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Chemical and Physical Stability of Citral and Limonene in Sodium Dodecyl Sulfate−Chitosan and Gum Arabic-Stabilized Oil-in-Water Emulsions

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Citral and limonene are the major flavor components of citrus oils. Both of these compounds can undergo chemical degradation leading to loss of flavor and the formation of undesirable off-flavors. Engineering the interface of emulsion droplets with emulsifiers that inhibit chemical reactions could provide a novel technique to stabilize citral and limonene. At present, emulsified flavor oils are usually stabilized by gum arabic (GA), which is a naturally occurring polysaccharide−protein complex. The objective of this study was to examine if citral and limonene were more stable in emulsions stabilized with a sodium dodecyl sulfate (SDS)−chitosan complex than GA. Citral degraded less in GA-stabilized than in SDS−chitosan-stabilized emulsions at pH 3.0. However, SDS−chitosan-stabilized emulsions were more effective at retarding the formation of the citral oxidation product, *p*-cymene, than GA-stabilized emulsions. Limonene degradation and the formation of limonene oxidation products, limonene oxide and carvone, were lower in the SDS−chitosan- than GA-stabilized emulsions at pH 3.0. The ability of an SDS−chitosan multilayer emulsifier system to inhibit the oxidative deterioration of citral and limonene could be due to the formation of a cationic and thick emulsion droplet interface that could repel prooxidative metals, thus decreasing prooxidant−lipid interactions.

KEYWORDS: Citral; limonene; flavor; multilayered emulsion; electrostatic deposition; oxidation

INTRODUCTION

Natural flavor ingredients have numerous uses in food products. Citrus oils are natural flavor ingredients with high consumer acceptance. Citrus oils are commonly added to foods and beverages in the form of an emulsion. Unfortunately, the flavor components in citrus oil emulsions are chemically unstable and the emulsions are susceptible to physical deterioration resulting in loss of product quality and a decrease in shelf life. Therefore, the formation of chemically and physically stable citrus oil emulsions for use in foods and beverages would be a major benefit for the food industry.

Citral (3,7-dimethyl-2,6-octadienal) and limonene [1-methyl-4-(1-methylethenyl)cyclohexene] are two of the most important flavor compounds in essential oils obtained from citrus fruits. Citral consists of neral and geranial, which are geometrical isomers. Citral will degrade rapidly at low pH and under oxidative stress (*1*−*5*). Acid-catalyzed cyclization of citral reduces the fresh lemon flavor of citrus oils and leads to the formation of compounds that produce undesirable off-flavors (*3*, *6*−*8*). Citral degradation at low pH starts with the isomerization of geranial to neral, which then forms the monoterpene alcohols, *p*-menthadien-8-ol and *p*-menthadien-4-ol, which in turn can oxidize to form *p*-cymene-8-ol and through a dehydration reaction produces aromatic compounds such as α-*p*-dimethyl-styrene, *p*-cymene, and *p*-cresol (*6*, *7*). *p*-Cresol and *p*-methylacetophenone are two of the most potent degradation products of citral (*3*, *8*).

Both *R*-(+)- and *S*-(−)-limonene (D- and L-forms, respectively) are found in essential oils from citrus peels, rosemary, eucalyptus, lavender, caraway, lemon grass, peppermint, tea tree, and pine (*9*). Limonene has a pungent, green, lemonlike odor (*10*) and makes up over 95% of peel oil from lemons (*9*). Limonene is susceptible to degradation by oxidation reactions. Limonene oxidation initially results in the formation of hydroperoxides. As with the oxidation of unsaturated fatty acids, limonene hydroperoxides can undergo scission reactions that lead to the formation of numerous products including perillyl alcohol, perillyl acetate, carveol acetate, limonene oxide, carvone, and carveol (*11*, *12*). As with citral degradation, limonene decomposition causes the loss of lemonlike odors and the formation of off-flavors described as flowery, piney, and minty (*13*).

Strategies to inhibit citral and limonene degradation include reduction of storage temperature and alteration of environmental

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conditions such as pH and the partial pressure of oxygen. Unfortunately, these strategies are not practical for many foods meaning that new protection technologies are needed to protect citrus oils from degradation. Many chemical reactions in emulsions occur through interactions between water and lipid components at the interface of the emulsion droplet. This means that the interfacial membrane of oil-in-water emulsions could be altered to inhibit chemical reaction rates if the interfacial membrane is able to inhibit interactions between lipid and aqueous phase components (*14*). Recently, it has been demonstrated that emulsions prepared with multiple layers of emulsifiers have improved stability to environmental stresses. Multilayered oil-in-water emulsions are produced by layer-by-layer (LbL) deposition techniques where polyelectrolytes absorb onto oppositely charged surfaces or colloidal particles due to electrostatic attraction (*15*−*17*). It has been shown that multilayer emulsions are more physically stable than conventional emulsions under conditions commonly found in foods (e.g., have better stability to particle aggregation at high salt concentrations, freeze−thaw cycling, thermal processing, and high calcium concentrations) (*15*, *16*, *18*−*24*). Multilayered oil-in-water emulsions are also more effective at inhibiting oxidative reactions than single-layer emulsions, since they can be prepared with a thick and cationic droplet interface that inhibits interactions between lipids and water-soluble prooxidants such as iron. Both liquid and spray-dried tuna oil-in-water emulsions coated by lecithin−chitosan layers were more oxidatively stable emulsions than emulsions coated with lecithin alone or than bulk tuna oil (*25*, *26*).

Gum arabic (GA), a polysaccharide−protein complex, is commonly used by the food industry to emulsify flavor oils (*27*, *28*). The adsorption of GA onto an emulsion droplet produces a relatively thick and negatively charged interfacial membrane that stabilizes the emulsion through steric and electrostatic interactions (*29*). GA is not an ideal emulsifier because it needs to be used at relatively high concentrations and because there are considerable fluctuations in its cost, availability, and reliability (*28*, *29*).

The objective of this study was to determine if emulsion droplet interfacial engineering using multiple layers of emulsifiers could inhibit citral and limonene degradation in a model oil-in-water emulsion system. Hexadecane was used as a nonoxidizible lipid carrier so that the degradation reactions of limonene and citral could be studied independent of the oxidation of other lipid molecules that exist in citrus oil. Sodium dodecyl sulfate (SDS) and chitosan were used to create multiple layers of emulsifiers. SDS was used as a representative of an anionic small molecule surfactant. Chitosan is a cationic food-grade polysaccharide that is water soluble at low pH. Having opposite electrical charges, SDS and chitosan can form SDS−chitosan complexes on the surface of emulsion droplets by an electrostatic LbL deposition technique. Limonene and citral stabilities in SDS- and SDS−chitosan-stabilized emulsions were compared to GA-stabilized emulsion since GA is commonly used to stabilize citrus oil emulsions in foods and beverages.

MATERIALS AND METHODS

Materials. (+)-Limonene (97% pure), citral (mixture of *cis* and *trans* isomers, 95% pure), dodecane, and tridecane were purchased from Acros Organics (Fair Lawn, NJ). GA was donated by TIC Gums (Seyal type). GA was stored at 4 °C and used without further purification. Chitosan (medium molecular weight, 75−85% deacetylation), SDS (99% pure), cumene hydroperoxide (CH, 80% pure), ferrous sulfate, sodium acetate, hydrochloric acid, sodium hydroxide, and all other chemicals (reagent or high-performance liquid chromatography grade) were obtained from Sigma Chemical Co. (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). Double-distilled water was used to prepare all solutions.

Methods. *SDS- and SDS−Chitosan-Stabilized Emulsions.* Oil-in-water multilayered emulsions were made in a two-step process based on an electrostatic LbL deposition technique by modifying the method of Aoki et al. (*30*). Oil-in-water multilayered emulsions were prepared by mixing 5% oil phase with 95 wt % aqueous phase. The oil phase consisted of 0.5 wt % citral or limonene (flavor oils), 0.35 wt % dodecane, and 0.1 wt % tridecane (internal standards) dissolved in *n*-hexadecane (inert carrier oil). The aqueous phase consisted of 100 mM sodium acetate buffer and 12.5 mM SDS at pH 3.0. For limonene emulsions, CH (final concentration, 100 mmol/kg oil) was dissolved in the oil phase and stirred for 15 min. These stock emulsions were diluted with 100 mM sodium acetate buffer (pH 3.0) containing either 0 or 2 wt % chitosan to form 3 wt % oil-in-water primary (0% chitosan) and secondary (0.2 wt % chitosan) emulsions. The SDS−chitosan-stabilized emulsions were sonicated for 2 min at an amplitude of 40% and a duty cycle of 0.5 s (model 500, Sonic Dismembrator, Fisher Scientific) and then homogenized through a two-stage high-pressure valve homogenizer (APV-Gaulin, Wilmington, MA) at 2000 psi for three passes to disrupt any flocs formed during their preparation.

GA-Stabilized Emulsions. GA-stabilized oil-in-water emulsions were prepared by mixing 5% oil (0.5 wt % citral or limonene plus 100 mM CH and the internal standards, 0.35 wt % dodecane, and 0.1 wt % tridecane in *n*-hexadecane) with 95 wt % aqueous phase (1.0 wt % GA and 100 mM sodium acetate buffer, pH 3.0). Hence, the final emulsifier-to-lipid ratio was 1:2. A coarse emulsion premix was prepared by homogenizing oil and aqueous phase using a high-speed blender (Biospec Products, Inc., Bartlesville, OK) at setting 2 for 2 min at room temperature. The coarse emulsion was then passed through a two-stage high-pressure valve homogenizer (APV-Gaulin) at 3000 psi for four passes. Finally, the GA-stabilized emulsions were diluted with 100 mM sodium acetate buffer (pH 3.0) to a final lipid concentration of 3 wt %.

Immediately prior to storage studies, ferrous sulfate was added to the limonene emulsions at a final concentration of 0.25 mM and stirred for 15 min. All emulsions were then stored in the dark in 4 mL amber vials (completely filled to eliminate headspace) and in 10 mL capped test tubes (for creaming index, CI) at 37 and/or 55 °C.

Characterization of Emulsion Physical Stability. The emulsion droplet size distribution as a function of storage time was measured using a laser light-scattering instrument (MalvernSizer; Malvern Instruments Ltd., Worcestershire, United Kingdom) (*31*). The laser light-scattering instrument measured the intensity of laser light scattered from a dilute emulsion and then reported the particle size distribution that gave the closest fit between theoretical calculations (Mie theory) and experimental measurements of intensity vs scattering angle. A refractive index ratio of 1.08 was used. Prior to each measurement, emulsions were vortexed, stirred, inverted, and vortexed again to ensure that they were homogeneous. To avoid multiple scattering effects, emulsions were then diluted (approximately 1:1000) with the same buffer as in their continuous phase. The emulsions were stirred continuously in the sample chamber (at an instrument stirring speed of approximately 50%) to ensure that they were homogeneous. Particle size measurements were reported as the weight-average mean diameter, d_{43}.

The susceptibility of emulsions to creaming was ascertained by measuring the height of the interface between the opaque droplet-rich layer at the top and the transparent or turbid droplet-depleted layer at the bottom of the test tube. Creaming results were reported as the CI = 100 × (height of interface)/(height of total emulsion) (*32*).

The electrical charge on the droplets was measured using ζ-potential measurements that were carried out by injecting diluted samples into the measurement chamber of a particle electrophoresis instrument (ZEM 5003, Zetamaster, Malvern Instruments Ltd.). The ζ-potential was determined by measuring the direction and velocity that the droplets moved in the applied electric field.

Characterization of Limonene and Citral Degradation. Citral degradation was monitored by measuring the decrease of citral isomers neral and geranial and the formation of the citral breakdown product, *p*-cymene. Limonene degradation was monitored by measuring the

decrease of limonene and the formation of the limonene breakdown products, limonene oxide, and carvone. Analysis of citral, limonene, and their degradation products was conducted on a gas chromatograph (GC; model GC-17A; Shimatzu, Avondale, PA) equipped with a capillary column (DB-5, J&W Scientific, Folsom, CA; 30 m × 0.25 mm i.d., 0.25 μm film thickness) with a glass injection splitter ratio of 7:1 and a flame ionizing detector. For citral, the oven temperature was programmed as follows: 80 → (3 °C/min) 90 → (2 °C/min) 110 → (25 °C/min) 200 °C. For limonene, the oven temperature was programmed as follows: from 80 → (3 °C/min) 110 → (20 °C/min) 200 °C. Injector and detector temperatures were 220 and 240 °C, respectively. Helium (12 mL/min) was used as the carrier gas. Oil in the emulsions (0.2 mL) was solubilized into 2.8 mL of methanol and vortexed for 15 s prior to injection. Emulsions stabilized with GA were centrifuged for 1 min at 2000*g* after addition to methanol to precipitate excess GA. Sample (1.0 μL) was injected into the GC, and compounds were identified by comparison of retention times with authentic standards. Concentrations were determined relative to the internal standards, dodecane and tridecane, as g compound/g oil.

Statistical Analysis. All experiments were repeated twice using duplicate measurements and reported as means ± standard deviations. Statistical analyses were performed using *t*-test and one way analysis of variance ($P \leq 0.05$) (*33*).

RESULTS

Citral Stability in Oil-in-Water Emulsions Stabilized by SDS, SDS−Chitosan, or GA at pH 3.0. The influence of interfacial composition and properties on the stability of citral in oil-in-water emulsions was determined. The ζ-potential of the SDS-stabilized droplets was highly negative (−98.3 ± 5.3 mV), whereas that of the SDS−chitosan-stabilized emulsion droplets was positive (+28.6 ± 1.1 mV), which can be attributed to absorption of cationic chitosan onto the surface of anionic SDS-coated droplets (*16*). Citral stability in the SDS- and SDS−chitosan-stabilized oil-in-water emulsions was compared to GA-stabilized emulsions under the same storage conditions. The net charge in the GA-stabilized droplets was slightly negative (−11.5 ± 0.7 mV). Storage studies were not conducted at pH 7.0 because the pK_a of chitosan ranges from 6.3 to 7.0 (*34*, *35*), and thus, at pH 7.0, the chitosan loses its charge and the emulsions become unstable to flocculation (*16*). pH 3.0 was used since these flavors are often used in acidified beverages.

The stability of citral in citral/hexadecane-in-water emulsions at pH 3.0 was determined by measuring the loss of citral's isomers (neral and geranial) during storage at 37 and 55 °C (**Figure 1**). Within an emulsifier system, neral and geranial degradation rates were similar at both incubation temperatures. At 37 °C, neral and geranial concentrations decreased 33.6 and 33.8% after 1 day of storage in the SDS-stabilized emulsion (**Figure 1a**). In the SDS−chitosan double-layer emulsion, neral and geranial concentrations decreased by 26 and 34%, respectively, after 1 day of storage. After 4 days of storage at 37 °C, over 95% of both neral and geranial was lost in both the SDS- and the SDS−chitosan-stabilized emulsions. Citral degradation was not observed in GA-stabilized emulsion stored at 37 °C until after 2 days of storage. After 4 days of storage, the neral and geranial concentrations in the GA-stabilized emulsions decreased by 64 and 63%, respectively. As expected, citral degraded faster at 55 °C with neral and geranial concentrations decreasing over 95% after 2 days of storage of both the SDS- and the SDS−chitosan-stabilized emulsions (**Figure 1b**). The GA-stabilized emulsion lost 54.4 and 48.4% of its neral and geranial after 2 days of storage at 55 °C.

After acid-catalyzed cyclization of citral, oxidation reactions can occur to produce a number of compounds that produce off-flavors. The impact of emulsifier type on citral degradation and

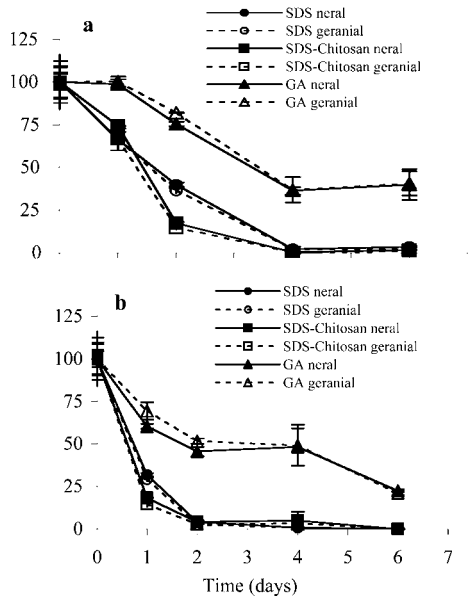

Figure 1. Degradation of citral isomers neral and geranial in 0.3 wt % citral/hexadecane oil-in-water emulsions stabilized with SDS, SDS–chitosan, and GA during storage at (**a**) 37 or (**b**) 55 °C at pH 3.0. Data markers represent averages ($n = 2$) ± standard deviations.

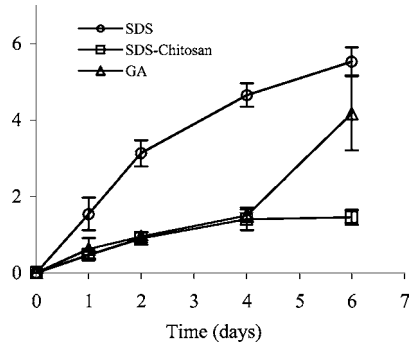

Figure 2. Detection of *p*-cymene in 0.3 wt % citral/hexadecane oil-in-water emulsions stabilized with SDS, SDS–chitosan, and GA during storage at 55 °C at pH 3.0. Data markers represent averages ($n = 2$) ± standard deviations.

oxidation was also followed by monitoring the formation of *p*-cymene. Very low *p*-cymene concentrations were detected in the emulsions stored at 37 °C (data not shown). However, *p*-cymene formation could be detected in all three emulsions during incubation at 55 °C after 1 day of storage (**Figure 2**). After 6 days of storage, *p*-cymene concentrations were lowest in the SDS−chitosan emulsion (1.5 mg/g oil; $p \leq 0.05$). *p*-Cymene concentrations in the SDS- and GA-stabilized emulsions were similar ($p \geq 0.05$) at 5.6 and 4.2 mg/g oil.

Physical Stability of Citral-Containing Emulsions. The initial mean particle diameters (d_{43}) of the SDS-, SDS−chitosan-, and GA-stabilized emulsions were 0.25, 0.41, and 1.1 μm, respectively, at both 37 (**Figure 3a**) and 55 °C (**Figure 3b**). Particle sizes did not change ($p \geq 0.05$) in any of the emulsions during storage, with the exception of the GA-stabilized emulsions stored at 55 °C for 6 days where an appreciable increase in mean particle size was observed (**Figure 3b**). Droplet creaming was not observed in the SDS-stabilized emulsion at either 37 or 55 °C during 6 days of storage. A small amount of droplet creaming was observed in the SDS−chitosan-stabilized emulsions after 2 days of storage with CIs ranging from 2 to 6% and 1 to 9% for 37 and 55 °C, respectively. GA-

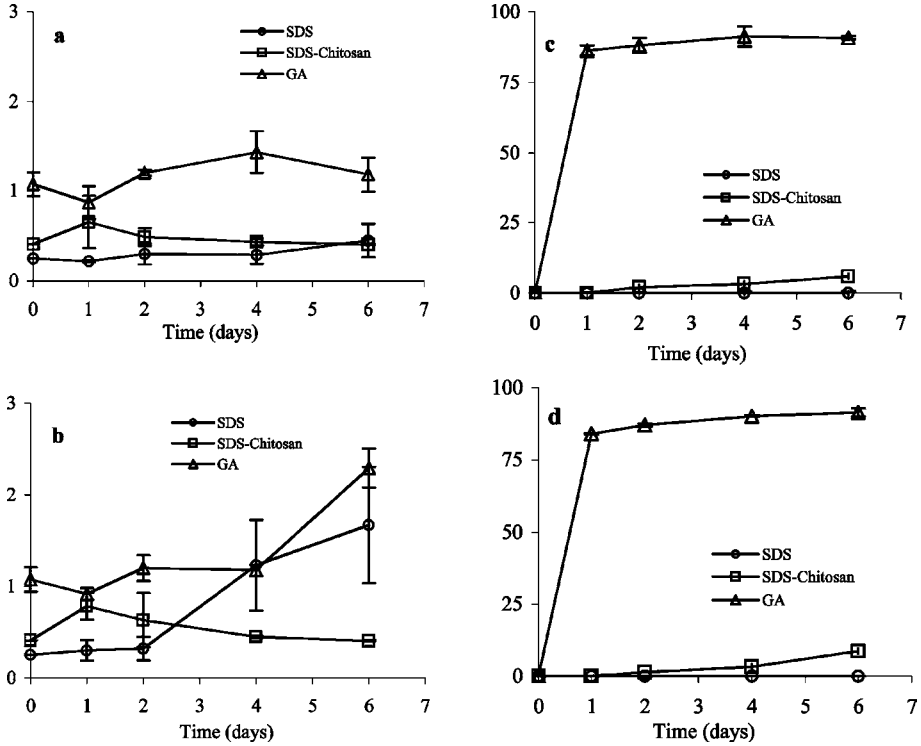

Figure 3. Mean particle diameter at (**a**) 37 and (**b**) 55 °C and CI at (**c**) 37 and (**d**) 55 °C in 0.3 wt % citral/hexadecane oil-in-water emulsions stabilized with SDS, SDS–chitosan, or GA at pH 3.0. Data markers represent averages ($n = 2$) ± standard deviations.

stabilized emulsions creamed rapidly with CIs over 84% being recorded after 2 days of storage (**Figure 3c**,**d**).

Chemical Stability of Limonene-Containing Emulsions. The stability of limonene was also determined in oil-in-water emulsions with different interfacial compositions and properties: SDS-, SDS−chitosan-, and GA-coated droplets. The ζ-potential of the primary emulsion containing SDS-coated droplets was −88.9 ± 5.1 mV, while that in the secondary emulsion containing SDS−chitosan-coated droplets was +32.6 ± 1.7 mV. The net charge on the GA-coated droplets was −15.2 ± 0.8 mV. Preliminary studies showed that at 55 °C limonene degradation was very slow and there were no limonene oxidation products detected (data not shown). Therefore, CH and ferrous sulfate (CH + Fe), final concentrations of 100 mmol/kg oil and 0.25 mM, respectively, were added to all emulsions to accelerate limonene oxidation. These prooxidants were chosen because the degradation of lipid hydroperoxides by iron is thought to be a major mechanism of oxidative reactions in oil-in-water emulsions. Limonene stability in all of the emulsions was monitored during storage in the dark at 55 °C for 14 days (**Figure 4**).

The limonene stability tended to be greater in the emulsions coated by SDS−chitosan layers than in those coated by SDS or GA layers. For example, limonene degradation was not observed in the SDS−chitosan emulsion until after 2 days of storage, whereas the limonene concentrations decreased by 33.1 and 15.4% after 1 day of storage in the SDS- and GA-stabilized emulsions, respectively (**Figure 4**). After 14 days of storage, 45.5 and 33.1% of limonene was lost in SDS- and SDS−chitosan-stabilized emulsions, respectively, as compared to over 75.0% in the GA-stabilized emulsions.

Limonene oxide and carvone formation were observed in all of the emulsions (**Figure 5**). Limonene oxide concentrations in the SDS-stabilized emulsions after 1, 6, and 14 days of storage were 1.1, 1.2, and 1.8 mg limonene oxide/g total lipid while in the SDS−chitosan-stabilized emulsions limonene oxide was 0.3, 0.8, and 2.4 mg limonene oxide/g total lipid, respectively

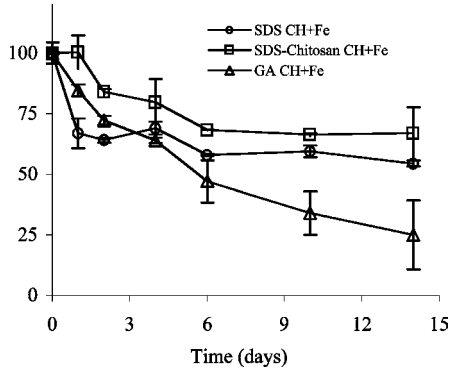

Figure 4. Degradation of limonene in 0.3 wt % limonene/hexadecane oil-in-water emulsions with CH and ferrous chloride (CH + Fe) stabilized with SDS, SDS–chitosan, and GA during storage at 55 °C at pH 3.0. Data markers represent averages ($n = 2$) ± standard deviations.

(**Figure 5a**). In GA-stabilized emulsions, limonene oxide concentrations after 1, 6, and 14 days of storage were 1.0, 2.1, and 3.0 mg limonene oxide/g total lipid, respectively. Limonene oxide formation tended to be lower in SDS−chitosan-stabilized emulsions than in SDS- and GA-stabilized limonene/hexadecane-in-water emulsions. For example, limonene oxide concentrations were significantly lower ($p \leq 0.05$) in SDS−chitosan-stabilized emulsions at day 6 as compared to SDS- and GA-stabilized emulsions. In SDS-stabilized emulsions, limonene oxide concentrations were significantly lower at day 6 as compared to GA-stabilized emulsions.

As with limonene oxide, the formation of the oxidation product carvone tended to be lower in the SDS−chitosan emulsions (0.5, 2.2, and 1.7 mg carvone/g total lipid at 1, 6, and 14 days of storage, respectively) as compared to SDS-stabilized (1.7, 2.5, and 2.2 mg carvone/g total lipid at 1, 6, and 14 days of storage, respectively) and GA-stabilized (1.1, 3.0, and 3.1 mg carvone/g total lipid at 1, 6, and 14 days of storage, respectively) emulsions (**Figure 5b**). However, there

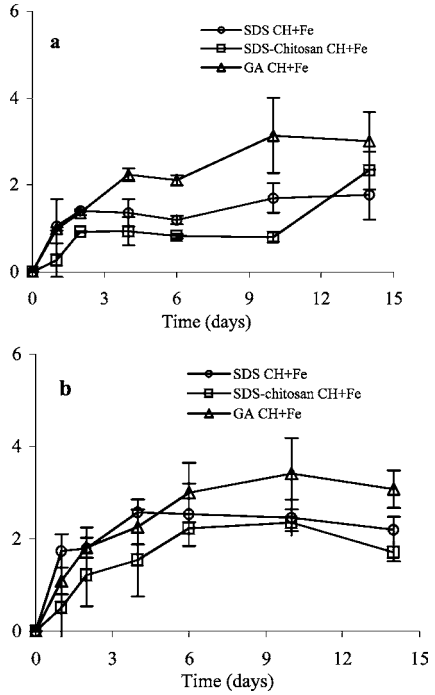

Figure 5. Formation of (**a**) limonene oxide and (**b**) carvone in 0.3 wt % limonene/hexadecane oil-in-water emulsions with CH and ferrous chloride (CH + Fe) stabilized with SDS, SDS–chitosan, and GA during storage at 55 °C at pH 3.0. Data markers represent averages ($n = 2$) ± standard deviations.

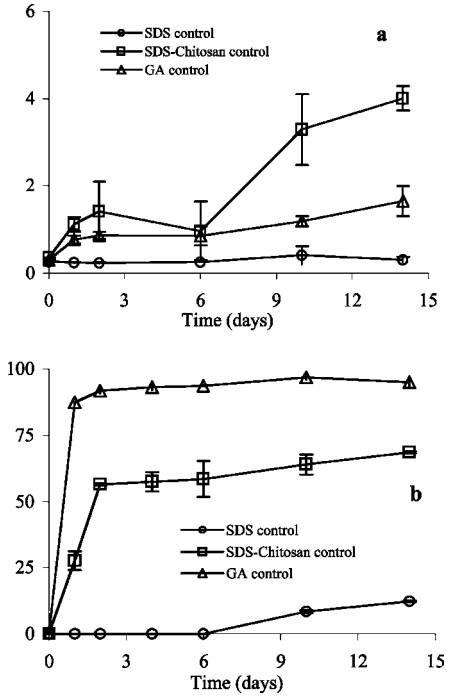

Figure 6. Mean particle diameter (**a**) and CI (**b**) in 0.3 wt % limonene/hexadecane oil-in-water emulsions stabilized with SDS, SDS–chitosan, and GA at pH 3.0 during storage at 55 °C. Data markers represent averages ($n = 2$) ± standard deviations.

were no statistical differences ($p \geq 0.05$) in carvone concentrations between the different emulsions systems.

Physical Stability of Limonene-Containing Emulsions. The physical stability of SDS-, SDS−chitosan-, and GA-stabilized hexadecane oil-in-water emulsions containing limonene was monitored in control emulsions without prooxidants (**Figure 6**) as well as in the emulsions with the addition of CH and ferrous sulfate (CH + Fe) (**Figure 7**). Overall, emulsions containing SDS−chitosan-coated and GA-coated droplets were physically less stable than those containing SDS-coated droplets during storage. The initial mean particle diameters (d_{43}) of the SDS-, SDS−chitosan-, and GA-coated droplets were 0.25, 0.37, and 0.99 μm, respectively (**Figure 7a**). The droplet diameter increased from 0.25 to 1.1 μm in the SDS-stabilized emulsions and from 0.37 to 3.5 μm in SDS−chitosan-stabilized emulsions during 14 days of storage. The emulsion droplet sizes in the GA-stabilized emulsions increased from 0.99 to 4.3 μm after 14 days of storage (**Figure 7a**). The droplet diameter increased from 0.25 to 0.41 μm in the control SDS-stabilized emulsion, from 0.31 to 4.0 μm in the SDS−chitosan-stabilized emulsion, and from 0.31 to 1.7 μm in the GA-stabilized emulsions during 14 days of storage (**Figure 6a**). Creaming was not observed in the SDS-stabilized emulsions until day 6 of storage (**Figures 6b** and **7b**). In the SDS−chitosan-stabilized emulsions, creaming was observed after 1 day of storage with CIs ranging from 37 to 75%. As seen in the previous experiments, GA-stabilized emulsions creamed rapidly with CIs over 85% after 1 day of storage (**Figures 6b** and **7b**).

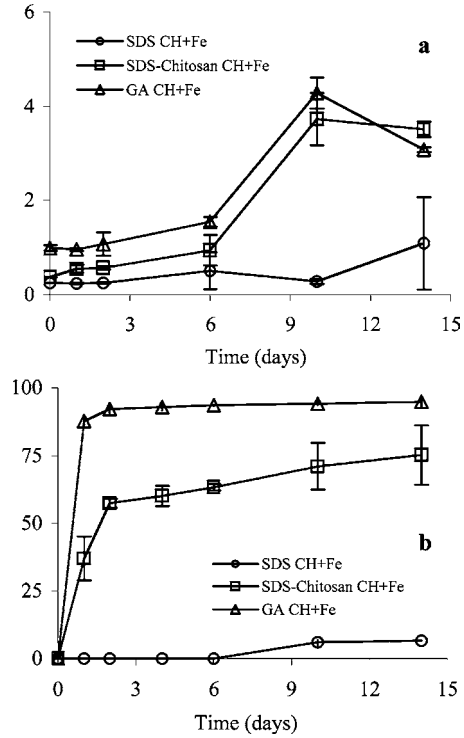

Figure 7. Mean particle diameter (**a**) and CI (**b**) in 0.3 wt % limonene/hexadecane oil-in-water emulsions with CH and ferrous chloride (CH + Fe) stabilized with SDS, SDS–chitosan, and GA at pH 3.0 during storage at 55 °C. Data markers represent averages ($n = 2$) ± standard deviations.

DISCUSSION

Chemical Stability of Emulsions. Acid-catalyzed citral degradation converts neral and geranial into monoterpene alcohols, which can undergo oxidative and dehydration reactions to produce compounds such as α-*p*-dimethyl-styrene, *p*-cymene, and *p*-cresol (*6*, *36*). Our results suggested that GA was more effective in inhibiting the loss of emulsified citral isomers than when SDS or SDS−chitosan was used to coat the oil droplets. However, when SDS−chitosan was used as an emulsifier, the formation of *p*-cymene (a citral degradation product) was less than in the GA- and SDS-stabilized emulsions. Very little is

known about the mechanism of acid-catalyzed citral degradation in oil-in-water emulsions. In emulsions, citral will partition into the lipid phase, the water phase, and the emulsion droplet interface since citral is amphiphilic and surface-active. Thus, citral would be expected to degrade by acid either at the emulsion droplet interface or in the water phase. Because citral is soluble in water (590 mg/L), it is possible that interfacial engineering of the emulsion droplet would not impact citral degradation if the reaction mainly occurred in the water phase and not at the emulsion droplet interface. The reason for the observed differences in acid-catalyzed degradation of citral when GA was used as an emulsifier instead of SDS or SDS−chitosan is unclear. One possible difference is the fact that much larger amounts of GA are needed to stabilize the emulsions as compared to SDS or SDS−chitosan. The large amounts of GA would result in a high concentration of nonadsorbed GA, which could effect the acid-catalyzed degradation of citral in the continuous phase of the emulsion.

In the models used in this study, limonene degradation was accelerated by the production of free radicals by the interaction of lipid-soluble CH with iron. The extent of oxidation was determined by measuring the loss of substrate (limonene) and the amount of reaction products (carvone and limonene oxide). The ability of SDS−chitosan to inhibit limonene degradation and carvone and limonene oxide formation indicates that this system was able to inhibit oxidative reactions. Inhibition of oxidation by the SDS−chitosan system suggests that this oxidative reaction occurs at the interface of the emulsions droplet and that a thick, cationic interfacial membrane could decrease oxidation rates possibly by inhibiting iron−lipid interactions. This is in agreement with lipid oxidation studies where chitosan-based multilayer-stabilized emulsions were observed to inhibit the oxidation of fish oils in liquid and dried emulsions (*25*, *26*). Here, it has been attributed to the electrostatic and steric repulsion between the cationic iron ions and the thick cationic droplet interface.

Physical Stability of Emulsions Containing Limonene. In the absence of pro-oxidants, limonene degradation in the emulsions was too slow to measure over the storage periods used in this work. For this reason, CH and iron (Fe) were added to the emulsions to accelerate the rate of limonene degradation. Iron is a multivalent cation that could potentially destabilize the emulsions through electrostatic screening and ion-binding effects (*27*). We therefore measured the physical stability of the emulsions containing limonene in the absence and presence of pro-oxidants to determine whether any observed emulsion instability was caused by limonene degradation or by addition of the pro-oxidants (**Figures 6** and **7**).

In the absence of pro-oxidants, we observed an increase in droplet aggregation (**Figure 6a**) and creaming instability (**Figure 6b**) during storage of the emulsions. The emulsion stabilized by SDS was the most stable, with the mean particle diameter remaining fairly constant throughout the 2 weeks of storage and only a small amount of creaming being observed at 10 and 14 days. These results suggest that the electrical charge on the SDS-coated droplets was sufficiently high to prevent droplet aggregation through electrostatic repulsion. On the other hand, the emulsions containing SDS−chitosan-coated droplets showed a significant increase in mean particle diameter and creaming instability after only 1 day of storage, suggesting that droplet aggregation had occurred. It should be noted that the SDS−chitosan-stabilized emulsions containing limonene (**Figure 6**) appeared to be considerably less stable than those containing citral (**Figure 3**). The physicochemical origin of this effect of lipid type on emulsion stability is currently unknown. There was evidence of a slight increase in the mean particle diameter of the GA-stabilized emulsions after 14 days of storage, and rapid creaming was observed after 24 h. As mentioned earlier, this rapid creaming can be attributed to the relatively large initial droplet diameter ($d_{43} \approx 1.1$ μm), since droplets of this size are known to cream relatively quickly in oil-in-water emulsions (*27*).

In the presence of pro-oxidants, we also observed an increase in droplet aggregation (**Figure 7a**) and creaming instability (**Figure 7b**) during storage in all of the emulsions. In addition, we observed some differences between the physical stability of some of the emulsions in the absence and presence of the pro-oxidants. There was no significant change in the mean particle diameter of the SDS-stabilized emulsion during 2 weeks of storage, and only a slight amount of creaming was observed after 10 and 14 days of storage in the presence of prooxidants (**Figure 7**). The physical stability of this emulsion was therefore similar in the presence and absence of pro-oxidants, which suggests that the levels of iron present were not sufficient to promote instability through electrostatic screening or ion-binding effects. In addition, it suggests that any lipid oxidation products formed due to limonene degradation were incapable of destabilizing the SDS-stabilized emulsion. The SDS−chitosan-stabilized emulsion behaved similarly in the absence and presence of pro-oxidants, which again suggests that pro-oxidants or oxidation products did not contribute significantly to its destabilization. It should be noted that the SDS−chitosan-stabilized emulsions turned slightly yellow during storage (data not shown). This yellow pigment formation could be due to Maillard or Michael addition type reactions between carbonyls resulting from limonene oxidation (e.g., ketones and epoxides) and amines from the chitosan. The formation of yellow pigmentation in spray-dried lecithin−chitosan-stabilized fish oils emulsion has also been observed during storage, which was attributed to interaction of amine groups from chitosan with aldehydes arising from oxidation of the fish oil (*26*).

There was an appreciable difference between the stability of the GA-stabilized emulsion in the presence and absence of pro-oxidants (**Figures 6** and **7**). In the presence of pro-oxidants, the emulsion was still highly unstable to creaming, which is to be expected because of the relatively large initial droplet diameter of the GA-coated droplets. On the other hand, the GA-stabilized emulsion containing pro-oxidants showed a rapid increase in mean particle diameter between 6 and 9 days of storage (**Figure 7a**), whereas the one containing no pro-oxidants did not (**Figure 6a**). In principle, this increase in instability could have been due to the presence of the pro-oxidants or due to the formation of limonene oxidation products. We postulate that the latter mechanism is more likely since one would expect ion-induced effects (such as electrostatic screening or ion binding) to occur fairly rapidly (within 24 h). It is possible that surface-active limonene oxidation products were formed during storage and that once these reached a certain level they partially displaced the GA molecules from the droplet surfaces, thereby inducing droplet aggregation.

In conclusion, engineering the emulsion droplet interface with a SDS−chitosan multiple-layer system was not effective at preventing the degradation of citral in oil-in-water emulsions as compared to GA; however, it was more effective than GA in preventing limonene degradation. In emulsions stabilized with SDS−chitosan, the formation of citral and limonene degradation products was less than in GA-stabilized emulsions as can be seen by an inhibition of *p*-cymene, limonene oxide, and carvone formation. This is in agreement with previous experiments that

showed that SDS−chitosan could inhibit oxidation of unsaturated fatty acids. While SDS−chitosan was effective in preventing some oxidative reactions, it produced physically stable citral but physically unstable limonene emulsions, as can be seen by larger particle sizes and increased creaming rates. Further work is needed to determine if multilayer emulsion systems formed from generally recognized as safe food ingredients would be effective at producing physically and chemically stable flavor oil emulsions.

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