JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Factors Affecting Lycopene Oxidation in Oil-in-Water Emulsions

CAITLIN S. BOON,[†] ZHIMIN XU,[§] XIAOHUA YUE,[§] D. JULIAN MCCLEMENTS,[†] JOCHEN WEISS,[†] AND ERIC A. DECKER*,[†]

Department of Food Science, Chenoweth Laboratory, University of Massachusetts, Amherst, Massachusetts 01003, and Department of Food Science, Louisiana State University, Baton Rouge, Louisiana 70803

Evidence that dietary lycopene decreases the risk for a number of health conditions has generated new opportunities for the addition of lycopene to functional foods. This work examined the potential of oil-in-water emulsions as a lycopene delivery system for foods. Oil-in-water emulsions containing lycopene were prepared using different kinds of surfactant (cationic, anionic, and nonionic) and oil types (corn oil, stripped corn oil, and hexadecane). The formation of fatty acid oxidation products and the degradation of lycopene and tocopherol were then monitored. Fatty acids and lycopene had greater stability in oil-in-water emulsions stabilized by cationic dodecyltrimethylammonium bromide (DTAB) or nonionic polyoxyethylene (23) lauryl ether than by anionic sodium dodecyl sulfate (SDS). Oxidative stability in the corn oil-in-water emulsions stabilized by SDS was in the following order: tocopherol < lycopene < fatty acids. When emulsions were prepared using different carrier oils, the lycopene stability was in the following order: nonstripped corn oil > hexadecane > tocopherol-stripped corn oil. Lycopene degradation rates were similar in emulsions with and without fatty acids, suggesting that lycopene loss was independent of the presence of fatty acids. These results suggest that the stability of lycopene in oil-in-water emulsions could be inhibited by altering the emulsion droplet interface and by the presence of tocopherols.

KEYWORDS: Lipid oxidation; emulsion; lycopene; carotenoid; corn oil; iron; surfactants; hexadecane

INTRODUCTION

Lycopene is a chromoplast carotenoid responsible for the red color of tomatoes, watermelon, guava, and grapefruit (*1, 2*). In recent years, a number of studies have suggested that dietary lycopene may decrease the risk for developing a number of health conditions due to its ability to act as an antioxidant and to stimulate cell-to-cell communication (*3*). The greatest evidence, thus far, for a medical condition that dietary lycopene affects is a reduced incidence of prostate cancer (*4*). However, a number of studies have provided limited evidence that lycopene intake may also be associated with a reduced risk of cardiovascular disease (*5*) as well as cancers of the cervix, colon, esophagus, stomach, and breast (*2, 6*). These findings have increased consumer interest in lycopene-containing food products and generated new applications for lycopene-containing food ingredients.

The antioxidant properties associated with the health benefits of lycopene present challenges in preventing the degradation of lycopene within food products (*7*). Because lycopene is lipid-soluble (*5*), one way of protecting it from oxidative degradation might be to incorporate it into the oil phase of oil-in-water emulsions. Emulsions are effective ingredient delivery systems for functional foods because they can be engineered with numerous antioxidative functions (*8*). Incorporation of antioxidative functionality into the aqueous phase, oil phase, and the surfactant-containing interfacial region of an oil-in-water emulsion can more effectively inhibit the oxidation of lipids in the core of the emulsion droplet compared to the oil in a bulk phase. An additional advantage emulsions as ingredient delivery systems is that their antioxidant protection systems can remain active once the emulsion is dispersed into water-based food (*9, 10*).

As a first step to understanding the potential for emulsions as oxidatively stable delivery systems for bioactive ingredients such as lycopene, one must understand the relative oxidative susceptibility of each lipid within the emulsion. Food emulsions contain a variety of anti- and pro-oxidative species that, when combined, influence the overall rate of degradation of both the fatty acids and bioactive components in emulsion droplets. In this work, various oil types were used to understand how oil composition might influence the rate of both fatty acid and lycopene oxidation. The results of this work should help to provide information on whether lipid oxidation or lycopene

* Corresponding author [e-mail edecker@foodsci.umass.edu; telephone (413) 545-1026; fax (413) 545-1262].
[†] University of Massachusetts.
[§] Louisiana State University.

10.1021/jf072929+ CCC: $40.75 © 2008 American Chemical Society
Published on Web 02/01/2008

degradation is the most important factor in determining the shelf life of a lycopene-containing delivery system, as well as determining which oil composition might offer greatest protection.

When considering the oxidative stability of unsaturated fatty acids in oil-in-water emulsions, the reaction of most concern is the breakdown of lipid hydroperoxides naturally present at oil droplet interfaces by endogenous iron or other reactive transition metals in the aqueous phase (*9*). As this reaction proceeds, additional lipid hydroperoxides and highly reactive peroxyl and alkoxyl radicals are formed. These radicals in turn react with other unsaturated fatty acids, thus propagating the oxidation reaction. Eventually, these reactions result in fatty acid decomposition to produce secondary oxidation products, such as aldehydes and ketones, which are associated with the off-flavors and odors of rancid food products (*9, 11, 12*). By reducing the interactions between lipid hydroperoxides and transition metals, the speed of the entire lipid oxidation process can be slowed (*9*).

A number of mechanisms have been used to reduce interactions between transition metals and lipid hydroperoxides in oil-in-water emulsions, including the manipulation of the interfacial region to form a physical and electrostatic barrier (*13–18*). Mancuso et al. and Mei et al. (*13, 14*) showed that surfactant type could influence the rate of oxidation. Specifically, these studies found that emulsions stabilized by anionic surfactants oxidized at the fastest rates, followed by nonionic surfactants, while cationic surfactants stabilized emulsions exhibited the slowest oxidation. The proposed reasoning for this observation was that cationic surfactants create a positively charged barrier around the oil droplets that repel cationic iron and other metals, whereas anionic surfactants attract iron to the droplet surface, where it can easily degrade lipid hydroperoxides (*9, 13, 14*).

The objective of this work was to gain a better understanding of how the properties of the emulsion droplet interface will influence the oxidative stability of lycopene and unsaturated fatty acids. By gaining a better understanding of the mechanisms of lycopene degradation and lipid oxidation in emulsions, new antioxidant technologies could be developed that could stabilize this important bioactive component in functional foods.

MATERIALS AND METHODS

Corn oil was purchased from a local supermarket. LycoVit Dispersion (11% lycopene) in sunflower oil was donated by BASF Corp. (Florham Park, NJ). Pure lycopene standard was a gift of LycoRed Ltd. (Beer Sheva, Israel). Dodecyltrimethylammonium bromide (DTAB), sodium dodecyl sulfate (SDS), imidazole, barium chloride dihydrate, ammonium thiocyanate, iron(II) sulfate heptahydrate, silicic acid, activated charcoal, and *n*-hexane were purchased from Sigma Chemical Co. (St. Louis, MO). Polyoxyethylene(23) lauryl ether (Brij 35), sodium acetate, hydrochloric acid (HCl), sodium hydroxide (NaOH), iso-octane, 2-propanol, methanol, and 1-butanol were purchased from Fisher Scientific (Fair Lawn, NJ). All chemicals were of analytical grade or purer.

Isolation of Corn Oil Triacylglycerols. Stripped corn oil was prepared by diluting 30 g of oil with 30 mL of hexane. This mixture was passed through a chromatographic column (3.0 cm diameter, 35 cm in length). The bottom layer of the column was packed with 22.5 g of silicic acid that had been washed three times with distilled water, filtered, and activated at 110 °C for 20 h. A middle layer of 5.625 g of activated charcoal was used as well as a top layer of 22.5 g of the washed, filtered, and activated silicic acid. The oil was eluted with 270 mL of *n*-hexane, and the solvent was removed in a Rotavapor RE 111 (Büchi, Flawil, Switzerland) at 38 °C. Traces of solvent were removed by flushing with nitrogen, and oil was stored at −80 °C until use.

Preparation and Storage of Emulsions. Oil-in-water emulsions were prepared using 5 wt % oil phase in a sodium acetate−imidazole buffer solution (10 mM each, pH 7.0) containing 30 mM surfactant (SDS, Brij 35, or DTAB). The oil phase of the emulsion was prepared by dispersing the LycoVit dispersion into corn oil, hexadecane, or stripped corn oil at a final concentration of 0.33 mg of lycopene/g of oil (stored at −80 °C until use). This amount of lycopene was chosen because it allowed for a reasonable range for quantifying lycopene using and integrating sphere. An aqueous phase was prepared and stirred overnight to ensure complete dispersion of the surfactant. Just prior to emulsion preparation, the oil phase was thawed using tap water. For corn oil emulsions, a coarse oil-in-water emulsion was prepared by blending the oil and aqueous phases for 2 min using a Biohomogenizer M133/1281-0 (Biospec Products, Inc., Bartlesville, OK). Due to the increased physical instability of coarse emulsions containing hexadecane, these samples were prepared by blending for 2 min with the Biohomogenizer followed by an additional step of sonicating for 2 min (using 0.5 s pulses) at 70% amplitude using a Fisher Scientific Sonic Dismembrator 500. The coarse emulsions were then homogenized through a two-stage high-pressure valve homogenizer (APV-Gaulin, Wilmington, MA) for four passes at 4000 psi (27.6 MPa). During each pass, the emulsions were collected in a beaker submerged in a cool water bath. After homogenization, the pH of each emulsion was adjusted to pH 7.0 using HCl or NaOH. During each step in emulsion preparation, samples were covered as much as possible to reduce light exposure.

Emulsions were split into individual samples for each treatment and analysis, stored in the dark at 15 °C, and rocked constantly.

Preparation and Storage of Pure Lycopene Emulsions. A series of lycopene degradation experiments were conducted to compare the degradation rates of pure lycopene to the LycoVit dispersion, which contains sunflower oil in addition to the lycopene. To create the oil phase, pure lycopene crystals (95% purity) were added to hexadecane at a concentration of 3 mg of lycopene/g of hexadecane. The oil phase was then sonicated for 85 s (using 0.2 s on, 0.5 s off pulses) at 25% amplitude using a Fisher Scientific Sonic Dismembrator 500. The emulsions were then prepared as described above for hexadecane emulsions. Due to the expense of pure lycopene, smaller quantities of these emulsions were made, and samples were stored in 23-G-20 capped glass fluorometer cells (Starna Cells, Inc., Atascadero, CA) and placed on rocker plates in the dark at 15 °C.

Physical Properties of Emulsions. Particle size distributions of the emulsion droplets were measured using a laser diffraction instrument (Malvern Mastersizer, Malvern Instruments, Worcestershire, U.K.). The mean particle diameters (D_{43}) of the DTAB, Brij 35, and SDS corn oil-in-water emulsions were found to be 0.36 ± 0.02, 0.41 ± 0.11, and 0.37 ± 0.01 μm, respectively. Hexadecane emulsions stabilized by SDS had a mean particle diameter (D_{43}) of 0.26 ± 0.00 μm. Particle size distributions were measured periodically and did not change over the course of the experiments. The electrical charge, or zeta potential (ζ), of the emulsion droplets was measured using a microelectrophoresis instrument (ZetaSizer Nano, Malvern Instruments). Zeta potential samples were prepared by diluting emulsions 1:100 with 10 mM sodium acetate−imidazole buffer (pH 7.0) and placing the dilutions into disposable capillary cells (Malvern Instruments).

Measurement of Lipid Oxidation. Lipid hydroperoxide concentrations were used as an indicator of primary lipid oxidation products. Lipid hydroperoxide concentrations were determined according to a modified version of Nuchi et al. (*19*). Emulsion samples (0.3 mL) were mixed with 1.5 mL of iso-octane/2-propanol (3:1, v/v), by vortexing (10 s, three times). This mixture was then centrifuged at 3400*g* for 2 min. A 100 μL volume of the resulting organic solvent phase was added to 2.8 mL of methanol/1-butanol (2:1, v/v). Thiocyanate/ferrous solution (30 μL) [prepared by mixing equal volumes of 0.144 M $FeSO_4 \cdot 7H_2O$ with 0.132 M $BaCl_2$ (acidic solution), centrifuging, and mixing equal volumes of the clear ferrous solution with 3.94 M ammonium thiocyanate] was added to the methanol/1-butanol mixture, vortexed, and incubated at room temperature for 20 min. Following the incubation period, sample absorbances were read at 510 nm using a Genesys 20 spectrophotometer (ThermoSpectronic, Waltham, MA). The hydroperoxide content was determined using a standard curve developed using known concentrations of cumene hydroperoxide.

Hexanal was measured as a secondary lipid oxidation product. Hexanal concentration was measured using a GC-17A Shimadzu gas chromatograph equipped with an AOC-5000 autosampler (Shimadzu, Kyoto, Japan). A 30 m × 0.32 mm Equity DB-1 column (Supelco, Bellefonte, PA) with a 1 μm film thickness was used for separations. Each sample was shaken and heated at 55 °C in the autosampler heating block for 13 min. A 50/30 μm DVB/Carboxen/PDMS solid-phase microextraction (SPME) fiber needle (Supelco) was injected into the sample vial for 1 min to adsorb volatiles and then injected into the 250 °C injector port for 3 min. The gas chromatograph ran for 10 min at 65 °C for each sample. Helium was used as a carrier gas, with a total flow rate of 15.0 mL/min. A flame ionization detector at a temperature of 250 °C was used. Hexanal concentrations were determined from peak areas using a standard curve made from authentic hexanal.

Tocopherol content of oil phase samples was determined by normal phase HPLC using refined vegetable oil preparation methods as described by Xu (*20*).

Lycopene Concentration. The absorbance of lycopene-containing emulsions was used to determine lycopene concentrations. Periodically, 7 mL sample volumes were placed in open glass fluorometer cells (20 × 10 mm) and measured using a Shimadzu UV-2101 PC UV–vis Scanning Spectrophotometer equipped with an ISR-Integrating Sphere Assembly (Shimadzu). For the pure lycopene degradation experiments, readings were taken using the same fluorometer cells in which the sample were stored. Lycopene content was determined using a standard curve created using various concentrations of corn oil emulsions with and without added lycopene. Concentrations were expressed relative to zero time lycopene concentrations because small differences in lycopene concentrations occurred in the different emulsions.

Statistical Analysis. All measurements were made using triplicate samples. Statistical Analysis Systems version 9.1 software (SAS Institute, Cary, NC; 2002) analysis of variance procedures (PROC GLM combined with the LS MEANS, SLICE, and PDIFF functions) were used to analyze results. In this analysis, surfactant type, oil type, chelator addition, and storage time were considered fixed effects. When significant interactions were found among the effects tested, a Bonferroni adjustment ($p \leq 0.05$/no. of comparisons) was used for declaring significance. To determine when secondary oxidation products exited lag phase, a one-tailed Dunnett's test was used to determine when hexanal concentrations were significantly higher than 0 h values.

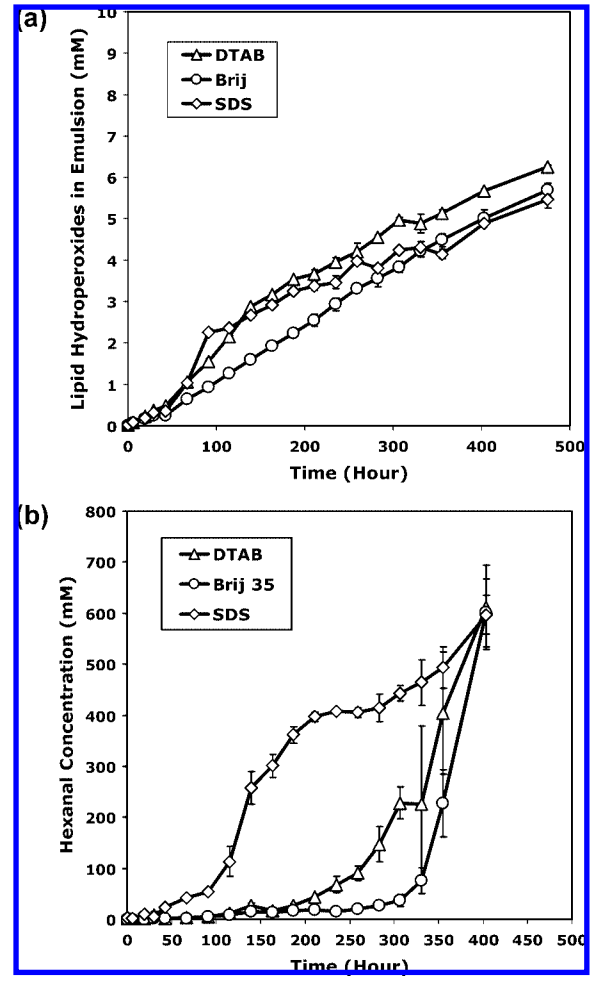

Figure 1. Development of lipid hydroperoxides (**a**) and hexanal (**b**) in corn oil oil-in-water emulsions stabilized by dodecyltrimethylammonium bromide (DTAB), polyoxyethylene(23) lauryl ether (Brij 35), or sodium dodecyl sulfate (SDS). Data points represent means ($n = 3$) ± standard deviations. Some error bars lie within the data points.

RESULTS AND DISCUSSION

When the chemical stability of functional food delivery systems is examined, it is essential to understand the stability of the bioactive ingredient as well as the overall oxidation profile of the lipid carrier. This is necessary to determine if the bioactive ingredient is degraded before lipid oxidation and thus the bioactive compound is lost prior to the product becoming rancid. Conversely, the bioactive lipid could oxidize more slowly than unsaturated fatty acids and thus the shelf life would be determined by rancidity development instead of the stability of the bioactive lipid.

Impact of Lycopene Addition to Corn Oil Emulsions. As a first step toward understanding the influence of lycopene on the oxidative profile of oil-in-water emulsions, two sets of emulsions were made. Both sets were stabilized by DTAB, Brij 35, or SDS. One set of emulsions was made with only corn oil as the lipid phase, whereas the other was made with corn oil containing 3 mg of lycopene ingredient/g of oil from a lycopene ingredient composed of 11% lycopene in sunflower oil. The development of primary and secondary oxidation products in corn oil only and in corn oil with added lycopene are shown in **Figures 1** and **2**, respectively. Formation of lipid hydroperoxides increased rapidly in oil-in-water emulsions stabilized with each of the emulsifiers in both emulsions without (**Figure 1a**) and with (**Figure 2a**) lycopene. Lipid hydroperoxide concentrations were lowest in Brij-stabilized emulsions, exhibiting significantly lower amounts than DTAB or SDS emulsions from 67 to 307 h in emulsions without lycopene and from 91 to 139 h in emulsions with lycopene. No consistent differences were found between emulsions stabilized by SDS and DTAB in lipid hydroperoxide formation. When the secondary lipid oxidation product, hexanal, was measured to monitor lipid oxidation in the emulsions without (**Figure 1b**) or with (**Figure 2b**) added lycopene, a lag phase was observed followed by a rapid rise in hexanal formation. Emulsions stabilized with SDS had much faster hexanal formation than the DTAB- and the Brij-stabilized emulsions both without (**Figure 1b**) and with lycopene (**Figure 2b**). In the SDS-stabilized emulsions without and with lycopene, the length of the lag phase of hexanal formation was only one testing point different (115 vs 139 h, respectively). DTAB- and Brij-stabilized emulsions had longer lag phases of hexanal formation, lasting 307 and 331 h, respectively in emulsions both with without lycopene (**Figures 1b** and **2b**).

The loss of lycopene was also measured in the emulsion with added lycopene (**Figure 3**). Lycopene degradation was fastest in SDS-stabilized emulsions and similar in DTAB- and Brij-stabilized emulsions. In all emulsions containing lycopene, the majority of lycopene was lost prior to the formation of hexanal. In the SDS-, DTAB-, and Brij-stabilized emulsions approximately 90, 74, and 71% of the lycopene was degraded during the lag phase, respectively. Antioxidants that are effective at

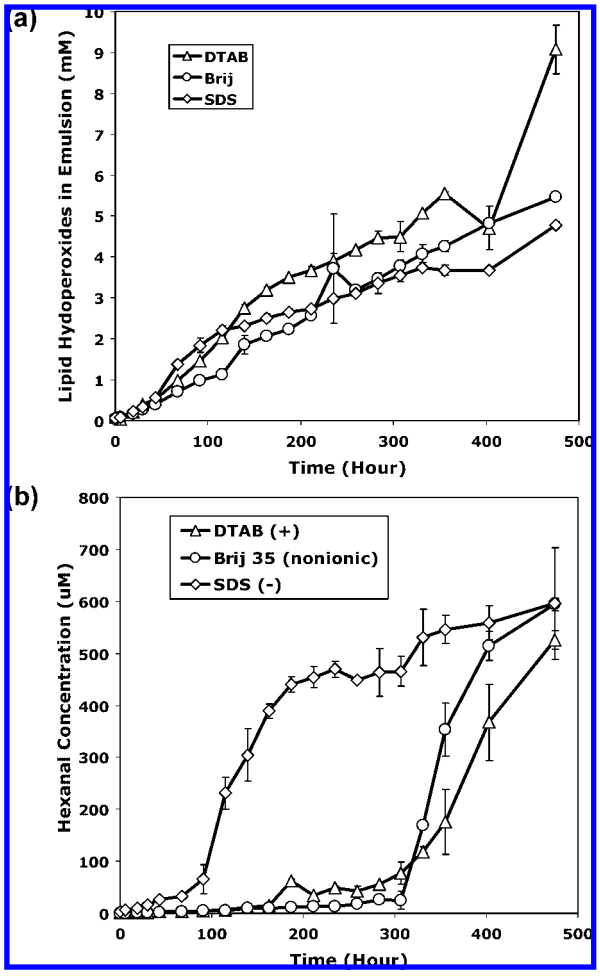

Figure 2. Development of lipid hydroperoxides (**a**) and hexanal (**b**) in corn oil oil-in-water emulsions containing lycopene stabilized by dodecyltrimethylammonium bromide (DTAB), polyoxyethylene(23) lauryl ether (Brij 35), or sodium dodecyl sulfate (SDS). Data points represent means ($n = 3$) ± standard deviations. Some error bars lie within the data points.

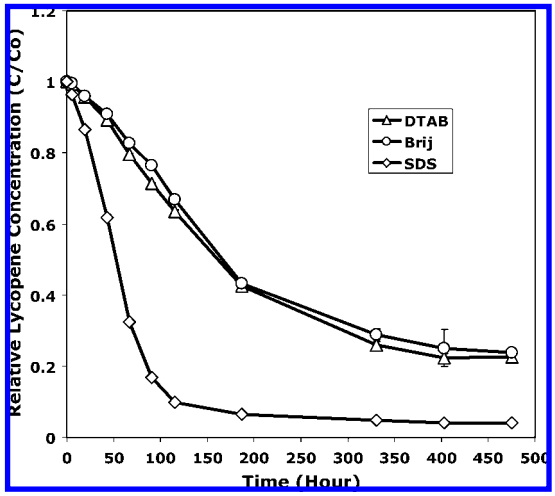

Figure 3. Relative lycopene concentration of dodecyltrimethylammonium bromide (DTAB), polyoxyethylene(23) lauryl ether (Brij 35), or sodium dodecyl sulfate (SDS) stabilized corn oil oil-in-water emulsions. Data points represent means ($n = 3$) ± standard deviations. Some error bars lie within the data points.

inhibiting lipid oxidation by scavenging free radicals do so by being preferentially oxidized, thus delaying the oxidation of fatty acids and increasing the lag phase of the formation of lipid oxidation products. Although lycopene was preferentially oxidized before fatty acids decomposed into hexanal, lycopene did not have a major impact on increasing the hexanal lag phase compared to emulsions without lycopene (**Figures 1** and **2**). This could occur if lycopene was interacting with free radicals, causing lycopene degradation, but resulting lycopene oxidation products (e.g., radicals) had sufficient energy to cause the oxidation of fatty acids, thus not being able to inhibit lipid oxidation. Several other studies have shown that carotenoids can be antioxidative (*21, 22*) or pro-oxidative in oil-in-water emulsions (*22–24*). Differences in the impact of carotenoids on oxidation rates in oil-in-water emulsions could be due to a number of factors including differences in storage conditions, concentrations and type of carotenoids, and the presence of other antioxidants and pro-oxidants.

Table 1. Zeta Potential of Emulsions Stabilized by Small Molecule Surfactants

surfactant	zeta potential (mV)
DTAB	+46.1 ± 15.4
Brij 35	−3.2 ± 0.7
SDS	−107.6 ± 3.3

Emulsions can be engineered to increase the oxidative stability of bioactive lipids within the emulsion droplet core. One such technique is to manipulate the surface charge of the emulsion droplet and thereby alter the ability of transition metals to absorb to the emulsion droplet interface where they can efficiently promote oxidative reactions. **Table 1** shows the zeta potential of emulsions stabilized by DTAB, SDS, and Brij 35. The oil-in-water emulsion droplets were found to be cationic when stabilized by DTAB and anionic when stabilized by SDS and Brij 35. SDS-stabilized emulsion droplets had a 14-fold higher negative charge density than emulsions stabilized by Brij 35. Zeta potential results were found to be similar to previous studies using Brij and SDS as surfactants (*25*).

In these experiments, formation of lipid hydroperoxides did not vary greatly among the emulsions stabilized with the different emulsifiers. However, the SDS-stabilized emulsions had a much faster formation of hexanal. This pattern of anionic emulsion droplets having low lipid hydroperoxides and high secondary oxidation products such as hexanal has also been observed in other emulsions systems (*13, 26, 27*). This pattern is thought to be due to the high concentrations of transition metals associated with the anionic emulsion droplet interface, which leads to rapid hydroperoxide decomposition, thus preventing hydroperoxide accumulation and causing rapid formation of hydroperoxide decomposition products such as hexanal.

The results from this study showed that cationic DTAB-stabilized emulsions droplets were not more oxidatively stable than emulsions stabilized with the slightly anionic Brij 35, a pattern not observed in other oil-in-water emulsion systems (*9, 13, 14, 17, 18, 28*). One possible reason for this discrepancy could be due to the higher levels of emulsifiers required to stabilize emulsions containing lycopene. When emulsions are made, surfactants absorb to the lipid surface until the surface becomes saturated. Remaining surfactant then partitions into the aqueous phase. If the concentration of surfactant in the aqueous phase was above the critical micelle concentration (CMC), the surfactant would form micelles. Brij 35 has a lower CMC [0.09 mM (*29*)] than SDS or DTAB [6–8 mM (*29*) and 13.5 mM (*30*)]. Because the emulsions in this work were prepared on an equal molar surfactant basis, it is more likely that Brij 35 would

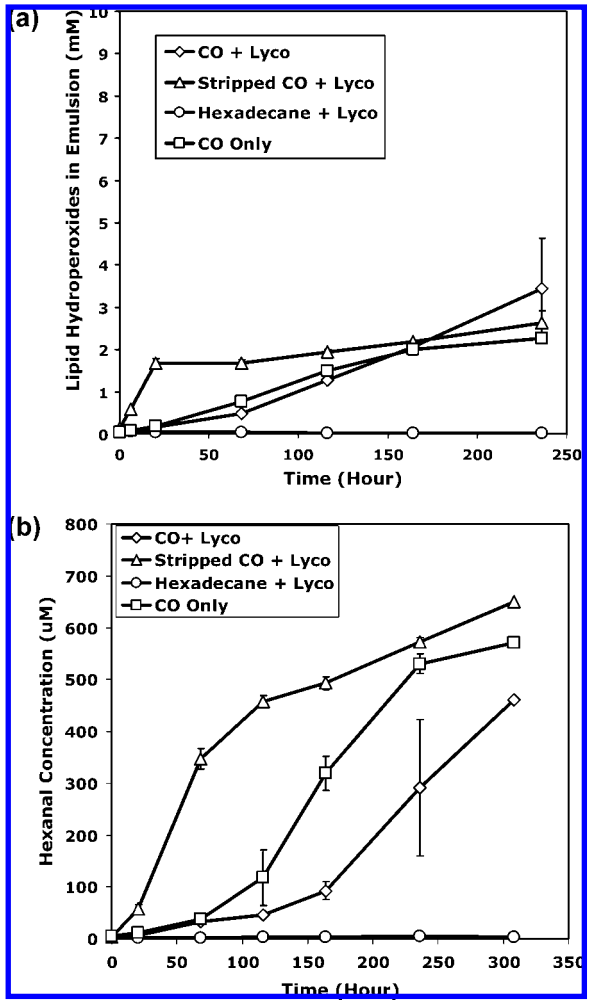

Figure 4. Development of lipid hydroperoxides (**a**) and hexanal (**b**) in sodium dodecyl sulfate-stabilized oil-in-water emulsions with oil phases composed of either corn oil only, corn oil with added lycopene, stripped corn oil with lycopene, or hexadecane with lycopene. Data points represent means ($n = 3$) $\pm$ standard deviations. Some error bars lie within the data points.

form more micelles in the continuous phase than SDS or DTAB. Surfactant micelles have been shown to inhibit lipid oxidation (*31, 32*), so this could explain why Brij 35- and DTAB-stabilized emulsions had similar oxidative stability.

Impact of Oil Type on Stability of Lycopene Emulsions. To obtain a better understanding of how fatty acids and antioxidants in the oil phase influence lycopene oxidation in oil-in-water emulsions, lycopene-containing SDS-stabilized emulsions were prepared with corn oil, corn oil stripped of its minor components, and hexadecane. Hexadecane, is a hydrocarbon that is fully saturated and, thus, is resistant to oxidation (*31*). **Figure 4a** shows the development of lipid hydroperoxides in the various treatments. The stripped corn oil samples oxidized most rapidly, forming significantly higher amounts of lipid hydroperoxides than all other samples within 20 h of emulsion preparation. Corn oil samples with and without added lycopene exhibited similar lipid hydroperoxide formation with significant differences in lipid hydroperoxide concentrations only occurring at the final testing time (236 h). The hexadecane emulsion had no detectable lipid hydroperoxide throughout the experiment, even though it contained a small amount of sunflower oil from the lycopene ingredient. Similar results were found when oxidation in the emulsions was analyzed by monitoring formation of the secondary oxidation product, hexanal (**Figure 4b**).

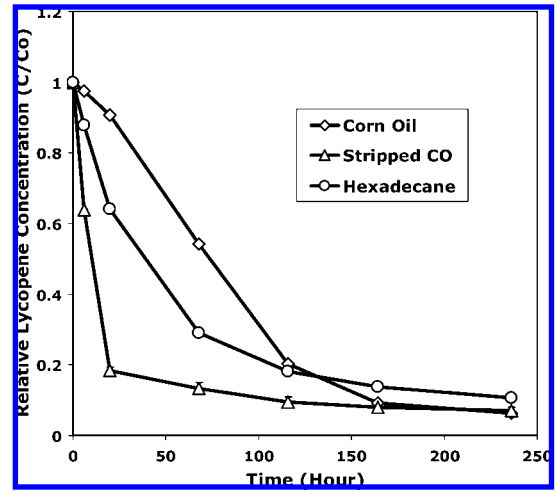

Figure 5. Relative lycopene concentration of sodium dodecyl sulfate-stabilized oil-in-water emulsions with oil phases composed of corn oil with added lycopene, stripped corn oil with lycopene, or hexadecane with lycopene. Data points represent means ($n = 3$) $\pm$ standard deviations. Some error bars lie within the data points.

Again, the stripped corn oil with added lycopene oxidized first, with no substantial lag phase before the rapid rise in hexanal concentration. The corn oil only and corn oil with lycopene emulsions exhibited similar lag phase behavior up to 68 h of storage, but during the exponential phase of hexanal formation, the corn oil only emulsions had a faster rate of hexanal formation than the corn oil with lycopene emulsions. This may suggest that lycopene was not very effective in extending the lag phase but that it may be able to quench alkoxy radicals to inhibit β-scission reactions that would produce hexanal. Hexadecane samples, again, showed no lipid hydroperoxide or hexanal formation.

Lycopene degradation in emulsions with different oil types was in the order of stripped corn oil > hexadecane > corn oil (**Figure 5**). Lycopene degradation was so fast in the stripped corn oil that lycopene concentrations were significantly lower than the other two treatments within 6 h of storage. The fact that lycopene degradation was faster in the stripped corn oil and hexadecane compared to corn oil suggests that antioxidants inherent in corn oil could be protecting the lycopene. Comparing lycopene degradation and hexanal development data shows that nearly 82 and 80% of the lycopene had been degraded before hexanal formation (**Figure 4b**) exited the lag phase in both stripped and nonstripped corn oil treatments, respectively.

The rapid lycopene oxidation observed in the hexadecane emulsions could be due to co-oxidation of the lycopene by oxidation of the sunflower oil in the lycopene ingredient. To test this, pure lycopene in hexadecane emulsions were made and lycopene degradation rates were compared when lycopene was added in the pure form or as lycopene in sunflower oil. The results of the degradation study in hexadecane can be seen in **Figures 6** and **7**. These results clearly indicate that both forms of lycopene exhibited nearly identical degradation profiles. This suggests that the sunflower oil in the lycopene dispersion did not play a major role in the oxidation of lycopene in hexadecane-in-water emulsions containing the lycopene dispersed in sunflower oil (**Figure 5**). Instead, lycopene degradation in the hexadecane system might be caused by interactions with other emulsion components. Iron, which is commonly found in oil-in-water emulsions, has previously been shown to promote carotenoid degradation. In this pathway, ferric iron participates in an electron-transfer reaction with carotenoids to form ferrous

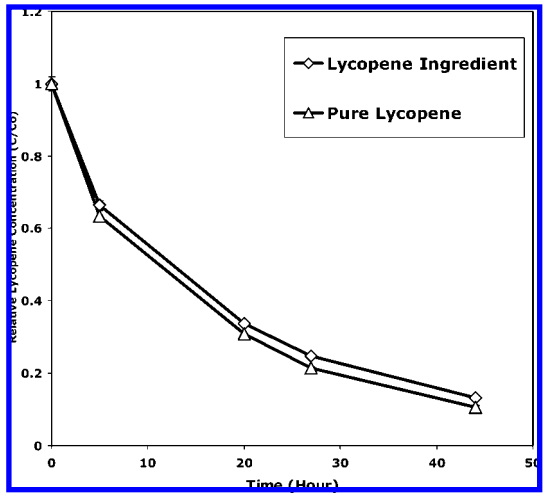

Figure 6. Relative lycopene concentration in sodium dodecyl sulfate-stabilized oil-in-water emulsions containing either pure lycopene or Lycovit lycopene ingredient in hexadecane. Data points represent means (n = 3) ± standard deviations. Some error bars lie within the data points.

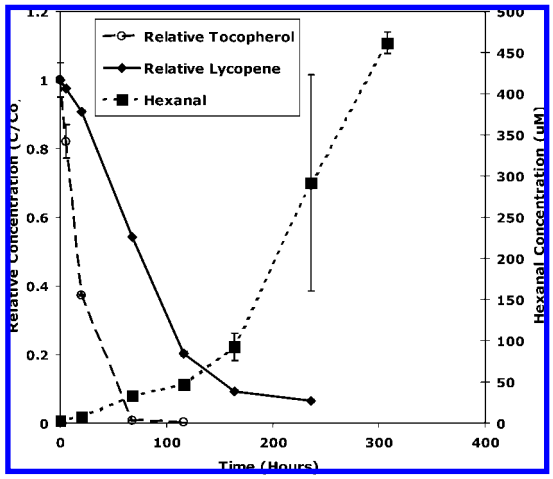

Figure 7. Relative tocopherol, relative lycopene, and hexanal concentrations in sodium dodecyl sulfate-stabilized corn oil-in-water emulsions with added lycopene. Data points represent means (n = 3) ± standard deviations. Some error bars lie within the data points.

iron and a carotenoid radical cation, which is thought to further degrade by additional electron transfer reactions, deprotonation, or reaction with oxygen (*33–40*). Iron is known to be a strong pro-oxidant in oxidation of unsaturated fatty acids in oil-in-water emulsions by the ability of ferrous ions to decompose lipid hydroperoxides into free radicals (*9, 41*). Therefore, in the corn oil based systems, iron may be recycling from ferric to ferrous during the reaction with lycopene and then from ferrous to ferric upon reaction with lipid hydroperoxides. This recycling pathway could decrease the stability of both the fatty acids and the lycopene.

In corn oil-in-water emulsions containing lycopene it is likely that other oil components are protecting lycopene because lycopene degrades much more quickly in stripped corn oil that has had its minor components such tocopherols and carotenoids removed. This can be seen in **Figure 6**, where total tocopherol concentrations in SDS-stabilized corn oil emulsions decreased prior to loss of lycopene and formation of hexanal. The rapid loss of tocopherols suggests that they are preferentially oxidized and thus could be protecting lycopene and unsaturated fatty acids from degradation. Tocopherols were completely oxidized after 68 h of incubation, at which time 46% of lycopene was lost and the hexanal concentrations were still in the lag phase (lag phase ended after 164 h). In addition to tocopherols, other minor components in the oil such as phospholipids could also be inhibiting fatty acid and lycopene degradation (*42*).

Overall, this study suggests that oxidation of lycopene in oil-in-water emulsions is influenced by both surfactant and oil type. Interfacial characteristics of the emulsion droplet were also found to impact oxidation and lycopene degradation. Negatively charged interfaces on emulsions droplets (e.g., SDS) were found to produce the fastest oxidation rates, suggesting that cationic transition metals could be promoting lycopene degradation because they are attracted to the emulsion droplet surface, where they can more readily interact with lycopene in the droplet interior. Lycopene was found to degrade more rapidly than unsaturated fatty acids but less rapidly than tocopherols. This suggests that in oil-in-water emulsions significant loss of lycopene could occur prior to the development of rancidity. Lycopene was also unstable in the absence of corn oil, suggesting that the co-oxidation of lycopene by unsaturated fatty acids was unnecessary for lycopene degradation. In fact, components of corn oil actually inhibited the degradation of lycopene because lycopene degradation was faster in oils stripped of their minor components than in unmodified oil. Because tocopherols are oxidized prior to lycopene, the preferential oxidation of tocopherols could be inhibiting the oxidation of lycopene. Overall, these results indicate that the stability of lycopene in oil-in-water emulsions could be altered by altering the physical properties of the emulsion droplet interface and by the addition of antioxidants such as tocopherols.

ABBREVIATIONS USED

DTAB, dodecyltrimethylammonium bromide; Brij 35, polyoxyethylene(23) lauryl ether; SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; HCl, hydrochloric acid; NaOH, sodium hydroxide.

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Received for review October 3, 2007. Revised manuscript received December 21, 2007. Accepted December 25, 2007. This research was partially funded by Grant 2004-02422 from NRI CSREES, USDA.

JF072929+