Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 106 (2008) 698–705
Food Chemistry
www.elsevier.com/locate/foodchem

Stability of citral in protein- and gum arabic-stabilized oil-in-water emulsions

Darinka Djordjevic [a], Luisito Cercaci [b], Jean Alamed [a], D. Julian McClements [a], Eric A. Decker [a,*]

[a] Department of Food Science, University of Massachusetts, Amherst, MA 01003, United States
[b] Dipartimento di Scienze degli Alimenti, Università di Bologna, Viale G. Fanin, 40, I-40127 Bologna, Italy

Received 5 March 2007; received in revised form 14 June 2007; accepted 18 June 2007

Abstract

Citral is a major flavor component of citrus oils that can undergo chemical degradation leading to loss of aroma and formation of off-flavors. Engineering the interface of emulsion droplets with emulsifiers that inhibit chemical reactions could provide a novel technique to stabilize citral. The objective of this study was to determine if citral was more stable in emulsions stabilized with whey protein isolate (WPI) than gum arabic (GA). Degradation of citral was equal to or less in GA- than WPI-stabilized emulsion at pH 3.0 and 7.0. However, formation of the citral oxidation product, *p*-cymene was greater in the GA- than WPI-stabilized emulsion at pH 3.0 and 7.0. Emulsions stabilized by WPI had a better creaming stability than those stabilized by GA because the protein emulsifier was able to produce smaller lipid droplets during homogenization. These data suggest that WPI was able to inhibit the oxidative deterioration of citral in oil-in-water emulsions. The ability of WPI to decrease oxidative reactions could be due to the formation of a cationic emulsion droplet interface at pH 3.0 which can repel prooxidative metals and/or the ability of amino acids in WPI to scavenge free radical and chelate prooxidative metals.

Keywords: Citral; Flavor; Emulsion; Protein; Oxidation

1. Introduction

Citrus oils are common flavor ingredients in foods. Citral (3,7-dimethyl-2,6-octadienal) is one of the most important flavor compounds in citrus oils. Citral consists of two geometrical isomers, neral and geranial, and is highly susceptible to acid-promoted and oxidative degradation (Clark, Powell, & Radford, 1977; Kimura, Nishimura, Iwata, & Mizutani, 1983a; Schieberle & Grosch, 1988a). It decomposes rapidly during storage by a series of cyclization and oxidation reactions (Tan, 1997; Ueno, Masuda, & Ho, 2004). Acid-catalyzed cyclization of citral reduces the intensity of the fresh lemon flavor due to decreased levels of citral and results in the formation of the variety of the undesirable off-flavors that limit the shelf-life of acidic citrus-flavored foods and beverages (Kimura, Nishimura, Iwata, & Mizutani, 1983b; Peacock & Kuneman, 1985; Schieberle, Ehrmeier, & Grosch, 1988; Schieberle & Grosch, 1988a). Proposed deterioration mechanisms of citral in acid solutions involve the isomerization of geranial to 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 to produce stable aromatic compounds such as α-*p*-dimethylstyrene, *p*-cymene, and *p*-cresol (Kimura et al., 1983b; Peacock & Kuneman, 1985). Among the most potent degradation products of citral are *p*-cresol and *p*-methylacetophenone (Schieberle et al., 1988; Schieberle & Grosch, 1988a). Reduction of storage temperature and increase in pH

* Corresponding author. Tel.: +1 413 545 1026; fax: +1 413 545 1262.
E-mail address: edecker@foodsci.umass.edu (E.A. Decker).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.06.033

significantly retarded the formation of some citral off-flavor products, such as α-*p*-dimethylstyrene and *p*-cymen-8-ol (Kimura et al., 1983b; Peacock & Kuneman, 1985). Studies of the stability of fresh and aged citrus oil-in-water emulsions at pH 2.0 have shown that geranial and neral dominated the overall flavor profile in fresh emulsions, whereas *p*-methylacetophenone and *p*-cresol were dominant off-flavors in aged emulsions (Schieberle & Grosch, 1988b).

Consumer demand for natural flavor ingredients and more complex and authentic aroma profiles have resulted in an increased demand for the incorporation of citrus oil and citral, into different food and beverage products. However, incorporation of chemically unstable citrus oil components into foods and beverages presents a challenge for the food industry because their chemical deterioration needs to be inhibited to minimize loss of product quality. Engineering the interfacial membranes of oil-in-water emulsions to alter chemical reaction rates could be an effective technique used to stabilize citral. Proteins are emulsifiers that can alter both emulsion droplet charge and interfacial membrane thickness in oil-in-water emulsions. The advantage of using proteins as emulsifiers is they are generally recognized as safe (GRAS) food additives that can form physically stable oil-in-water emulsions that vary in surface charge. When pH values are below the isoelectric point (pI) of the protein, the emulsion droplet interfacial proteins will have a positive charge that repels transition metals thus decreasing metal–lipid interactions and increasing oxidative stability (Hu, McClements, & Decker, 2004; McClements & Decker, 2000).

At present, emulsified flavor oils are usually stabilized by gum arabic, which is a naturally occurring polysaccharide–protein complex (McClements, 2004; Tan, 2004). Gum arabic adsorbs to the surface of the oil droplets created during homogenization, where it forms a relatively thick and negatively charged interfacial layer around droplets that stabilizes them against aggregation through steric and electrostatic interactions (Chanamai, 2002). Nevertheless, there have been many efforts to find alternative sources of natural emulsifiers to stabilize emulsified flavor oils because gum arabic has to be used at relatively high concentrations and because there are considerable fluctuations in its cost, availability and reliability (Chanamai, 2002; Tan, 2004).

Whey protein isolate (WPI) is commonly used as an emulsifier in the food industry to stabilize oil-in-water emulsions. The major component of WPI is β-lactoglobulin, which is a globular protein with a molecular weight of ~18.3 kDa and an isoelectric point (pI) of ~5.0 (Swaisgood, 1996). At acidic pH, the interfacial layer around oil droplets stabilized by WPI is relatively thin (~2 nm) (Dickinson & McClements, 1995) and positively charged ($\zeta \sim +29$ mV at 100 mM NaCl at pH 3) (Harnsilawat, Pongsawatmanit, & McClements, 2006). The positive charge on protein-coated droplets at acidic pH has previously been shown to improve the oxidative stability of emulsified polyunsaturated lipids, which has been attributed to its ability to repel cationic transition metals away from the droplet surfaces, thereby decreasing iron–lipid interactions (Hu et al., 2004; McClements & Decker, 2000). An additional benefit to WPI stabilized emulsion is that they are stable to thermal processing operations such as those used in pasteurization (Djordjevic, Kim, McClements, & Decker, 2004).

The objective of this study was to determine if protein-stabilized oil-in-water emulsions could inhibit citral degradation in a model emulsion system. Hexadecane was used as a non-oxidizible lipid carrier so that the degradation reactions of citral could be studied independently of the oxidation of other lipid molecules that exist in citrus oil. Whey protein isolate (WPI) was used in protein-stabilized oil-in-water emulsions. Citral stability in WPI-stabilized emulsions was compared to gum arabic (GA)-stabilized emulsion since gum arabic is commonly used to stabilize citrus oil emulsions in foods and beverages.

2. Materials and methods

2.1. Materials

Citral (mixture of *cis* and *trans* isomers, 95% pure), dodecane and tridecane were purchased from Acros Organics (Fair Lawn, NJ). Whey protein isolate (WPI, 97.7 wt% protein) and gum arabic (GA) were donated by Davisco Foods International (Le Sueur, MN) and Kraft Foods (TIC Gums, Seyal type), respectively. Both WPI and GA were stored at 4 °C and used without further purification. Sodium citrate, hydrochloric acid, sodium hydroxide and all other chemicals (reagent or HPLC grade) were obtained from Sigma Chemical Company (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA). Double-distilled water was used to prepare all solutions.

2.2. Methods

2.2.1. Preparation of oil-in-water emulsions

Oil-in-water emulsions were prepared by mixing 10% oil (2 wt% citral plus the internal standards, 1.4 wt% dodecane and 0.4 wt% tridecane dissolved in *n*-hexadecane) with an aqueous phase consisting of 10 mM sodium citrate buffer (adjusted to pH 3.0 or pH 7.0) and WPI or GA, at a final emulsifier-to-oil ratio of 1:10 for WPI (for example, 1.0 wt% protein for 10 wt% oil) and 1:2 for GA. WPI and GA were dissolved in the sodium citrate buffer by stirring at room temperature overnight to ensure complete dispersion. Prior to the homogenization step, the aqueous phase was stirred for 1 h at room temperature and its pH value was readjusted to pH 3.0 or 7.0 using 1.0 M and/or 12.0 M HCl or 1.0 M and/or 5.0 M NaOH. 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 tem-

perature. The coarse emulsion was then passed through a two-stage high-pressure valve homogenizer (APV-Gaulin, Wilmington, MA) at 3000 psi for four passes. All emulsions were stored in dark in 4 ml amber vials and in 10 ml capped test tubes (for creaming index) at 4, 20, 37 and/or 55 °C.

2.2.2. Physical stability of protein-stabilized oil-in-water emulsions

Emulsion droplet size distribution as a function of storage time was measured using a laser light scattering instrument (MalvernSizer; Malvern Instruments Ltd., Worcestershire, UK) (McClements, 2001). The laser light scattering instrument measures the intensity of laser light scattered from a dilute emulsion, and then reports the particle size distribution that gives the closest fit between theoretical calculations (Mie theory) and experimental measurements of intensity versus 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 the continuous phase. The emulsions were stirred continuously in the sample chamber (at an instrument stirring speed approximately 50%) to ensure that they were homogeneous. Particle size measurements were reported as weight-average mean diameters, 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 Creaming Index (CI) = 100 × (height of interface)/(height of total emulsion) (Demetriades & McClements, 2000). The electrical charge on the droplets was established 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., Worcestershire, UK). The ζ-potential was determined by measuring the direction and velocity that the droplets moved in the applied electric field.

2.2.3. Measurement of citral degradation in oil-in-water emulsions

Citral degradation was monitored by measuring the decrease of citral isomers neral and geranial and the formation of the citral breakdown product, *p*-cymene. Analysis of citral and its degradation products were conducted on a gas chromatograph (model GC-17A; Shimatzu; Avondale, PA) equipped with a capillary column (DB-5, J&W Scientific, Folson, California; 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 (FID). Oven temperature was programmed as follows: 80 °C (3 °C/min) → 90 °C (2 °C/min) → 110 °C (25 °C/min) → 200 °C. Injector and detector temperatures were 220 °C and 240 °C, respectively. Helium (12 ml/min) was used as the carrier gas. Emulsions (0.1 ml) were dissolved in 2.9 ml 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 gum arabic. 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.

2.3. Statistical analysis

All experiments were conducted twice in duplicate and reported as means ± standard deviations. Statistical analyses were performed using *t*-test and one-way analysis of variance (ANOVA, $p \leqslant 0.05$) (Sigma Stat, 1992–1997).

3. Results and discussion

3.1. Influence of emulsifier and pH on chemical stability of emulsified citral

The stability of citral in citral/hexadecane-in-water emulsions stabilized with whey protein isolate (WPI; Fig. 1a) and gum arabic (GA; Fig. 1b) at pH 3.0 was determined by measuring the loss of citral's isomers, neral and geranial during storage at 4–55 °C. The composition of neral and geranial in the citral used in this study was 50.3% and 49.7%, respectively. Since the concentrations of the two isomers differed, stability was expressed as relative to day 0 concentrations. In the WPI-stabilized emulsions, no major differences ($p > 0.05$) in neral and geranial degradation rates were seen at all incubation temperatures. As expected, neral and geranial degradation rates increased with increasing incubation temperature as was previously reported (Ikenberry & Saleeb, 1993a; Ikenberry & Saleeb, 1993b). For example, after 6 days of incubation, neral and geranial concentrations, respectively, were 16.8% and 17.1% (4 °C); 23.2% and 26.2% (20 °C); 73.7% and 74.3% (37 °C); and 96.2% and 96.6% (55 °C) less than day 0 neral and geranial concentrations at pH 3.0.

Degradation of citral isomers in emulsions stabilized with gum arabic (GA) also increased with increasing incubation temperatures at pH 3.0 with citral degradation being slower than in the WPI-stabilized emulsions (Fig. 1a and b). At 4 °C, no significant differences ($p \geqslant 0.05$) in neral and geranial concentrations compared to day 0 were observed during the entire incubation period in the GA-stabilized emulsions. Unlike the WPI-stabilized emulsions, neral degradation was faster than geranial ($p \leqslant 0.05$) in the GA-stabilized emulsions. For example, after 6 days of storage, neral and geranial concentrations were 10.4% and 3.7% (20 °C); 35.5% and 27.8% (37 °C); and 49.7% and 36.5% (55 °C), respectively, less than day 0 concentrations at pH 3.0. After 28 days of storage neral was still significantly ($p \leqslant 0.05$) lower than geranial at incubation temperatures from 20 °C to 55 °C [*e.g.* neral and geranial concentrations, respectively, were 24.8% and

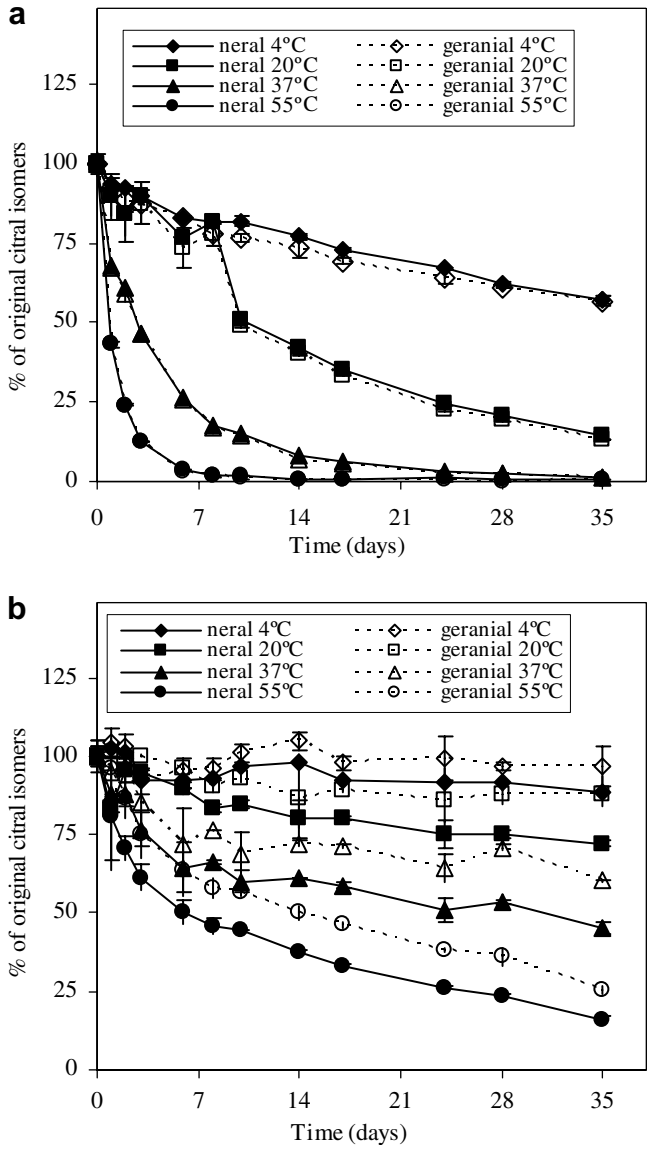

Fig. 1. Degradation of citral isomers neral and geranial in 2 wt% citral/hexadecane oil-in-water emulsions stabilized with (a) whey protein isolate (WPI) or (b) gum arabic (GA) during storage at 4, 20, 37 and 55 °C at pH 3.0. Data markers represent average ± standard deviations.

12.2% (20 °C); 46.4% and 29.1% (37 °C); and 76.5% and 63.9% (55 °C), lower than day 0 values at pH 3.0, Fig. 1b].

Citral stability was greater ($p \leqslant 0.02$) in GA vs. WPI-stabilized citral/hexadecane-in-water emulsions stored at incubation temperatures of $\geqslant 20$ °C at pH 3.0 (Fig. 1). For example, in emulsions stored for 6 days at 20 °C, neral and geranial concentrations 23.2% and 26.2% lower, respectively, than day 0 values in the WPI-stabilized emulsions compared to 10.4% and 3.7% lower, respectively, than day 0 values in the GA-stabilized emulsions at pH 3.0. Similarly, in emulsions stored for 6 days at 55 °C, neral and geranial concentrations 96.2% and 96.6% lower, respectively, than day 0 values in the WPI-stabilized emulsions compared to 49.7% and 36.5% lower, respectively, than day 0 values in the GA-stabilized emulsions at pH 3.0.

The stability of citral in citral/hexadecane-in-water emulsions stabilized with whey protein isolate WPI (Fig. 2a) and gum arabic GA (Fig. 2b) at pH 7.0 was also determined during storage from 4 °C to 55 °C. In both the WPI and GA-stabilized emulsions, no major differences ($p \geqslant 0.05$) in neral and geranial degradation rates were seen at all incubation temperatures at pH 7.0. Neral and geranial degradation rates again increased with increasing incubation temperature in both emulsion systems. Citral stability was similar in GA than WPI-stabilized citral/hexadecane-in-water emulsions stored at all incubation temperatures at pH 7.0. For example, in emulsions stored for 6 days at 20 °C, neral and geranial concentrations 18.9% and 17.0% lower, respectively, than day 0 values in the

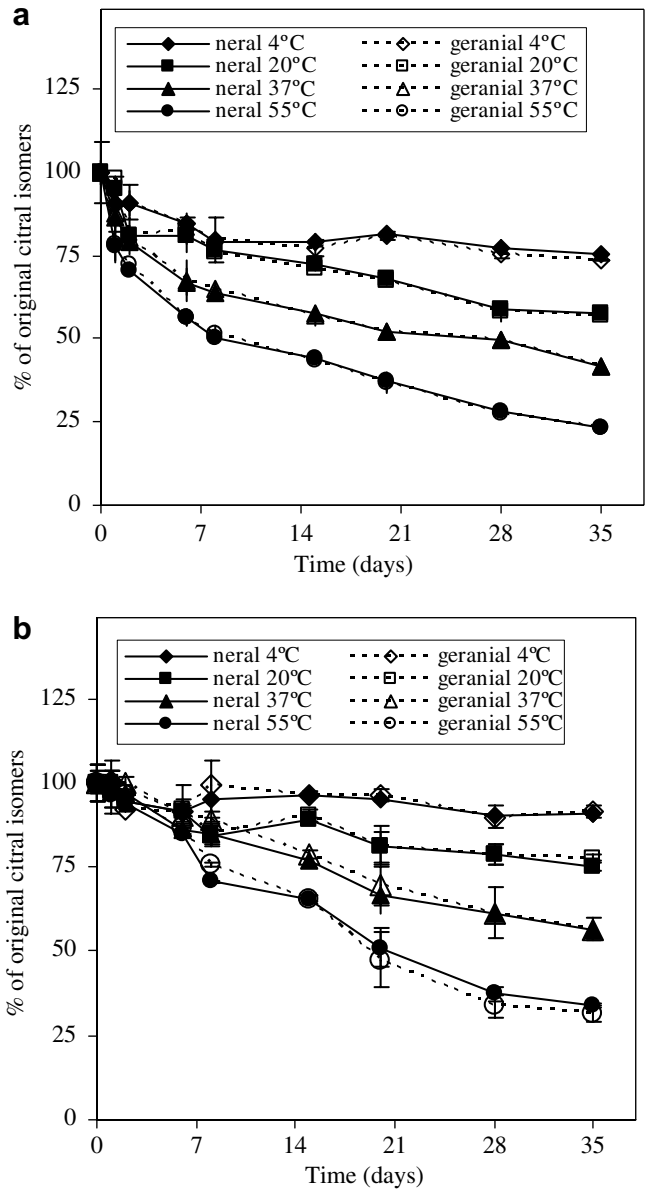

Fig. 2. Degradation of citral isomers neral and geranial in 2 wt% citral/hexadecane oil-in-water emulsions stabilized with (a) whey protein isolate (WPI) or (b) gum arabic (GA) during storage at 4, 20, 37 and 55 °C at pH 7.0. Data markers represent average ± standard deviations.

WPI-stabilized emulsions at pH 7.0 compared to 8.7% and 8.1% lower, respectively, than day 0 values in the GA-stabilized emulsions at pH 7.0. Similarly, in emulsions stored for 6 days at 55 °C, neral and geranial concentrations were 43.5% and 43.6% lower, respectively, than day 0 values in the WPI-stabilized emulsions at pH 7.0 compared to 15.4% and 13.6% lower, respectively, than day 0 values in the GA-stabilized emulsions at pH 7.0.

Citral was much more stable ($p \leqslant 0.003$) in the WPI-stabilized emulsions at pH 7.0 than pH 3.0 (Figs. 1a and 2a). For example, in WPI-stabilized emulsions stored for 6 days at 55 °C, neral and geranial concentrations were 43.5% and 43.6% lower than day 0 at pH 7.0 compared to 96.2% and 96.6% at pH 3.0. Citral degradation was also faster ($p \leqslant 0.01$) at pH 3.0 than 7.0 in the GA-stabilized emulsions during incubation at 55 °C (Figs. 1b and 2b). After 6 days of storage, neral and geranial concentrations decreased 49.7% and 36.5%, respectively, at pH 3.0 compared to 15.4% and 13.6%, respectively, at pH 7.0.

After acid-catalyzed cyclization of citral, oxidation reactions can occur to produce a number of compounds that produce off-flavors. It has been reported that of these oxidation products, *p*-cymene is produced in greater concentrations than α,4-dimethylstyrene during the degradation of citral at pH 3.0 stored at 40 °C (Ueno et al., 2004). Therefore the impact of emulsifier type on citral degradation and oxidation was also followed by monitoring the formation of *p*-cymene. In WPI- and GA-stabilized citral/hexadecane-in-water emulsions at pH 3.0, *p*-cymene formation was not observed at temperatures ⩽37 °C (data not shown). At 55 °C, *p*-cymene was detected in both emulsions after 1 day of storage with higher levels of *p*-cymene being detected in the GA- than WPI- stabilized emulsions after 10 days of storage (Fig. 3a). After 35 days of storage, *p*-cymene concentrations in the GA-stabilized emulsions were 2.1 times greater than in the WPI-stabilized emulsions. Since the formation of *p*-cymene is due to oxidation, its lower concentrations in the WPI-stabilized emulsion could be due to the ability of the cationic protein interface to inhibit oxidative reactions by electrostatic repulsion of transition metals (Hu, McClements, & Decker, 2003).

In the GA-stabilized emulsions at pH 7.0 stored at 55 °C, *p*-cymene was detected after 1 day of storage but was not detected in the WPI-stabilized emulsions until after 15 days of storage with no further increases in *p*-cymene concentrations being observed in either emulsion with prolonged storage (Fig. 3b). At pH 7.0, *p*-cymene formation was much lower than at pH 3.0 for both WPI- and GA-stabilized emulsions. For example, after 35 days of storage at 55 °C, *p*-cymene concentrations were 93% and 99% lower, respectively, at pH 7.0 than 3.0. These lower *p*-cymene concentrations in the GA- and WPI-stabilized emulsions were likely due to the lower citral degradation rates at pH 7.0. It is thought that this decrease in the citral oxidation rate with increased pH is partly the result of the reduced formation and consequently slower oxidation of citral cyclization products menthadienols (*e.g.* *p*-mentha-1(7), 2-dein-8-ol and *p*-mentha-1,5-dien-8-ol) (Peacock & Kuneman, 1985). It is unclear why *p*-cymene formation was slower in the WPI- than GA-stabilized emulsions since at pH 7.0 the WPI emulsions are anionic which can promote oxidative reactions (Hu et al., 2003). It is possible that the antioxidant properties of some of the amino acids in the WPI (Elias, McClements, & Decker, 2005) could be inhibiting the formation of *p*-cymene.

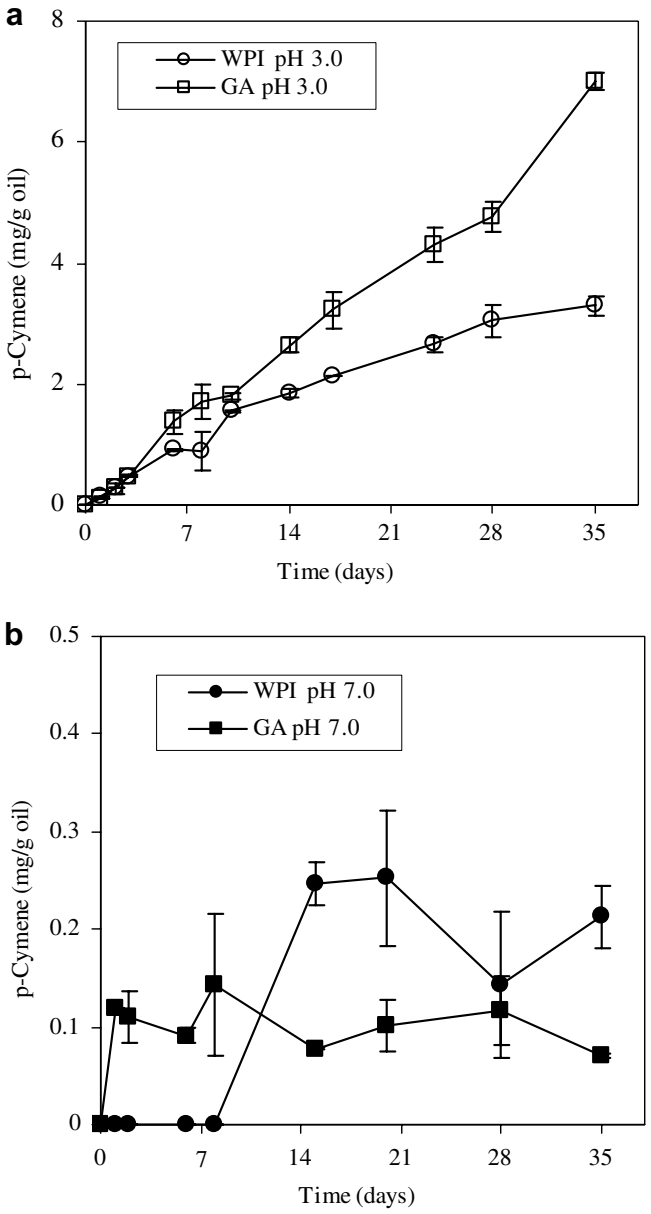

Fig. 3. Detection of *p*-cymene in 2 wt% citral/hexadecane oil-in-water emulsions stabilized with whey protein isolate (WPI) or gum arabic (GA) stored at 55 °C at (a) pH 3.0, (b) pH 7.0. Data markers represent average ± standard deviations.

3.2. Influence of emulsifier and pH on physical stability of emulsified citral

The physical stability of WPI- and GA-stabilized citral/hexadecane oil-in-water emulsions at pH 3 and 7 was

monitored at 37 °C. The initial mean droplet diameters (d_{43}) of the WPI-stabilized emulsions were 0.53 μm at pH 3 and 0.28 μm at pH 7 (Fig. 4), with both emulsions having a monomodal size distribution (Fig. 5). This result suggested that the whey protein was more effective at producing small droplets during homogenization at pH 7 than at pH 3. At pH 3, the measured mean particle diameter of the WPI-stabilized emulsions did not increase dramatically during storage, with d_{43} ranging from 0.53 to 0.81 μm during the first 35 days. On the other hand, at pH 7, the mean particle diameter increased considerably after 1 day of storage, going from 0.28 to 6.3 μm, with no further major changes occurring during the remainder of the incubation time. Similar increases in mean particle size have been reported at neutral pH in hydrocarbon oil-in-water emulsions stabilized by β-lactoglobulin, which is the major globular protein in WPI (Kim, Decker, & McClements, 2002; Kim, Decker, & McClements, 2004). This affect was attributed to droplet flocculation caused by an increase in the surface hydrophobicity of the lipid droplets when the adsorbed proteins underwent surface denaturation (Kim et al., 2002, 2004). The observed increase in mean particle diameter could be attributed to the formation of a small population of very large particles ($d > 10$ μm), as seen in the particle size distribution data (Fig. 5). These large particles are probably the result of the droplet flocculation mentioned above. The creaming stability measurements indicated that the emulsions were more stable to visible creaming at pH 7 than at pH 3, which can be attributed to the fact that the majority of the particles in the pH 7 emulsion had smaller diameters than those in the pH 3 emulsion, and therefore creamed more slowly (Fig. 6).

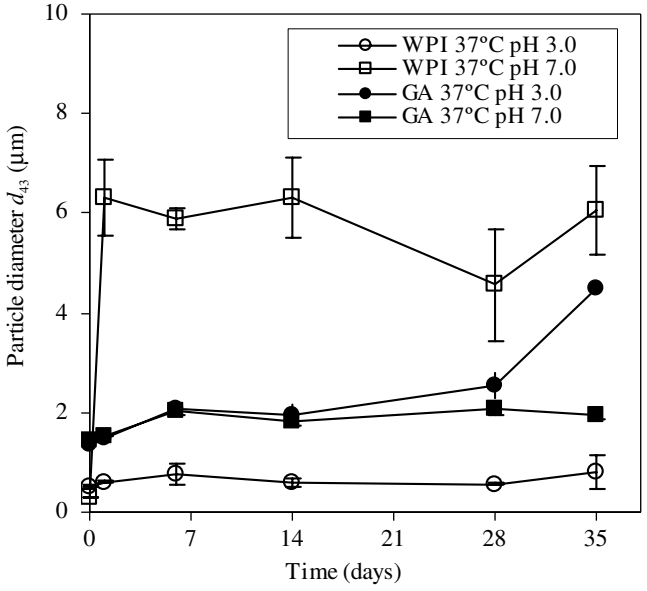

Fig. 4. Mean particle diameter of 2 wt% citral/hexadecane oil-in-water emulsions stabilized with whey protein isolate (WPI) or gum arabic (GA) during storage at 37 °C at pH 3.0 and pH 7.0. Data markers represent average ± standard deviations.

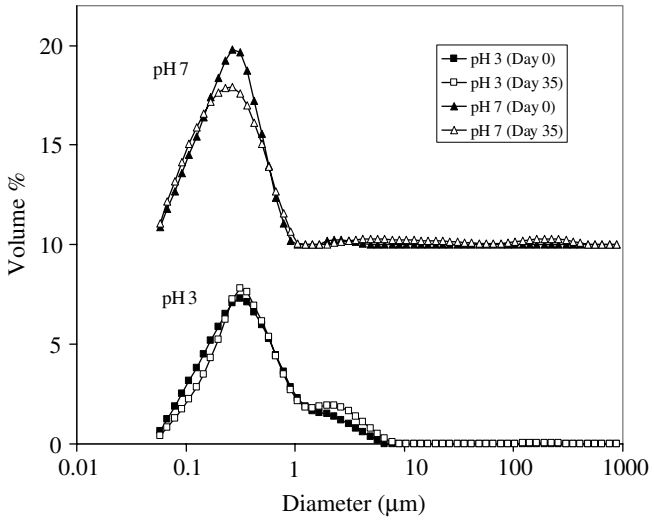

Fig. 5. Emulsion droplet size distribution of 2 wt% citral/hexadecane oil-in-water emulsions stabilized with whey protein isolate (WPI) during storage at 37 °C at pH 3.0 and pH 7.0. Data for the pH 7.0 emulsions were increased by 15% to more easily view the droplet size distributions. Data markers represent average ± standard deviations.

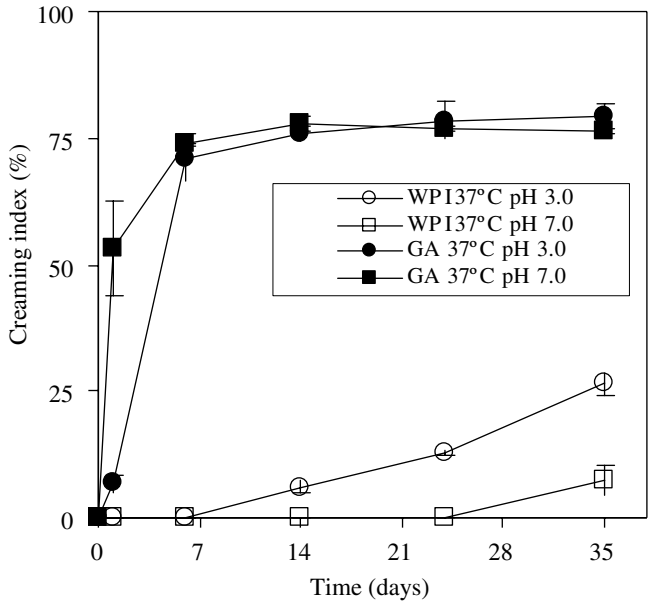

Fig. 6. Creaming index CI for 2 wt% citral/hexadecane oil-in-water emulsions stabilized with whey protein isolate (WPI) or gum arabic (GA) during storage at 37 °C at pH 3.0 and pH 7.0. Data markers represent average ± standard deviations.

The initial mean particle sizes in the GA-stabilized emulsions were higher than those in the WPI-stabilized emulsions, with $d_{43} = 1.4$ μm at pH 3 and 1.5 μm at pH 7 (Fig. 4), and the distributions were initially monomodal at both pH (Fig. 7). In addition, the creaming stability of the GA-stabilized emulsions was considerably worse than that of the WPI-stabilized emulsions (Fig. 6). These results indicate that whey protein was more effective at producing small droplets during homogenization than GA, possibly

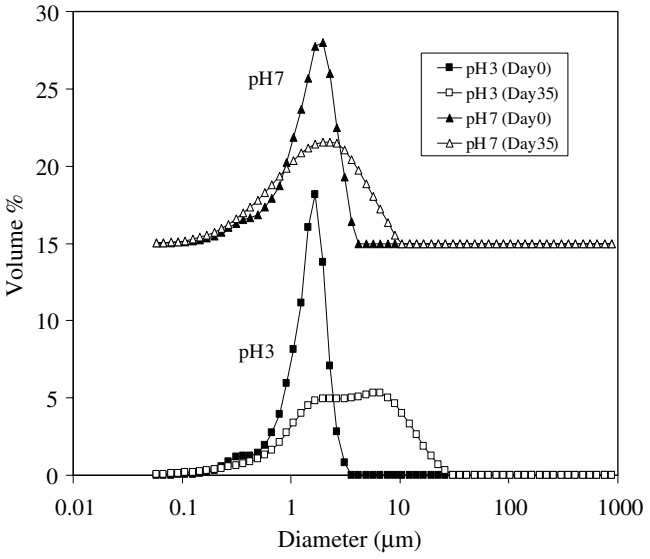

Fig. 7. Mean particle diameter of 2 wt% citral/hexadecane oil-in-water emulsions stabilized with gum arabic (GA) during storage at 37 °C at pH 3.0 and pH 7.0. Data markers represent average ± standard deviations.

because the small globular protein molecules adsorbed more rapidly to the lipid droplet formed within the homogenizer than the larger polysaccharide molecules (McClements, 2004). There was a gradual increase in the mean particle diameter in the GA-stabilized emulsions during storage (Fig. 4), which could be attributed to a broadening in the particle size distribution (Fig. 7). This effect may have been due to individual droplet growth (Ostwald ripening) or droplet aggregation (flocculation or coalescence).

4. Conclusions

In conclusion, use of WPI as an emulsifier was unable to decrease the rate of acid catalyzed degradation of citral. However, WPI was more effective than GA at decreasing the formation of the citral oxidation product, *p*-cymene. These data suggest that WPI was able to inhibit the oxidative deterioration of citral in oil-in-water emulsions. The ability of WPI to decrease the oxidative deterioration of citral could be due to the formation of a cationic emulsion droplet interface at pH 3.0 which can repel prooxidative metals in addition to the ability of WPI to act as a free radical scavenger and metal chelator. Emulsions stabilized by WPI had a better creaming stability than those stabilized by GA because the protein emulsifier was able to produce smaller lipid droplets during homogenization. Nevertheless, there was some evidence of droplet flocculation in the emulsions stabilized by WPI during storage, particularly at pH 7.

References

Chanamai, R. A. D. J. M. (2002). Comparison of gum arabic, modified starch, and whey protein isolate as emulsifiers: influence of pH, CaCl2 and temperature. *Journal of Food Science, 67*, 120–125.

Clark, B., Powell, C., & Radford, T. (1977). Acid-catalyzed cyclization of citral. *Tetrahedron, 33*(17), 2187–2191.

Demetriades, K., & McClements, D. J. (2000). Influence of sodium dodecyl sulfate on the physicochemical properties of whey protein-stabilized emulsions. *Colloids and Surfaces A – Physicochemical and Engineering Aspects, 161*(3), 391–400.

Dickinson, E., & McClements, D. J. (1995). *Advances in food colloids*. London: Chapman & Hall.

Djordjevic, D., Kim, H.-J., McClements, D. J., & Decker, E. A. (2004). Physical stability of whey protein-stabilized oil-in-water emulsions at pH 3: Potential ω-3 fatty acid delivery systems (Part A). *Journal of Food Science, 69*, C351–C355.

Elias, R. J., McClements, D. J., & Decker, E. A. (2005). Antioxidant activity of cysteine, tryptophan, and methionine residues in continuous phase beta-lactoglobulin in oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 53*(26), 10248–10253.

Harnsilawat, T., Pongsawatmanit, R., & McClements, D. J. (2006). Influence of pH and ionic strength on formation and stability of emulsions containing oil droplets coated by β-lactoglobulin – alginate interfaces. *Biomacromolecules, 7*(6), 2052–2058.

Hu, M., McClements, D. J., & Decker, A. E. (2004). Emulsion droplets engineered to improve the oxidative stability of *n*-3 fatty acids in functional foods. *Lipid Technology, 16*(4), 79–82.

Hu, M., McClements, D. J., & Decker, E. A. (2003). Impact of whey protein emulsifiers on the oxidative stability of salmon oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 51*(5), 1435–1439.

Ikenberry, D. A., & Saleeb, R. K. (1993a). The effect of temperature and acidity on the stability of specific lemon flavor components. I Data and methodology. In G. Charalambous (Ed.) *Food flavors, ingredients and composition* (pp. 339–354). Amsterdam, The Netherlands: Elsevier Science Publishers.

Ikenberry, D. A., & Saleeb, R. K. (1993b). The effect of temperature and acidity on the stability of specific lemon flavor components, II Kinetics. In G. Charalambous (Ed.) *Food flavors, ingredients and composition* (pp. 355–370). Amsterdam, The Netherlands: Elsevier Science Publishers.

Kim, H. J., Decker, E. A., & McClements, D. J. (2002). Impact of protein surface denaturation on droplet flocculation in hexadecane oil-in-water emulsions stabilized by beta-lactoglobulin. *Journal of Agricultural and Food Chemistry, 50*(24), 7131–7137.

Kim, H. J., Decker, E. A., & McClements, D. J. (2004). Comparison of droplet flocculation in hexadecane oil-in-water emulsions stabilized by beta-lactoglobulin at pH 3 and 7. *Langmuir, 20*, 5753–5758.

Kimura, K., Nishimura, H., Iwata, I., & Mizutani, J. (1983a). Studies on the deterioration mechanism of lemon flavor. 3. Identification of acidic substances from deteriorated citral and effects of anti-oxidant on their formation. *Agricultural and Biological Chemistry, 47*(7), 1661–1663.

Kimura, K., Nishimura, H., Iwata, I., & Mizutani, J. (1983b). Deterioration mechanism of lemon flavor. 2. Formation mechanism of off-odor substances arising from citral. *Journal of Agricultural and Food Chemistry, 31*(4), 801–804.

McClements, D. J. (2001). Emulsion droplet size determination. In R. E. Wrolstad, T. E. Acree, H. An, E. A. Decker, M. H. Penner, & D. S. Reid, et al. (Eds.) *Current protocols in food analytical chemistry* (Vol. 1). New York, NY: John Wiley & Sons Inc.

McClements, D. J. (2004). *Food emulsions: Principles, practices, and techniques* (2nd ed.). Boca Raton, FL: CRC Press.

McClements, D. J., & Decker, E. A. (2000). Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. *Journal of Food Science, 65*(8), 1270–1282.

Peacock, V. E., & Kuneman, D. W. (1985). Inhibition of the formation of a-*p*-dimethylstyrene and *p*-cymen-8-ol in a carbonated citral-containing beverage system. *Journal of Agricultural and Food Chemistry, 33*, 330–335.

Schieberle, P., Ehrmeier, H., & Grosch, W. (1988). Aroma compounds resulting from the acid catalyzed breakdown from citral. *Zeitschrift fur Lebensmittel-Untersuchung und-Forschung, 187*, 35–39.

Schieberle, P., & Grosch, W. (1988a). Identification of potent flavor compounds formed in an aqueous lemon oil/citric acid emulsion. *Journal of Agricultural and Food Chemistry, 36*, 797–800.

Schieberle, P., & Grosch, W. (1988b). Quantitative analysis of important volatile flavor compounds in fresh and stored lemon oil/citric acid emulsions. *Lebensmittel-Wissenschaft & Technologie, 21*(3), 158–162.

Sigma Stat. (1992–1997). *Statistical software for Windows copyright.* Chicago IL: SPSS Inc.

Swaisgood, H. E. (1996). Characteristics of milk. In O. R. Fennema (Ed.) *Food chemistry* (Vol. 14). New York, NY: Marcel Dekker.

Tan, C.-T. (1997). Beverage emulsions. In S. E. Friberg & K. Larsson (Eds.), *Food emulsions* (3rd ed., pp. 491–524). New York, NY: Marcel Dekker Inc..

Tan, C. T. (2004). Beverage emulsions. In S. L. Friberg & K. Sjoblom (Eds.) (4th ed.) *Journal of food emulsions* (Vol. 12). New York: Marcel Dekker.

Ueno, T., Masuda, H., & Ho, C.-T. (2004). Formation mechanism of *p*-methylacetophenone from citral via a *tert*-alkoxy radical intermediate. *Journal of Agricultural and Food Chemistry, 52*(18), 5677–5684.