

## Citral Stability in Oil-in-Water Emulsions with Solid or Liquid Octadecane

LONGYUAN MEI, SEUNG JUN CHOI, JEAN ALAMED, LULU HENSON,  
 MICHAEL POPPLEWELL, D. JULIAN McCLEMENTS, AND ERIC A. DECKER\*

Department of Food Science, University of Massachusetts, Amherst, Massachusetts 01003

Citral stability in oil-in-water emulsions at pH 3.0 with solid or liquid octadecane was determined. Citral degradation was faster in anionic sodium dodecyl sulfate (SDS)-stabilized emulsions than non-ionic polyoxyethylene (23) lauryl ether (Brij)-stabilized emulsions. Crystallization of octadecane in both Brij- and SDS-stabilized emulsion droplets resulted in faster degradation of citral. Crystallization of octadecane in emulsion droplets increased citral partitioning into the aqueous phase, with 41–53% of the total citral in the aqueous phase when octadecane was solid compared to 18–25% when octadecane was liquid. This research suggests that factors that increase partitioning of citral out of the droplets of oil-in-water emulsions increase citral degradation rates. These results suggest that the stability of citral could be increased in oil-in-water emulsions by technologies that decrease its partitioning and exposure to acidic aqueous phases.

**KEYWORDS:** Citral; degradation; flavor; emulsions; octadecane; SDS; Brij

### INTRODUCTION

Citrus flavors, especially lemon, lime, and orange, are among the most popular flavors in foods (1). Citrus flavors are most commonly derived from citrus oils obtained from the peel of the citrus fruit or as a byproduct obtained during the concentration of juice. Because citrus oils are not fully water-soluble, they are added to many food and beverages as oil-in-water emulsions. In citrus-flavored beverages, these emulsions are commonly stabilized by gum arabic or modified starch.

One of the most important citrus flavor components is citral. Citral is a monoterpene aldehyde that is composed of a mixture of two geometric *cis* and *trans* isomers, geranial and neral. Because citral is one of the main contributors to lemon flavor and aroma, its concentration is the most important factor in determining the value and quality of lemon oils (2–4). However, lemon oil is an unstable food ingredient because it deteriorates at low pH and under oxidative conditions (5).

Citral decomposes rapidly during storage at acidic pH by a series of cyclization and oxidation reactions (6–9). These reactions decrease the level of fresh lemon-like aroma of citral and also generate many off-odor compounds that limit the application of citral in the beverage, food, and fragrance industries. Under acidic conditions, the formation of off-odor compounds from citral degradation is also affected by the temperature, light, and oxygen availability (10–12). Deterioration mechanisms of citral in acid solutions was studied by Kimura et al. (8), Peacock and Kuneman (12), and Schiebrle et al. (9). In this context, Kimura et al. (8) and Peacock and Kuneman (12) proposed that geranial in acidic solution rapidly isomerizes into neral, followed

by the formation of the monoterpene alcohols, *p*-menthadien-8-ol and *p*-menthadien-4-ol. These intermediate monoterpene alcohols oxidize to *p*-cymene-8-ol, which undergoes a dehydration reaction and becomes converted into more stable aromatic compounds, such as  $\alpha$ -*p*-dimethyl-styrene, *p*-cymene, and *p*-cresol (8). According to Schiebrle et al. (9), oxidation of  $\alpha$ -*p*-dimethyl styrene can lead to the formation of *p*-methylacetophenone.

Emulsions can present unique opportunities to alter chemical reaction rates because of the presence of an interfacial membrane that can inhibit reactions between lipid- and aqueous-phase components. Emulsions are stabilized by an interfacial membrane that can be altered to protect lipids from chemical deterioration. For example, the formation of a cationic barrier and/or a thick interfacial membrane that inhibits iron–lipid hydroperoxide interactions has been used to inhibit lipid oxidation (13). Also, in the pharmaceutical and food industries, it has been well-known that colloidal systems, such as nano- and microemulsions, are powerful delivery systems to encapsulate, protect, and deliver lipophilic functional components. Recently, the pharmaceutical and food industries have attended solid lipid nanoparticles because of their higher ability to encapsulate, protect, and deliver lipophilic functional components compared to nano- and microemulsions. Because citral degradation can occur by both acidic pH and oxidative conditions, it could be possible that manipulation of the interfacial membrane of an emulsion could increase citral stability in oil-in-water emulsions. An additional strategy that could improve citral stability would be to entrap it in a solid lipid matrix within an emulsion droplet, so that it would not be exposed to aqueous-phase components. Therefore, the objectives of this study were to determine the influence of the surfactant type and the physical state of lipids on the stability of citral in oil-in-water emulsions.

\*To whom correspondence should be addressed. Telephone: (413) 545-1026. Fax: (413) 545-1262. E-mail: edecker@foodsci.umass.edu.

## MATERIALS AND METHODS

**Materials.** Citral (95%) was obtained from Acros (Geel, Belgium). Octadecane (99%), Brij 35 [polyoxyethylene (23) lauryl ether], and sodium dodecyl sulfate (SDS, minimum of 98.5%) were obtained from Sigma-Aldrich, Inc. (St. Louis, MO). All other chemicals were reagent-grade or purer and were obtained from Fisher Scientific (Pittsburgh, PA).

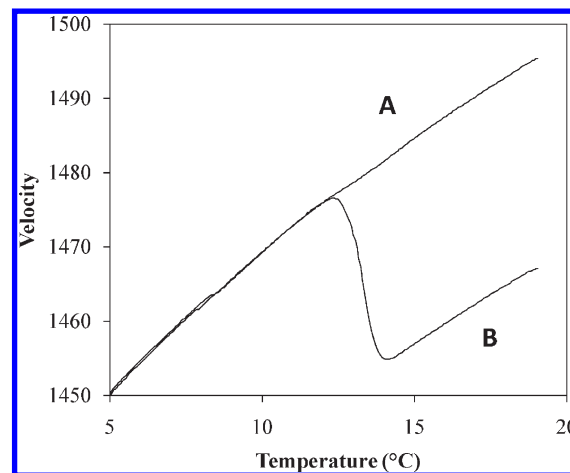
**Methods. Emulsion Preparation.** Octadecane was heated to 35 °C to ensure that it was completely liquid. Citral was dissolved in the heated octadecane at 2 wt %. A coarse oil-in-water emulsion consisting of 5.0 wt % octadecane/citral and 95.0 wt % of an aqueous phase consisting of 17 mM surfactant (SDS or Brij 35) and 10 mM sodium acetate/imidazole buffer (pH 3.0) was made by homogenizing the lipid and aqueous phases for 2 min using a two-speed hand-held homogenizer (Biospec Products, Inc., Bartlesville, OK) at the highest speed setting. The size of the droplets in the coarse emulsion was then further reduced by sonication for 1 min at a frequency of 20 kHz, amplitude of 50%, and a duty cycle of 0.5 s (model 500, sonic dismembrator, Fisher Scientific, Pittsburgh, PA). Care was taken to ensure that all samples were maintained at 35 °C to prevent lipid crystallization during emulsion preparation.

Emulsions were subjected to different thermal treatments to produce droplets containing either liquid or solid octadecane. To produce emulsions with liquid octadecane, samples were cooled from 35 to 20 °C. To produce emulsions with solid octadecane, the samples were cooled from 35 to 5 °C and then heated to 20 °C. The emulsions were then kept in a temperature-controlled room (20 °C) for the duration of the experiments. At this temperature, octadecane in droplets could be either solid or liquid depending upon its thermal history.

**Emulsion Characterization.** The solid/liquid status of the lipid droplets (octadecane) in oil-in-water emulsions was confirmed using an ultrasonic resonator system that measures ultrasonic velocity versus temperature (ResoScan System, Germany). The ultrasonic velocity of solid hydrocarbons is much higher than that of liquid hydrocarbons, so that phase transitions can be easily monitored by ultrasonic velocity temperature scanning. Emulsions believed to contain either solid droplets ("pre-cooled" emulsion) or liquid droplets ("non-cooled emulsion") were prepared as described above and then placed in the measurement chamber of the ultrasonic resonator at 20 °C. The samples were then scanned from 20 to 5 °C at a rate of 345 mK/min, and the change in ultrasonic velocity was recorded. Initially at 20 °C, the ultrasonic velocity of the pre-cooled emulsion was much higher than that of the non-cooled emulsion (Figure 1), which indicated that the lipid phase had crystallized in the pre-cooled emulsion. In addition, there was a steady decrease in the ultrasonic velocity with a decreasing temperature in the pre-cooled emulsion, indicating that no further phase transitions occurred; i.e., the lipid was already crystalline. On the other hand, there was a sharp increase in the ultrasonic velocity around 14 °C upon cooling the non-cooled emulsion, indicating that the lipid phase changed from liquid to solid at this temperature. These results confirm the fact that the octadecane was solid in the emulsions that had been cooled from 35 to 5 °C and then warmed back to 20 °C but that they were liquid in the emulsions that were cooled directly from 35 to 20 °C.

The particle size distribution of the emulsions was determined using a laser diffraction particle size analyzer (Mastersizer X., Malvern Instruments, Worcestershire, U.K.). The mean particle size was reported as the volume-surface mean diameter,  $d_{32}$  ( $= \sum n_i d_i^3 / \sum n_i d_i^2$ ), where  $n_i$  is the number of particles with diameter  $d_i$ . The mean particle diameter in all emulsions ranged from 0.25 to 0.33  $\mu\text{m}$  and did not change during the course of the studies. The electrical charge or  $\zeta$  potential of the emulsion droplets was measured using a microelectrophoresis instrument (ZetaSizer Nano, Malvern Instruments, Worcestershire, U.K.).  $\zeta$ -Potential samples were prepared by diluting emulsions 1:100 with 10 mM sodium acetate/imidazole buffer (pH 7.0) and placing the diluted emulsions into disposable capillary cells (Malvern Instruments, Worcestershire, U.K.).

**Measurement of the Physical Location of Citral in Oil-in-Water Emulsions.** Partitioning of citral in the different phases of the oil-in-water emulsions was measured by separating the aqueous phase and oil droplets by centrifuging the emulsion at 15000g for 1 h at 20 °C in plastic tubes that were surrounded by water in an ultracentrifugation tube. A syringe was used to penetrate the plastic centrifuge tube to collect the



**Figure 1.** Ultrasonic velocity versus temperature scans of SDS-stabilized octadecane oil-in-water emulsions containing citral after 29 days of storage at 20 °C. Samples include (A) "liquid" emulsions that were cooled from 35 to 20 °C and (B) "solid" emulsions that were cooled from 35 to 5 °C and then heated to 20 °C. All emulsion contained 5% octadecane and 0.1% citral and were stabilized by 17 mM SDS in 10 mM sodium acetate/imidazole buffer (pH 3.0).

lower aqueous phase, and citral concentrations were determined by gas chromatography (see below).

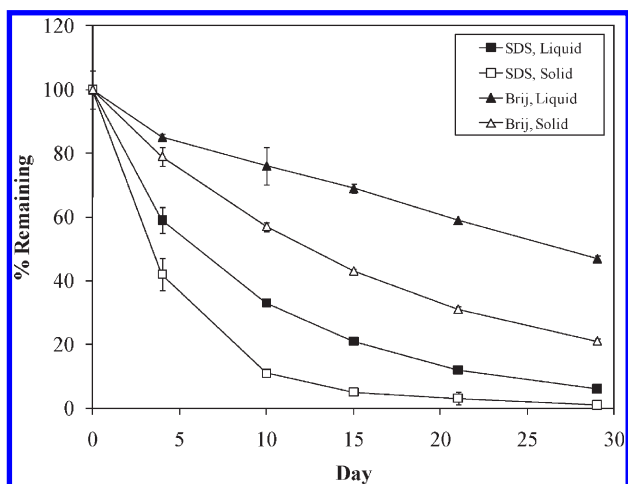
**Measurement of Citral.** Citral isomers were measured with a Shimadzu GC-17A gas chromatograph equipped with a flame ionization detection and chromatography data system (version 4.3) software, as previously described (14). Separations were performed on a 30 m  $\times$  0.32 mm DB5 fused silica capillary column (J & W Scientific, Folsom CA). Oven temperature was programmed as follows: 100 °C for 0.2 min, increase from 100 to 170 °C at 35 °C/min, hold at 170 °C for 0.2 min, increase from 170 to 240 °C at 70 °C/min, and hold at 240 °C for 0.8 min. The inlet pressure was programmed as follows: hold at 120 kPa for 2.4 min, increased from 120 to 170 kPa at 50 kPa/min, and hold at 170 kPa for 1.1 min. Carrier gas was helium, and the split ratio was 5:1. The injector temperature was 250 °C, and the detector temperature was 270 °C. Samples (0.1 mL) were vortexed into 1 mL of reagent alcohol, of which 1.0  $\mu\text{L}$  was injected into the gas chromatograph. The two isomers of citral, neral and geranial, were identified by a comparison of retention times to authentic standards. Citral concentrations were expressed as the sum of neral and geranial. Neral and geranial concentrations were determined using peak areas and a standard curve made with authentic standards.

**Statistics.** All experiments were conducted in triplicate samples and were repeated at least 2 times. Data are presented as mean  $\pm$  standard deviation. Data results were analyzed by analysis of variance (ANOVA) using SPSS (SPSS, Inc., Chicago, IL). The differences between mean values were compared using Duncan's multiple range test with significance defined as  $p < 0.05$ .

## RESULTS AND DISCUSSION

After preparation of the emulsions at 35 °C, one-half of the sample was cooled to 20 °C to prepare the emulsion with liquid octadecane, while the other half was cooled to 5 °C and then reheated to 20 °C to produce the emulsion with solid octadecane. To verify whether the octadecane was either solid or liquid, the emulsions were analyzed by ultrasound (Figure 1 shows the ultrasound scan for solid and liquid SDS-stabilized emulsions after 29 days of storage). Ultrasonic velocity decreased linearly as the emulsion with the solid octadecane was cooled from 19 to 5 °C, indicating that the octadecane did not undergo a phase transition upon cooling, thus verifying that the octadecane was solid (15, 16). Cooling the emulsion with the presumably liquid octadecane resulted in a large increase in ultrasonic velocity at 14 °C, indicating that the octadecane was crystallizing. These

results indicate that the emulsion cooled to 5 °C and reheated to 20 °C contained solid octadecane, while the emulsion maintained at 20 °C contained liquid octadecane. The above ultrasonic

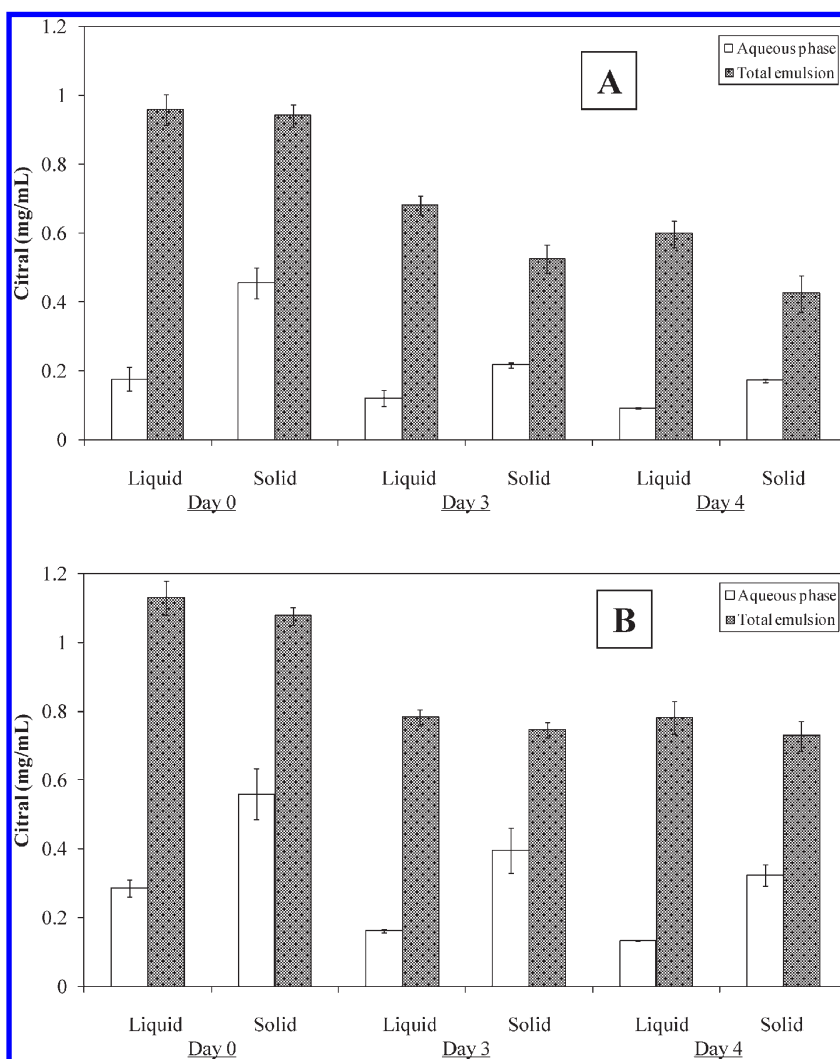


**Figure 2.** Stability of SDS- and Brij 35-stabilized octadecane/citral in oil-in-water emulsions (pH 3.0) containing liquid or solid octadecane during storage at 20 °C. Data represent means  $\pm$  standard deviations ( $n = 3$ ). Some error bars lie within data points.

velocity measurement did not change after 0, 15, or 29 days of storage at 20 °C, indicating that the emulsion remained either solid or liquid throughout the study. The particle size of the emulsions ranged from 0.25 to 0.33  $\mu\text{m}$  and did not change during storage (data not shown).

**Figure 2** shows the stability of citral during storage in SDS- or Brij-stabilized emulsions containing solid or liquid octadecane. The two citral isomers, neral and geranial, degraded in all of the emulsions at similar rates (data not shown); therefore, only total citral concentrations are reported. Because emulsions were prepared separately, slight differences in initial citral concentrations were observed. Therefore, citral degradation was calculated as a decrease in the percentage of the sum of both isomers compared to 0 time values (100%). In both the SDS- and Brij-stabilized emulsions, citral degradation at pH 3.0 was faster in emulsions containing solid octadecane. In comparison to 10 days of storage, the remaining citral in SDS-stabilized emulsions was 37% for liquid octadecane and 11% for solid octadecane. In Brij-stabilized emulsions, citral was 77 and 59% lower than 0 time values for liquid and solid octadecane, respectively, after 10 days of storage.

Citral is soluble in water at 590 mg/L and 25 °C (17), and the isomer structures suggest that they could be surface-active. Therefore, neral and geranial would be expected to partition into several different locations in oil-in-water emulsions, including the core of the lipid droplet, the lipid–water interface, and the



**Figure 3.** Total and aqueous-phase citral concentrations in oil-in-water emulsions stabilized by (A) Brij 35 or (B) SDS containing solid or liquid octadecane during storage at 20 °C. Data represent means  $\pm$  standard deviations ( $n = 3$ ). Some error bars lie within data points.



aqueous phase. To determine the physical location of citral in the SDS- and Brij-stabilized emulsions, emulsions were centrifuged to cream the emulsion droplets and isolate the aqueous phase. **Figure 3** shows aqueous-phase citral concentrations in the SDS- and Brij-stabilized emulsions containing liquid and solid octadecane. In the presence of both surfactants, aqueous-phase citral was higher in emulsions with solid octadecane. This could be due to the formation of pure octadecane crystals during cooling, which would result in the formation of pools of concentrated citral. These concentrated pools of citral would increase the partitioning of citral into the aqueous phase compared to liquid emulsions, where the citral would be diluted throughout the octadecane. In both the SDS- and Brij-stabilized emulsions, crystallization of octadecane resulted in aqueous-phase citral concentrations that approached (SDS = 550 mg of citral/L) or exceeded (Brij = 630 mg of citral/L) the water solubility limit of citral (590 mg/L). Changes in the physical location of citral by octadecane crystallization in oil-in-water emulsions has also been reported by Mikhalev et al. (18).

The proportion of citral in the aqueous phase remained relatively constant in all of the emulsions, even though total citral concentrations decreased during storage. For example, in SDS-stabilized emulsions, aqueous-phase citral concentrations during the entire storage time ranged from 41 to 48% and from 15 to 18% for solid and liquid emulsions, respectively. In Brij-stabilized emulsions, aqueous-phase citral concentrations during storage ranged from 44 to 53% and from 17 to 25% for solid and liquid emulsions, respectively.

Citral degradation was faster in the SDS- than Brij-stabilized octadecane-in-water emulsions at all time points (**Figure 2**), even though aqueous-phase citral concentrations were consistently higher in the Brij- than SDS-stabilized emulsions (**Figure 3**). For example, remaining citral in the SDS-stabilized liquid octadecane after 10 days of storage was 37% compared to 77% in Brij-stabilized emulsions with liquid octadecane (**Figure 2**). The higher degradation in the SDS-stabilized octadecane-in-water emulsions could be due to several factors. The surface charge of SDS- and Brij-stabilized octadecane-in-water emulsions at pH 3.0 was  $-106$  and  $-3$  mV, respectively. The high negative charge density of SDS could potentially attract cations, such as iron, which could increase the oxidative degradation of citral. Alternately, the larger polar head group of Brij could provide a steric barrier that could decrease reactions between citral and water-phase components. The higher aqueous-phase citral concentrations in the Brij- compared to SDS-stabilized octadecane-in-water emulsions could be due to solubilization of citral into surfactant micelles, as seen for surfactant solubilization of antioxidants out of emulsion droplets (19). Both surfactants were used at equal molar concentrations (17 mM), but the number of surfactant micelles could differ as a function of the concentration of surfactant absorbed onto the emulsion droplet and the critical micelle concentration (cmc) of the surfactant. The cmc of SDS is higher ( $> 1$  mM) compared to Brij 35 ( $< 0.1$  mM), suggesting that the Brij-stabilized emulsions could have more surfactant micelles in the aqueous phase. Solubilization of citral into Brij micelles could also help explain why aqueous-phase citral concentrations were higher than the reported water solubility limit of citral.

In conclusion, solidification of octadecane in oil-in-water emulsion droplets at pH 3.0 caused an increase in aqueous-phase citral concentrations, which in turn accelerated acid-promoted citral degradation. Citral degradation was faster in anionic SDS-stabilized emulsions than non-ionic Brij-stabilized emulsions.

These results suggest that the stability of citral could be increased in oil-in-water emulsions by technologies that decrease its partitioning and exposure to acidic aqueous phases.

## LITERATURE CITED

- (1) Tan, C.-T. Beverage emulsions. In *Food Emulsion*; Friberg, S. E., Larsson, K., Eds.; Marcel Dekker, Inc.: New York, 1997; pp 491–524.
- (2) Shaw, P. E. Review of quantitative analysis of citrus essential oils. *J. Agric. Food Chem.* **1979**, *27*, 246–257.
- (3) Staroscik, J. A.; Wilson, A. Quantitative analysis of cold-press lemon oil by glass capillary gas chromatography. *J. Agric. Food Chem.* **1982**, *30*, 507–509.
- (4) Veriotti, T.; Sacks, R. High-speed GC and GC/time-of-flight MS of lemon and lime oil samples. *Anal. Chem.* **2001**, *73*, 4395–4402.
- (5) Schieberle, P.; Grosch, W. Identification of potent flavor compounds formed in an aqueous lemon oil/citric acid emulsion. *J. Agric. Food Chem.* **1988**, *36*, 797–800.
- (6) Baines, D. A.; Jones, R. A.; Webb, T. C.; Campion-Smith, I. H. The chemistry of terpenes-I: The effect of hydrogen ion concentration and oxygen upon the acid catalyzed cyclization of citral. *Tetrahedron* **1970**, *26*, 4901–4913.
- (7) Clark, B.; Powell, C.; Radford, T. Acid-catalyzed cyclization of citral. *Tetrahedron* **1977**, *13*, 2187–2191.
- (8) Kimura, K.; Hishimura, H.; Iwata, I.; Mizutani, J. Deterioration mechanism of lemon flavor. 2. Formation mechanism of off-odor substances arising from citral. *J. Agric. Food Chem.* **1983**, *31*, 801–804.
- (9) Schieberle, P.; Ehrmeier, H.; Grosch, W. Aroma compounds resulting from the acid catalyzed breakdown from citral. *Z. Lebensm.-Unters.-Forsch.* **1988**, *187*, 35–39.
- (10) Ikenberry, D. A.; Saleeb, R. K. The effect of temperature and acidity on the stability of specific lemon flavor components. I. Data and methodology. In *Food Flavors, Ingredients and Composition*; Charalambous, G., Ed.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1993; pp 339–354.
- (11) Liang, C.-P.; Wang, M.; Simon, J. E.; Ho, C.-T. Antioxidant activity of plant extracts on the inhibition of citral off-odor formation. *Mol. Nutr. Food Res.* **2004**, *48*, 308–317.
- (12) Peacock, V. E.; Kuneman, D. W. Inhibition of the formation of  $\alpha$ -p-dimethylstyrene and *p*-cymen-8-ol in a carbonated citral-containing beverage system. *J. Agric. Food Chem.* **1985**, *33*, 330–335.
- (13) Decker, E.; McClements, D. J. Transition metal and hydroperoxide interactions: An important determinant in the oxidative stability of lipid dispersions. *Inform* **2001**, *12*, 251–255.
- (14) Djordjevic, D.; Cercaci, L.; Alamed, J.; McClements, D. J.; Decker, E. A. Stability of citral in protein- and gum arabic-stabilized oil-in-water emulsions. *Food Chem.* **2008**, *106*, 698–705.
- (15) Dickinson, E.; McClements, D. J.; Povey, M. J. W. Ultrasonic investigation of the particle-size dependence of crystallization in *n*-hexadecane-in-water emulsions. *J. Colloid Interface Sci.* **1991**, *142*, 103–110.
- (16) McClements, D. J.; Povey, M. J. W.; Dickinson, E. Absorption and velocity dispersion due to crystallization and melting of emulsion droplets. *Ultrasonics* **1993**, *31*, 433–437.
- (17) United Nation Environment Programme. Screening Information Data Set (SIDS) Initial Assessment Report for 13th SIDS Initial Assessment Meeting (SIAM), Bern, Switzerland, Nov 6–9, **2001** (<http://www.chem.unep.ch/irptc/sids/OECD/SIDS/5392-40-5.pdf>).
- (18) Mikhalev, O.; Karpov, I.; Kazarova, E.; Alfimov, M. The effect of crystallization of the dispersion phase in emulsions on the rate of interfacial reactions. *Chem. Phys. Lett.* **1989**, *164*, 96–100.
- (19) Richards, M. P.; Chaiyasit, W.; McClements, D. J.; Decker, E. A. Ability of surfactant micelles to alter the partitioning of phenolic antioxidants in oil-in-water emulsions. *J. Agric. Food Chem.* **2002**, *50*, 1254–1259.

Received for review July 30, 2009. Revised manuscript received October 13, 2009. Accepted November 2, 2009.