Appl Biol Chem (2016) 59(3):471–474
DOI 10.1007/s13765-016-0181-8

Online ISSN 2468-0842
Print ISSN 2468-0834

NOTE

Effect of cosolvent and surfactant on the solubility and stability of citral in a beverage model

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Received: 20 January 2016 / Accepted: 17 February 2016 / Published online: 14 March 2016

Abstract To evaluate how triacetin and surfactant affect the solubilization and stability of citral, solutions containing citral were prepared with polyoxyethylene stearyl ethers and triacetin. Triacetin had a significant impact on the increment of saturation concentration of citral, and the citral solubility increased as surfactants were added. However, an interaction effect between triacetin and surfactant on citral solubility was not observed. At pH 3, the degradation rate constant of citral gradually decreased with a proportion of the triacetin concentration. Additionally, the chemical degradation of citral was considerably retarded in micelles built with surfactant. The surfactant with the smaller hydrophilic head exhibited higher citral stabilization ability than the one with the larger hydrophilic head; however, a negative interaction effect between triacetin and surfactant on citral stability in an acidic environment was observed.

Keywords Chemical degradation · Citral · Polyoxyethylene stearyl ethers · Solubility · Storage stability · Triacetin

Introduction

Citral is a widely used flavor additive in foods and beverages (Schieberle and Grosch 1998). The reduction of the fresh citrus aroma of citral occurs due to its rapid decomposition through a series of cyclization and oxidation reactions (Kimura et al. 1983; Schieberle and Grosch 1998; Ueno et al. 2004). An acidic pH generally accelerates the cyclization and oxidation of citral. Because of the acidity of most foods and beverages, the adjustment of pH to a natural level, where the cyclization and oxidation of citral rarely occur, is not an effective way to preserve the original freshness of citral for a desired period (Djordjevic et al. 2007).

The isolation of labile food flavors from a harsh environment is a possible way to increase their stability, which could be easily achieved by incorporating them into colloidal systems. Many reports present scientific findings that the incorporation of labile food flavors into emulsions and micelles drastically increased their stability in an acidic environment (Djordjevic et al. 2008; Choi et al. 2009; Given Jr. 2009; Yang et al. 2012; Maswal and Dar 2013). Therefore, these micellar systems have been extensively studied as potential encapsulation systems for food flavors (Asker et al. 2011; Park et al. 2015).

Triacetin (glycerol triacetate) is a colorless and slightly viscous liquid (Acrctander 1969). Applied especially for food flavors into liquid- or emulsion-type foods, triacetin is a commonly used solvent to dissolve the flavor compounds to a required concentration. In addition, the incorporation of triacetin into oil-in-water emulsions improved the stability of flavor compounds at an acidic condition, indicating that triacetin has a positive effect on the chemical stability of flavor compounds in an acidic beverage model (Choi et al. 2009).

Therefore, as part of our continuing work into the micellar systems for the increment of the stability of labile food flavors, we report the results of a study designed to evaluate the effect of triacetin on the efficacy of micellar

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system. In particular, this work determined the effect of triacetin on the solubility and stability of citral in micelles.

Materials and methods

Materials

Citral, polyoxyethylene (20) stearyl ether, polyoxyethylene (100) stearyl ether, and triacetin were purchased from Sigma-Aldrich (St. Louis, MO, USA). The number (20 or 100) following the 'polyoxyethylene' part refers to the total number of oxyethylene groups found in the molecule. Surfactant solutions were prepared by dissolving polyoxyethylene (20) stearyl ether (S20) or polyoxyethylene (100) stearyl ether (S100) in 10 mM phosphate buffer (pH 7 ± 0.1) to achieve a 20× concentration of their critical micelle concentrations (CMC); triacetin solutions were prepared by dissolving triacetin in 10 mM phosphate buffer (pH 7.0 ± 0.1) to 0.1, 1, and 3 % (w/w).

Solubility and stability experiments

Citral was added into sample solution to a 4× higher concentration of its saturation concentration (≈590 mg/L) in water and dissolved by stirring for 15 h at 20 °C. To determine the concentration of dissolved citral in solution after its dissolution, a 10 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 30 mL of the sample solution was collected.

To determine the stability of citral in sample solution, citral was directly added to the surfactant or triacetin solutions, finally obtaining a half concentration of the citral's saturation concentration in each solution, which was later dissolved by stirring for 12 h at 4 °C. Next, the sample solutions were adjusted to a final pH of 3.0 ± 0.1 using 0.1 N hydrochloric acid and they were subsequently stored under quiescent conditions at 4 °C for up to 14 days. Citral concentration was measured using a gas chromatograph (7890A, Agilent Technologies, Santa Clara, CA, USA) with a flame ionizing detector, as described previously (Hong et al. 2016).

The degradation rate constant (k) of citral in sample solutions was calculated, assuming the following 1st order reaction:

$$C_t = C_0 e^{-kt},$$

where C_0 is the initial citral concentration, and C_t is the citral concentration remaining at time t. The values of k were determined by performing a linear regression on the plots of ln (C_t/C_0) versus t.

Statistical analysis

All experiments were performed in triplicate, and mean values are reported with standard deviations. Analysis of variance (ANOVA) was performed, and the mean separation was analyzed by Duncan's multiple range test ($p < 0.05$). All statistical analyses were conducted using SPSS for Windows 12.0 (SPSS Inc., Chicago, IL, USA).

Results and discussion

Citral solubilization

The independent effects of surfactant and triacetin on citral solubility are presented in Table 1. Without surfactant, citral solubility increased with triacetin concentration. Based on the study on the investigation of the effect of triacetin on gas/liquid partition coefficient of citral (Reineccius et al. 2005), triacetin as a solubilizing agent is able to increase the saturation concentration of citral in aqueous solution. The increase of citral solubility in the presence of triacetin can be explained by the hydrophobic interactions of these molecules.

Compared to buffer solution, surfactants significantly affected citral solubility. Because surfactants built micellar structure with the hydrophobic cores over their CMCs,

Table 1 Influence of triacetin and surfactant on the saturation concentration of citral in an aqueous solution at pH 7

Surfactant	Solubilized citral concentration (mg/mL)			
	Triacetin (%)			
	0	0.1	1	3
No	$^{c}479.2 \pm 0.9^{x}$	$^{b}501.2 \pm 4.3^{x}$	$^{a}526.5 \pm 13.2^{x}$	$^{ab}515.4 \pm 16.5^{x}$
S20	$^{c}567.3 \pm 0.8^{y}$	$^{b}624.6 \pm 7.4^{y}$	$^{a}651.6 \pm 16.5^{y}$	$^{b}629.0 \pm 7.8^{y}$
S100	$^{b}647.0 \pm 6.1^{z}$	$^{a}701.1 \pm 24.0^{z}$	$^{a}727.4 \pm 11.8^{z}$	$^{a}723.2 \pm 37.1^{z}$

The values having different superscripts (a, b, and c) in each row are significantly different ($p < 0.05$) by Duncan's multiple range test

The values having different superscripts (x, y, and z) in each column are significantly different ($p < 0.05$) by Duncan's multiple range test

micelles could solubilize water-insoluble compounds into their hydrophobic cores, thereby enabling flavors to be solubilized to a considerably higher level than their saturation concentration in an aqueous solution (Maswal and Dar 2013; Park et al. 2015). The solubilization capacity of S20 micelles was greater than that of S100 micelles. The large number of citral molecules was solubilized into the micellar hydrophobic cores, but the considerable amount of citral molecules could be solubilized on the micelle–water interface due to hydrogen bonding between –OH groups of citral and oxyethylene groups of surfactants (Bhat et al. 2008). Therefore, during the experimental design, because of the 5× greater oxyethylene content in S100 than in S20 with the same hydrophobic tail, the higher solubilization power of S100 was expected than S20. The hypothesis, however, seems to contradict the higher solubilization power of S20 than S100. This contradiction may be explained by the micelle aggregation number or the proposed number of surfactant molecules per micelle (Bhat et al. 2008). It could be assumed that the aggregation number of S20 micelles is greater than that of S100 micelles, indicating that the S20 micelles have the larger volume of the micellar hydrophobic core. Whereas the greater oxyethylene content of S100 enhanced the citral solubility to a certain level, S20 micelles offered the large volume of the micellar hydrophobic core for citral to be solubilized. The effect of micellar volume (aggregation number) could predominate over the effect of the number of oxyethylene groups on micelle surfaces, in keeping with the observation that micellar size increases with a decrease in oxyethylene content (Barry and El Eini 1976).

There was no interaction effect between triacetin and surfactant on the increment of the citral's saturation concentration. It was expected that the incorporation of triacetin into the micellar hydrophobic cores increased the micellar volume which citral molecules were solubilized into. It was also anticipated that the hydrophilic corona regions of micelles could change to a considerably more appropriate condition by the solubilization of triacetin in the micelles' palisade layer. Therefore, although it is not clear how triacetin or surfactant increases the citral solubility, no interaction effect between the variables on citral solubilization suggests that their solubilization mechanisms work independently to increase the citral solubility.

Citral degradation

The changes in the citral concentration of sample solutions were monitored during 14 days of storage (Fig. 1). Table 2 shows the degradation rate constant (k) of citral at different pH values with or without triacetin and surfactant (Hong et al. 2016). The k values of surfactant solutions were always lower than those in a buffer solution. The surfactant

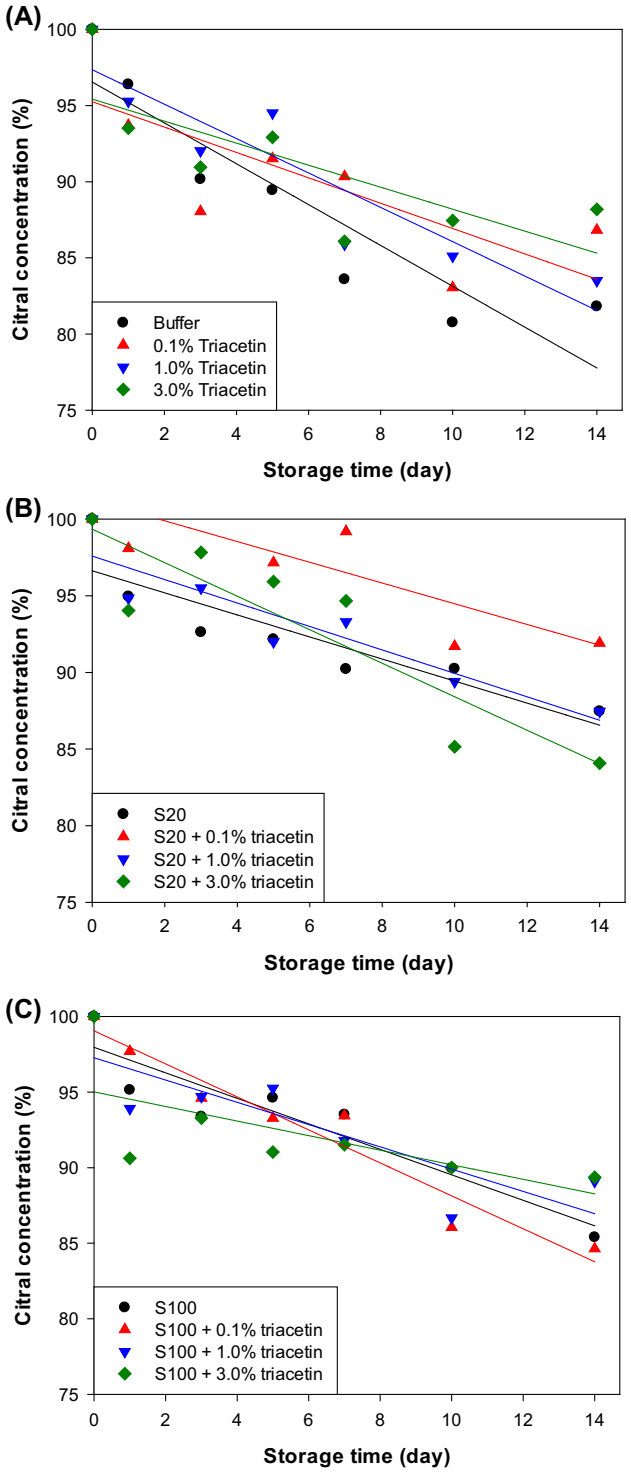

Fig. 1 Time dependence of the citral concentration remaining in sample solutions containing triacetin (**A**), S20 and triacetin (**B**), and S100 and triacetin (**C**) at pH 3 and 4 °C. *Error bars* are omitted for clarity

concentrations were 20 times their CMCs, suggesting that most of the surfactant molecules could form micelles. Nonpolar citral molecules were partitioned into a hydrophobic

Table 2 Degradation rate constants (k) determined by modeling the chemical degradation of citral with triacetin and surfactant at pH 3

Surfactant	Triacetin (%)			
	0	0.1	1	3
No	0.0401	0.0384	0.0369	0.0283
S20	0.0289	0.0362	0.0361	0.0328
S100	0.0309	0.0427	0.0327	0.0294

environment and were sequestered from the acidic environment, which could result in the improved chemical stability of citral (Choi et al. 2009; Maswal and Dar 2013; Park et al. 2015). The k values for the S100 micelles were slightly higher than that of S20 micelles, which agrees with the previous report (Hong et al. 2016). Because the size of the hydrophilic head of S20 is considerably smaller than that of S100, there is a large difference in the thickness of the hydrophilic corona region between both micelles (Park et al. 2015). Most of the citral molecules were located into the micelles' hydrophobic cores. Concurrently, a fairly large amount of citral molecules were still located within the hydrophilic corona region and not perfectly isolated from the environment that rapidly degraded citral (Maswal and Dar 2013). With triacetin alone, the k value decreased with triacetin concentration, suggesting that triacetin could retard the degradation of citral by interfering directly with the chemical reaction (Choi et al. 2009). However, these results show that the mixed systems were less stable than the solutions containing triacetin or surfactant alone. Interestingly, the highest k value was observed at a low concentration of triacetin. Although no interaction between surfactant and triacetin on citral solubilization was observed, a negative interaction effect was observed between them regarding citral stability. However, how triacetin improves the stability of citral, as well as how triacetin and surfactant together reduce the citral stability, are currently unknown. To elucidate the stabilization mechanism of triacetin and the destabilization mechanism of triacetin and surfactant, the application of analytical methods that are able to distinguish between different molecular environments of citral warrants further study.

Acknowledgments This work was financially supported in part by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2013R1A1A1008321), and by the High Value-Added Food Technology Development Program (313021-3) funded by the Ministry of Agriculture, Food and Rural Affairs, Republic of Korea.

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