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Protection of Ascorbic Acid from Copper(II)−Catalyzed Oxidative Degradation in the Presence of Fruit Acids: Citric, Oxalic, Tartaric, Malic, Malonic, and Fumaric Acids

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PROTECTION OF ASCORBIC ACID FROM COPPER(II)–CATALYZED OXIDATIVE DEGRADATION IN THE PRESENCE OF FRUIT ACIDS: CITRIC, OXALIC, TARTARIC, MALIC, MALONIC, AND FUMARIC ACIDS

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Vitamin C (ascorbic acid) is sensitive to oxygen and heat, and can be degraded during unsuitable conditions of cooking and preservation methods of food. The nutritional quality of food may be adversely affected due to transition metal–catalyzed oxidative degradation of ascorbic acid. The effect of Cu(II) complexes formed with protective organic acids widely found in fruits on the autoxidation of ascorbic acid was investigated, where ascorbic acid was quantified with the Cupric Reducing Antioxidant Capacity assay. The Cu(II)-catalyzed oxidation at pH 4.5 followed first-order kinetics with respect to ascorbic acid concentration. The rate constants of ascorbic acid oxidation increased with Cu(II) concentration for a fixed level of organic acid. The catalytic oxidation of ascorbic acid was inhibited in the presence of stable Cu(II)-organic acid binary complexes, and accordingly, the inhibitive potency of citric acid (highest) was followed by oxalic acid, malonic acid, malic acid, tartaric acid, and fumaric acid in this order. The rate-limiting step of ascorbic acid oxidation was the formation of a ternary transition complex between Cu(II), hydrogen ascorbate, and carboxylic acid. The inhibitive activity of Cu(II)-ligand complexes increased as the binary complex stability increased. The presence of fruit acids in foodstuffs may help protect nutritional ascorbic acid values by preventing metal–catalyzed oxidation reactions.

Keywords: ***Ascorbic acid, Cu(II)–catalyzed oxidation, Antioxidant protection, Fruit acids, Food stability.***

INTRODUCTION

Ascorbic acid (AA), commonly known as Vitamin C, is one of the most important water-soluble vitamins in the human diet, because it helps the body in forming connective tissue, bone, teeth, blood vessel walls, and it assists the body in assimilating iron and amino acids.[1] A diet deficient in vitamin C may cause a person to develop scurvy. Vitamin C lowers the incidence of mortality from two of the most prevalent diseases, cardiovascular disease and cancer.[2] AA is an essential vitamin for human health. It is widely found in

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vegetables and fruits, and is capable of quenching free radical species. It also prevents worsening of taste and colour in various foodstuffs and fruits. It can either be derived from natural sources or be chemically synthesized. Since humans cannot synthesize vitamin C in their bodies, they have to supply it from nutritional sources. All fresh vegetables, fruits, and meat contain certain amounts of vitamin C. Since this vitamin is sensitive to air oxygen and heat, it can be relatively rapidly degraded during unsuitable conditions of cooking and preservation of food. When transition metal ions, such as Fe(III) and Cu(II), are reduced by ascorbate, their lower oxidation states (e.g., Fe(II) and Cu(I), respectively) may give rise to undesired Fenton reactions with oxygen or hydrogen peroxide producing hydroxyl radicals that cause tissue damage and adversely affect the nutritional quality of beverages,[3] as exemplified with the following reactions:[4]

$$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^{\bullet} + OH^{-},$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^{-}.$$

Copper is an important transition metal found in many food and pharmaceutical products.[5] Ascorbic acid is a natural component of many foods and is often added to food and beverages as a vitamin supplement and antioxidant. AA is a good indicator of the retention of nutritional quality of fruits and vegetables, because it is highly sensitive to temperature, humidity, and air.[6] Vitamin C in a fortified formula was shown to decrease more rapidly with increasing water activity of the medium.[7] An additional advantage of AA is its synergistic protective action on food plant flavonoids from oxidative degradation.[8] Vitamin C regenerates vitamin E by reducing vitamin E radicals formed when vitamin E scavenges the oxygen radicals. This interaction between vitamin C and vitamin E radicals can take place not only in homogeneous solutions but also in liposomal membrane systems.[9] Some evidence suggests that ascorbate protects against lipid peroxidation by regenerating the reduced form of α-tocopherol.[10] AA content is taken as an indication of fruit freshness and retention of other components, because contrary to other organic acids and sugars, vitamin C is quite unstable with respect to the activity of ascorbic acid oxidase enzyme and to the reaction with oxygen in the presence of heavy metal ions and light.[11,12] Thus, AA—due to its oxidation to dehydroascorbic acid by molecular oxygen—is the most affected hydrophilic antioxidant during processing of fruits and vegetables.[12] Therefore, mechanisms governing its protection in plant food are worthy of exploration.

Transition metal ions, especially in their lower oxidation states, are capable of forming reactive oxygen species (ROS) in the presence of molecular oxygen through Fenton-like reactions.[4] When natural defenses of the organism (of enzymatic, non-enzymatic, or dietary origin) are overwhelmed by an excessive generation of ROS, a situation of oxidative stress occurs in which cellular and extracellular macromolecules (proteins, lipids, and nucleic acids) can suffer oxidative damage causing tissue injury.[13] Generally, the stability of AA in pharmaceutical formulations increases if traces of transition metal ions that catalyze AA autoxidation are sequestered. Ligands with strong complexing groups are able to sequester metal ions that are responsible for the catalytic oxidation of AA.[14]

It is known that metal ions affect the degradation rate of antioxidants like lycopene during the processing of tomato products.[15] Traces of transition metal ions like Cu(II) and Fe(III) also act as catalysts in the autoxidation of ascorbic acid. Because of the biochemical importance of AA in the food and pharmaceutical industries, Cu(II)-catalyzed oxidation of

AA by molecular oxygen has been extensively studied.[16,17] It has been found that the catalytic efficiency of Cu(II) complexes depends on the nature of ligands and on the coordination geometry of the metal ion.[18] The complexation of Cu(II) by certain ligands, such as aminopolycarboxylates of EDTA, CDTA, NTA, and citrate, acetate, tartarate, phthalate, histidine and glutathione, modifies its catalytic activity.[16,17,19–22] Khan and Martell[17] showed that the stability of the cupric chelate is closely related to its catalytic activity in AA oxidation, i.e., the higher the chelate stability, the greater inhibition of AA autoxidation will be observed.

It is important to maintain ascorbic acid stability in beverages, and the rate of consumption of dissolved oxygen at a fixed temperature in packaged orange juices was shown to be directly dependent on the concentration of L-ascorbic acid.[23] The principal organic acids used to enhance beverage flavours are citric, tartaric, fumaric, and phosphoric acids.[24] Citric acid is the most widely used acid while malic and tartaric acid are important natural compounds found in fruits that are used along with fumaric acid in fruit-flavoured drinks.[24] Oxalic acid has a high affinity to form metal-chelate complexes with multivalent cations, especially copper ions.[25] Tartaric, malic, and fumaric acids all enhanced Fe(II) and Fe(III) uptake by human epithelial cell lines, but tartaric acid with two hydroxyl groups was superior to malic acid, which has only one.[26] Citric acid acts as a sequestering agent of metallic ions, primarily due to its ability to chelate these ions, which can accelerate lipid oxidation processes of fats and oils, browning, complex formation (turbidity), and decolourations.[27] Washing with 1% citric acid followed by an anti-browning treatment with 1.5% sodium ascorbate was the most effective treatment for preserving the colour, antioxidant, and antimicrobial quality of mushrooms.[28]

The objective of this study was to determine the effects of organic acids widely found in fruits, namely citric, oxalic, tartaric, malic, malonic, and fumaric acids, on the kinetics of uncatalyzed and Cu(II)-ion catalyzed autoxidation of AA in aerated and acetate-buffered solution at 25°C and $I = 0.1$ ionic strength (adjusted with KNO_3) using Cu(II)-Nc as the reagent for spectrophotometric AA assay.[29] Investigations were carried out at a pH of 4.5 (adjusted with acetic acid/acetate buffer) common in fruit juice-based soft drinks. Besides quantifying AA in the presence of flavonoids,[30] the Cu(II)-Nc reagent was applied to the assay of a wide variety of antioxidants (including flavonoids, vitamin E, β-carotene, uric acid, and bilirubin), forming the basis of the CUPRAC (CUPric Reducing Antioxidant Capacity) method used to measure the total antioxidant capacity[31–33] and applied to certain food plants.[34]

MATERIALS AND METHODS

Chemicals and Instruments

All chemicals were of analytical reagent grade. L-ascorbic acid (mentioned as ascorbic acid: AA in the text) was used without purification. AA, fumaric acid, citric acid, malic acid, malonic acid, tartaric acid, oxalic acid, neocuproine (Nc: 2,9-dimethyl-1,10-phenanthroline), $CuCl_2.2H_2O$, sodium acetate, ammonium sulfate, and ethanol were purchased from Sigma Aldrich (Steinheim, Germany); acetic acid, $CuSO_4.5H_2O$, KNO_3 from E. Merck, Darmstadt, Germany. The Cu-carboxylic acid chelates were prepared in 0.01 M acetate buffer at pH = 4.5, prepared according to Hsieh and Harris.[35] Cu(II)-Nc (neocuproine) reagent was prepared from $CuCl_2$ and neocuproine, and were used for AA assay as described elsewhere.[29] Deionized distilled water was used throughout.

The CUPRAC spectrophotometric determination of AA was carried out using a mixture of Cu(II) and neocuproine as the chromogenic oxidant.[29] The pH of the medium was 7.0 (adjusted with ammonium acetate buffer), and AA was determined by measuring the absorbance of the reduction product, the Cu(I)-neocuproine chromophore, at 450 nm against a reagent blank. Preliminary experiments combined with previous experience for the CUPRAC method[29,31,32] confirmed that the spectrophotometric assay of AA was not adversely affected from the presence of citric, oxalic, tartaric, malic, malonic, and fumaric acids tested in this work as possible autoxidation inhibitors.

The absorbances were measured and spectra taken with a Perkin Elmer Lambda 25 UV-Vis spectrophotometer (Perkin Elmer Inc., Waltham, MA, USA) using a pair of matched quartz cuvettes of 1 cm thickness. The pH measurements were made using a Selectra pH 2001 pH-meter (JP Selecta S.A., Abrera, Barcelona, Spain). The ionic strength of the reaction medium was maintained at $I = 0.1$ (adjusted with KNO_3) in order to keep the activity coefficients of the revelant species constant during the kinetic process.[3] All experiments were carried out at 25°C. The aqueous solutions in which catalyzed oxidation of AA was carried out were 100% saturated with oxygen at room temperature; the oxygen concentrations were measured at a level of 8.1 mg/L with the aid of an HI 9146-04 oxygen meter (PCE Instruments UK Limited, Southampton, UK).

Sample Preparation and Kinetic Measurements

All stock solutions were prepared daily unless otherwise stated, and double distilled water was used throughout. AA solution during the time of measurements was protected from daylight. For the determination of undegraded AA with the Cu(II)-Nc reagent, the following solutions were used: 7.5×10^{-3} M Nc solution in 96% (v/v) ethanol, 1.0×10^{-2} M $CuCl_2$ aqueous solution, and 1 M ammonium acetate aqueous solution.

The stock solution of 1.57×10^{-4} M of copper(II) sulfate, 5.67×10^{-3} M of AA, 1.0×10^{-2} M acetate buffer (at pH = 4.5), and 1.0×10^{-2} M of the corresponding fruit acids were prepared in water. In 100 mL flasks of kinetic measurement, the final concentrations of constituents in the mixture were 0.1 M KNO_3, 0.0015 M acetate buffer, 1.0×10^{-3} M fruit acid, 0.785×10^{-7}–6.28×10^{-7} M copper(II) sulfate, and added lastly was 5.67×10^{-4} M ascorbic acid. Reaction was timed with a chronometer when AA was added to the sample solution. During the reaction period, a stream of air (flowrate: 60 L/h) was passed through the flask and the solution was saturated with oxygen. The purged air was presaturated with water vapour passed through a wash bottle. Since the rate of reaction was slow compared to the rate of dissolution of oxygen, the reaction solution was saturated with oxygen at all times. After the addition of ascorbic acid, an aliquot of 0.6 mL was taken every 5–10 min (for a total period not exceeding 40 min) for spectrophotometric measurement, and the pH of the sample solution was recorded. The rate of oxidation was measured by quantifying the residual AA during the course of oxidation. All tests were made in triplicate, and the results were presented as arithmetic means. Uncatalyzed and Cu(II)-fruit acid chelate-catalyzed AA (5.67×10^{-4} M) oxidations were investigated in mixture solutions containing Cu(II) at 0, 0.785, 1.57, 3.14, 4.71, 6.28×10^{-7} M, and carboxylic acid at 1.0×10^{-3} M.

Procedure for AA Determination

The method used to determine the ascorbic acid concentration is based on the oxidation of AA to dehydroascorbic acid with a Cu(II)-Nc reagent in an ammonium

acetate-containing medium at pH 7.0, where the maximum absorption wavelength of the formed bis(neocuproine) copper(I) chelate is 450 nm.[29] For this purpose, one mL of $CuCl_2$ solution was placed in a test tube, and then 1 mL of Nc, and 1 mL ammonium acetate to bring the final pH to 7.0; 1.4 mL of water and 0.6 mL AA solution were finally added by mixing in this order. After 2 min, the absorbance at 450 nm was recorded against a reagent blank. All solutions during the course of Cu(II)-catalyzed autoxidation of AA in the absence or presence of fruit acid chelators were analyzed for AA in this manner. Since flavonoids were absent in the tested solutions of this work, the conventional CUPRAC procedure was used in AA determinations without a need for preliminary extraction of flavonoids as their La(III) complexes.[30]

Statistical Analysis

Descriptive statistical analyses were performed using Excel software (Microsoft Office 2002, Microsoft Corp., Redmond, WA, USA) for calculating the means and the standard error of the mean. Results were expressed as the mean ± standard deviation(s). Using SPSS software for Windows (version 13, IBM Corp., Armonk, NY, USA), the data were evaluated by ANOVA.[36]

RESULTS AND DISCUSSION

Reaction Mechanisms

The consecutive pK_a (i.e., Log acidity constant) values of ascorbic acid are 4.1 and 11.79, respectively.[37] Basically, the undissociated (H_2A) and monoanionic (HA^-) forms of ascorbic acid predominate in solution at the working pH of 4.5. This pH was selected first because it is a common pH for fruit juice-based beverages, and second because lower and higher pH experiments yielded much slower and faster degradation reactions of AA, respectively. The oxidation of ascorbic acid in oxygen-saturated aqueous solution was assumed to follow a first order reaction.[16,17,38] The reaction rate can be expressed as:

$$-d[H_2A]/dt = k[H_2A] \tag{1}$$

where $[H_2A]$ is the concentration of the remaining ascorbic acid (proportional to A_{450} recorded in the Cu(II)-Nc spectrophotometric assay) during the course of autoxidation, t is the time and k is the first order rate constant that can be calculated from a plot of log $[H_2A]$ vs. time. This plot gives a straight line with a slope–$k/2.303$. If k_d is defined as the difference between the first order rate constants in the presence and absence of cupric ion (as defined by Khan and Martell),[16] then the specific rate constant (K) independent of the cupric ion (catalyst) concentration is expressed with the equation:

$$K = k_d/[\text{Cu(II)}]. \tag{2}$$

The effectiveness of the tested fruit acids in retarding Cu(II)-catalyzed oxidative degradation of AA could only be compared on the basis of their specific rate constants; therefore, such a comparison was undertaken to yield more realistic and correct results.

Kinetics and Rate Constants

In order to follow the uncatalyzed and Cu(II)-catalyzed oxidation of ascorbic acid in acetate-buffered solution, the log C_{AA} vs. time data were analyzed with a linear regression approach. Kinetic curves for copper-catalyzed autoxidation of AA without any fruit acid at Cu(II) concentrations of 0.785, 1.57, 3.14, 4.71, and 6.28 $\times$ 10^{-7} (10^{-7} being the common exponent of all concentrations) are given in Fig. 1. The corresponding curves in the presence of citric acid as Cu(II)-complexing agent, i.e., for the Cu(II)−citric acid−ascorbic acid system at pH 4.5 using 1.0 $\times$ 10^{-3} M citric acid and the same variable concentrations of Cu(II) as in Fig. 1, are depicted in Fig. 2a. Analogic curves are given for other fruit acids, such as oxalic acid (Fig. 2b), malonic acid (Fig. 2c), malic acid (Fig. 3a), tartaric acid (Fig. 3b), and fumaric acid (Fig. 3c), in order to observe the inhibitive effects of these carboxylic acids on the Cu(II)-catalyzed autoxidation of AA. The exposure times in the experimental design were varied between 25 and 40 min, until AA did not remain in solution (practically, this corresponded to an end point where the final detectable absorbance at 450 nm could be observed for the fastest degraded solution among a series). The experiment without protective fruit acids (Fig. 1) were completed earlier than those with fruit acids (Figs. 2 and 3), because AA degraded faster in the former case.

The rate of change of log C_{AA} with time (i.e., slope of linear curves in Figs. 2 and 3) increased as the Cu(II) concentration was increased for a given fixed concentration (1.0 mM) of the fruit acid or for its absence (Fig. 1), showing the prooxidant behavior of Cu(II) in the presence of O_2 and the inhibition of AA autoxidation with Cu(II)-carboxylic acid chelate formation. The specific rate constants (K) independent of Cu(II) concentration (calculated with the aid of Eq. (2)) were found from the slopes of k_d vs. [Cu(II)] curves, as shown in Fig. 4 for the tested carboxylic acid systems with Cu(II) + AA at pH 4.5.

The specific rate constant (K) found with the help of regression analysis[3,36] of curves presented in Fig. 4 are tabulated in Table 1. The K values found for all Cu(II)-chelate systems formed with the tested fruit acids were lower than that of Cu(II) alone. Generally, for all fruit acids, K values significantly decreased as the fruit acid concentration was increased (data not shown), confirming inhibition of Cu-catalyzed autoxidation with increased Cu-binding by organic acids widely found in fruits. The order of K values for the studied carboxylic acids in pH 4.5 acetate buffer was: citric acid < oxalic acid <

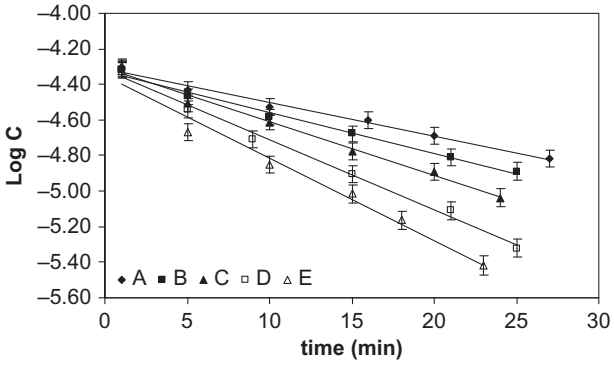


Figure 1 Kinetic curves for copper-catalyzed autoxidation of AA, without fruit acid; Cu(II) at [(A: 0.785, B: 1.57, C: 3.14, D: 4.71, E: 6.28) $\times$ 10^{-7} M].

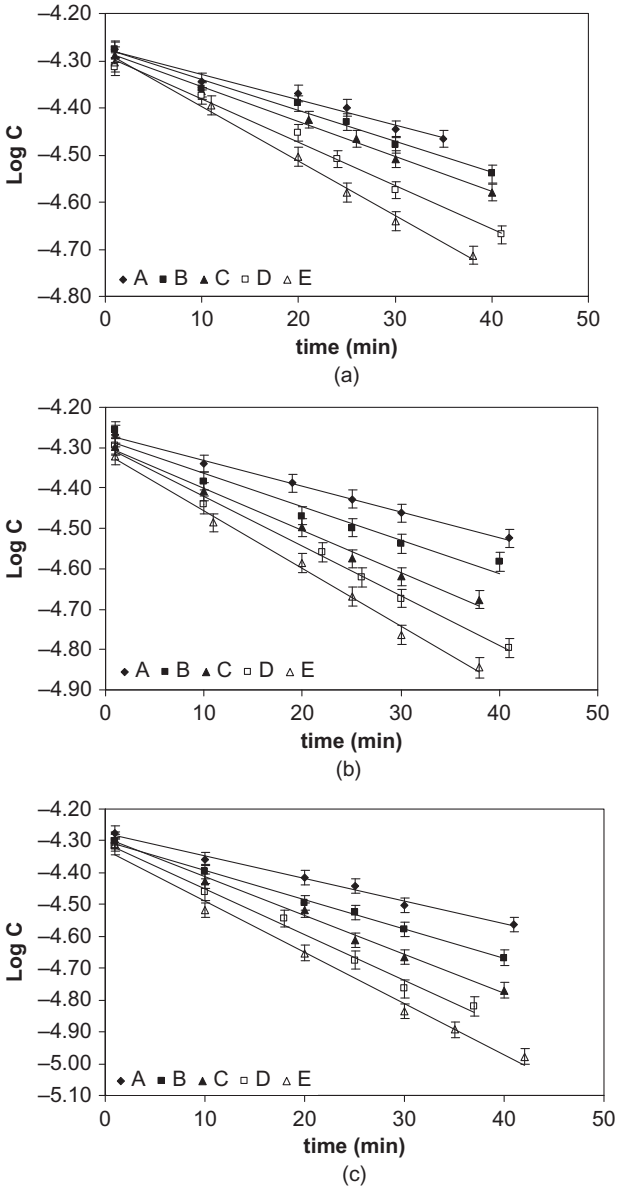


Figure 2 Kinetic curves for copper-catalyzed autoxidation of AA: (a) citric acid at 1.0×10^{-3} M; (b) oxalic acid at 1.0×10^{-3} M; (c) malonic acid at 1.0×10^{-3} M; Cu(II) at [(A: 0.785, B: 1.57, C: 3.14, D: 4.71, E: 6.28) $\times$ 10^{-7} M].

malonic acid < malic acid < tartaric acid < fumaric acid < no fruit acid (only acetate buffer). In a literature report, the effect of natural chelating agents on the solubility of mineral elements in oat bran were studied, and citric acid was found to be much more potent than malic acid for metal binding affinity.[39]

Enzymatic browning is ubiquitous in fruits and vegetables, adversely affecting color, taste, nutrition, and safety.[40] Enzymatic and non-enzymatic browning reactions of amino

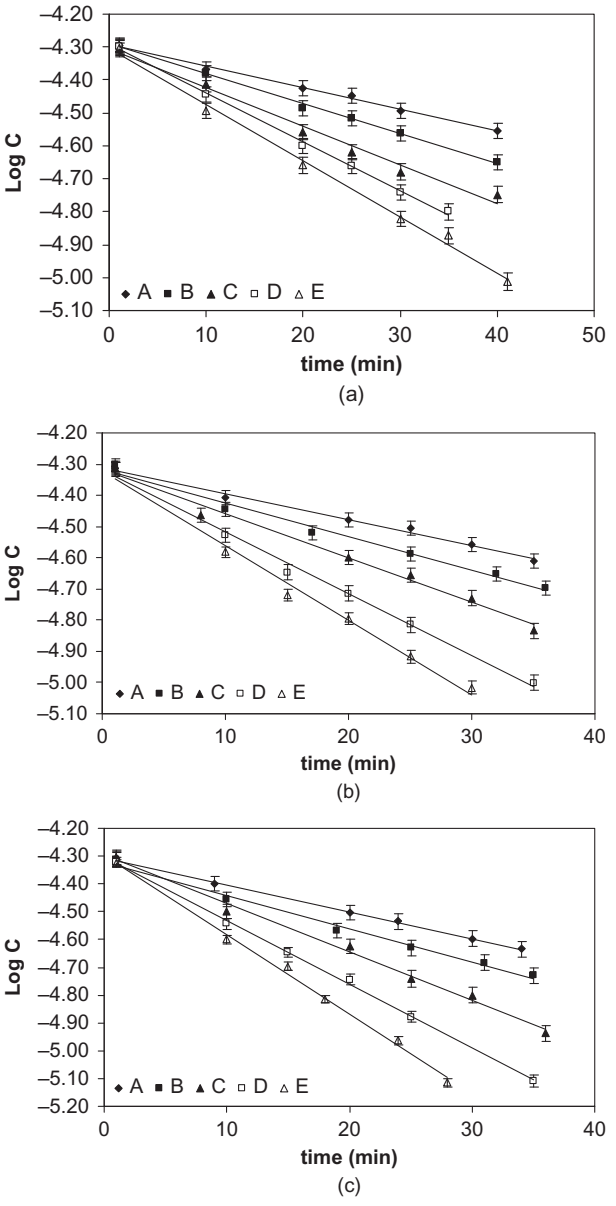


Figure 3 Kinetic curves for copper-catalyzed autoxidation of AA: (a) malic acid at 1.0×10^{-3} M; (b) tartaric acid at 1.0×10^{-3} M; (c) fumaric acid at 1.0×10^{-3} M; Cu(II) at [(A: 0.785, B: 1.57, C: 3.14, D: 4.71, E: 6.28) $\times 10^{-7}$ M].

acids and proteins with carbohydrates, oxidized lipids, and oxidized phenols cause deterioration of food during storage and processing.[41] In respect to the inhibitory effects of various antibrowning agents on apple slices, oxalic, tartaric, and malonic acids constituted the best inhibitor group that showed only a slight change in colour, whereas citric and malic acids belonged to a medium inhibitor group, followed by weak inhibitors of acetic

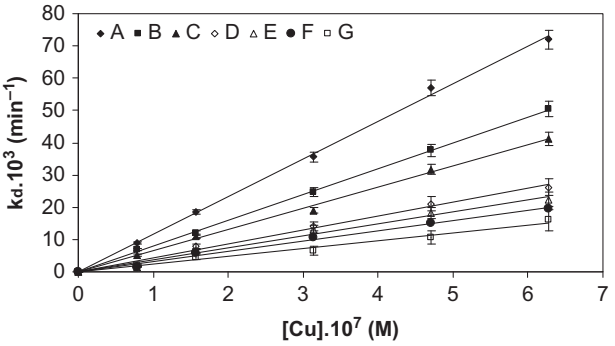


Figure 4 k_d as function of Cu(II) concentration in 1.0×10^{-3} M carboxylic acid containing solutions buffered to pH 4.5 with acetate. [A: without fruit acid, B: fumaric acid, C: tartaric acid, D: malic acid, E: malonic acid, F: oxalic acid, G: citric acid]. k_d: difference between first-order rate constant in the presence and absence of Cu(II); slopes of lines yield; K: specific rate constants.

Table 1 The specific rate constants at pH 4.5 for autoxidation of ascorbic acid catalyzed (inhibited) by fruit acid-Cu(II) complexes (fruit organic acids each at 1 mM concn.).

Name of organic acid	Specific rate constant $K \pm s_k$ (M^{-1} min^{-1})
Citric acid	$(2.42 \pm 0.16) \times 10^4$ ($r^2 = 0.9827$)
Oxalic acid	$(3.19 \pm 0.14) \times 10^4$ ($r^2 = 0.9922$)
Malonic acid	$(3.74 \pm 0.23) \times 10^4$ ($r^2 = 0.9848$)
Malic acid	$(4.32 \pm 0.20) \times 10^4$ ($r^2 = 0.9913$)
Tartaric acid	$(6.56 \pm 0.17) \times 10^4$ ($r^2 = 0.9972$)
Fumaric acid	$(7.99 \pm 0.09) \times 10^4$ ($r^2 = 0.9995$)

For Cu(II) alone (without fruit acid, in pH: 4.5 acetate buffer), $K \pm s_k$ (M^{-1} min^{-1}) = $(11.64 \pm 0.25) \times 10^4$ ($r^2 = 0.9982$).
s_k = The standard deviation of K values, r^2 = linear regression coefficient (squared).
K = Specific rate constant independent of concentration, $K = k_d/[\text{Cu(II)}]$.

and fumaric acids.[42] Citric acid showed a synergistic antibrowning effect when used along with oxalic acid.[42] However, it should be borne in mind that the mechanism of antibrowning activity based on polyphenol oxidase enzyme inhibition is quite different from that of catalytic (inhibitive) AA autoxidation involving transition metal ion chelation. Khan and Martell[17] proposed the following mechanism for Cu(II) chelate (ML^{n+})-catalyzed oxidation of ascorbic acid (H_2A):

$$H_2A \leftrightarrow HA^- + H^+$$

$$HA^- + ML^{n+} \leftrightarrow MHLA^{(n-1)+}$$

$$MHLA^{(n-1)+} \rightarrow MHLA^{\bullet(n-1)+}$$

$$MHLA^{\bullet(n-1)+} \xrightarrow{\text{rapid}} A^{\bullet-} + ML^{(n-1)+}$$

$$A^{\bullet-} + ML^{n+} \xrightarrow{\text{rapid}} A + ML^{(n+1)+}$$

where the catalyst ML^{n+} may be regenerated by oxidation of $ML^{(n-1)+}$ with dissolved O_2 (here, H_2A denotes ascorbic acid, and A, the 2-e oxidized product, dehydroascorbic acid). In this multi-step process, the slow (rate-determining) step is the formation of $MHLA^{(n\text{-}1)+}$ ternary transition complex from monohydrogen ascorbate (HA^-) and Cu(II)-chelate (ML^{n+}). If the binary M-L chelate is too stable, the ternary transition complex may not form. Weak bonding in the transition complex may lead to easy deformation of this complex during electron transfer. In this respect, Khan and Martell[17] postulated that the autoxidation rate of AA in the presence of cupric chelates would decrease with increasing stability of the metal complexes, and found a linear relationship in the catalytic activity of chelates as a function of logarithmic stability constant of the corresponding complex. Again, in accordance with this hypothesis, the copper complexes of Cu(II)-phenanthroline, Cu(II)-dipyridyl,[17] and Cu(II)-thiourea[43] were completely inactive in catalyzing ascorbic acid autoxidation. In the case of thiourea, it was shown that copper(II) is both reduced and complexed by thiourea to form relatively stable Cu(I)-thiourea complexes.[44] Thus, as the stability of Cu(II)-L increases, the tendency of CuL to form a mixed ligand chelate with hydrogen ascorbate (HA^-) decreases.[17]

The logarithm of first cumulative stability constants (Log β_1) at different ionic strengths of the studied fruit acid complexes with Cu(II) were as follows: oxalic acid (6.7),[45] tartaric acid (3.2),[20] citric acid (6.03),[46] malic acid (3.6),[47] malonic acid (5.0),[48] and fumaric acid (2.51).[45] A theoretical analysis comprising these considerations is given below:

For 1:1 complexes, we have:

$$\mathrm{M} + \mathrm{L} \leftrightarrow \mathrm{ML}$$

$$\beta_1 = [\mathrm{ML}]/([\mathrm{M}][\mathrm{L}]) \tag{3}$$

and for 1:2 complexes, we have:

$$\mathrm{M} + 2\mathrm{L} \leftrightarrow \mathrm{ML_2}$$

$$\beta_2 = [\mathrm{ML_2}]/([\mathrm{M}][\mathrm{L}]^2) \tag{4}$$

where [L] is $C_{H2L}.\alpha_L$, (where C_{H2L}: total carboxylic acid concentration; α_L: relative abundance of L).

For diprotic organic acids (H_2L), $\alpha_L = K_{a1}.K_{a2}/([H^+]^2 + K_{a1}[H^+] + K_{a1}K_{a2})$, and for triprotic organic acids (H_3L), $\alpha_L = K_{a1}.K_{a2}K_{a3}/([H^+]^3 + K_{a1}[H^+]^2 + K_{a1}K_{a2}[H^+] + K_{a1}K_{a2}K_{a3})$. Thus,

$$\beta_1 = [\mathrm{ML}]/([\mathrm{M}]\ C_{H2L} \cdot \alpha_L) \qquad \text{and } \beta_1' = \beta_1\alpha_L \tag{5}$$

$$\beta_2 = [\mathrm{ML_2}]/([\mathrm{M}]\ C_{H2L}^2 \cdot \alpha_L^2) \quad \text{and } \beta_2' = \beta_2\alpha_L^2 \tag{6}$$

where $\beta_1'$and β_2' are the conditional first and second cumulative stability constants of the ML and ML_2 complexes, respectively, calculated at the working pH. Specific inhibition (I_s) of copper-catalyzed autoxidation of ascorbic acid by the tested carboxylic acids are given in Table 2. The inhibition ratio, I, can be defined as:

Table 2 Specific inhibition (I_s) of copper−catalyzed autoxidation of AA at pH = 4.5 by the tested fruit acids, with the relevant acidity and stability constants.

Fruit acid	pK_{a1}	pK_{a2}	pK_{a3}	Log β_1[a]	Log β_2[a]	α_L	I_s
Oxalic acid	1.2	4.2	—	6.16[c] (5.98)	8.50[c] (8.15)	6.7×10^{-1}	726
Tartaric acid	3.2	4.8	—	3.2 (2.72)	5.11 (4.15)	3.3×10^{-1}	436
Citric acid	2.99[b]	4.3[b]	5.65[b]	6.03 (4.64)	10.4 (7.66)	5.8×10^{-1}	792
Malic acid	3.46	5.1	—	3.6 (2.87)	—	1.88×10^{-1}	629
Malonic acid	2.8	5.7	—	5.0 (3.76)	—	5.8×10^{-2}	679
Fumaric acid	3.03	4.44	—	2.51 (2.23)	—	5.24×10^{-1}	314

[a]The values in parentheses are the corresponding conditional stability constants at pH = 4.5, i.e., Log β_1' and Log β_2', respectively, calculated with the aid of Eqs. (5) and (6).
[b]Taken from Buffle et al.[50]
[c]Taken from Dean.[49]

$$I = (K_{\text{Cu(alone)}} - K_{\text{(complex)}})/K_{\text{Cu(alone)}} \tag{7}$$

The specific inhibition (I_s) is then defined as the mean inhibition ratio per unit concentration (1 M) of fruit acid, i.e., $I_s = I/C_{H2L}$.[3] The data depicted in Table 2 clearly show that the catalytic autoxidation-degradation of AA can be effectively inhibited by the tested organic carboxylic acids. An excellent correlation existed between the conditional first cumulative stability constants (Log β_1') of the Cu(II)-fruit acid complexes and specific inhibition (I_s) such that:

$$I_s = 104 \text{ Log } \beta_1' + 211;\ r = 0.798. \tag{8}$$

Although there were not enough data for correlating the conditional second cumulative stability constants (Log β_2'), it was also apparent from Table 2 that the higher Log β_2' yielded the higher I_s. Acetic acid, being a monoprotic acid, has $\alpha_L = K_a/(K_a + [H^+])$, and the α_L value at pH = 4.5 is 0.344; this makes the conditional stability constant of the tabulated value of Log $\beta_1 = 2.16$[49] as Log $\beta_1' = 1.70$. All the tested fruit acids having higher Log β_1' than acetic acid yielded higher I_s than the buffer medium and, therefore, a stronger protective effect for AA. Inspection of values tabulated in Table 2 makes it clear that tartaric acid had the second lowest value of Log $\beta_1' = 2.72$. In accordance with Eq. (8), the experimentally found inhibitive effect of citric acid—as reflected in I_s values—on oxidative degradation of AA was highest, followed by oxalic acid, malonic acid, malic acid, tartaric acid, and fumaric acid, in this order (i.e., tartaric acid had the second lowest potency, conforming to the order of its Log β_1'). Thus, it was shown in this work that the presence of the tested carboxylic acids in fruits may help protect nutritive values by protecting ascorbic acid, an essential freshness parameter of fruits, from oxidative degradation.

CONCLUSIONS

The Cu(II)-catalyzed autoxidation of ascorbic acid (AA) was first-order with respect to AA concentration. The rate constants of AA oxidation increased with Cu(II) concentration for a fixed level of organic acid. The effect of the concentrations of organic acid and Cu(II) on the autoxidation of AA can be interpreted with the plausible formation of a ternary transition complex formed between monohydrogen ascorbate and Cu(II)-organic

acid chelate. Since the catalytic oxidation of AA was inhibited in the presence of stable Cu(II)-organic acid complexes, the highest conditional stability constant was encountered in the citric acid complex of Cu(II), resulting in a stronger inhibition with this complex. The inhibitive potency of citric acid was followed by oxalic acid, malonic acid, malic acid, tartaric acid, and fumaric acid in this order. Since the tested organic fruit acids had a stronger complexation ability for AA than acetate buffer, they were found to better protect AA than acetic acid in the presence of Cu(II). The inhibitive activity and therefore the AA protective effect of Cu(II)-ligand complexes increased as the complex stability (specifically, the conditional first cumulative stability constant of the binary complex at the working pH) increased. The presence of fruit acids in foodstuffs may help protect nutritional ascorbic acid values by preventing transition metal-catalyzed oxidation reactions.

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