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Stability of Citral in Oil-in-Water Emulsions Prepared with Medium-Chain Triacylglycerols and Triacetin

Seung Jun Choi,[†] Eric Andrew Decker,[†] Lulu Henson,[§] L. Michael Popplewell,[§] and David Julian McClements*,[†]

[†]Department of Food Science, University of Massachusetts, Amherst, Massachusetts 01003, and [§]International Flavors and Fragrances, Union Beach, New Jersey 07735

Citral is widely used in the beverage, food, and fragrance industries for its characteristic flavor profile. However, it chemically degrades over time in aqueous solutions due to an acid-catalyzed reaction, which leads to loss of desirable flavor notes and formation of off-flavor notes. The objective of this research was to examine the impact of organic phase composition [triacetin and medium-chain triacylglycerols (MCT)] on the oil−water partitioning and chemical degradation of citral in oil-in-water emulsions. MCT was present as emulsion droplets ($d \approx 900$ nm), whereas triacetin was present as microemulsion droplets ($d \approx 10$ nm). In the absence of organic phase, the rate of citral degradation increased as the aqueous phase pH was reduced from 7 to 3. The percentage of citral within the aqueous phase increased with increasing triacetin concentration at both pH 3 and 7, which was attributed to a reduction in MCT droplet concentration. There was no significant change in the particle size distribution of the emulsions during storage, independent of triacetin concentration and pH, which indicated that they were physically stable. Both 5 wt % MCT as emulsion droplets and 5 wt % triacetin as microemulsion droplets were able to appreciably slow citral degradation at pH 3. These results may have important implications for understanding and improving the chemical stability of citral in beverage emulsions.

KEYWORDS: Citral; triacetin; chemical degradation; flavor; emulsion; microemulsion

INTRODUCTION

Citral (3,7-dimethyl-2,6-octadienal) is one of the most important flavor compounds in citrus oil and is widely used in foods and beverages. Citral is a monoterpene aldehyde that is composed of a mixture of two geometric cis- and trans-isomers, geranial and neral, in a 3:2 ratio (*1*, *2*). Citral decomposes rapidly during storage at acidic pH by a series of cyclization and oxidation reactions (*3*, *4*). Acid-catalyzed cyclization of citral decreases the level of fresh-like aroma of citral and also generates off-odor compounds that limit citral's application in foods and beverages (*1*, *5*−*7*). Under acidic conditions, the formation of off-odor compounds from citral degradation is also affected by temperature and oxygen availability (*5*, *6*, *8*). The mechanism of citral deterioration in acidic solutions has been studied previously (*1*, *5*, *6*). Kimura et al. (*5*) and Peacock and Kuneman (*6*) 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 is converted into more stable aromatic compounds such as α-*p*-dimethylstyrene, *p*-cymeme, and *p*-cresol (*5*, *6*). According to Schieberle and Grosch (*1*), oxidation of α-*p*-dimethylstyrene can lead to the formation of *p*-methylacetophenone. *p*-Cresol and *p*-methylacetophenone are two of the most potent degradation products of citral (*1*, *7*).

The rate of chemical degradation of a labile component can be altered appreciably when it is incorporated into a multiphase heterogeneous material such as an emulsion because of partitioning of the labile component and other ingredients between different physicochemical environments: aqueous phase, oil phase, and interfacial region (*9*). If a labile component can be located within an environment where it is isolated from other components that promote its chemical degradation, then it may be possible to retard the degradation rate. Because citral degradation occurs predominantly in acidic aqueous solutions, it may be possible to alter its degradation rate by altering its partitioning between the oil and aqueous phases.

Triacetin (glycerol triacetate) is an organic component that is used as a flavor solvent in the food industry and as an antifungal agent in the perfumery and pharmaceutical industries. We hypothesized that the incorporation of triacetin into oil-in-water emulsions would alter the partitioning of citral between the oil and water phases and, thereby, alter its stability to chemical degradation. This hypothesis was tested by measuring the impact of triacetin on the physical and chemical stability of citral in model Brij-stabilized oil-in-water emulsions containing medium-chain triacylglycerols.

MATERIALS AND METHODS

Materials. Citral (mixture of cis- and trans-isomers, 95% pure, of plant origin) and Brij 35 were purchased from Acros Organics (Fair Lawn, NJ).

*Author to whom correspondence should be addressed [telephone (413) 545-1019; fax (413) 545-1262; e-mail mcclements@foosci.umass.edu].

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Brij 35 [polyoxyethylene (23) lauryl ether] is a nonionic surfactant containing polyethyleneoxide chains as the hydrophilic part and *n*-alkyl chains as the hydrophobic part. Medium-chain triacylglycerols (MCT, NEOBEE 1053) were obtained from Stepan Co. (Northfield, IL). Triacetin was obtained from International Flavors and Fragrances (Union Beach, NJ). All other chemicals were of reagent grade or purer and were obtained from Fisher Scientific (Pittsburgh, PA).

Preparation of Brij-Stabilized Emulsions. Oil-in-water emulsions were prepared by mixing 5 wt % lipid phase (triacetin/MCT) with 95 wt % aqueous phase (10 mM sodium citrate buffer, 1 wt % Brij 35, pH 3 or 7). A range of different triacetin-to-MCT mass ratios were used to prepare the lipid phases. A coarse emulsion premix was prepared by homogenizing the lipid and aqueous phases together using a high-speed blender (Tissu-Tearor, Biospec Products, Bartlesville, OK) for 2 min at room temperature. For reduction of droplet size, the premixed emulsions were sonicated (Sonic Dismembrator 500, Fisher Scientific) for 2 min at an amplitude of 70% and a duty cycle of 0.5 s at 4 °C. Citral (0.05 wt %) was then added to the emulsions, after which they were stirred for 1 h at room temperature.

Characterization of Physical Stability of Brij-Stabilized Oil-in-Water Emulsions. The particle size distribution of the emulsions as a function of storage time was measured using a laser light scattering instrument (Mastersizer, Malvern Instruments, Worcestershire, U.K.). To avoid multiple scattering effects, emulsions were diluted to a droplet concentration of approximately ≈0.005 wt % using buffer solution at the pH of the sample and stirred continuously throughout the measurements to ensure the samples were homogeneous. Particle size was reported as 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), and volume-weighted mean diameter, $d_{43} = \sum n_i d_i^4 / \sum n_i d_i^3$. For some samples, the particle size of the emulsions was also measured at 25 °C using a dynamic light scattering instrument (Nano-ZS, Malvern Instruments). The particle size from this instrument was reported as the scattering intensity-weighted mean diameter, *z*-average.

Measurement of Citral in Oil-in-Water Emulsions. Citral degradation was monitored by measuring the decrease of citral isomers neral and geranial using a gas chromatograph (GC-17A, Shimadzu, 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 5:1 and a flame ionizing detector. The oven temperature program was started from 100 °C by an increase of 3 °C/min to 145 °C and then an increase of 20 °C/min to a final temperature of 300 °C held for 10 min; the injector was 250 °C; the flame ionization detector was 250 °C. Helium (9 mL/min) was used as the carrier gas.

To determine the concentration of citral in the continuous phase, emulsions containing citral were centrifuged at 15000*g* at 4 °C for 1 h. After centrifugation, a 3 mL disposable syringe with a 21 gauge needle was pressed against the wall of the glass tube and gently pushed to the bottom of the tube, where approximately 2 mL of the continuous phase was removed. The continuous phase removed was filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA); 0.1 mL of the filtered material was dissolved in 0.9 mL of reagent alcohol and vortexed for 15 s prior to injection into the GC. An aliquot of sample (1.0 μL) was injected into the GC, and the two isomers of citral were identified by comparison to retention times with authentic standards. To determine the concentration of citral in the whole emulsions, the emulsions (0.1 mL) were dissolved directly in 0.9 mL of reagent alcohol and vortexed for 15 s before injection into the GC.

Citral's two isomers (neral and geranial) were identified by comparison of retention times with authentic standards. Concentrations for the isomers were determined relative to the internal standard (dodecane), and citral concentration was expressed as the sum of the isomers. The recovery for citral's isomers was determined by comparing the sum of peak areas for citral's isomers with a peak area of internal standard (dodecane). We got almost 100% recovery.

RESULTS AND DISCUSSION

Impact of Solution pH on Citral Stability. Initially, the influence of solution pH on the chemical degradation of citral was examined, so as to establish conditions under which oil/water partitioning experiments could be carried out without having changes in citral concentration due to chemical degradation. The saturation concentration of citral in an aqueous citrate buffer solution was determined to be 560 mg/L (≈0.056 wt %) at 20 °C, which is in good agreement with the value of 590 mg/L in water at 25 °C reported in the literature (*10*). An initial citral concentration of 0.05 wt % was therefore used to study the impact of pH on the chemical degradation of dissolved citral in aqueous solutions, because this concentration was below the saturation level. The change in citral concentration in aqueous solutions during storage at 20 °C is shown in **Figure 1**. The rate of citral degradation was modeled by assuming a first-order reaction: $C(t) = C_0 \exp(-kt)$, where C_0 is the initial citral concentration, C is the citral concentration remaining at time t, and k is the degradation rate constant. The values of k and r^2 (the correlation coefficient) were determined by carrying out a linear regression on plots of $\log(C(t)/C_0)$ versus t (**Table 1**). There was little change in citral concentration over 7 days of storage at pH 7, indicating that it was stable to chemical degradation at this pH. The rate of citral degradation increased with decreasing pH (**Figure 1**; **Table 1**). After 1 week of storage, the percentages of the initial citral remaining in the system were about 100, 88, 78, 62, and 25% for pH 7, 6, 5, 4, and 3, respectively.

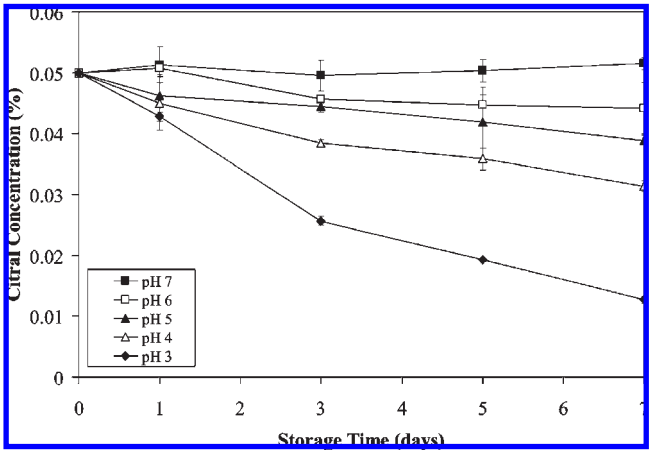

Figure 1. Degradation of citral (initial concentration of 0.05 wt %) in 20 mM sodium citrate buffers having various pH values.

Table 1. Parameters Determined by Modeling the Chemical Degradation of Citral as a Function of pH (See **Figure** 1) Assuming a First-Order Reaction

pH	k (min^{-1})	r^2
3	−0.085	−0.997
4	−0.028	−0.988
5	−0.014	−0.984
6	−0.009	−0.922
7	0.001	0.362

Physical Location of Citral in Oil-in-Water Emulsions. The purpose of these experiments was to measure the partitioning of citral in emulsions containing different ratios of MCT and triacetin at pH 3 and 7. As mentioned earlier, pH 7 was used because citral is relatively stable to chemical degradation so that measured changes in citral concentration could be attributed to physical partitioning, rather than chemical degradation. The citral concentrations in the aqueous phase and in the entire emulsion (aqueous phase plus oil phase) were measured after the emulsions had been prepared (**Figure 2**). At both pH values, the percentage of citral in the aqueous phase increased with increasing triacetin concentration in a similar manner. These results suggest that triacetin favored the partitioning of citral into the aqueous phase, and so additional experiments were performed to establish the origin of this effect.

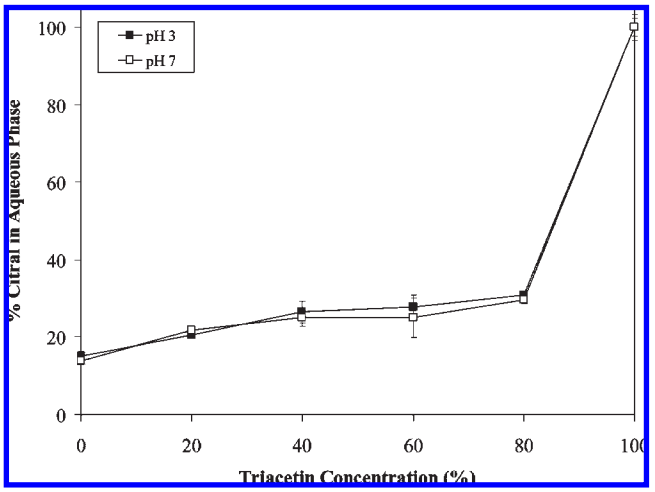

Figure 2. Impact of pH and organic phase triacetin concentration (5 wt % total organic phase: triacetin + MCT) on the percentage of citral present in the aqueous phase.

Physical Location of Triacetin in Oil-in-Water Emulsions. The overall appearance of mixed systems containing a total concentration of 5 wt % organic phase (triacetin + MCT) changed appreciably with triacetin concentration. For ≤0−80 wt % triacetin in the organic phase, the systems were optically opaque milky-white dispersions, but for 100 wt % triacetin in the organic phase the systems were transparent and colorless, which suggested that they contained particles that were too small to scatter light strongly. The particle size distributions (**Figure 3**) and mean particle diameters (**Figure 4**) of colloidal dispersions containing different triacetin concentrations were therefore measured. These figures suggest that conventional emulsions containing relatively large lipid droplets (d = 850−900 nm) were formed in the systems containing 0−80 wt % triacetin in the organic phase, but that microemulsions (d = 10 nm) were formed in the systems containing 100 wt % triacetin in the organic phase. Interestingly, 5 wt % triacetin was immiscible with buffer solution in the absence of surfactant, forming a milky colloidal dispersion after sonication that rapidly separated into an oily layer on top of an aqueous phase upon standing. On the other hand, 5 wt % triacetin spontaneously formed a transparent colorless liquid when it was added to an aqueous buffer solution containing 1 wt % Brij 35. This observation suggests that the triacetin formed a microemulsion only in the presence of the nonionic surfactant molecules. Presumably, triacetin molecules were incorporated between surfactant molecules within the microemulsion structure.

The fact that triacetin could form a microemulsion in the aqueous phase meant that it was important to establish the physical location of the triacetin itself within the different systems. That is, was it predominantly in the lipid droplets or in the surrounding aqueous phase? The location of the triacetin was established by measuring its concentration in the aqueous phase and within the total system using gas chromatography (**Figure 5**). These measurements clearly show that the majority of triacetin was located within the aqueous phase (presumably as microemulsions), rather than in the droplet phase. Consequently, the concentration of droplets within the mixed systems decreased as the triacetin concentration increased, which has important consequences for understanding the impact of triacetin on citral location (**Figure 2**). The observed increase of citral in the aqueous phase with increasing triacetin concentration can therefore be at least partly attributed to the fact that the droplet concentration decreased. For example, the total MCT droplet concentrations

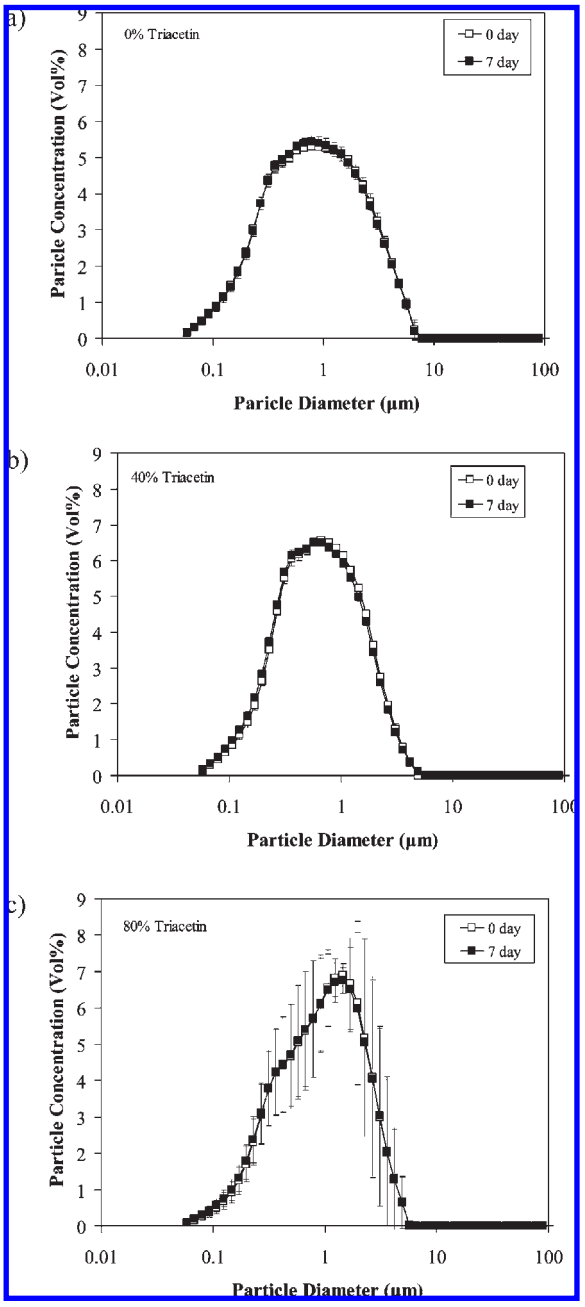

Figure 3. Impact of storage on the droplet size distribution of MCT/triacetin emulsions containing (**a**) 0% triacetin, (**b**) 40% triacetin, and (**c**) 80% triacetin in the organic phase (pH 3).

present in the systems were 5, 4, 3, 2, 1, and 0 wt % for triacetin concentrations in the organic phase of 0, 20, 40, 60, 80, and 100 wt %, respectively. This information could be used to calculate the oil−water partition coefficients of citral in the emulsions (**Figure 6**): log P = 2.02, 1.97, 1.95, 2.10, and 2.35 for emulsions at pH 3 and log P = 2.07, 1.193, 1.99, 2.17, and 2.37 for emulsions at pH 7 with triacetin concentrations in the organic phase of 0, 20, 40, 60, and 80 wt %, respectively. These calculations indicate that the partitioning of citral between the MCT droplets and the surrounding aqueous phase was fairly similar at all triacetin concentrations, with log P being around 2.

Physical Stability of Citral-Containing Emulsions. For practical applications it is important that colloidal dispersions containing citral remain physically stable throughout their shelf life. The physical stability of Brij-stabilized citral/MCT/triacetin systems at pH 3 and 7 was therefore determined by monitoring their

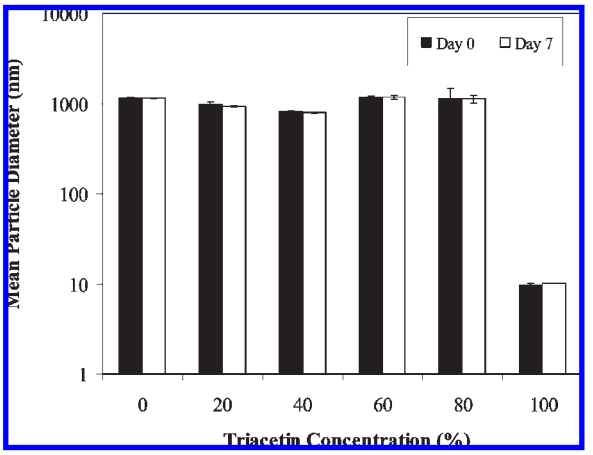

Figure 4. Impact of storage time and triacetin concentration on mean particle diameter of MCT/triacetin systems (pH 3). The mean particle diameter was measured by static light scattering (d_{43}) for the 0−80% triacetin systems and by dynamic light scattering (*z*-average) for the 100% triacetin system.

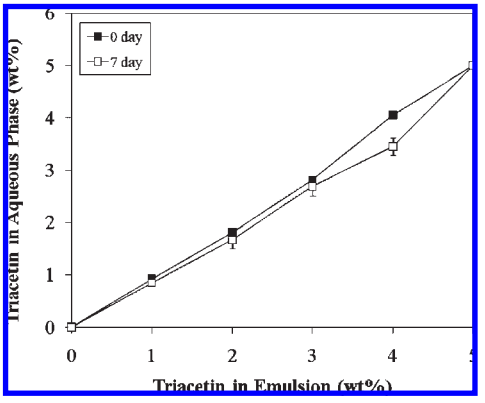

Figure 5. Location of triacetin within triacetin/MCT systems at pH 3 and 7.

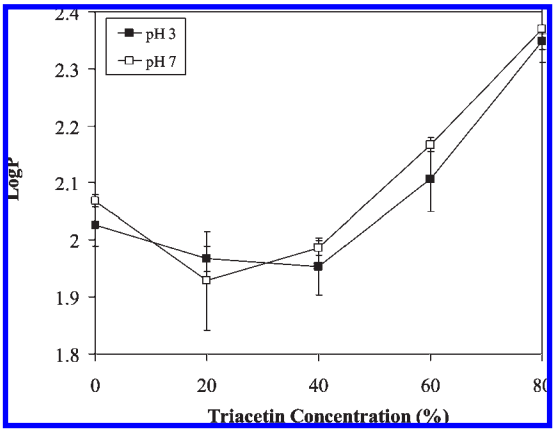

Figure 6. Impact of triacetin concentration within triacetin/MCT systems on the oil−water partition coefficient (log *P*) at pH 3 and 7.

particle size distribution during 7 days of storage at 20 °C. The initial mean droplet diameters of the Brij-stabilized emulsions were around 0.96 μm (d_{43}) and 0.45 μm (d_{32}), independent of pH, with both emulsions having monomodal particle size distributions (**Figure 3**). At both pH 3 and 7, there was no significant change in the particle size distribution or mean particle diameter of the emulsions during 7 days of storage, independent of the triacetin concentration present, which suggested that all of the

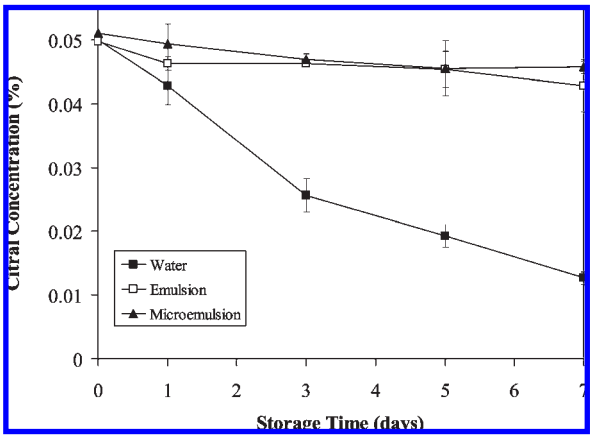

Figure 7. Degradation of citral in aqueous buffer solutions, MCT emulsions, and triacetin microemulsions during storage at pH 3 and 20 °C.

emulsions were stable to Ostwald ripening, flocculation, and coalescence.

Chemical Stability of Citral in Brij-Stabilized MCT/Triacetin Emulsions. The chemical degradation of citral in MCT/triacetin emulsions stabilized with Brij 35 at pH 3 and 7 was determined by measuring the loss of citral's isomers (neral and geranial) during storage for 7 days at 20 °C (**Figure 7**). The chemical stabilities of citral at pH 3 in aqueous buffer solution, emulsion (5 wt % MCT), and microemulsion (5 wt % triacetin) are compared in **Figure 7**. This figure clearly shows that both the emulsion and microemulsion are able to stabilize the citral from chemical degradation. The most likely reason for the ability of the emulsion to stabilize citral against chemical degradation is the fact that the citral partitions into the MCT oil droplets and is therefore protected from the aqueous acidic environment; that is, < 20% of citral is in the aqueous phase (**Figure 2**). On the other hand, there are no oil droplets for the citral to partition into in the microemulsion system, and all of the citral is present within the aqueous phase (**Figure 2**). It is possible that the citral molecules are incorporated within nonpolar regions of the triacetin/Brij 35 microemulsion structures, thereby stabilizing them against acid-catalyzed degradation. Alternatively, either the triacetin or the Brij 35 may be able to retard the degradation of citral by interfering directly with the chemical reaction.

The impact of triacetin concentration on the chemical degradation rates of citral at pH 3 and 7 is shown in **Figure 8**. For comparison, the degradation rates of citral at pH 3 and 7 in aqueous solutions are −0.085 and 0.001 day^{-1}, respectively (**Table 1**). At pH 7, there was little evidence of chemical degradation of the citral during 7 days of storage. At pH 3, the rate of chemical degradation was greatest for 0% triacetin in the organic phase (the emulsion system) and 100% triacetin in the organic phase (the microemulsion system) and less for the 20−80% triacetin in the organic phase (mixed systems). These results show that the mixed systems were even more stable than the emulsion and microemulsion systems, which are in turn more stable than the citral in water systems. The physicochemical origin of the improved stability of the citral in the mixed systems is currently unknown. In the mixed systems there would be a mixture of oil droplets and microemulsion droplets that the citral molecules could partition into, rather than just one or the other. It is possible that the concentration of citral that is molecularly dispersed in the water (rather than trapped within oil or microemulsion droplets) was reduced in the mixed systems, but further work using analytical methods that can distinguish between different molecular environments of citral, for example, NMR, is needed to

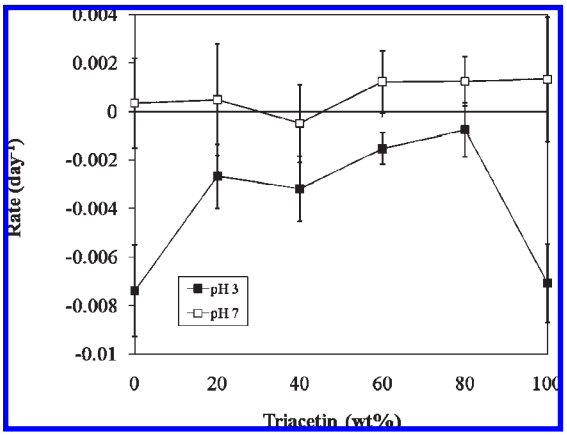

Figure 8. Impact of triacetin concentration on citral degradation rates in systems containing organic phases with different triacetin and MCT ratios (total organic phase of 5 wt %).

elucidate this fact. Citral trapped in droplets or microemulsions is presumably more chemically stable because it is not in direct contact with water.

Conclusions. This study has shown that the chemical stability of citral can be increased by adding either medium-chain triacylglycerols or triacetin to the aqueous phase. The MCT was present as oil droplets (d = 800−900 nm) that acted as sinks for the citral, thereby protecting it from chemical degradation in the acidic aqueous phase. The triacetin was present as microemulsion particles (d = 10 nm) that may have been able to protect the citral by incorporating it within their hydrophobic internal regions. The concentration of the organic phase (5 wt %) used in these studies was relatively high compared to that in finished beverage emulsions (< 0.1 wt %). Consequently, this strategy may not work to protect citral in finished beverage products because the majority of the citral may partition into the aqueous phase. However, it may work to stabilize citral during storage in beverage concentrates, which do have relatively high organic phase concentrations (> 20 wt %). In addition, beverage emulsion products are stored for much longer than the periods used in this study, may experience temperature fluctuations, and may contain other additives that could affect the rate of citral degradation. Consequently, further studies are needed to elucidate the impact of other factors on citral stability in model beverage emulsions.

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