Copper(II) Ascorbate: A Novel Food Preservation System

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The addition of ascorbic acid (0.05–0.40%) and trace amounts of copper gluconate (10–65 ppm) to foods and beverages effectively removed oxygen dissolved in the food within 1–5 min and depleted the headspace oxygen within a few days. Unlike other cations tested, copper catalyzed ascorbate-mediated reduction of oxygen to water without the concomitant generation of hydroxyl radical and other activated oxygen species. Therefore, copper(II) ascorbate protected foods against lipid peroxidation, discoloration, and other oxidative damage. Furthermore, copper(II) ascorbate inhibited bacterial growth and thereby increased the microbiological stability of high-moisture foods.

Keywords: *Antioxidant; ascorbic acid; copper; dehydroascorbic acid; food preservative; iron; oxidation; oxygen; radical*

INTRODUCTION

The ubiquity of oxygen and transition metal catalysts in food products poses a continuous challenge to the food technologist and process engineer. The presence of iron in close vicinity with molecular oxygen causes the formation of various activated oxygen species, such as hydroxyl radical, hydrogen peroxide, and superoxide anion radical. These highly reactive chemicals in turn lead to the indiscriminate oxidation of vitamins, lipids, and food polymers, decreasing both initial consumer acceptance of the food and available shelf life.

The various approaches to combat oxidative action inside food and beverage packages have been reviewed previously (Rice, 1986). In short, to mitigate these detrimental effects, many food products are formulated with an antioxidant, i.e., a metal sequestrant or a radical scavenger. The efficacy of chelating agents such as EDTA or pyrophosphate is strongly system-dependent. In some foods the addition of EDTA has been shown to even accelerate food deterioration by solubilizing iron (Mahoney and Graf, 1986). One of the most effective chelating antioxidants is phytic acid since it occupies all six iron coordination sites and therefore precludes the availability of a reactive aquo site (Graf et al., 1984, 1987; Graf and Eaton, 1990). However, while phytic acid is in use in several foreign countries, it is not FDA-approved in the United States.

The addition of radical scavengers effectively prolongs shelf life of most foods. Unlike metal sequestrants, though, they fail to inhibit the formation of radical species but merely react with them in competition with other food ingredients. Therefore, radical scavengers are being consumed during storage and their efficacy decreases over time. Furthermore, some of the most effective scavengers are currently falling into disfavor and may be banned in the near future due to alleged carcinogenic properties. Examples include BHA and BHT.

Additional protection against oxidative damage is often afforded by physical removal of headspace oxygen through vacuum processing or modified atmosphere packaging. However, these methods are only partially effective due to high levels of oxygen dissolved in the food. Similarly, oxygen depletion using reactive iron pouches, such as Ageless or FreshMax, suffer from the same shortcomings due to their low rate of oxygen consumption inside the food itself.

To circumvent these common problems encountered during actual food formulation, we designed a chemical oxygen removing system using GRAS- and FDA-approved ingredients. The present paper describes the chemical properties and food applications of a novel copper(II) ascorbate food preservation system containing a combination of ascorbic acid (0.05–0.40%) and copper gluconate (10–65 ppm).

MATERIALS AND METHODS

Materials. Materials were purchased from the following sources: ascorbic acid, citric acid, EDTA, ovalbumin, sodium ascorbate, sodium phytate, and Tris (Sigma Chemical Co.); copper gluconate (Pfizer Chemical Co.). All other chemicals were of analytical grade. Ingredients used for salsa and guacamole were of food grade and FDA approved.

Methods. *Dissolved Oxygen.* The rate of oxygen consumption in solution was determined in a thermostated 2.0-mL vessel using an O_2-selective Clark electrode and a Yellow Springs Instrument oxygen meter model 53. The concentration of dissolved oxygen in millimeters of mercury was monitored continuously on a strip-chart recorder. First-order rate constants were calculated from linear regression analyses of kinetic semilog plots.

Headspace Oxygen. Headspace O_2 was determined by withdrawing an air aliquot with a hypodermic syringe and injecting the sample into a Toray oxygen analyzer.

Refrigerated Mexican Salsa. Ten kilograms of control and copper(II) ascorbate salsa were formulated as shown in Table 1. The batches were heated to 80 °C and then cooled to 24 °C. Sodium ascorbate and copper gluconate were added and blended into the batch. Aliquots of 176 g were placed into round 7.9-cm-diameter O_2-impermeable plastic containers with 48 mL of headspace. An aluminum foil cover was heat-sealed onto the tubs. The samples were stored under refrigeration for 18 months.

Guacamole. The guacamole was formulated as shown in Table 2. Each batch was blended very gently by hand to minimize physical damage to avocados and tomatoes. Copper gluconate was added immediately before the food product was packaged to avoid any premature oxidation of ascorbic acid to dehydroascorbic acid. Aliquots of 160 g were placed into 6-oz O_2-impermeable plastic containers which provided a headspace of ap-

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Table 1. Mexican Salsa Formulas

ingredient	control (g)	copper(II) ascorbate (g)
diced tomatoes	6364	6354
diced onions	869	869
diced green chili peppers	844	844
tomato paste	723	723
water	685	685
diced green onions	217	217
vinegar	98	98
sucrose	73	73
salt	49	49
jalapeño puree	29	29
sodium ascorbate	0	20
carrageenan	20	20
garlic powder	15	15
potassium sorbate	5	0
sodium benzoate	5	0
cumin	2	2
Mexican oregano	2	2
copper gluconate	0	0.4

Table 2. Guacamole Formulas[a]

ingredient	control (g)	copper(II) ascorbate (g)
avocados	632.0	627.0
tomatoes	218.8	218.8
preservative-free lemon juice	76.3	76.3
red onions	49.2	49.2
olive oil	21.2	21.2
ascorbic acid	0	5.0
garlic cloves	2.5	2.5
copper gluconate	0	0.04

[a] a_w = 0.92, pH 4.1.

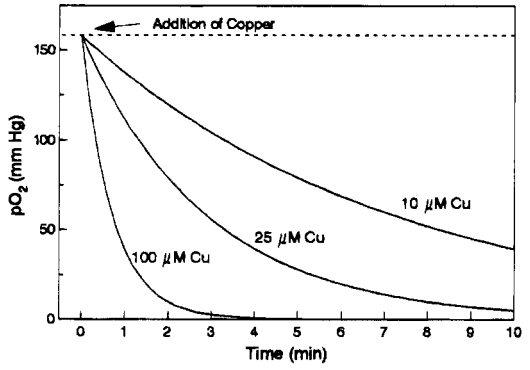

Figure 1. Oxygen utilization by copper(II) ascorbate. The oxygen tension was measured in 1 mM sodium ascorbate, 10–100 μM copper gluconate, and 50 mM Tris, pH 7.4 at 25 °C, using a Clark electrode. The dashed line denotes oxygen utilization by ascorbic acid alone.

proximately 50 mL. The dishes were heat-sealed with aluminum foil and stored in the refrigerator for 90 days. At the end of this storage period all samples were subjected to a chemical, microbiological, and sensory evaluation.

Statistical Analysis. Data collected from this study were evaluated by analysis of variance and least significant difference using the Statistical Analysis System (SAS Institute, Cary, NC). The Statgraphics software package was used for the generation of regression curves.

RESULTS

Copper(II) ascorbate effects rapid removal of oxygen from an aqueous solution as demonstrated in Figure 1. In the absence of added copper, ascorbic acid remains fairly stable, as demonstrated by the lack of oxygen utilization.

Table 3. Effect of Copper Chelating Agents on the Rate of Oxygen Utilization

chelating agent	first-order rate constant k^a (min^{-1})
none	1.5 ± 0.2
glycine	2.2 ± 0.2
histidine	1.5 ± 0.1
EDTA	0.03 ± 0.01
phytic acid	1.8 ± 0.2

[a] Dissolved oxygen was determined at 25 °C using a Clark electrode in solutions containing 40 ppm of copper gluconate, 0.2% sodium ascorbate, 2 mM chelating agent, and 50 mM Tris, pH 7.4.

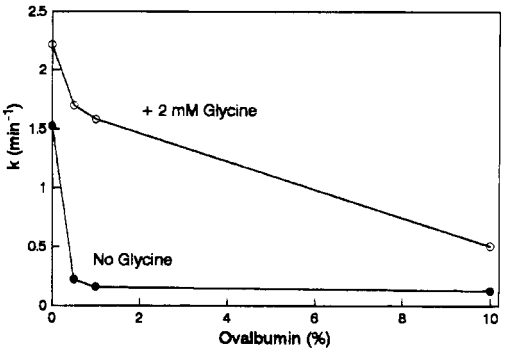

Figure 2. Inhibitory effect of ovalbumin on O_2 utilization by copper(II) ascorbate in the absence and presence of 2 mM glycine. Dissolved O_2 was determined at 25 °C using a Clark electrode in solutions containing 40 ppm of copper gluconate, 0.2% sodium ascorbate, 50 mM Tris, pH 7.4, and increasing concentrations of ovalbumin. First-order rate constants were calculated as described under Methods.

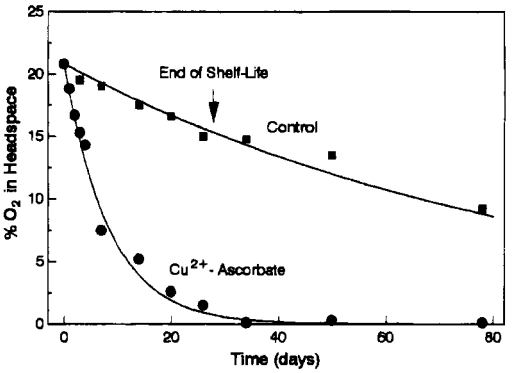

Figure 3. Effect of 40 ppm of copper gluconate and 0.2% sodium ascorbate on oxygen depletion in the headspace of airtight containers containing Mexican salsa. All samples were stored at 5 °C. The solid lines were calculated from the first-order rate equation using the following rate constants: k = 0.12 day^{-1} (control); k = 0.011 day^{-1} (copper(II) ascorbate).

Copper chelating agents exhibited variable effects on copper(II) ascorbate as summarized in Table 3. Whereas EDTA almost completely inhibited the oxygen removal, histidine and phytic acid failed to significantly affect the catalytic rate. The amino acid glycine, however, accelerated the reaction by almost 50%. Furthermore, it substantially obliterated the inhibitory effect of ovalbumin by competing for free copper with a high affinity as shown in Figure 2. Several proteins tested were found to avidly bind copper ions and thereby completely suppress copper(II) ascorbate mediated oxygen consumption.

The effectiveness of copper(II) ascorbate was tested in several actual food systems. Figure 3 shows the large effect of 0.2% sodium ascorbate and 40 ppm of copper gluconate on the shelf-life stability of Mexican salsa. Oxygen utilization from the headspace of the control salsa samples represents oxidation of the salsa, leading

Table 4. Effect of Copper(II) Ascorbate on Guacamole Stability[a]

attribute	control	copper(II) ascorbate
color	dark green/brown	light green
odor	sour, tart, spoiled	fresh
flavor	note determined	fresh
pressure in container	ballooned	vacuum
texture	gas bubbles	uniform
syneresis	+	−
visible mold growth	+	−
total plate count (organisms/g)	4×10^5	6×10^3

[a] The copper(II) ascorbate sample contained 0.50% ascorbic acid and 0.004% copper gluconate. Both samples were stored in O_2-impermeable plastic containers at 4 °C and evaluated after 90 days.

to a number of undesirable organoleptic changes. Although copper(II) ascorbate effected O_2 depletion in the headspace only slowly due to the high product viscosity, it removed all dissolved O_2 within a few minutes of production. This complete deoxygenation afforded protection against discoloration, off-flavor generation, syneresis, and microbial growth for more than 400 days, whereas the control reached the end of its acceptable shelf life after 27 days. After 390 days, no growth of yeast, molds, or lactics (<10/g) had occurred in the copper(II) ascorbate samples, although both potassium sorbate and sodium benzoate were omitted from the formula—unlike in the control samples. Similar results were obtained on frozen pizza sauce.

The shelf life of guacamole is extremely short due to both chemical and microbiological instability of avocado when exposed to oxygen. Copper(II) ascorbate was found to remove all dissolved oxygen within a few minutes and completely inhibited polyphenol oxidase-catalyzed browning of guacamole for at least 90 days as shown in Table 4. Growth of microorganisms gives rise to large gas pockets in guacamole, ballooning of the container, and off-flavors. Copper(II) ascorbate strongly inhibited bacterial, yeast, and mold growth, maintained a uniform product texture, and achieved a visible vacuum in the packaging container due to removal of all headspace oxygen (Table 4).

DISCUSSION

The mechanism of oxygen removal by copper(II) ascorbate involves the catalytic four-electron reduction of O_2 without the concomitant generation of reactive radical species. The reduction of 0.5 mol of oxygen to 1 mol of water requires the stoichiometric oxidation of 1 mol of ascorbic acid to dehydroascorbic acid as shown in

$$AA + {}^1/_2O_2 \rightarrow DHAA + H_2O \quad (1)$$

where AA is ascorbic acid and DHAA is dehydroascorbic acid.

Dehydroascorbic acid may become oxidized further to CO_2 and other byproducts. The formation of these secondary metabolites renders the use of copper(II) ascorbate unsuitable for clear beverages, since these types of products assume a slight reddish-brown hue after extended shelf life.

While iron also catalyzes O_2 removal, the two transition metals use different catalytic mechanisms for this conversion. Very low levels of copper rapidly deoxygenate an aqueous ascorbate solution to form water and dehydroascorbic acid without the development of detectable amounts of intermediate radical species. Iron, however, catalyzes the redox transfer between oxygen and ascorbic acid at a much slower rate via the well-understood Haber–Weiss cycle. This mechanism involves activation of oxygen and generation of the highly reactive hydroxyl radical, $^{\bullet}OH$. Activated iron species, such as ferryl and perferryl ions, are also invoked to explain subsequent lipid peroxidation and other oxidative events. The role of metals in oxygen radical reactions has been thoroughly reviewed elsewhere (Aust et al., 1985), but the mechanistic differences between iron- and copper-catalyzed oxygen reduction by ascorbic acid are not understood.

In many foods ascorbic acid has been shown to exacerbate oxidative damage due to its activation of oxygen and iron as discussed above, particularly when high concentrations of iron are maintained in a soluble form by EDTA (Mahoney and Graf, 1986). In fact, the combination of iron, EDTA, and ascorbic acid is a potent oxidant system used in many organic oxidation reactions. Nevertheless, ascorbic acid also is a radical scavenger and has been employed for the preservation of some seafoods (Deng et al., 1978). High endogenous copper levels in many marine organisms may possibly potentiate the protective action of ascorbic acid in these foods.

Several O_2-absorbing pouches are currently on the market, including Ageless from Mitsubishi and FreshMax. However, all of these sachets suffer the same drawbacks due to their low rate of oxygen removal. Direct addition of copper(II) ascorbate to the food was determined to effect oxygen depletion in the headspace at twice the rate of sachets. Furthermore, dissolved oxygen is removed within a few minutes by the direct addition of copper(II) ascorbate, while it is removed at the same rate as headspace oxygen in the sachet method.

This large difference in the rate of oxygen removal arises from the opposite driving forces in the two systems. In the direct addition method, the dissolved oxygen tension is zero after a few minutes. This sets up a steep oxygen gradient across the air–liquid interface which rapidly drives oxygen from the headspace into the solution. In the pouch method, however, the oxygen tension in solution and headspace remain virtually identical throughout shelf life. Slow oxygen removal from the headspace by the sachet creates an infinitesimal gradient which forces reequilibration between headspace and solution by slow movement of oxygen from solution to headspace.

The direct addition of copper(II) ascorbate provides immediate protection against oxidative damage. After 1–5 min, the O_2 within the food becomes depleted, and the food remains completely deoxygenated despite the residual oxygen content in the headspace, since every O_2 molecule is removed instantaneously as it impinges on the food surface. In the sachet method, however, the oxygen content decreases only slowly over an extended time period. After 10 days, certain types of food may still contain 1% O_2, which is sufficient to support peroxidation of lipids, enzymatic browning, and growth of yeasts and molds.

Another advantage of the direct addition method is the bactericidal effect of copper(II) ascorbate, whereas the pouch method can inhibit only strict aerobes by virtue of its oxygen deprivation. The microcidal properties of copper(II) ascorbate, particularly in combination with hydrogen peroxide, are well-known and have been

utilized previously in water disinfectant applications (Ragab-Depre, 1982).

Copper(II) ascorbate offers strong commercial viability as a novel food preservation system (Graf, 1993, 1994) in colored beverages and high-moisture foods. The addition of copper(II) ascorbate affords a foolproof and rapid method for detecting leaks and misformulated batches, since samples that contain both copper and ascorbic acid develop a strong visible vacuum after 24 h. Furthermore, its low cost, FDA approval, processing flexibility, and high efficacy provide development opportunities to food and beverage corporations.

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