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# Copper-Catalyzed Oxidation of a Structured Lipid-Based Emulsion Containing α-Tocopherol and Citric Acid: Influence of pH and NaCl

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The effects of salt and pH on copper-catalyzed lipid oxidation in structured lipid-based emulsions were evaluated. Ten percent oil-in-water emulsions were formulated with a canola oil/caprylic acid structured lipid and stabilized with 0.5% whey protein isolate. α-Tocopherol and citric acid were added to the emulsions to determine how changes in pH or the addition of NaCl affected their antioxidant activity. The peroxide values and anisidine values of emulsions stored at 50 °C were measured over an 8-day period. Increased lipid oxidation occurred in the pH 7.0 emulsions and when 0.5 M NaCl was added to the pH 3.0 samples. Adding α-tocopherol, citric acid, or a combination of the two compounds slowed the formation of hydroperoxides and their subsequent decomposition products in pH 3.0 emulsions.

**KEYWORDS: Anisidine value; citric acid; copper; emulsions; lipid oxidation; peroxide value; structured lipids; α-tocopherol; whey protein isolate**

## INTRODUCTION

The ability of structured lipids (SLs) to combine the beneficial characteristics of component fatty acids into one triacylglycerol (TAG) molecule enhances the role fats and oils play in food, nutrition, and health applications (*1*). SLs might provide the most effective means of delivering desired fatty acids for nutritive or therapeutic purposes and for targeting specific diseases and metabolic conditions (*2*). Structured TAGs that contain medium-chain fatty acids (MCFAs) might provide a vehicle for rapid hydrolysis and absorption because of their smaller molecular size and greater water solubility in comparison to long-chain TAGs (*3*). The combination of an increased absorption rate for MCFAs and beneficial long-chain fatty acids (LCFAs) in one TAG should make SLs very attractive to the medical community and functional food manufacturers. However, the food industry has been slow to incorporate SLs into their product formulations. Before SLs are accepted by the food industry, a better understanding of the physicochemical mechanisms of lipid oxidation is needed for SL-based products.

Lipid oxidation is of great concern to the food industry because it causes changes in the quality attributes of foods, such as taste, texture, shelf life, appearance, and nutritional profile (*4*). Although substantial amounts of fats are consumed as food emulsions, most oxidation studies to date have been carried out in bulk oils. Food emulsions contain an array of components such as salt, sugar, metals, and emulsifiers that affect the rate of oxidation and can interfere with added antioxidants (*5*). Salts act as prooxidants or antioxidants depending on the nature of the system involved (*4*), so their effects must be evaluated on a case-by-case basis. Contradictory results have been reported regarding the effects of pH on lipid oxidation. Mancuso et al. (*6*) reported that oxidation increases with increasing pH, whereas other researchers have found the opposite effect (*7*, *8*). Iron-catalyzed lipid oxidation has been the subject of many recent studies (*9*−*13*). However, the rate of iron-mediated oxidation is much lower than that with an equal concentration of copper, and most foods contain 3.1−31 $\mu$M $Cu^{2+}$ (*14*). Therefore, more studies on copper-catalyzed lipid oxidation in various emulsion systems are needed.

The most commonly used method of retarding lipid oxidation in fatty foods is the addition of antioxidants (*15*). Antioxidants are classified according to their mechanisms of action as either primary or secondary antioxidants. Primary antioxidants are capable of accepting free radicals so that they can delay the initiation step or interrupt the propagation step of autoxidation (*4*). Tocopherols are highly potent primary antioxidants that are widely employed in the food industry because they are able to react with the lipid hydroperoxyl and with the alkoxyl radicals formed by the metal-catalyzed decomposition of hydroperoxides (*16*). Secondary antioxidants can retard lipid oxidation through a variety of mechanisms, including chelating metals, replenishing hydrogen to primary antioxidants, scavenging oxygen, and deactivating reactive species (*4*). Citric acid can chelate metal ions by forming bonds between the metal and the carboxyl or hydroxyl groups of the citric acid molecule. This secondary antioxidant is very effective in retarding the oxidative deterioration of lipids in foods and is commonly added to vegetable oils after deodorization (*17*). Combinations of chelators and radical scavengers often result in synergistic inhibition of lipid oxida-

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tion, because multicomponent antioxidant systems can inhibit oxidation at many different phases of oxidation. Citric acid can have a "sparing" effect on tocopherol. The chelator decreases the number of free radicals generated in a system by inhibiting metal-catalyzed oxidation (*18*). The physical state of lipid systems has been shown to affect antioxidant activities, and further studies are needed to better understand the effect of pH on interfacial lipid oxidation (*19*).

The objective of this study was to determine the effects of salt and pH on copper-catalyzed lipid oxidation in structured lipid-based emulsions. α-Tocopherol, citric acid, and a combination of the two compounds were incorporated into the emulsions to determine their effects on lipid oxidation and how changes in pH or the addition of NaCl affected their antioxidant activity.

## MATERIALS AND METHODS

**Materials.** Canola oil was purchased from a local supermarket. Caprylic acid (purity > 98%), citric acid, α-tocopherol, and cupric sulfate were purchased from Sigma Chemical Company (St. Louis, MO). An *sn*-1,3-specific immobilized lipase originating from *Rhizomucor miehei* (IM 60) was obtained from Novo Nordisk A/S (Bagsvaerd, Denmark). Whey protein isolate (WPI) (#27361) was provided by Land O'Lakes (St. Paul, MN). All other reagents were purchased from Fisher Scientific (Fair Lawn, NJ).

**Structured Lipid Production.** The SL was produced in a packed-bed bioreactor using optimal conditions previously reported (*20*) for reacting canola oil and caprylic acid. The product was purified using a KDL-4 short-path distillation unit (UIC Inc., Joliet, IL). The oil was passed through the distillation apparatus three times under the following conditions: holding temperature, 25 °C; heating oil temperature, 185 °C; cooling water temperature, 15 °C; pressure, <0.01 Torr. The purified SL product contained the following fatty acids (mol %): 37.3% C8:0, 1.8% C16:0, 1.7% C18:0, 47.3% C18:1, 8.9% C18:2, and 3.0% C18:3 as determined by gas chromatography of methyl esters (*21*).

**Emulsion Preparation.** Ten percent oil-in-water emulsions were prepared with the canola oil/caprylic acid SL, 10 mM phosphate buffer, and 0.5% whey protein isolate (WPI). α-Tocopherol was premixed into the SL, while citric acid was added to the buffer. Total antioxidant addition levels were 0.02% of the oil weight in each emulsion. Mixed antioxidant systems contained equal parts of tocopherol and citric acid. NaCl (0.5 M) was added to the appropriate emulsion samples. The pH of the emulsions was adjusted to 3.0 or 7.0 by addition of HCl or NaOH, respectively. The emulsions were passed through a high-pressure valve homogenizer (Emulsiflex, C5, Avestin, CA) six times at 10 000 psi. All samples were held on ice during processing. Sodium azide (1 mM) was added to the emulsions to slow microbial growth. Cupric sulfate (50 μM) was added to the emulsions immediately prior to storage. Particle size distribution was measured by integrated light scattering (Mastersizer S, Malvern Instruments, Malvern, U.K.) using standard optical parameters to ensure that similar apparent particle diameters ($D_{3,2}$ values ranged from 0.46 to 1.85 μm) were achieved in the emulsions during homogenization. This was done to eliminate the potential for droplet size differences to affect lipid oxidation rates.

**Oxidation Experiments.** Emulsion samples were allowed to oxidize in a 50 °C covered water bath for 8 days. The primary and secondary oxidation products were measured in the emulsion samples after 0, 1, 2, 4, and 8 days of storage. Oil was extracted from the emulsions by adding isooctane/2-propanol (3:2, v/v), vortexing three times for 10 s each, and centrifuging for 5 min at 1000 rpm. The clear upper layer was collected, and the solvent was evaporated under nitrogen. Peroxide values (PVs) were determined using the International Dairy Foundation method described in detail by Shantha and Decker (*22*). Anisidine values (AVs) were determined according to AOCS Official Method Cd 18-90 (*23*). This method determines the amount of aldehyde (principally 2-alkenals and 2,4-alkadienals) present in the oil (*24*).

**Statistical Analysis.** All experiments were performed on duplicate samples. Statistical analyses were conducted with the SAS (*25*) software package. Analyses of variance were performed by ANOVA procedures.

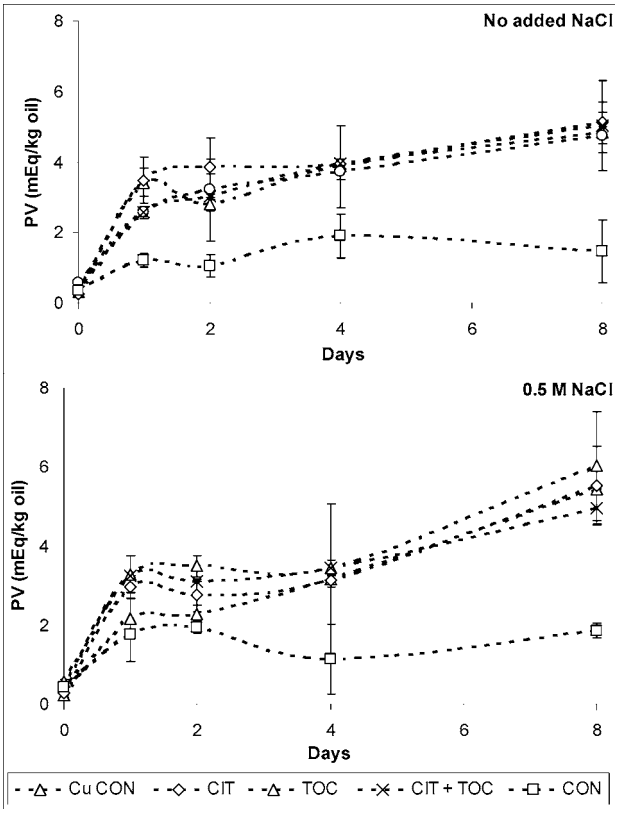


**Figure 1.** Copper-catalyzed changes in peroxide values (PVs) over time in structured lipid-based emulsions at pH 7.0. Cu CON, contained 50 μM cupric sulfate; CIT, contained 50 μM cupric sulfate and citric acid (0.02 wt % of oil); TOC, contained 50 μM cupric sulfate and α-tocopherol (0.02 wt % of oil); CIT + TOC, contained 50 μM cupric sulfate, citric acid (0.01 wt % of oil), and α-tocopherol (0.01 wt % of oil); CON, contained no added cupric sulfate. Data shown are the averages of duplicate samples held at 50 °C. Error bars on chart represent standard deviations.

Significant differences ($p < 0.05$) were determined by the least-squares difference method.

## RESULTS AND DISCUSSION

Control (CON) samples that did not contain copper were included in this experiment to verify that the addition of 50 μM cupric sulfate affected the rate of lipid oxidation of the SL-based emulsions. Significantly ($p < 0.05$) greater PVs and AVs were observed in the copper-catalyzed control (Cu CON) samples compared to their CON counterparts on all days of storage (excluding day 0) for emulsions at pH 7.0 (**Figures 1** and **3**). The addition of copper significantly ($p < 0.05$) increased the PV of the emulsions at pH 3.0 on days 4 and 8 of the study (**Figure 2**). The AVs were significantly ($p < 0.05$) increased by the addition of copper to the pH 3.0 emulsions on all days of storage (excluding day 0) (**Figure 4**). The significant increase in lipid oxidation due to copper addition was expected because transition metals are known to accelerate lipid oxidation reactions by hydrogen abstraction and peroxide decomposition, which results in the formation of free radicals (*18*).

**Effect of pH and NaCl on Oxidation.** The pH of the emulsions had a significant ($p < 0.05$) effect on the primary (PV) and secondary oxidation (AV) products on days 1, 2, 4, and 8 of the study. On the final day of analysis, the hydroperoxide levels were greater in the copper-catalyzed emulsions at pH 3.0 than in their pH 7.0 counterparts (**Figures 1** and **2**). However, the final anisidine values were higher in the pH 7.0 emulsions (**Figures 3** and **4**). Similar pH effects on lipid

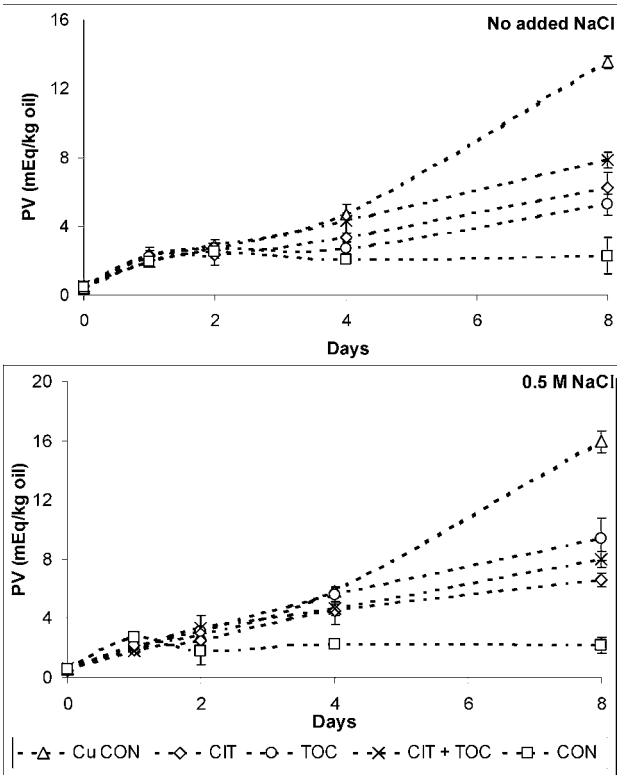


**Figure 2.** Copper-catalyzed changes in peroxide values (PVs) over time in structured lipid-based emulsions at pH 3.0. (See Figure 1 for sample abbreviations.) Data shown are the averages of duplicate samples held at 50 °C. Error bars on chart represent standard deviations.

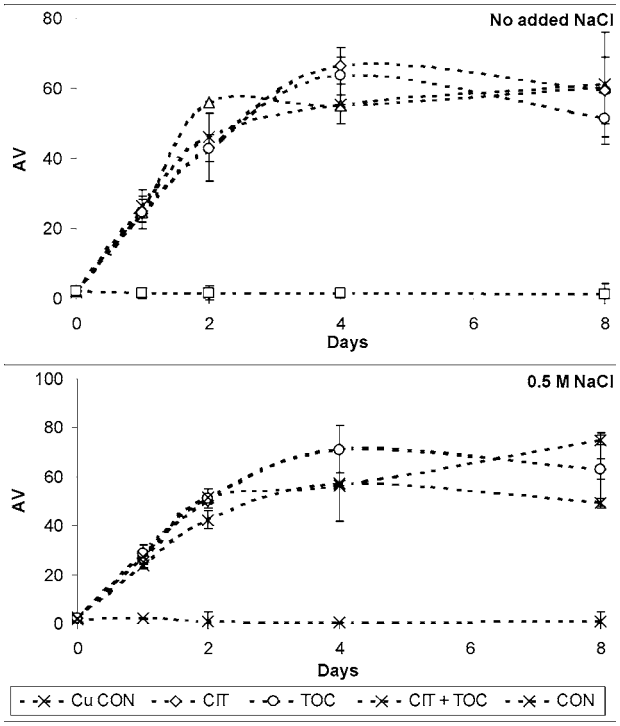


**Figure 3.** Copper-catalyzed changes in anisidine values (AVs) over time in structured lipid-based emulsions at pH 7.0. (See Figure 1 for sample abbreviations.) Data shown are the averages of duplicate samples held at 50 °C. Error bars on chart represent standard deviations.

oxidation have been reported for salmon-oil-based emulsions. Lipid peroxide formation in this system was greater at pH 3.0 than at 7.0, although differences in thiobarbituric acid reactive

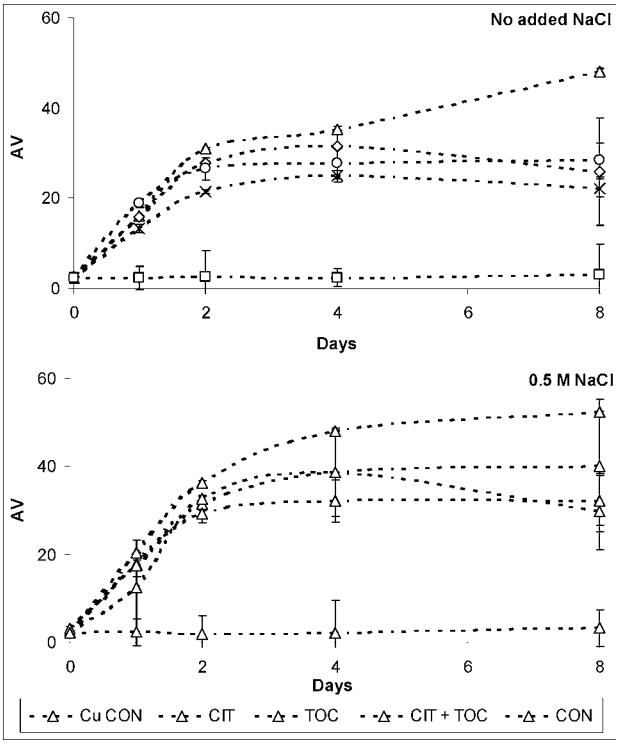


**Figure 4.** Copper-catalyzed changes in anisidine values (AVs) with time in structured lipid-based emulsions at pH 3.0. (See Figure 1 for sample abbreviations.) Data shown are the averages of duplicate samples held at 50 °C. Error bars on chart represent standard deviations.

substances (TBARS) formation were not detected between the two pH's (*26*).

When taken alone, the hydroperoxide data indicate that low pH further promotes copper-catalyzed lipid oxidation in emulsions. Hydroperoxides decompose rapidly in the presence of heat or transition metals via the Fenton reaction (*15*). Therefore, when the results of the primary and secondary oxidation studies are combined, it seems more likely that the lower peroxide values at pH 7.0 were due not to decreased lipid oxidation rates, but rather to copper's increased ability to decompose the lipid hydroperoxides into secondary products at pH 7.0. The emulsion droplets carry a negative charge at pH 7.0, because the anionic whey proteins are above their isoelectric point (pI) of ~5.0 (*27*). When the surface charge of dispersed lipids as micelles is negative, metal-catalyzed oxidation rates are much higher than they are at positively charged interfaces (*18*). This effect presumably exists because of the electrostatic attraction between the positively charged metal and the negatively charged emulsion droplet membrane (*28*). Whey proteins are below their isoelectric point (pI) in the pH 3.0 systems (*27*), thereby producing positively charged emulsion droplets. Hence, at pH $< 5.0$, the positively charged copper ions can no longer bind to the emulsion droplets, which explains the decreased secondary oxidation rates in the pH 3.0 emulsions compared to their pH 7.0 counterparts. Because of this phenomenon, food manufacturers might be able to lower the pH below the pI in protein-stabilized emulsions as a strategy for decreasing copper-catalyzed lipid oxidation. A similar strategy was proposed previously for iron-catalyzed lipid oxidation (*9*).

Sodium chloride did not significantly affect the hydroperoxide formation rate after 8 days of storage at 50 °C at either pH (**Figures 1** and **2**). However, the effect on secondary oxidation was statistically significant ($p < 0.05$) (**Figures 3** and **4**). At pH 3.0, the addition of 0.5 M NaCl generally resulted in an

increase in secondary oxidation in the emulsions containing copper. Previous work on emulsions containing iron ($Fe^{2+}$) indicated that concentrations of 87 and 170 mM NaCl increased lipid oxidation (*29*). NaCl stimulation of oxidation could be due the ability of chloride ions to increase the catalytic activity of metals or the NaCl-induced changes in the physical properties of the emulsion droplets, such as reduction in the thickness of the double layer (*29*). Because salt is a ubiquitous ingredient in foods, processors must be mindful of its ability to promote lipid oxidation in the presence of metals and incorporate antioxidants into their product formulations accordingly.

**Effect of α-Tocopherol and Citric Acid on Oxidation.** Radical scavengers and metal sequestrants were the antioxidant types utilized for this study. The former class, which includes tocopherols, does not block the initial generation of radicals, but merely reacts with them to form less reactive radicals. The second category consists of chelating agents, such as citric acid, that either precipitate the metal or suppress its reactivity by occupying all coordination sites (*14*).

The activity of the antioxidants in this study varied according to the pH of the emulsion, but was not influenced by the NaCl addition. At pH 3.0, the addition of tocopherol, citric acid, or a combination of the two compounds resulted in significantly ($p < 0.05$) lower PVs and AVs compared to the Cu CON samples (**Figures 2** and **4**). Although antioxidant systems containing antioxidants with different mechanisms of action are currently hailed as the most effective strategy for inhibiting lipid oxidation (*18*), tocopherol and citric acid did not have synergistic effects on hydroperoxide or aldehyde formation in this study. Instead, all antioxidants (tocopherol, citric acid, and a combination of the two) had similar effects on the rate of lipid oxidation (**Figures 2** and **4**). At pH 7.0, none of these added antioxidants significantly affected the oxidation rates.

Huang et al. (*19*) reported that the oxidative stability of α-tocopherol was highest at pH 3.0 and lowest at pH 7.0 in Tween 20 micelle solutions. Their study showed that α-tocopherol was 90% depleted in the pH 7.0 systems after 8 days of storage at 60 °C, whereas only 20% depletion occurred in the pH 3.0 systems. Additionally, the hydrogen-donating ability of α-tocopherol at the oil−water interface was increased by protonation of its phenolic hydroxyl group at low pH. These authors concluded that the antioxidant activity of α-tocopherol depends on both its hydrogen-donating activity and its depletion rate (*19*). A similar rate of tocopherol depletion might have occurred in the pH 7.0 SL-based emulsions and led to the decreased antioxidant effect of α-tocopherol on day 8 in the pH 7.0 emulsions compared to the pH 3.0 emulsions.

The increased efficacy of citric acid at low pH might have resulted from the increased solubility of transition metals in those environments (*27*). Additionally, the activity of copper can be controlled by binding to proteins (*18*). Therefore, in the pH 7.0 systems, the copper ions might have been bound to the negatively charged emulsion droplets and, therefore, unable to complex with the citric acid. However, when the pH was lowered to 3.0, the copper was released from the proteins and became exposed to the citric acid. This might account for the increased antioxidant activity observed in the pH 3.0 emulsions that contained citric acid.

Copper-catalyzed lipid oxidation in SL-based emulsions appears to be influenced by the system pH and the presence of NaCl. Emulsions at pH 7.0 had greater rates of lipid oxidation than their pH 3.0 counterparts. NaCl addition increased secondary oxidation of the acidic emulsions. Adding α-tocopherol, citric acid, or a combination of the two compounds to the pH 3.0 emulsions successfully slowed the formation of hydroperoxides and their subsequent decomposition into secondary carbonyl compounds. The results of this study will aid in understanding the complexity of lipid oxidation in real food emulsions prepared with canola oil/caprylic acid structured lipids and might expedite the incorporation of structured lipids into food products.

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