, and 5.0% Oleic Acid and 1.0% Methyl Oleate at pH 7.0

sample	ζ (mV)	sample	ζ (mV)	sample	ζ (mV)
control	−9.54	1.0% oleic acid	−27.50	1.0% methyl oleate	−12.2
		2.5% oleic acid	−44.95		
		5.0% oleic acid	−53.70		

emulsions, different concentrations of oleic acid (1.0, 2.5, and 5.0% of oil concentration) were added during emulsification of the Tween 20-stabilized soybean oil-in-water emulsions at pH 7.0. Tween 20 was chosen because it is a nonionic surfactant so it would have a lower impact on emulsion droplet surface charge than ionic emulsifiers. Because commercial free fatty acids can contain lipid hydroperoxides, they were purified prior to addition to the emulsions.

The droplet surface charge or zeta potential (ζ) of the emulsions with various concentrations of oleic acid is shown in **Table 1**. Control emulsions had a surface charge of −9.54 mV compared to −27.50, −44.95, and −53.70 mV when the emulsions contained 1.0, 2.5, and 5.0% oleic acid, respectively. Tween 20-stabilized oil-in-water emulsions have been previously reported to be negatively charged (*23*−*25*). It is unclear if this negative charge is due to impurities in the Tween 20. The decrease in surface charge with increasing oleic acid concentrations suggested that acid groups of the oleic acid were migrating to and concentrating at the lipid−water interface of the emulsion droplet.

The rates of lipid oxidation of 1.0% stripped soybean oil-in-water emulsions with added oleic acid were followed by monitoring lipid hydroperoxide formation as an indictor of primary oxidation products and hexanal formation as an indicator of secondary oxidation products (**Figure 1**). Increasing oleic acid concentrations significantly increased both lipid hydroperoxides and headspace hexanal formation. After 6 h of storage, 5.0% oleic acid exhibited a significantly higher hydroperoxide concentration than all other emulsions. After 1 day of storage, there were dramatic increases in hydroperoxide formations in all of the emulsions containing added oleic acid (**Figure 1a**). A similar trend was observed for hexanal formation (**Figure 1b**). From this study, free fatty acids were clearly shown to act as powerful prooxidant in oil-in-water emulsions with as little as 1.0% oleic acid in the oil phase accelerating both lipid hydroperoxide and hexanal formation.

Effect of Methyl Oleate and Oleic Acid on the Physical and Chemical Properties of Oil-in-Water Emulsions. The ability of the free fatty acids to accumulate at the oil−water interface can decrease emulsion droplet surface charge (**Table 1**). Because cationic metals are strong prooxidants in oil-in-water emulsions, negatively charged emulsion droplets could attract metals, thus accelerating lipid oxidation rates. However, Miyashita and Takagi (*8*) also postulated that free fatty acids could promote lipid oxidation via acid-catalyzed hydroperoxide decomposition into free radicals. To determine if a free carboxylic acid group was necessary for the prooxidant activity of fatty acids, both oleic acid and methyl oleate were added to the stripped soybean oil-in-water emulsions at 1.0% of the oil content. **Table 1** shows that the droplet surface charge was −9.54, −27.50, and −12.20 mV for emulsions with no added fatty acids, oleic acid, and methyl oleate, respectively. **Figure 2** shows that whereas free oleic acid accelerated both lipid hydroperoxide and headspace hexanal formation, the presence of methyl oleate did not change oxidation rates compared to the control with no added fatty acids. These data show that a free carboxylic acid group is necessary for the prooxidant activity of fatty acids in oil-in-water emulsions, which

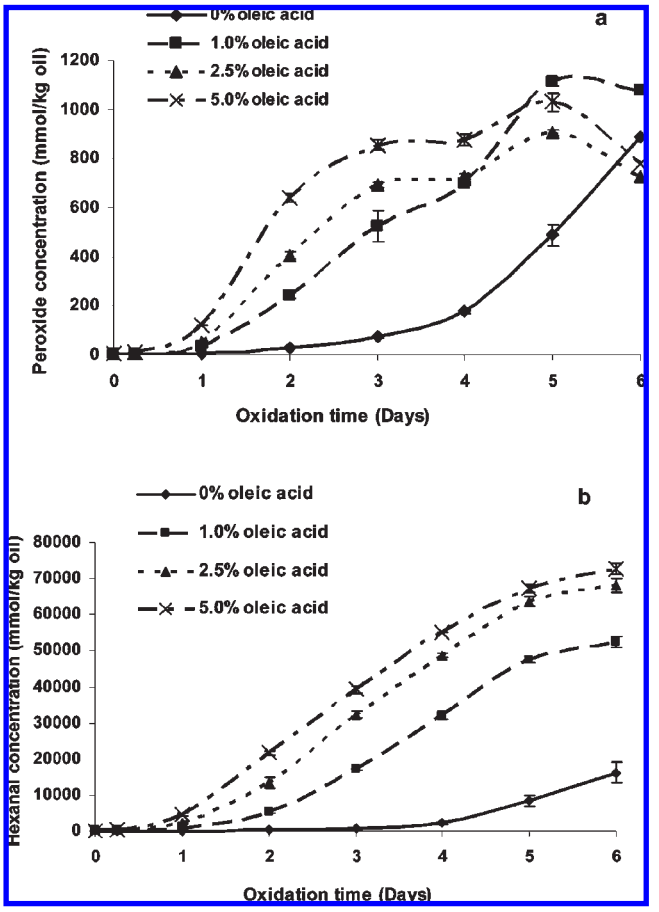

Figure 1. Formation of lipid hydroperoxide concentration (**a**) and hexanal (**b**) in 1.0% stripped soybean oil-in-water emulsions at pH 7.0 without (control) and with addition of 1.0, 2.5, and 5.0% oleic acids (oil wt) during storage at 15 °C in the dark for 6 days.

is in agreement with Miyashita and Takagi (*8*), Mistry and Min, (*9*), and Frega et al. (*11*), who found similar results in bulk oils.

Effect of pH on Physical and Chemical Properties of Oil-in-Water Emulsions Containing Oleic Acid. Transition metals are found abundantly in nature and thus can end up in foods from a variety of sources, including water, packaging, processing equipment, and ingredients including fats and oils (*26*). Transition metals such as iron primarily accelerate lipid oxidation by promoting the decomposition of lipid hydroperoxides into highly reactive alkoxy and peroxy radicals, which can abstract hydrogen from fatty acids, thus further propagating oxidation (*7*). One of the most important factors that influence the prooxidant activity of iron is its physical location, which dictates its ability to interact with lipid hydroperoxides. For example, iron−lipid hydroperoxide interactions and thus lipid oxidation increase dramatically in negatively charged emulsion droplets, where iron is attracted to the emulsion droplet surface (*27*, *28*).

The pK_a of medium- and long-chain (C ≥ 10) fatty acids in aqueous solution is approximately 4.8−5.0 (*13*−*15*). If the ability of free fatty acids to promote lipid oxidation in oil-in-water emulsions is due to their ability to make emulsion droplets more negatively charged, then when the pH of the emulsions is below the pK_a of the free fatty acids, the emulsion droplet charge would decrease as the acid group became protonated and lipid oxidation rates would decrease. To test this hypothesis, the emulsion droplet charge and oxidative stability of 1.0% stripped soybean

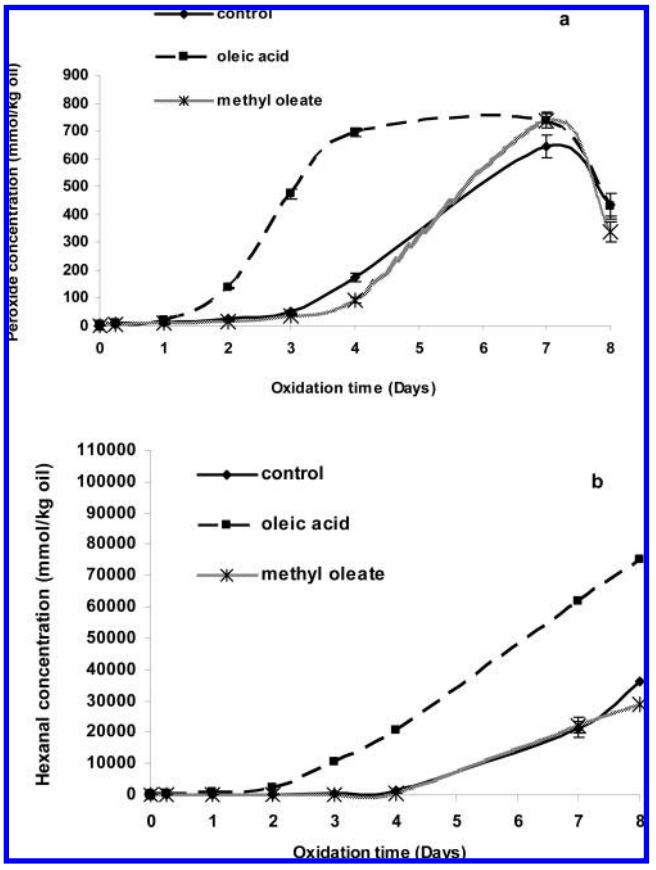

Figure 2. Formation of lipid hydroperoxide concentration (**a**) and hexanal (**b**) in 1.0% stripped soybean oil-in-water emulsions without (control) and with addition of 1.0% oleic acids and 1.0% methyl oleate (oil wt) at pH 7.0 during storage at 15 °C in the dark for 8 days.

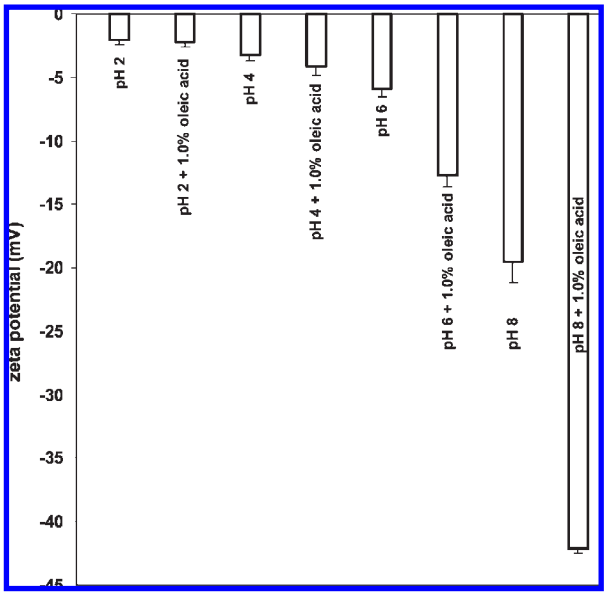

Figure 3. Droplet surface charge or zeta potential (ζ) of 1.0% stripped soybean oil-in-water emulsions with addition of 1.0% oleic acids (oil wt) at pH 2.0, 4.0, 6.0, and 8.0.

oil-in-water emulsions with and without 1.0% oleic acid (wt % in oil) were measured over the pH range of 2.0−8.0.

The droplet surface charge of the emulsions as a function of pH is shown in **Figure 3**. The surface charge ranged from −2.22 to −42.10 mV and from −2.05 to −19.50 mV from pH 2.0 to 8.0 for emulsions with and without oleic acid, respectively. A significant increase in emulsion droplet charge in the presence of oleic acid occurred at pH values above 4.0. The large increase in droplet charge above the pK_a of the free fatty acids is likely due to the deprotonation of the fatty acids producing a highly polar anionic carboxylic acid, which can then migrate to the oil−water interface.

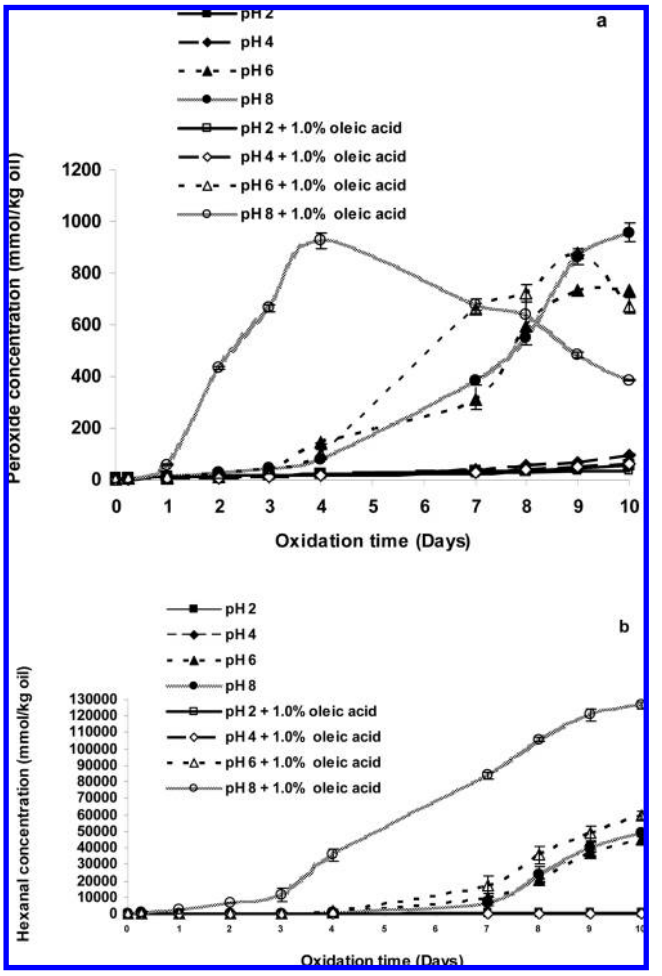

Figure 4. Formation of lipid hydroperoxide concentration (**a**) and hexanal (**b**) in 1.0% stripped soybean oil-in-water emulsions with addition of 1.0% oleic acids (oil wt) at pH 2.0, 4.0, 6.0, and 8.0 during storage at 15 °C in the dark for 10 days.

The influence of pH on the oxidative stability of stripped soybean oil-in-water emulsions with and without 1.0% oleic acid (wt % in oil) is shown in **Figure 4**. As the pH of the emulsions without added fatty acids was decreased from 8.0 to 6.0, both hexanal and hydroperoxide formation decreased. In emulsions without oleic acid at 2.0 and 4.0, the concentrations of lipid hydroperoxides (< 55 and 95 mmol/kg of oil, respectively) and headspace hexanal (< 850 and 300 mmol/kg of oil, respectively) were low throughout the storage study. Addition of oleic acid only showed a prooxidant effect at pH 6.0 and 8.0. Overall, oxidation rates in all of the emulsions decreased as the negative charge of the emulsion droplet decreased (**Table 1**). Added oleic acid was only prooxidative at pH values above the pK_a, at which the free fatty acids were able to make the emulsion droplet more negatively charged. These data further support the notion that an unprotonated carboxylic acid group is necessary for the prooxidant activity of fatty acids. It is unclear why the negative charge of the emulsions droplet without added fatty acids decreased. It is

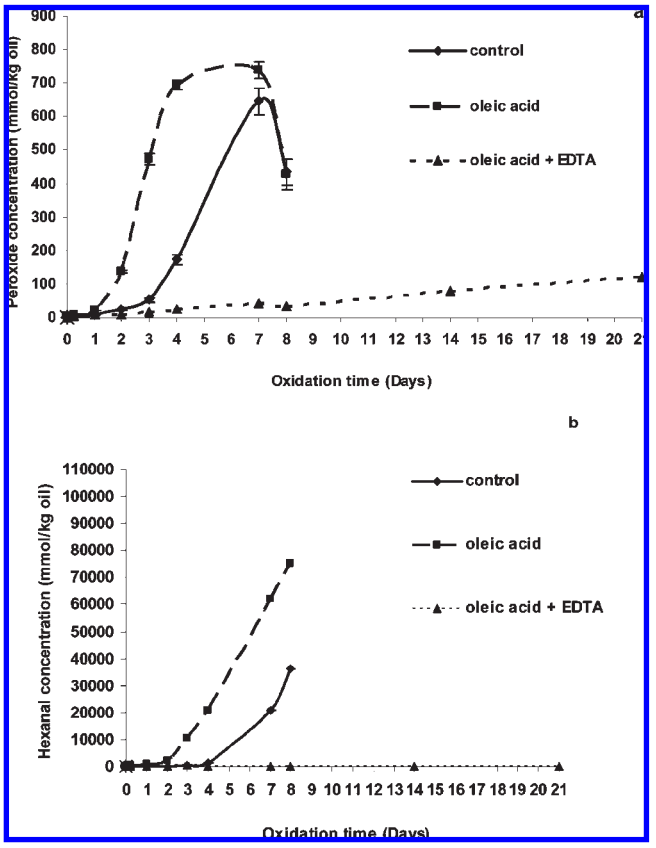

Figure 5. Formation of lipid hydroperoxide concentration (**a**) and hexanal (**b**) in 1.0% stripped soybean oil-in-water emulsions with addition of 1.0% oleic acids (oil wt) without and with 200 μm EDTA at pH 7.0 during storage at 15 °C in the dark for 21 days.

possible that this could be due to the presence of free fatty acids in Tween 20.

Effect of EDTA and Fatty Acid Hydroperoxides. From the above data it is unclear if the prooxidant activity of free fatty acids in oil-in-water emulsions is due to acid-catalyzed decomposition of preexisting lipid hydroperoxides or the ability of surface active free fatty acids to concentrate at the emulsion droplet oil−water interface, where they can make the droplet more anionic, thus attracting transition metals that promote the oxidation. To better understand which mechanism is most prevalent, EDTA was added to the emulsions to evaluate the role of transition metals in the prooxidant activity of free fatty acids. There was no significant difference in emulsion droplet charge in stripped soybean oil-in-water emulsions in the presence of 200 μM EDTA (data not shown). However, lipid oxidation was significantly inhibited by EDTA with only small amounts of hydroperoxides being formed and almost complete suppression of headspace hexanal formation (**Figure 5**). Because EDTA did not change the surface charge of the emulsion droplets, these data strongly suggest that the prooxidant mechanism of free fatty acids is due to their ability to attract prooxidant metals to the surface of the emulsion droplet, where they can interact with oxizable lipids versus the acid-catalyzed decomposition of preexisting lipid hydroperoxides.

Hydroperoxides are primary products of lipid oxidation that are normally found in fats and oils due to formation during processing, transportation, and storage (*29*, *30*). Most lipid-containing foods contain hydroperoxides; even high-quality lipids still contain about 10−100 nmol/g of lipid. In oil-in-water emulsions, another source of hydroperoxides comes from the surfactant, for example, phospholipids and Tween 20, which were found to have 4−35 μmol of

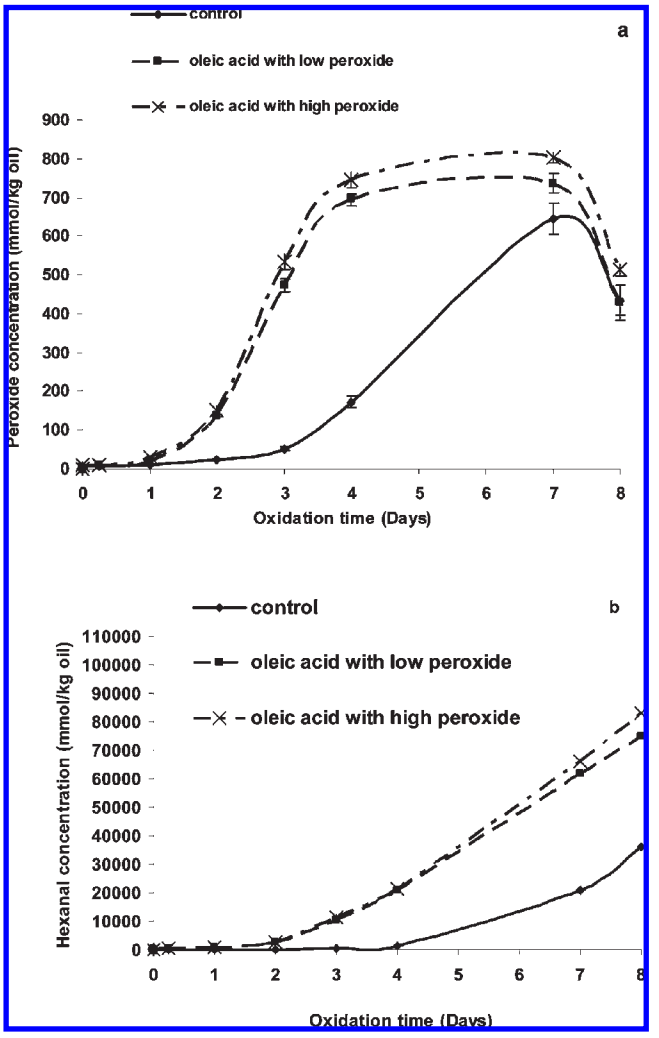

Figure 6. Formation of lipid hydroperoxide concentration (**a**) and hexanal (**b**) in 1.0% stripped soybean oil-in-water emulsions with addition of 1.0% oleic acids low and high in hydroperoxides (oil wt) at pH 7.0 during storage at 15 °C in the dark for 8 days.

hydroperoxides/g of surfactant (*31*). Lipid hydroperoxides are strong prooxidants due to their ability to decompose into free radicals in the presence of light, metals, and high temperatures (*4*, *31*). Hydroperoxides on free fatty acid, methyl esters, and triacylglycerols increase the surface activity of the parent molecules, presumably due to the presence of oxygen, which increases polarity. Fatty acid hydroperoxides are more surface active than methyl esters or acylglycerol hydroperoxides (*12*). Therefore, it is possible that the presence of free fatty acid hydroperoxides could increase oxidation rates in oil-in-water emulsions because the free fatty acid hydroperoxides would concentrate at the oil−water interface, where they can readily interact with iron bound to the emulsion droplet surface.

In the previous experiments in this study, oleic acid was purified so that hydroperoxide concentrations were low (<0.05 mmol/kg of fatty acid). To determine if oleic acid hydroperoxides accelerated lipid oxidation in oil-in-water emulsion, oleic acid oxidized at 55 °C in the dark was blended with purified oleic acid and added to the 1.0% stripped soybean oil-in-water emulsions at 5.0 mmol of hydroperoxides/kg of oleic acid (keeping total oleic acid concentration at 1.0% of the oil). The zeta potential of emulsion droplets with oleic acid low in hydroperoxides (-31.3 ± 0.5) was not significantly different ($p < 0.05$) from emulsion droplets with oleic acid high in hydroperoxides (-29.2 ± 0.4). According to the lipid oxidation results shown in **Figure 6**, neither lipid hydroperoxide

nor hexanal formation rates were different in oil-in-water emulsions with low or high oleic acid hydroperoxide concentration. Many studies have shown that lipid oxidation rates are very dependent on the concentration of lipid hydroperoxides. Studies from Nuchi and co-workers (*12*, *31*) found that both Tween 20 and linoleic acid hydroperoxides decreased the lag phase of hexanal formation in oil-in-water emulsions. Therefore, it was somewhat surprising that the addition of oleic acid hydroperoxides did not increase oxidation rates. However, the amount of hydroperoxides in the original emulsions was 35 μM/kg emulsion. Therefore, the added oleic acid hydroperoxides increased total hydroperoxides concentrations by only 1.5%. Even though the oleic acid hydroperoxide level is low in comparison to hydroperoxides on the triacylglycerols, it does show that highly surface active oleic acid hydroperoxides are not more prooxidative than hydroperoxides on the triacylglycerols.

In summary, oil-in-water emulsions containing free fatty acids are extremely susceptible to lipid oxidation when the pH of the emulsion is higher than the pK_a of the free fatty acids. Under these conditions the carboxylic acid groups on the free fatty acids are negatively charged. Because the charged free fatty acids are surface active, they migrate to the oil−water interface of emulsion droplets, where they decrease the negative charge of the emulsion droplet. Inhibition of lipid oxidation in oil-in-water emulsions containing free fatty acids by EDTA indicates that the most likely mechanism for the prooxidant activity of free fatty acids is the attraction of cationic transition metals to the emulsion droplet surface, where they can interact with lipid and promote oxidation. These results indicate that the oxidative stability of oil-in-water emulsions could be greatly improved by maintaining low levels of free fatty acids.

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Received April 17, 2009. Revised manuscript received June 15, 2009. Accepted June 18, 2009.