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Role of Proteins in Oil-in-Water Emulsions on the Stability of Lipid Hydroperoxides

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The purpose of this research was to better understand the mechanisms by which proteins affect the rates of lipid oxidation in order to develop protein-stabilized emulsion delivery systems with maximal oxidative stability. This study evaluated the affect of pH and emulsifier concentration on the stability of cumene hydroperoxide in hexadecane-in-water emulsions stabilized by β-lactoglobulin (β-Lg). Emulsions prepared with 0.2 wt % β-Lg (at pH 7.0) showed a 26.9% decrease in hydroperoxide concentrations 5 min after 0.25 mM ferrous ion was added to the emulsion. EDTA, but not continuous phase β-Lg, could inhibit iron-promoted lipid hydroperoxide decomposition. Lipid hydroperoxides were more stable to iron-promoted degradation at pH values below the p*I* of β-Lg, where the emulsion droplet would be cationic and thus able to repel iron away from the lipid hydroperoxides. Heating the β-Lg-stabilized emulsions to produce a cohesive protein layer on the emulsion droplet surface did not alter the ability of iron to decompose lipid hydroperoxides. These results suggest that proteins at the interface of emulsion droplets primarily stabilize lipid hydroperoxides by electrostatically inhibiting iron−hydroperoxide interactions.

KEYWORDS: Lipid oxidation; emulsions; lipid hydroperoxides; interfacial protein; β-lactoglobulin

INTRODUCTION

The oxidative deterioration of lipids negatively affects the quality of foods, influencing the products' flavor, odor, and nutritive value, oftentimes resulting in an unacceptable consumer product (*1*). The rate at which oxidation takes place is dependent on several factors, such as temperature, the presence of prooxidants and antioxidants, and the molecular nature of the lipid as well as how it is dispersed within a product (*1*, *2*). Lipids are often present in food products in the form of an emulsion. Many common food products (e.g., milk, salad dressing, peanut butter, yogurt) exist as oil-in-water emulsions; in these products, the lipid portion is dispersed as miniscule droplets within an aqueous continuous phase (*3*). The susceptibility of these emulsified lipids to oxidation depends not only upon the factors previously mentioned but also upon the surrounding molecular environment and interactions with other molecules within the immediate vicinity (*4*). Recent studies have highlighted transition metal based catalysis as a primary mechanism for lipid oxidation in emulsion systems (*5*−*8*).

Iron, a transition metal, is a strong prooxidant that is ubiquitous in all food systems. Transition metals that are in close proximity to surface-active lipid hydroperoxides at the emulsion droplet interface will promote hydroperoxide degradation. Iron can decompose hydroperoxides (LOOH) into alkoxyl (LO$^{\bullet}$) and peroxyl (LOO$^{\bullet}$) radicals by the following mechanisms:

$$Fe^{2+} + LOOH \rightarrow Fe^{3+} + LO^{\bullet} + OH^{-}$$

$$Fe^{3+} + LOOH \rightarrow Fe^{2+} + LOO^{\bullet} + H^{+}$$

In lipid systems, these highly reactive radicals abstract hydrogen from unsaturated fatty acids (LH) within their immediate vicinity, forming new radicals that can further promote oxidation, eventually leading to rancidity. The ability of iron to break down lipid hydroperoxides can depend largely on its physical location relative to the interface of the emulsion droplet. This ability may be hindered by the presence of bulky proteins or surfactants on the droplet interface (*5*).

Globular whey proteins are commonly used as emulsifiers because of their ability to adsorb to the oil−water interface of emulsion droplets, thereby improving emulsion stability (*9*−*11*). β-Lactoglobulin (β-Lg) was chosen as a model globular protein emulsifier for this study because of the extensive research available on its structure and behavior in emulsion systems. In its native state, the protein contains disulfide bonds and a partially buried free sulfhydryl group, as well as hydrophobic residues buried within its globular folds (*12*). Globular whey proteins undergo various degrees of conformational changes during emulsification. Immediately following adsorption to the oil−water interface, the adsorbed protein retains a conformation similar to its native state (*13*). However, following adsorption, the conformation of the protein begins to change due to its new molecular environment. Whereas the protein was once completely immersed in an aqueous environment, one side of the protein is now in contact with hydrophobic

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lipid molecules. Consequently, the globular protein begins to partially unfold and rearrange itself along the interface, often exposing once-buried disulfide, thiol, and nonpolar amino acid groups in the process (*13*, *14*). Once exposed, these residues are available to interact with other proteins at the interface through hydrophobic interactions, disulfide bond formation, or thiol−disulfide interchange reactions (*15*, *16*).

In addition to the effects incurred by adsorption, the conformation of globular proteins, as well as other protein emulsifiers, can be altered by pH or thermal denaturation. Because metal-promoted lipid hydroperoxide decomposition is so important to the oxidative stability of lipids, the objective of this study was to elucidate the effects of interfacial protein manipulation on the stability of lipid hydroperoxides in protein-stabilized oil-in-water emulsions. To study hydroperoxide stability, a model emulsion was prepared with hexadecane and cumene hydroperoxide. Hexadecane is a nonoxidizable lipid and was used to prevent further formation of lipid hydroperoxides (through autoxidation) when cumene hydroperoxide decomposed into free radicals. Ferrous ion was added to the continuous phase within the emulsion to accelerate the decomposition of hydroperoxides (*6*). Hydroperoxides, as surface active molecules, have the tendency to migrate toward the surface of the emulsion droplet (*17*). In this location, there is greater opportunity for them to interact with and be decomposed by prooxidants. This simple model was used to help better understand how different factors alter the ability of these lipid hydroperoxides to attain close enough contact with prooxidative metals to be decomposed, a reaction that produces free radicals and can accelerate the oxidation of unsaturated fatty acids.

MATERIALS AND METHODS

Materials. B-Lg (BioPURE; ≥95.0 wt % protein) was obtained from Davisco Food International, Inc. (Eden Prairie, MN). The β-Lg content of the powder was ≥90.0 wt % of total protein. Protein concentration is as listed by the manufacturer and was used without further purification. Cumene hydroperoxide, hexadecane, imidazole, ferrous sulfate, ammonium thiocyanate, and barium chloride were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium acetate, isooctane, 2-propanol, methanol, 1-butanol, hydrochloric acid, and sodium hydroxide were obtained from Fisher Scientific (Fair Lawn, NJ). Ethylenediaminetetraacetic acid (EDTA) disodium salt was purchased from Chempure Ultra (Houston, TX). All chemicals were of ACS reagent or HPLC grade. Double-distilled water was used in all experiments.

Methods. *Preparation and Characterization of Emulsions.* A β-Lg-stabilized hexadecane-in-water emulsion containing cumene hydroperoxide was used in all experiments. β-Lg (0.2 wt %) was dispersed in an imidazole−sodium acetate (5 mM each; pH 7.0) buffer solution and stirred for 2 h to ensure dissolution. The emulsifier solution was adjusted to pH 7.0 with 0.1−1 M HCl or NaOH. A coarse emulsion consisting of 5 wt % hexadecane (mixed with 22 mM cumene hydroperoxide) and 95 wt % emulsifier solution was prepared by blending both phases for 2 min using a two-speed hand-held biohomogenizer (Biospec Products Inc., Bartlesville, OK). The coarse emulsion was further homogenized by passing the emulsion through a two-stage high-pressure valve homogenizer (APV Lab 1000, Albertslund, Denmark) three times at 5000 psi. After homogenization, the emulsions were adjusted with 0.1 or 1 M HCl or NaOH to pH 3.0, 4.0, 6.0, or 7.0, depending on the experiment. For experiments testing EDTA, crystalline EDTA (0.5−10 mM) was added to the emulsions prior to the addition of ferrous sulfate.

For experiments that used thermal denaturation to form a cohesive protein layer, 0.2 and 1.0 wt % β-Lg emulsions were placed in test tubes and held at 80.0 °C for 30 min in a GP 200 water bath (NESLAB Instruments, Inc., Newington, NH). Following heating, emulsions were placed in tap water and cooled to room temperature (25 °C). To prepare emulsions with increased continuous phase β-Lg concentrations, 0.3 wt % β-Lg was initially dispersed in 5 mM imidazole−sodium acetate buffer (pH 7.0). This aqueous solution was blended with hexadecane (containing 22 mM cumene hydroperoxide) in a 92.5 wt % aqueous/7.5 wt % lipid ratio. These emulsions were homogenized as previously described. Following homogenization, the hexadecane phase was diluted to 5 wt % with a β-Lg-in-buffer solution to obtain continuous phase β-Lg concentrations of 0.0−1.3 wt %. Emulsions were adjusted to pH 3.0 or 7.0 using 0.1−1 M HCl or NaOH.

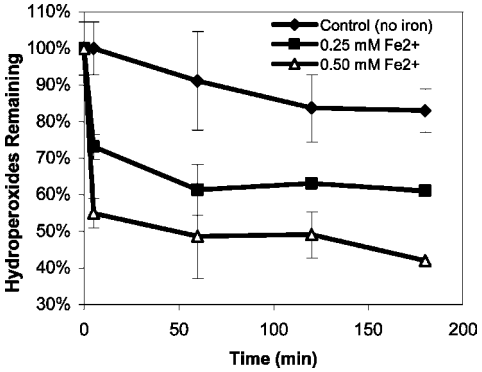

Figure 1. Effect of iron concentration on cumene hydroperoxide decomposition in 0.2 wt % β-Lg-stabilized hexadecane-in-water emulsions at pH 7. Data points represent means ($n = 3$) ± standard deviation.

The particle size of the emulsions was measured using a laser light scattering instrument (Malvern Mastersizer, Malvern Instruments, Ltd., Worcestershire, U.K.). Aside from the experiment that looked at the effect of pH on oxidative stability, the particle size (d_{32}) of all emulsions ranged from 0.26 to 0.37 μm. Droplet charge (zeta potential, ζ) was determined by injecting diluted hexadecane-in-water emulsions into the measurement chamber of a ZEM5003 Zetamaster (Malvern Instruments).

Hydroperoxide Stability Studies. To induce hydroperoxide degradation, 0.25−0.5 mM ferrous sulfate was added to each emulsion (not including a negative, no-iron control sample). After mixing, emulsion samples were incubated in an Innova 4080 incubator shaker (New Brunswick Scientific, New Brunswick, CT) at 37 °C while shaking at 98 rpm for the duration of each experiment (90−240 min). Cumene hydroperoxide degradation was measured with a modified thiocyanate method (*18*), where 0.3 mL of emulsion was added to 1.5 mL of isooctane/2-propanol (3:1) and vortexed three times vigorously for periods of 10 s each. Samples were then centrifuged for 2 min at 3400*g* (Centrific centrifuge, Fisher Scientific), and 0.2 mL of the upper solvent layer was vortexed with methanol:1-butanol (2:1; 2.8 mL) and thiocyanate/Fe^{2+} solution (30 μL). The thiocyanate/Fe^{2+} solution was made immediately prior to use by combining equal volumes (1 mL each) of 3.94 M thiocyanate solution and Fe^{2+} solution [taken from the supernatant of a previously mixed/centrifuged solution of 1 mL of 0.144 M $FeSO_4$ with 1 mL of 0.132 $BaCl_2$ (in 0.4 M HCl)]. After incubation of the samples at room temperature for 19 min, the absorbance of each sample was measured at 510 nm in a UV−vis scanning spectrophotometer (Shimadzu UV-2101PC, Kyoto, Japan). Hydroperoxide concentrations were determined using a cumene hydroperoxide calibration curve.

Statistical Analysis. All samples were measured in triplicate. Differences between treatments were analyzed using Student's *t* test (*19*).

RESULTS AND DISCUSSION

Cumene Hydroperoxide Degradation As Affected by Fe^{2+} Concentration. The effect of two concentrations of ferrous ion (0.25 and 0.50 mM) on hydroperoxide degradation was compared in emulsions at pH 7.0 to determine an appropriate concentration for subsequent experiments (**Figure 1**). Following the addition of iron, hydroperoxide concentrations were measured at 5, 60, 120, and 180 min. The most significant decrease

in hydroperoxide concentration occurred within the first 5 min following iron addition; the remaining measurements show a continual slight decrease in total hydroperoxides over time. The addition of 0.25 mM ferrous ion yielded a 26.9 ± 3.4% decrease in hydroperoxides after 5 min, whereas the addition of 0.50 mM ferrous ion resulted in a decrease of 45.0 ± 4.0% of total hydroperoxides. A rapid decrease in hydroperoxides suggests that the majority of ferrous ion added was oxidized to ferric ion within the first 5 min. This trend is supported by Mancuso et al. (*6*), who found that ferrous ion (0.5 or 1.0 mM) was completely oxidized to ferric within 2 min after its addition to sodium dodecyl sulfate (SDS) stabilized hexadecane-in-water emulsions containing cumene hydroperoxide.

Ferrous ion (Fe^{2+}) decomposes hydroperoxides at a rate $>10^7$ times faster than ferric ion (Fe^{3+}) (*20*). Its higher reactivity could also be associated with its increased solubility, as Fe^{2+} is more water soluble than Fe^{3+} (*21*). However, once Fe^{2+} has oxidized, the resulting Fe^{3+} may remain bound to the negatively charged protein-stabilized droplet interface because of electrostatic interactions. Because of its close proximity to hydroperoxides at the interface of the emulsion droplet, this ferric ion may be decomposing hydroperoxides, although at a slower rate than Fe^{2+}, during the later stages of incubation; the observed slow decrease in hydroperoxides after 5 min of incubation may be the result of this effect. This hypothesis is supported by the work of Mancuso et al (*6*), who found that Fe^{3+} could decompose cumene hydroperoxides in emulsion droplets which were stabilized by anionic SDS, but not by droplets stabilized by cationic dodecyltrimethylammonium bromide (DTAB) or nonionic Tween 20. This suggests that Fe^{3+} needs to be bound to the surface of the emulsion droplet to be able to efficiently decompose hydroperoxides.

On the basis of the results from this experiment, which showed significant hydroperoxide decomposition following the addition of both 0.25 and 0.50 mM Fe^{2+}, it was decided that 0.25 mM Fe^{2+} would be adequate for promoting hydroperoxide decomposition in subsequent experiments.

Fe^{2+}-Induced Cumene Hydroperoxide Degradation As Affected by EDTA. EDTA decreased ferrous ion-induced hydroperoxide degradation of cumene hydroperoxides in hexadecane-in-water emulsions (0.2 wt % β-Lg; pH 7.0) in a concentration-dependent manner (**Figure 2**). The emulsion without EDTA showed a 21.5 ± 3.0% decrease in hydroperoxide concentration 5 min after 0.25 mM ferrous ion was added to the emulsion. The percent of hydroperoxides that were decomposed at 5 min following iron addition decreased as EDTA concentration increased: 15.1 ± 2.7% (0.5 mM EDTA), 12.9 ± 3.9% (1.0 mM EDTA), and 4.9 ± 8.6% (10.0 mM EDTA). After 5 min of incubation, hydroperoxide decomposition continued in the samples containing all concentrations of EDTA. Mei et al. (*8*) reported that although high concentrations of EDTA added to emulsions removed >97% of iron from the surface of an SDS-stabilized emulsion droplet, low levels of lipid oxidation still occurred. Therefore, the decomposition of hydroperoxides in the EDTA-containing emulsion could be due to small amounts of iron not chelated by EDTA (e.g., iron in the lipid phase or at the emulsion droplet interface) or hydroperoxide decomposition by nonmetal pathways (e.g., light degradation).

Fe^{2+}-Induced Cumene Hydroperoxide Degradation As Affected by Continuous Phase β-Lg Concentration. Figure 3 shows the affect of adding β-Lg to the continuous phase of a 0.2 wt % β-Lg-stabilized emulsion on the degradation of cumene hydroperoxides. Recent studies in our laboratory have shown

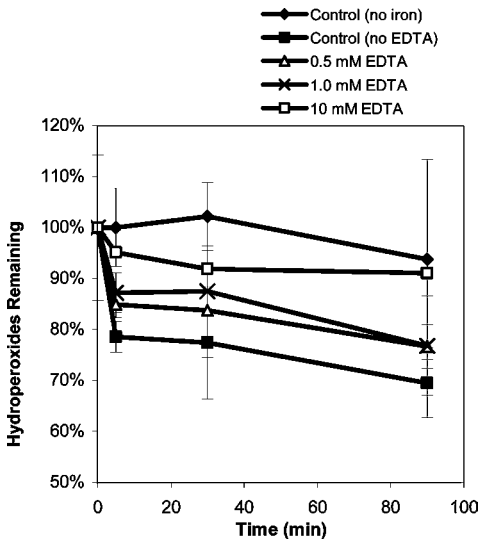

Figure 2. Effect of EDTA concentration on 0.25 mM Fe^{2+}-induced decomposition of cumene hydroperoxide in 0.2 wt % β-Lg-stabilized hexadecane-in-water emulsions at pH 7.0. Data points represent means ($n = 3$) ± standard deviation.

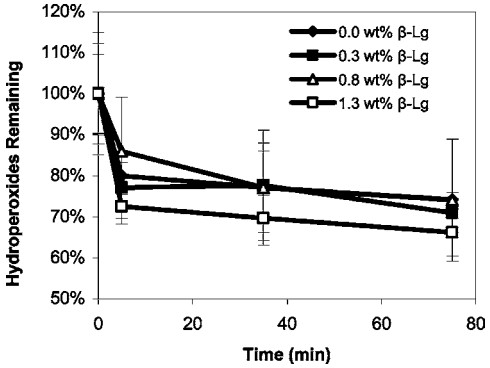

Figure 3. Effect of continuous phase β-Lg on 0.25 mM Fe^{2+}-induced decomposition of cumene hydroperoxide in 0.2 wt % β-Lg-stabilized hexadecane-in-water emulsions at pH 7.0. Data points represent means ($n = 3$) ± standard deviation.

that increasing the concentration of continuous phase protein in an emulsion system at pH 7 can inhibit lipid oxidation due to free radical scavenging and metal chelation (*22*). At pH 7, the excess, continuous phase proteins would be anionic, hence being able to chelate positively charged ferrous ions. This would thus inhibit hydroperoxide decomposition either by preventing cationic prooxidants from attaining close proximity to hydroperoxides at the emulsion droplet interface or by decreasing the reactivity of iron.

Increasing the concentration of β-Lg in the continuous phase of the β-Lg-stabilized emulsion at pH 7.0 following homogenization did not significantly ($p \geq 0.05$) affect the amount of hydroperoxide degradation that occurred upon the addition of 0.25 mM ferrous iron. Five minutes after the addition of ferrous iron, the emulsions containing continuous phase β-Lg concentrations of 0.0, 0.3, 0.8, and 1.3 wt % showed 20.0 ± 3.2, 22.9 ± 8.7, 14.9 ± 13.2, and 27.5 ± 2.8% decreases in total hydroperoxide concentrations, respectively. This suggests that the negatively charged proteins are not strong enough chelators to completely remove transition metals from the surface of the emulsion droplet. Prior studies that showed continuous phase β-Lg to have an antioxidative effect were performed in fish oil-in-water emulsions, where small amounts endogenous transition

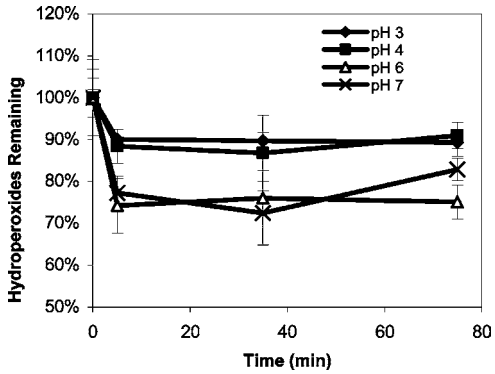

Figure 4. Effect of pH on 0.25 mM Fe^{2+}-induced decomposition of cumene hydroperoxide in 0.2 wt % β-Lg-stabilized hexadecane-in-water emulsions. Data points represent means ($n = 3$) ± standard deviation.

metals were the main prooxidants (*22*, *23*). This is in contrast to the hexadecane−cumene hydroperoxide model system, where exogenous ferrous ion was added. It is likely that the high concentrations of exogenous iron added to the system overwhelmed the chelating capacity of β-Lg, thus preventing it from inhibiting hydroperoxide degradation.

Fe^{2+}-Induced Cumene Hydroperoxide Degradation As Affected by pH. Cumene hydroperoxides were more stable to iron-promoted degradation at pH values below the p*I* of β-Lg (p*I* = 5.3) (**Figure 4**) (*12*). At pH 3.0 and 4.0, emulsions showed respective 9.9 ± 2.3 and 11.6 ± 4.1% decreases in hydroperoxides 5 min after the addition of 0.25 mM ferrous ion. At pH 6.0 and 7.0, the degradation of total hydroperoxides increased to 25.8 ± 6.6 and 22.7 ± 4.0%, respectively. The decrease in degradation that was observed at pH values below the protein's p*I* is likely due to electrostatic repulsion of iron by the positively charged droplets, which inhibited iron interactions with the hydroperoxides. Similar findings were reported by Hu et al. (*24*), who studied the influence of pH on lipid hydroperoxide and headspace propanal formation in 0.2 wt % WPI-stabilized 5 wt % salmon oil-in-water emulsions. The whey protein stabilized emulsions had greater oxidative stability at pH values below the p*I* of the protein stabilizer.

The particle diameter of the emulsion droplets was constant in all pH-adjusted emulsions (0.31 μm) with the exception of pH 4.0 (6.19 μm). The increase in emulsion droplet size at pH 4.0 is likely due to a decrease in charge density as the pH approached the p*I* (5.3) of β-Lg, thereby causing droplet coalescence or flocculation. The droplet charge density (ζ-potential) at pH 4.0 is +24.5 mV, which is smaller when compared to the charge at other pH values (pH 3.0, +53.4 mV; pH 6.0, −40.3 mV; pH 7.0, −62.5 mV). Although pH 6.0 is also close to the p*I* of the protein, the magnitude of the droplet charge appears to be sufficient to decrease droplet interactions. Emulsions were not prepared at pH 5.0, because the low droplet charge density would result in an unstable emulsion.

Role of a Cohesive Interfacial Protein Layer on Fe^{2+}-Induced Cumene Hydroperoxide Degradation. Heating β-Lg-stabilized oil-in-water emulsions above β-Lg's thermal denaturation temperature (~70 °C) forces rearrangement of protein molecules beyond what occurs following adsorption to the surface of the emulsion droplet (*13*). These rearrangements can result in changes in both intra- and intermolecular bonds in the proteins, including hydrophobic interactions and disulfide exchange, making the protein interface more cohesive and preventing these proteins from being desorbed by surfactants (*25*). An increase in protein−protein interactions at the droplet surface could affect iron−hydroperoxide interactions by forming a thicker, more cohesive layer. Thus, experiments were performed to gain a better understanding of this process, in the hope that a cohesive layer would decrease the interactions between oxidizable lipids inside the emulsion droplets (e.g., hydroperoxides) and prooxidants (e.g., iron) in the continuous phase.

β-Lg-stabilized emulsions at pH 3.0 or 7.0 were heated for 30 min at 80.0 °C to denature interfacial proteins and induce intra- and intermolecular protein bonding. At pH 7.0, the cumene hydroperoxide concentration immediately after heating was 1.05 ± 0.12 mmol/L of emulsion, as compared to 1.36 ± 0.18 mmol/L of emulsion for the unheated control. At pH 3.0, the cumene hydroperoxide concentration subsequent to heating was 1.17 ± 0.17 mmol/L of emulsion, as compared to 1.27 ± 0.14 mmol/L of emulsion for the unheated control. Decreases in cumene hydroperoxide concentrations during thermal processing could be due to heat- and/or endogenous transition metal-induced hydroperoxide decomposition. The fact that thermal processing decreased cumene hydroperoxide concentrations less at pH 3.0 than at pH 7.0 suggests that endogenous metals are involved in the decomposition because metal-promoted decomposition would be expected to be lower at pH 3.0, at which the emulsion droplets are cationic. Because of the loss in hydroperoxides that occurred during heating, results were reported as the decrease in hydroperoxides (e.g., millimoles of hydroperoxides per liter of emulsion) in each emulsion after the addition of iron.

After thermal processing, 0.25 or 0.50 mM Fe^{2+} was added to determine if heat-induced changes in protein structure could inhibit hydroperoxide decomposition. As seen before, iron-induced hydroperoxide decomposition was less at pH 3.0 than at pH 7.0, presumably due to the cationic emulsion droplet interface at pH 3.0 (**Figure 5**) At pH 7.0 (**Figure 5a**), the decreases in hydroperoxide concentrations were not significantly ($p \geq 0.05$) different between the heat-treated and non-heat-treated samples at both 0.25 and 0.50 mM Fe^{2+} concentrations. Similarly, at pH 3.0 (**Figure 5b**), there was not a significant ($p \geq 0.05$) difference in hydroperoxide decomposition in heated or non-heated emulsions at either iron concentration. These results suggest that heat-induced changes in interfacial protein were not able to protect the hydroperoxides.

Experiments were also conducted with thermally processed (30 min at 80.0 °C) 0.2 wt % β-Lg-stabilized emulsions (pH 3.0 or 7.0) containing 0.8 wt % β-Lg added to the continuous phase. In this experiment, thermal treatment would denature β-Lg, again causing intra- and intermolecular bonds to form in proteins at the surface of the emulsion droplet via interactions including disulfide exchange and hydrophobic interactions. In addition, heating would denature the continuous phase proteins. Because heat denaturation of whey proteins increases their antioxidant activity (*26*), the heat denaturation of the continuous phase proteins might increase cumene hydroperoxide stability. At pH 7.0, the cumene hydroperoxide concentration immediately after heating in the presence of 0.8 wt % continuous phase β-Lg was 0.68 ± 0.06 mmol/L of emulsion, compared to 1.80 ± 0.08 mmol/L of emulsion for the unheated control. At pH 3.0, the cumene hydroperoxide concentration of the emulsion immediately after heating with continuous phase β-Lg was 1.00 ± 0.17 mmol/L of emulsion, as compared to 1.86 ± 0.20 mmol/L of emulsion for the unheated control. Decreases in cumene hydroperoxide concentrations during thermal processing were again less at pH 3.0 than at pH 7.0, suggesting that endogenous metals were less active in the cationic emulsion droplets.

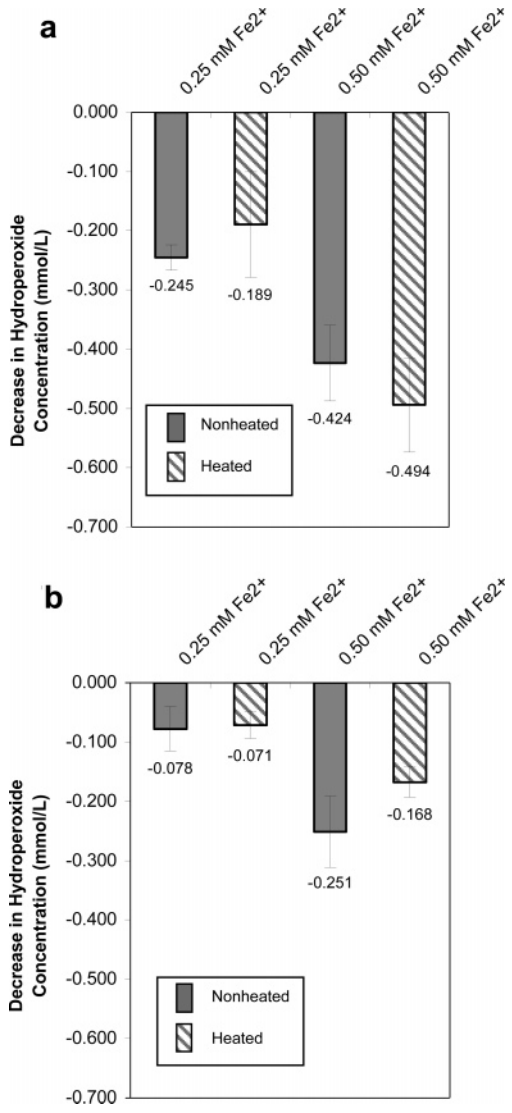

Figure 5. Effect of heating a 0.2 wt % β-Lg-stabilized hexadecane-in-water emulsions [(**a**) pH 7.0; (**b**) pH 3.0] at 80.0 °C for 30 min on Fe^{2+}-induced decomposition of cumene hydroperoxide. Hydroperoxide concentrations were measured 5 min after the addition of iron. Data points represent means ($n = 3$) ± standard deviation.

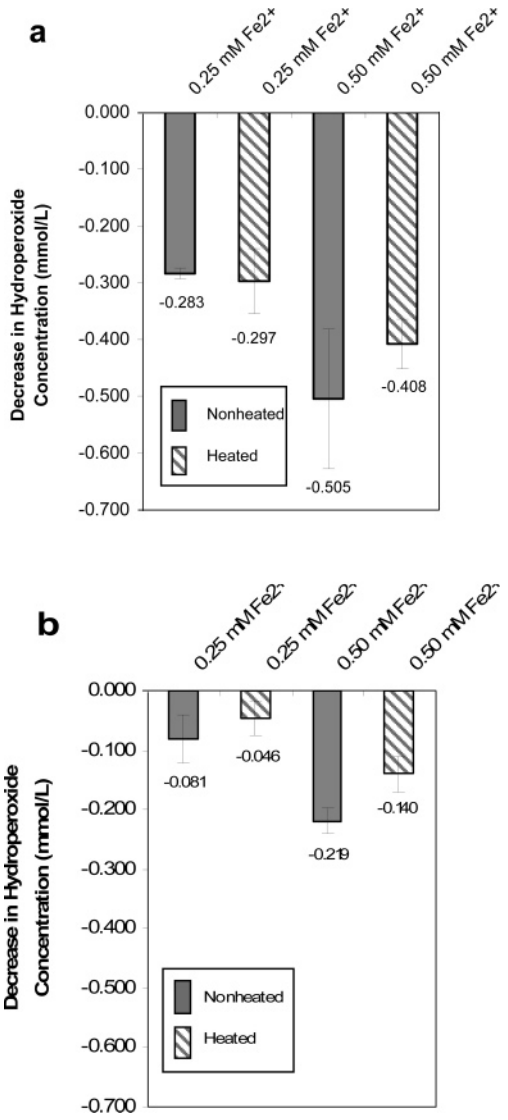

Figure 6. Effect of heating (80.0 °C for 30 min) a 0.2 wt % β-Lg-stabilized hexadecane-in-water emulsions containing 0.8 wt % β-Lg in the continuous phase on Fe^{2+}-induced decomposition of cumene hydroperoxide [(**a**) pH 7.0; (**b**) pH 3.0]. Hydroperoxide concentrations were measured 5 min after the addition of iron. Data points represent means ($n = 3$) ± standard deviation.

In the presence of continuous phase proteins, heating did not significantly influence hydroperoxide degradation at pH 7.0 or 3.0 when 0.25 mM Fe^{2+} or 0.50 mM Fe^{2+} was added to the system ($p \geq 0.05$) (**Figure 6**). No significant differences in hydroperoxide decomposition were seen in the absence of added iron at pH 3.0 or 7.0. These data follow the result seen in the prior experiment, suggesting that heat-induced cross-linking of proteins around the interface to create a more cohesive network of proteins does not increase hydroperoxide stability. In addition, adding continuous phase protein to the emulsion system prior to heating did not have an influence on hydroperoxide degradation, supporting the trend reported from **Figure 3**, which failed to show continuous phase β-Lg as being able to inhibit hydroperoxide degradation.

Conclusions. This study elucidated the influence of continuous phase and interfacial proteins on lipid hydroperoxide stability in oil-in-water emulsions. In β-Lg-stabilized emulsions, lipid hydroperoxide degradation was inhibited by EDTA but not continuous phase protein. Interfacial proteins were able to inhibit iron-promoted lipid hydroperoxide decomposition at pH values below the pI of β-Lg, at which the droplets would be cationic and capable of electrostatically repelling iron away from the emulsion droplet. Heat-induced physical changes in both interfacial and continuous phase proteins did not show a significant impact on lipid hydroperoxide stability at either pH 3.0 or pH 7.0. These results suggest that proteins at the interface of emulsion droplets primarily stabilize lipid hydroperoxides by electrostatically inhibiting iron−hydroperoxide interactions.

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