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Effect of Ubiquinol-10 on Citral Stability and Off-Flavor Formation in Oil-in-Water (O/W) Nanoemulsions

Qin Zhao, Chi-Tang Ho, and Qingrong Huang*

Department of Food Science, Rutgers University, 65 Dudley Road, New Brunswick, New Jersey 08901, United States

ABSTRACT: The effects of different concentrations of ubiquinol-10 ($Q_{10}H_2$) on citral's stability were systematically investigated and compared in citral-loaded oil-in-water (O/W) nanoemulsions. Solid phase microextraction gas chromatography (SPME-GC) was employed to monitor the degradation of citral and the formation of off-flavor compounds throughout storage at 25 and 45 °C. The optimum concentration of $Q_{10}H_2$ in the current formulation was determined to be around 0.10 wt % in the system ($Q_{10}H_2$/citral ratio 1:1), which can effectively protect citral from chemical degradation and oxidation. Results suggested, however, that a low concentration of $Q_{10}H_2$ may induce the majority of the ubisemiquinone ($Q_{10}^{\bullet-}$)/ubiquinone (Q_{10}) redox transition, which possibly endowed $Q_{10}H_2$ with pro-oxidant properties. Further increase in $Q_{10}H_2$ concentration beyond a certain value also hindered its effect due to the complex properties of radicals involved and the overall environment encountered. With appropriate concentrations of $Q_{10}H_2$ presented in the system, major citral oxidation off-flavor compounds (*p*-cresol, *α,p*-dimethylstyrene, *p*-methylacetophenone), and some of the lipid degradation products can be inhibited to lower levels. In contrast, ubiquinone-10 (Q_{10}) had a negligible effect on citral's chemical stability and off-flavor generation.

KEYWORDS: *citral degradation, lipid oxidation, coenzyme Q_{10}, oil-in-water nanoemulsion*

INTRODUCTION

Citral, one of the most important flavoring compounds with strong lemon aroma and high consumer acceptance, is widely used as an additive in the food, beverage, and perfumery industries. However, the long existing problem that limits its application is that citral can easily undergo acid-catalyzed cyclization and oxidation in a low-pH environment and under oxidative stress.[1,2] The degradation of citral leads to major loss of the lemon-like aroma and also the generation of many undesired off-flavors.[3−6] The complicated degradation mechanism of citral is not completely established and understood so far. Among all types of degradation products from citral, some of the acid-catalyzed isomerization compounds such as *p*-cymene, *p*-cymen-8-ol, and its dehydration product, *α,p*-dimethylstyrene, were previously postulated to contribute to the major potent off-odors,[3,4] but later studies further claimed some autoxidation compounds such as *p*-cresol and *p*-methylacetophenone, which have lower odor thresholds of 0.3−1.0 and 2.7−10.8 ng/L in air, respectively, to be more responsible for the undesired odorant.[5,6] Ueno et al. also proposed a formation mechanism of *p*-methylacetophenone from citral via a *tert*-alkoxy radical intermediate under acidic aqueous conditions.[7]

To protect citral from rapid degradation and minimize the major potent off-flavor generation, many strategies have been developed and applied by using food chemistry and engineering principles. Major methods include spray-dry encapsulation,[8] oil-in-water (O/W) emulsion/nanoemulsion systems,[9] engineering the interface of emulsion droplets with different emulsifiers,[10] multilayer coatings,[11,12] surface charges,[13] using solid lipids as oil phase,[14] using micelles and reverse micelles to entrap citral in the oil phase,[15] etc. More recently, the addition of antioxidants, especially naturally and commercially available ones, into systems loaded with citral has drawn increasing attention, especially because people are becoming more and more familiar with functional foods claiming additional nutritional supplements and health benefits nowadays. Peacock and Kuneman[4] added isoascorbic acid to a carbonated beverage system containing citral and found an inhibition effect in the formation of *α,p*-dimethylstyrene and *p*-cymen-8-ol. Liang et al.[16] studied the antioxidant activities of four plant extracts (grape seed, pomegranate seed, green tea, and black tea) and showed significant inhibition from all four plant extracts on the formation of *p*-cymene, *p*-cresol, and *p*-methylacetophenone, but promotion of the generation of *α,p*-dimethylstyrene under citrate buffer solutions (pH 3) containing 100 ppm citral. Ueno et al.[17] also investigated the ability of black tea theaflavins inhibiting the formation of *p*-cresol and *p*-methylacetophenone in acidic buffer solutions of pH 3. Recently, Yang et al.[18] systematically investigated the effects six different natural antioxidants on the stability of citral in oil-in-water nanoemulsions and found that *β*-carotene, tanshinone, and black tea extract could greatly enhance citral's chemical stability during storage as well as inhibit some of the potent off-flavor compounds. However, many of the above-mentioned antioxidants are not commercially available or need extensive extraction and separation work. The cost-ineffective issue also currently hinders their real application in the food industry. Some carotenoids and tea extracts have their own unique taste profiles and intense colors that will also pose a problem in lemon-flavored beverages. Therefore, it is necessary to find antioxidants that can effectively inhibit citral degradation and off-flavor formation.

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Ubiquinone (2,3-dimethoxy-5-methyl-6-multiprenyl-1,4-benzoquinone), also widely known as coenzyme Q_{10}, comprises a group of lipid-soluble, vitamin-like compounds essential for the electron transport chain in mitochondria for energy (ATP) production.[19] The predominant form of ubiquinone in animals and humans is ubiquinone-10 (known as CoQ_{10}), which contains 10 isoprenoid units as the hydrophobic side chain. CoQ_{10} is an essential and potent antioxidant in the human body that scavenges free radicals generated under oxidative stress. It exists in three redox states (Figure 1):[20] the fully oxidized

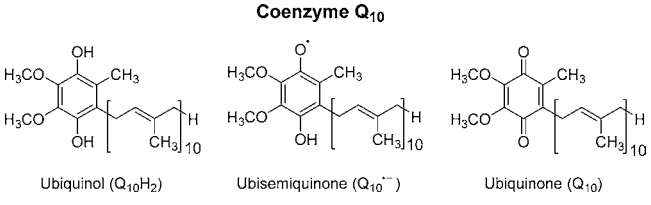

Figure 1. Chemical structures of three redox states of coenzyme Q_{10}.

ubiquinone (Q_{10}); the partially reduced ubisemiquinone ($Q_{10}^{\bullet-}$); and the fully reduced ubiquinol ($Q_{10}H_2$). These compounds can be recycled in vivo by the mitochondrial respiratory chain. Ubiquinol ($Q_{10}H_2$) is the "activated" form responsible for the antioxidant and health-promoting properties. In contrast to other antioxidants, this compound inhibits both the initiation and the propagation of lipid and protein oxidation[21] and is capable of regenerating other antioxidants such as α-tocopherol.[20] Recent studies also revealed its function in lipoprotein protection, gene expression involved in human cell signaling, metabolism, and transport. Although ubiquinol can be biosynthesized in vivo, age-related decline in the human body will cause accelerated aging and diminished energy levels. Therefore, ubiquinol is now a commercialized nutraceutical in many dietary supplements on the market and is gaining more and more attention as well as consumer acceptance. Furthermore, the addition of ubiquinol as a functional nutraceutical in beverage products, such as energy drinks, is now a promising and appreciable trend.

The antioxidant property of ubiquinol-10 ($Q_{10}H_2$) in inhibiting citral's degradation and off-flavor generation is not yet validated and elucidated. It is generally accepted that there are free radicals produced during citral degradation as well as lipid oxidation in the emulsion system. The aim of this work was then to see the effect of $Q_{10}H_2$ as an antioxidant in the oil-in-water nanoemulsion system to protect citral from chemical degradation and off-flavor generation. The effect of different concentrations of $Q_{10}H_2$ in the formulation was tested and discussed.

MATERIALS AND METHODS

Materials. NEOBEE 1053 medium-chain triacylglycerol (MCT) consisting of 55% caprylic and 44% capric triglyceride was obtained from Stepan Co. (Northfield, IL, USA). Alcolec PC75 (phosphatidylcholine enriched) soy lecithin containing ca. 76% unsaturated and 24% of saturated fatty acids was a gift from American Lecithin Co. (Oxford, CT, USA). $Q_{10}H_2$ (95%, UV) was purchased from Hangzhou Joymore Technology Co., Ltd. China. All other chemicals and supplies were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification and treatment.

Emulsion Preparation and Storage. The O/W nanoemlusions were prepared by using 10 wt % MCT as the oil phase, 85 wt % pH 3.0 buffer solution (10 mM citric acid/sodium hydroxide/sodium chloride) as the water phase, and 5 wt % PC75 soy lecithin as the emulsifier, which can be dispersed in water phase. For each emulsion sample, 0.1 wt % (1000 ppm) citral and 0.01 wt % (100 ppm) undecane (internal standard) were dissolved in the lipid phase, and $Q_{10}H_2$ with different concentrations (0.01, 0.05, 0.1, and 0.2 wt %) were also added into the oil phase before homogenization, respectively. Then the aqueous phase and oil phase were thoroughly mixed and homogenized using an Ultra-Turrax T-25 high-speed homogenizer (IKA Works Inc., Wilmington, DE, USA) at 24000 rpm for 5 min followed by a high-pressure homogenizer (EmulsiFlex-C3, Avestin Inc., Ottawa, Canada) for six cycles at a pressure of 150 MPa. Ten grams of each emulsion sample was weighed and stored in a 20 mL amber glass vial (Supelco Analytical) with a screw cap (PTFE/silicone septum, Supelco Analytical) designed for solid phase microextraction (SPME) immediately after preparation. All of the vials with emulsion samples were divided into two groups, with one stored at 25 °C and the other stored at 45 °C, both under dark conditions throughout the experiments.

Particle Size Measurements. The mean hydrodynamic emulsion particle size and distributions were measured using a BIC 90 plus particle size analyzer equipped with a Brookhaven BI-9000 AT digital correlator (Brookhaven Instruments Corp., Holtsville, NY, USA) based on dynamic light scattering. The light source is a solid-state laser operating at 658 nm with 30 mW power, and the signals were detected by a high-sensitivity avalanche photodiode detector. Emulsion samples stored at 25 and 45 °C were diluted 100× with Milli-Q water and well mixed prior to the measurement to prevent multiple scattering effects. All of the measurements were conducted in triplicate at a fixed scattering angle of 90° at 25 ± 1 °C. The mean diameter of each sample was determined by Cumulant analysis of the intensity−intensity autocorrelation function, $G(q, t)$.

Measurement of Citral. An Agilent 6850 gas chromatograph was used to quantify citral's two isomers and various degradation products during the storage. The GC was equipped with a J&W DB-5MS capillary column (30 m × 0.25 mm i.d.; 0.25 μm film thickness) and connected with a flame ionization detector (FID). The FID temperature was set at 250 °C. The oven temperature profile was programmed as follows: a 4 °C/min increment from 60 to 150 °C in the first stage, then a further increase to 230 °C at the rate of 20 °C/min, and finally a hold at 230 °C for 5 min with total program timing of 31.5 min. The flow rate of hydrogen as the flame gas was controlled at 40.0 mL/min, air flow at 45 mL/min, and helium as the carrier gas flow at 45.0 mL/min. The injection port was equipped with a 0.75 mm inner diameter SPME injection sleeve to minimize the broadening effect. For the SPME extraction, a manual sampling SPME fiber holder with a 65 μm polydimethylsiloxane/divinylbenzene fiber (needle size, 23 ga) was employed. The SPME fiber was exposed in the headspace of the amber glass vials with emulsion samples under constant magnetic stirring for 30 min for adsorption equilibrium at either 25 or 45 °C according to the storage condition. Then, it was inserted into the injection sleeve immediately and held for 5 min for complete desorption. Internal standard (undecane) was used to quantify citral's two isomers and the degradation products.

GC-Mass Analysis of Citral's Degradation Products. An Agilent 6890 gas chromatograph equipped with an Agilent 5973 mass detector and a J&W DB-5MS capillary column (30 m × 0.25 mm i.d.; 0.25 μm film thickness) was used. The temperature programming and gas flow rates were kept the same as for the above-described GC measurements. The ionization voltage was held at 70 eV, and the ion temperature was 280 °C. Authentic compounds for major degradation products of citral (*p*-cresol, α,*p*-dimethylstyrene, *p*-metha-1,5-dien-8-ol, and *p*-methylacetophenone) were purchased from Sigma-Aldrich for matching the mass spectrum and retention index.

Statistical Analysis. All experiments were conducted twice in duplicate, and all data were expressed as the mean ± standard deviation. When appropriate, data were analyzed using a *t* test by SigmaPlot 12.0 software; significant difference was defined at $p < 0.05$.

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RESULTS AND DISCUSSION

Physical Stability of Citral-Loaded Emulsions with and without $Q_{10}H_2$. The physical stability of O/W nanoemulsions under the storage temperatures of 25 and 45 °C was evaluated by the particle size profile of each sample in 10 day intervals throughout the storage time. The mean particle sizes of the different emulsion formulations were calculated by cumulant method and are shown in Figure 2. After high-speed and high-pressure homogenization processing in the same conditions, fresh nanoemulsions with particle sizes in the range of 98−120 nm were obtained. Among these, the control (without $Q_{10}H_2$) had the smallest particle size of 98 nm. The addition of $Q_{10}H_2$ to the oil phase had various impacts on the particle size. During the storage period, the particle size of emulsions stored at 25 °C increased very slightly. All of the emulsions had an increment of 25−40 nm in particle sizes during the 40 day storage time. The particle sizes of emulsions stored at 45 °C, in contrast, showed a faster and sharper increase due to the greater thermodynamic moving rate of particles. An approximately 70−95 nm increment range was observed for all of the tested emulsion samples after 40 days. All of the samples showed a similar trend of increment profile, and none of them were observed as having phase separation or creaming during the storage period at either temperature. Visual observation also indicated good kinetic stability of all citral-loaded lecithin-stabilized nanoemulsions.

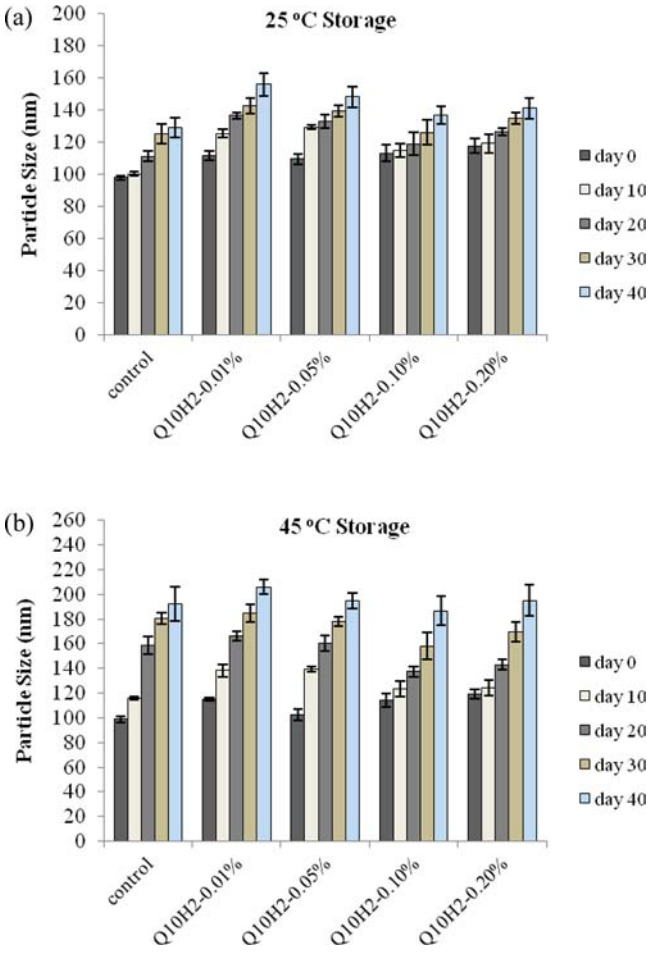

Figure 2. Mean emulsion particle size changes for citral-loaded emulsions with different concentrations of $Q_{10}H_2$ stored at 25 °C (a) and 45 °C (b). Data represent the mean ± standard deviation (n = 3).

Stability of Citral in Emulsions with and without $Q_{10}H_2$. The two isomers of citral, neral and geranial, naturally occur in the ratio of 2:3. In this study, the degradation rates of both neral and geranial were measured to indicate citral's loss during storage at 25 and 45 °C for 40 days. From the degradation rate profiles, neral and general behaved similarly in terms of degradation trends. Under the storage temperature of 25 °C (Figure 3), the control (without $Q_{10}H_2$) had 53.8% neral and 49.3% geranial left after 15 days. After 40 days of storage, there were 29.2% neral and 27.3% geranial left. Both neral and geranial showed a relatively fast degradation rate at earlier stages (days 0−15), and slower degradation rates were observed afterward. Adding different concentrations of $Q_{10}H_2$ into the citral-loaded emulsions caused different and complicated effects. Data that show the average percentage of citral remaining and statistical significance compared to control are presented in table format (Table 1). With a 0.01 wt % concentration of $Q_{10}H_2$ ($Q_{10}H_2$/citral ratio 1:10) in the system, surprisingly, more rapid degradations of both neral and geranial were observed in contrast with control. Only 47.9% neral and 42.4% geranial remained after 15 days, whereas after 40 days, only 19.3% neral and 16.0% geranial were left. However, when the concentrations of $Q_{10}H_2$ were increased to 0.05 wt % ($Q_{10}H_2$/citral ratio 1:2) and 0.10 wt % ($Q_{10}H_2$/citral ratio 1:1),

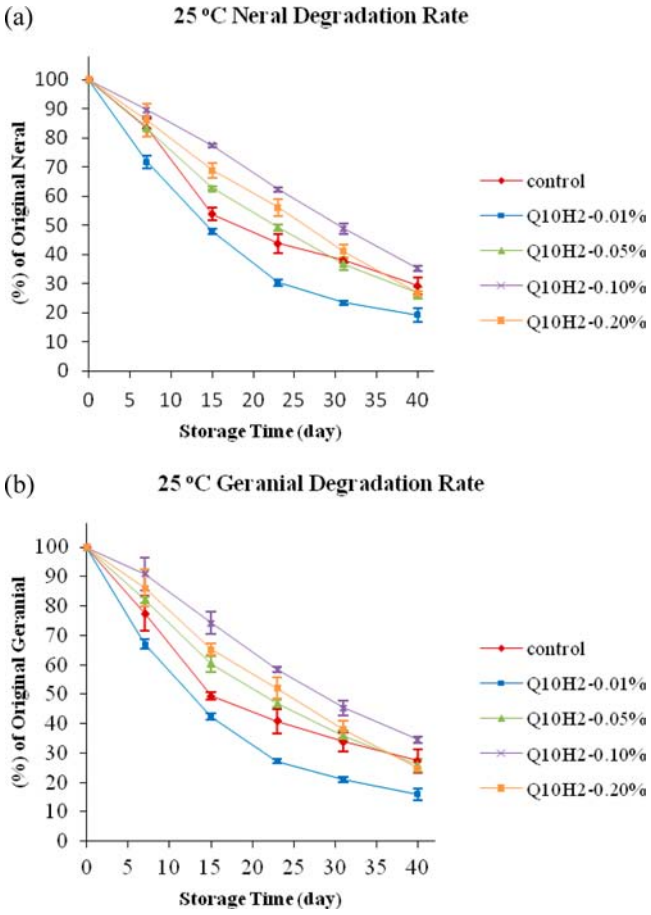

Figure 3. Degradation profiles of neral (a) and geranial (b) in emulsions with different concentrations of $Q_{10}H_2$ stored at 25 °C in comparison with the control.

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Table 1. Average Percentages of Neral and Geranial Retained in Various $Q_{10}H_2$ Formulations during 25 °C Storage

sample		day 7	day 15	day 23	day 31	day 40
control	neral	83.48 ± 2.94	53.75 ± 2.17	43.75 ± 3.16	37.90 ± 0.84	29.24 ± 2.84
	geranial	77.43 ± 6.03	49.31 ± 1.35	40.77 ± 4.16	33.78 ± 3.29	27.29 ± 3.86
$Q_{10}H_2$-0.01%	neral	71.70 ± 2.18[a]	47.91 ± 0.90	30.42 ± 1.08[a]	23.45 ± 0.66[a]	19.26 ± 2.37
	geranial	67.03 ± 1.66[a]	42.36 ± 1.21[a]	27.11 ± 0.07[a]	20.94 ± 0.76[a]	15.98 ± 1.98
$Q_{10}H_2$-0.05%	neral	83.13 ± 0.03	62.73 ± 0.57[a]	49.21 ± 1.07[a]	36.75 ± 2.18	26.76 ± 1.87
	geranial	82.10 ± 1.10	60.24 ± 2.73[a]	46.87 ± 1.20	35.87 ± 1.78	25.88 ± 2.16
$Q_{10}H_2$-0.10%	neral	89.58 ± 2.10	77.34 ± 0.53[a]	62.24 ± 0.58[a]	48.89 ± 1.81[a]	35.28 ± 0.84[a]
	geranial	90.86 ± 5.69	74.23 ± 3.70[a]	58.38 ± 0.86[a]	45.37 ± 2.49[a]	34.58 ± 1.07
$Q_{10}H_2$-0.20%	neral	86.12 ± 5.68	68.80 ± 2.65[a]	56.19 ± 2.88[a]	41.05 ± 2.25	26.64 ± 0.74
	geranial	86.02 ± 6.25	65.21 ± 2.11[a]	52.02 ± 3.65[a]	38.02 ± 2.93	24.75 ± 0.10

[a]Statistically significant difference from control.

the inhibition effects of both neral and geranial's degradations were observed, especially at early storage time. Specifically, the sample containing 0.05 wt % $Q_{10}H_2$ retained 62.7% of neral and 60.2% of geranial on day 23, and 0.10 wt % $Q_{10}H_2$ greatly inhibited citral from degradation compared to the control. Around 77.3% neral and 74.2% geranial were still left after 15 days, which showed significant difference compared to control and the best effect among all of the tested formulations. However, the emulsions with $Q_{10}H_2$ in the formulations showed more linear citral degradation rates. At the end of the 40 day storage period, 26.8% of neral and 25.9% geranial were left in the sample containing 0.05 wt % $Q_{10}H_2$, which was slightly lower than the control. The sample containing 0.10 wt % $Q_{10}H_2$ retained 35.3% neral and 34.6% geranial after 40 days of storage. Compared to the control values, these were increases of about 20.9% neral and 26.7% retention. Complicated results were observed when the $Q_{10}H_2$ concentration was further increased to 0.20% ($Q_{10}H_2$/citral ratio 2:1). From the quantification of the GC data, there was 56.2% neral and 52.0% geranial on day 15 remaining and 26.6% neral and 24.8% geranial left after 40 days, which was slightly better than the 0.05 wt % $Q_{10}H_2$ sample but less effective than the 0.10 wt % sample during the early stage of the storage period. Overall, the sample with 0.10 wt % $Q_{10}H_2$ formulated into the system showed the best effect to inhibit degradation of both neral and geranial at the 25 °C storage conditions.

From the degradation results at 25 °C, it can be tentatively concluded that the concentration of $Q_{10}H_2$ played an important role in protecting citral from degradation in the O/W nanoemulsions. With low concentration of $Q_{10}H_2$ (0.01 wt %) in the formulation, it was ineffective in protecting the citral from degradation. Adversely, it promoted citral degradation at a faster rate than the control. By increasing the $Q_{10}H_2$ concentration to higher levels, inhibition effects were observed. However, beyond a certain level (e.g., 0.20 wt % in our formulations), the inhibition will be adversely suppressed. One possible reason may be the complexity of the citral-loaded emulsion system, which involved both lipid oxidation and citral degradation/oxidation over the storage period. $Q_{10}H_2$ as an antioxidant could theoretically scavenge free radicals and retard oxidation. However, it has been implied before that the antioxidant functions of ubiquinol are mainly encompassed by the $QH_2 \rightarrow Q^{\bullet-}$ redox transition, whereas their pro-oxidant character will also arise from O_2 reduction coupled to the $Q^{\bullet-} \rightarrow Q$ reaction.[22] The context of the redox transitions of ubiquinol conducted by Rich et al.[23] and Swallow et al.[24] allowed one to use relevant reduction potentials to evaluate the reactivity of ubiquinols with nitrogen- and oxygen-containing free radicals on thermodynamic grounds, from which the E (QH_2 /$Q^{\bullet-}$, $2H^+$) and E ($Q^{\bullet-}$/Q) values are +190 and −220 mV, respectively. Overall, it appears that the ubiquinol/ubisemiquinone transition may be associated with antioxidant functions, whereas the ubisemiquinone/ubiquinone redox reaction may be endowed with pro-oxidant properties. Therefore, when a low concentration of $Q_{10}H_2$ is presented in the system, most of them were autoxidized into $Q_{10}^{\bullet-}$ and were further fully coupled by O_2 to form Q_{10} and $O_2^{\bullet-}$ at the early stage of storage, from which most of the $Q_{10}H_2$ was "sacrificed and wasted". The superoxide radicals formed can further oxidize $Q_{10}H_2$ or other compounds to form different radicals, which can have possible pro-oxidant properties and thus promote citral degradation and oxidation. An increased level of $Q_{10}H_2$ worked better as a real antioxidant, probably due to the majority of $QH_2/Q^{\bullet-}$ transition occurring at early stages to facilitate its antioxidant properties other than the minor $Q^{\bullet-}$/Q redox reaction when $Q_{10}H_2$ was abundant in the early stage. The phenomenon of further increasing the concentration of $Q_{10}H_2$ to 0.20 wt % showed a decreased inhibition effect was also interesting. It seems that the antioxidant property of $Q_{10}H_2$ is not proportionally linked with its dosage, whereas it is mostly dependent on the complexity of the system involved and environment encountered. As we know, quenching of an oxidizing radical always produces another radical and so may produce a pro-oxidant. Whether or not the overall effect of different concentrations of ubiquinol-10 worked as antioxidant or pro-oxidant depends on a combination of the properties (reduction potential and lifetime) of the various radicals involved in the whole process in the specific environment. Clearly, it is not easy to predict and, hence, our observations may be only a reported phenomenon that specifically occurred in our tested systems. Although it is difficult to elucidate the detailed mechanism, many previous studies and reviews[25−27] also addressed the similar phenomena on other antioxidants such as carotenoids. Thus, the importance of antioxidnat concentration should be emphasized with a switch from anti- to pro-oxidation observed in several systems as its concentration increases beyond a certain value.

While at 45 °C (Figure 4) storage temperature, both neral and geranial showed more rapid degradation rates in all of the tested formulations. Although data were partially overlapped and showed no statistical significance, minor differences of both neral and geranial retentions can still be observed. After the first 3 days of storage, around 65−72% neral and 60−65% geranial were left in different formulations. The sample with 0.01 wt % $Q_{10}H_2$ showed a relatively faster degradation rate than others after day 10, and the 0.10 wt % $Q_{10}H_2$ sample had a slightly slower rate of degradation for both neral and geranial compared to the control. The other two $Q_{10}H_2$ concentrations also showed a minor effect at early storage period. However, at later stages (i.e., after day 10) they were proven to be not effective in protecting citral from degradation and had even a slight promotion effect. After 20 days of storage at 45 °C, only about 5−14% of neral and 4−12% geranial were left among all of the formulations. At the end of the 40 days, almost all of the neral and geranial were degraded. Under high-temperature conditions, the degradation was more rapid and complicated. The purpose of the high-temperature storage was to investigate the off-flavor compounds produced by citral degradation; the detailed results and discussions will be given in a later section.

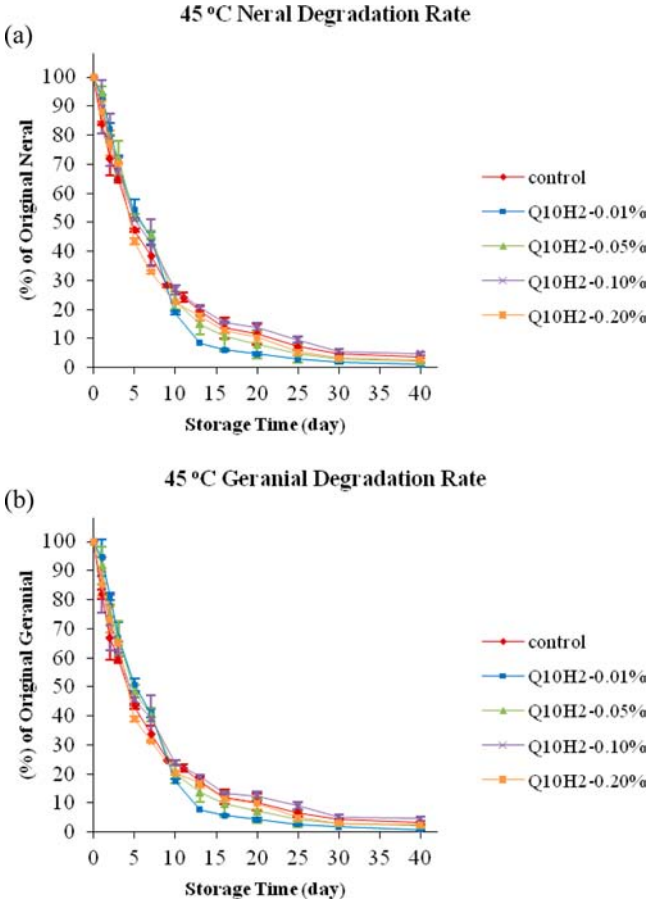

Figure 4. Degradation profiles of neral (a) and geranial (b) in emulsions with different concentrations of $Q_{10}H_2$ stored at 45 °C in comparison with the control.

Due to different formulations, extraction methods, and storage conditions, it is difficult for direct and quantitative comparisons between our data and previous work, but the lecithin-stabilized emulsions did show better protection on citral's chemical degradation under similar storage time and conditions. For instance, Djordjevic et al.[11] prepared sodium dodecyl sulfate−chitosan (SDS-CS) and gum arabic (GA) stabilized emulsions to test their efficacy on the stability of citral. Almost all of the neral and geranial were lost after only 6 days of storage at 37 °C in the SDS-CS stabilized emulsion, and around 35% of neral and geranial were left for the GA-stabilized one. In contrast, our formulation with lecithin PC75 as the emulsifier showed better protection at the even harsher conditions of 45 °C storage temperature, in which, after 7 days, there were still around 40% neral and 36% geranial left for the sample without antioxidant.

Comparison between Ubiquinol-10 and Ubiquinone-10. A previous study[20] indicated that ubiquinone-10 (Q_{10}), unlike $Q_{10}H_2$, exerts no antioxidant activity to inhibit lipid peroxidation in vitro. To systematically investigate the effect of Q_{10} on the inhibition of citral's degradation, a set of experiments were also conducted to compare 0.10 wt % Q_{10} in the emulsion formulation and the sample with same concentration of $Q_{10}H_2$ as previously tested. Similar emulsion particle size distributions and profiles were recorded for the formulation with 0.10 wt % Q_{10}. The freshly prepared citral nanoemulsion had a mean particle size of 107.0 ± 1.6 nm. After 40 days of storage, a slight increment of 26 nm was observed at 25 °C. In the 45 °C storage condition, the emulsion particle size increased from about 75 nm to a value of 182.0 ± 9.6 nm (data not shown), which was in the same range as the control and other formulations with differing concentrations of $Q_{10}H_2$. To investigate the effect of Q_{10} on citral's stability, GC measurements of both neral and geranial degradation rates during the storage time were also obtained under 25 °C (Figure 5) and 45 °C (Figure 6) storage conditions. With the incorporation of 0.10 wt % Q_{10}, no significant difference was observed in neral and geranial degradation compared with control under 25 °C, and the same concentration of $Q_{10}H_2$ was more effective, indicating Q_{10} could not protect citral from chemical degradation, although it will neither promote the degradation. While under 45 °C storage condition, citral degraded slightly faster in the sample with Q_{10} than in the control.

Evaluation of the Major Citral Degradation Compounds. Citral was completely degraded after 40 days of storage at 45 °C. Four major citral degradation products (*p*-cresol, *α*,*p*-dimethylstyrene, *p*-mentha-1,5-dien-8-ol, and *p*-methylacetophenone) were detected and quantified throughout the storage period with mass spectra and retention indices that agree with those of authentic compounds purchased from Sigma-Aldrich. Among them, three were oxidation products; only *p*-mentha-1,5-dien-8-ol was the acid-catalyzed reaction product.[17] Moreover, some other degradation products such as *p*-cymene, *p*-cymen-8-ol, and many monoterpene alcohols could not be detected. Therefore, it can be concluded that encapsulation of citral in the oil phase of nanoemulsion can effectively isolate protons in the acidic aqueous phase, thus inhibiting acid-catalyzed degradation reactions as we previously observed.[18] The detailed generation profiles of the four detected off-flavors during the storage are shown in Figure 7. However, for the acid-catalyzed degradation product, *p*-mentha-1,5-dien-8-ol (c), adding $Q_{10}H_2$ could not effectively inhibit its formation; instead, the control showed minimum levels throughout the storage time. For the three oxidation products, different concentrations of $Q_{10}H_2$ had different effects on their generations. For *p*-cresol (a), only 0.10 wt % of $Q_{10}H_2$ slightly inhibited the formation of it compared to control.

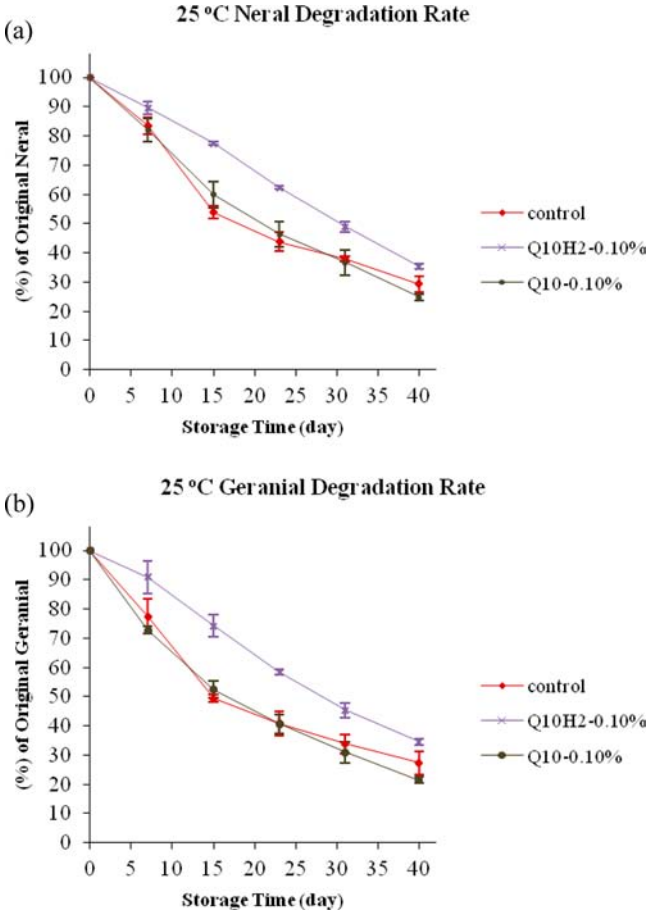

Figure 5. Degradation profiles of neral (a) and geranial (b) in emulsions with 0.10 wt % of Q_{10} stored at 25 °C in comparison with the control.

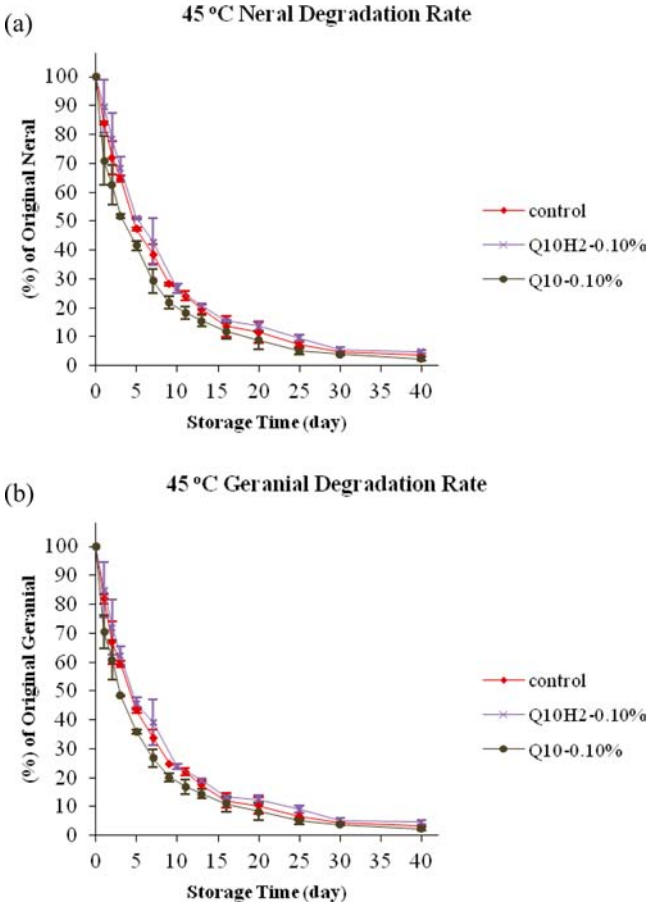

Figure 6. Degradation profiles of neral (a) and geranial (b) in emulsions with 0.10 wt % of Q_{10} stored at 45 °C in comparison with the control.

Others, especially the sample with 0.01 wt % $Q_{10}H_2$, actually increased the formation of *p*-cresol to a higher level of 7.7 ± 0.26 ppm on day 30 in contrast with 3.4 ± 0.38 ppm of the control. For *p*-methylacetophenone (d), similar results were observed, with the 0.10 wt % $Q_{10}H_2$ showing the minimum detectable levels throughout the storage period. The 0.05 wt % sample was fluctuating around comparable with the control and showed a negligible difference. Low (0.01%) and high (0.20%) concentration samples both promoted the generation of *p*-methylacetophenone to higher levels. Finally, it seems that the generation of *α*,*p*-dimethylstyrene (b) can be inhibited to certain levels by adding appropriate concentrations of $Q_{10}H_2$ (0.05, 0.10, and 0.20%), among which, 0.10% always showed the best performance. Only the 0.01% sample had slightly higher levels than the control. The observed results were in good accordance with the citral degradation profiles though. The sample (0.10% $Q_{10}H_2$) with the slowest degradation rate of both neral and geranial performed best in the off-flavor generation. Although some concentrations of $Q_{10}H_2$ (0.05 and 0.20%) showed some protection effect on citral's degradation under 25 °C, it turns out that they could not effectively inhibit the potent off-flavors *p*-cresol and *p*-methylacetophenone under 45 °C. Also, from the degradation profiles of both neral and geranial under 45 °C, faster degradation rates were observed of the emulsions with certain concentrations of $Q_{10}H_2$; only the 0.10% sample showed the inhibited rate compared with the control. These results, on the other hand, demonstrated the importance of the concentration of $Q_{10}H_2$ in the emulsion system to protect citral from degradation and the relevant off-flavor generation.

Effect of Ubiquinol-10 on Lipid Oxidation. Although lipid oxidation is not the main topic of this work, some of lipid degradation products were detected during the storage tests due to the incorporation of MCT and lecithin in the emulsion formulations, such as 2-heptanone, 1-octen-3-ol, and butanoic acid. The concentrations of the above-mentioned degradation compounds on day 30 (45 °C storage condition) are shown in Figure 8. Incorporation of $Q_{10}H_2$ decreased the level of 2-heptanone, with the sample containing 0.10% showing the minimum. For 1-octen-3-ol, only the 0.10% sample showed a decreased level. Other formulations all had relatively higher values than the control. The data of butanoic acid showed the sample with 0.01% $Q_{10}H_2$ had higher amounts than the control, and others all worked better than the control. These results are complicated and need more interpretations and support to better define the effect of $Q_{10}H_2$ and the role of its concentrations on lipid oxidation. It is widely accepted that addition of antioxidants into emulsions would retard lipid oxidation through inactivating free radicals, scavenging oxygen, and other oxidative molecules, whereas the concentration of antioxidant in specific systems and the potential switch from anti- to pro-oxidant at critical levels should draw more attention according to this research.

In summary, the effects of different concentrations of $Q_{10}H_2$ on citral's stability were systematically investigated and

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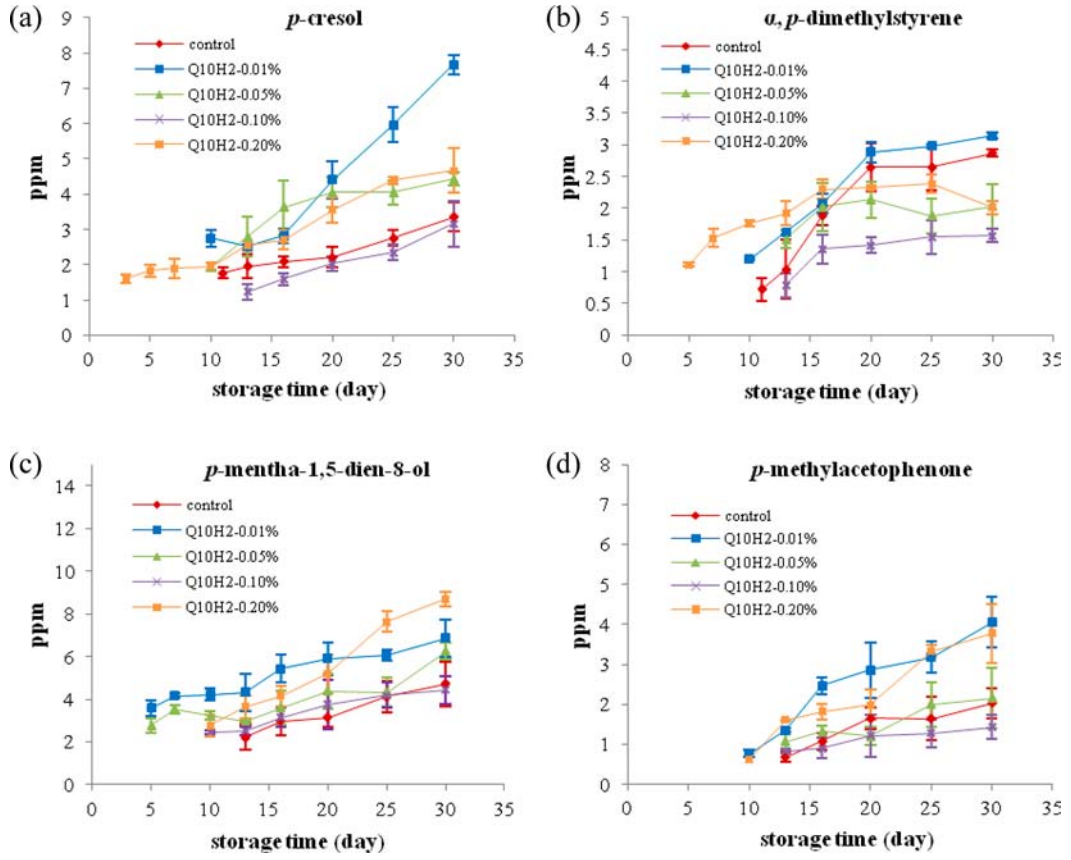

Figure 7. Generation profiles of four major citral degradation off-flavors in the emulsions stored at 45 °C: (a) *p*-cresol; (b) *α,p*-dimethylstyrene; (c) *p*-mentha-1,5-dien-8-ol; (d) *p*-methylacetophenone.

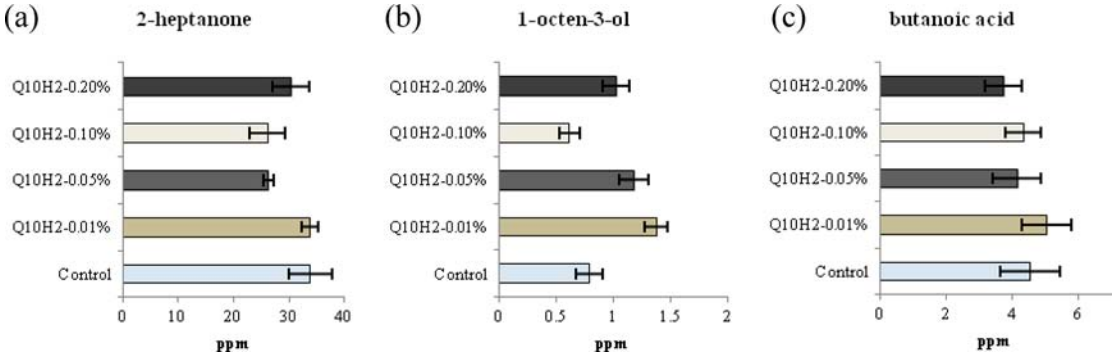

Figure 8. Concentrations of lipid degradation products from the emulsions stored at 45 °C for 30 days: (a) 2-heptanone; (b) 1-octen-3-ol; (c) butanoic acid.

compared in citral-loaded O/W nanoemulsions. Among all of the tested formulations, the optimum concentration of $Q_{10}H_2$ was determined to be 0.10 wt % ($Q_{10}H_2$/citral ratio 1:1), which can effectively protect citral from chemical degradation and oxidation in the system. However, 0.01 wt % $Q_{10}H_2$ was proven to have no protection effect and may induce the $Q_{10}^{\bullet-}/Q_{10}$ redox transition, which gave $Q_{10}H_2$ pro-oxidant properties. Further increasing the $Q_{10}H_2$ concentration beyond a certain value (e.g., 0.20 wt %) also hindered its efficacy. Major off-flavor compounds from citral degradation were monitored throughout the storage, and the major oxidation products, that is, *p*-cresol, *p*-methylacetophenone, *α,p*-dimethylstyrene, and some of the lipid degradation products could be properly inhibited with the optimum $Q_{10}H_2$ concentration. The oxidized form of Q_{10} was determined to have no protection effect on citral's chemical stability and off-flavor generation. This study provided detailed and quantitative data for reference of the CoQ_{10} incorporated emulsion systems in inhibiting citral's degradation and preventing the corresponding off-flavor generation. Besides the chemical antioxidant property in protecting sensitive flavor compounds, CoQ_{10} can also serve as a functional ingredient to improve human health. Thus, new strategies can be inspired for the food industry to develop multifunctional food products with improved sensory and human health.

AUTHOR INFORMATION

Corresponding Author

*(Q.H.) Phone: (848) 932-5514. Fax: (732) 932-6776. E-mail: qhuang@aesop.rutgers.edu.

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Notes

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