Effect of Sucrose on Oxygen Uptake of Ascorbic Acid in a Closed Aqueous System

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The effect of sucrose on the oxygen uptake kinetics of ascorbic acid (A) in a closed aqueous system was studied at different temperatures (26.5, 30, and 33 °C) and pH levels (3.0–5.0). The reactions generally followed a pseudo-first-order reaction with respect to dissolved oxygen. The activation energy of A oxidation in sucrose solutions was greater than that in solutions containing no sucrose at all pH levels. Approximately 1 mol of oxygen was used per mole of A at the early stage of the noncatalyzed oxidation of A in various solutions. Two effects of sucrose on the A oxidation in the closed homogeneous system were suggested. First, a pH-independent physical hindrance effect retarded A oxidation and, second, a pH-dependent catalytic effect accelerated oxidation due to metal impurities found even in analytical grade sucrose.

Sucrose is commonly present as a sweetening ingredient in fruit juices and drinks containing ascorbic acid. The effect of sugar on the stability of ascorbic acid in liquid media has been studied. Sucrose has long been reported to inhibit ascorbic acid oxidation in Cu-catalyzed or noncatalyzed reactions, and the mechanism of this protective effect was attributed to either the viscosity or the Cu-binding power of the sugar (Birch and Pepper, 1983; Lin and Agalloco, 1979; Kyzlink and Curda, 1970; Miller and Joslyn, 1949a,b; Chamrai, 1941). Recently, Hsieh and Harris (1987a) found that in an open-air system at pH 3.2 sucrose accelerated the degradation of ascorbic acid when its viscosity effect was diminished by vigorous shaking. This destructive effect of sucrose was attributed to the trace amounts of metal impurities found in sucrose because metal ions, e.g., cupric ion, have been shown to have great catalytic effect on the oxidation of ascorbic acid even at trace levels (Silverblatt et al., 1943) and it is very difficult to completely eliminate the metal impurities and their catalytic effect from water and food ingredients in liquid systems. Furthermore, the study (Hsieh and Harris, 1987a) shows that the destructive effect of sucrose on ascorbic acid was much more pronounced in copper-catalyzed solutions containing 1–5 ppm of added copper. Sucrose (10%) did not show any Cu-binding ability, as was postulated in early literature, but enhanced the catalytic effect on ascorbic acid oxidation by its physical bulking effect and decreased water activity in solutions which increased cupric ion activity (Hsieh and Harris, 1987b). These results shed new light on former studies that illustrated the effect of sucrose on ascorbic acid oxidation in a heterogeneous oxygen-diffusion mediated system. To clearly define the reaction mechanism of the effect of sucrose on ascorbic acid oxidation, it is also important to investigate the effect of sucrose in a closed homogeneous nondiffusion mediated aqueous system. Therefore, the present research was designed to study oxygen uptake in the early stages of ascorbic acid oxidation in a closed homogeneous aqueous system which simulated the practical quiescent condition of canned or bottled liquid food systems. The specific objectives of this research were to use kinetic analysis to reveal the effects of sucrose on the oxidation of ascorbic acid in solutions with reference to pH, temperature, and acetate buffer. The molar ratio of consumed oxygen to ascorbic acid in the early stage of the oxidation was calculated. An understanding of the physical and chemical roles of sucrose on ascorbic acid stability under various conditions provides an important basis for practical application in food processing, storage, and formulation.

MATERIALS AND METHODS

Sucrose (Sigma, containing <0.2 ppm of copper impurities) and all reagents were of analytical grade and used without further purification. All solutions were prepared using double-distilled deionized water. Sodium acetate–acetic acid buffers (0.1 M) were selected because they could be used over the whole pH range of the experiments. The following buffers were used: buffer I, pH 3.2, 0.262 g of sodium acetate and 5.551 mL of glacial acetic acid per liter; buffer II, pH 4.5, 3.173 g of sodium acetate and 3.516 mL of glacial acetic acid per liter; buffer III, pH 6.0, 7.806 g of sodium acetate and 0.275 mL of glacial acetic acid per liter.

The sucrose solutions were prepared by dissolving the required amount of sucrose in water or in 0.1 M acetate buffer (w/v) at the desired pH. The percent sucrose was selected to simulate the concentrations commonly found in beverages. Prior to the addition of ascorbic acid to the reaction mixture, the sucrose solutions were brought to desired working temperature in a water bath.

The pH values of solutions reported in the text were those observed before and after the addition of ascorbic acid and cooled at 25 °C. The experiments were carried out under a constant laboratory illumination condition.

Oxygen Uptake Kinetics. Oxygen uptake of ascorbic acid in solutions was studied in a closed system in which the oxygen and ascorbic acid were homogeneously distributed in the solution throughout the reaction. Experiments were conducted at various temperature (26.5, 30, and 33 °C) levels. These temperatures represent environmental conditions under which food and beverages containing sucrose might be held. A pH range of 3–5 was chosen because most foods that would be at risk for ascorbic acid loss would be at these levels. The rate of oxygen uptake by ascorbic acid was determined by measuring the dissolved oxygen concentration in solutions at time intervals. An oxygen electrode was calibrated and used to monitor the dissolved oxygen level in sample solutions. The procedures of oxygen uptake measurement were as described by Hsieh and Harris (1991). Sample solutions were presaturated with oxygen by bubbling compressed air into solutions for 1 h. The aerated solutions were placed in a constant-temperature water bath at the desired temperature

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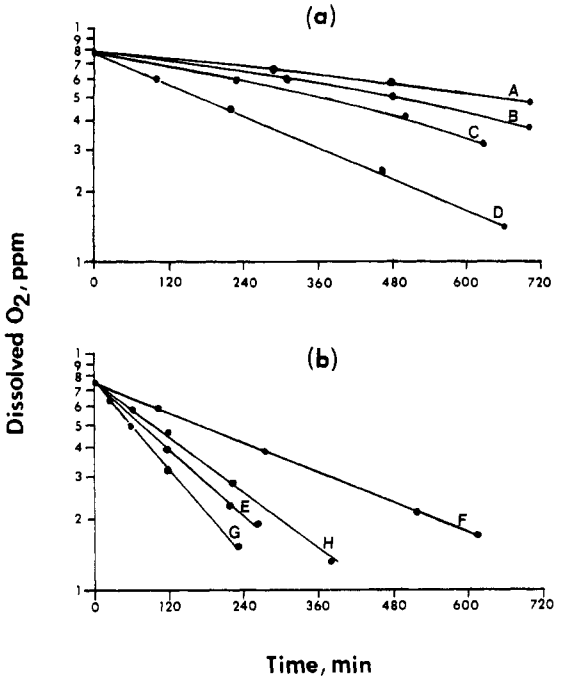

Figure 1. Semilogarithmic plot of the dissolved oxygen concentration vs time in various solutions containing ascorbic acid at 30 °C. (A) Water; (B) 10% sucrose in water; (C) buffer I; (D) 10% sucrose in buffer I; (E) buffer II; (F) 10% sucrose in buffer II; (G) buffer III; (H) 10% sucrose in buffer III.

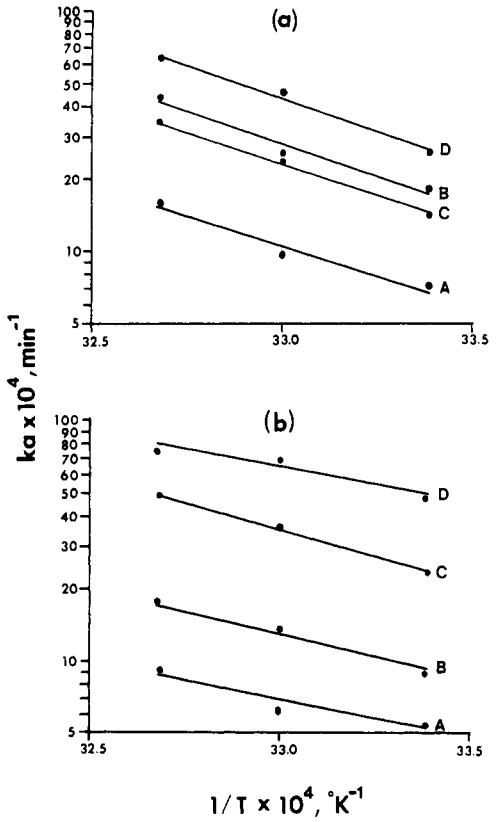

Figure 2. Arrhenius plots showing temperature dependence of the ascorbic acid oxidation in various solutions containing (a) 10% sucrose and (b) no sucrose. (A) Unbuffered solution (pH 3.0); (B) buffer I (pH 3.2); (C) buffer II (pH 4.5); (D) buffer III (pH 6.0).

for another hour to bring the dissolved oxygen to equilibrium with air. Approximately 300 mL of the air-saturated solution was poured in a BOD bottle, and the initial dissolved oxygen was measured. An excess amount (2 g) of L-ascorbic acid powder was added to each bottle, which was then immediately filled with the same solution and tightly closed with a glass stopper and a plastic cap. The bottle was rotated three times, allowing ascorbic acid to be quickly dissolved and evenly distributed in the solution. Afterward, the sample bottle was again placed in the water bath, and the water level was maintained high enough to cover the BOD bottle to the neck. Timing of the reaction began when the ascorbic acid was added. Linear regression analysis was applied to kinetic data with a commercial computer statistical program.

Table I. Rate Constants for the Oxidation of Ascorbic Acid at Various Temperature and pH Levels in Various Solutions

solution	pH[a]	temp, °C	K_a[b] × 10^4 min^{-1}	R^2	K[c] × 10^2 M^{-1} min^{-1}
water	3.00	26.5	5.40	0.9976	1.43
		30.0	6.33	0.9900	1.67
		33.0	8.96	0.9958	2.37
sucrose[d] in water	2.98	26.5	7.19	0.9815	1.90
		30.0	9.61	0.9914	2.54
		33.0	15.94	0.9954	4.21
buffer I[e]	3.20	26.5	9.07	0.9970	2.40
		30.0	13.06	0.9998	3.45
		33.0	17.69	0.9980	4.67
sucrose in buffer I	3.17	26.5	18.03	0.9992	4.76
		30.0	25.00	0.9793	6.60
		33.0	43.29	0.9998	11.43
buffer II[f]	4.25	26.5	23.36	0.9847	6.17
		30.0	35.41	0.9996	9.35
		33.0	48.42	0.9994	12.78
sucrose in buffer II	4.21	26.5	14.16	0.9992	3.74
		30.0	24.17	0.9994	6.38
		33.0	34.17	0.9998	9.02
buffer III[g]	5.00	26.5	47.44	0.9990	12.52
		30.0	69.25	0.9994	18.28
		33.0	74.29	0.9998	19.61
sucrose in buffer III	4.97	26.5	25.90	0.9996	6.84
		30.0	46.07	0.9992	12.16
		33.0	63.46	0.9998	16.75

[a] Values were measured after the addition of ascorbic acid. [b] Pseudo-first-order rate constants. [c] Second-order rate constants. [d] 10% sucrose concentration (w/v). [e] 0.1 M sodium acetate–acetic acid buffer of pH 3.2. [f] 0.1 M sodium acetate–acetic acid buffer of pH 4.5. [g] 0.1 M sodium acetate–acetic acid buffer of pH 6.0.

Table II. Activation Energy and Statistical Parameters Obtained from the Arrhenius Plots for Ascorbic Acid Oxidation at Various pH Levels

solution	pH[a]	activation energy, kcal/mol	R^2	F
water	3.00	13.998	0.9358	13.87
sucrose[b] in water	2.98	22.074	0.9582	22.93
buffer I[c]	3.20	18.719	0.9998	35470.00
sucrose in buffer I	3.17	24.296	0.9620	25.37
buffer II[d]	4.25	16.981	0.8545	23.11
sucrose in buffer II	4.21	24.772	0.9948	190.00
buffer III[e]	5.00	12.786	0.8968	8.70
sucrose in buffer III	4.97	25.253	0.9872	78.04

[a] Values were measured after the addition of ascorbic acid. [b] 10% sucrose concentration (w/v). [c] 0.1 M sodium acetate–acetic acid buffer of pH 3.2. [d] 0.1 M sodium acetate–acetic acid buffer of pH 4.5. [e] 0.1 M sodium acetate–acetic acid buffer of pH 6.0.

Molar Ratio between Oxygen and Ascorbic Acid. The molar ratio of consumed oxygen to ascorbic acid reacted in solutions was studied in a separate experiment by measuring the initial and the end point concentrations of dissolved oxygen and residual ascorbic acid for each 300-mL sample containing 60 mg of ascorbic acid in unbuffered or 0.1 M acetate buffered solutions at various pH levels. Samples were maintained at 30 °C. Since the reaction mechanism may be different when the oxygen concentration drops below the 2 ppm level (Khan and Martell, 1967), the reaction was monitored from the initial saturated dissolved oxygen concentration (9.2 ppm) down to 2 ppm. The concentration of residual ascorbic acid was determined by the

Table III. Molar Ratio between Ascorbic Acid (A) and Dissolved Oxygen (DO)[a]

solution	pH[b]	initial A concn, mg/L	final A concn, mg/L	initial DO, mg/L	final DO, mg/L	mol of A used[c] ×10000	mol of O_2 used[d] ×10000	O_2:A ratio
water	3.49	200	174	8.42	2.90	0.148	0.163	1.10
sucrose[e] in water	3.47	200	157	8.10	0.96	0.243	0.223	0.92
buffer I[f]	3.18	200	166	8.21	2.25	0.193	0.186	0.96
sucrose in buffer I	3.17	200	164	8.41	2.05	0.205	0.199	0.97
buffer II[g]	4.45	200	165	8.27	1.65	0.199	0.207	1.04
sucrose in buffer II	4.43	200	167	8.26	1.53	0.188	0.210	1.12
buffer III[h]	5.85	200	163	8.01	1.00	0.210	0.219	1.04
sucrose in buffer III	5.83	200	163	7.99	1.29	0.210	0.209	1.00

[a] Measurements were made in 300 mL of sample at 30 °C. [b] Measured after the addition of ascorbic acid. [c] Calculated by the formula (initial A − final A)/(mol wt of A). [d] Calculated by the formula (initial DO − final DO)/(mol wt of DO). [e] 5% sucrose concentration (w/v). [f] 0.1 M sodium acetate–acetic acid buffer of pH 3.2. [g] 0.1 M sodium acetate–acetic acid buffer of pH 4.5. [h] 0.1 M sodium acetate–acetic acid buffer of pH 6.0.

official titration method with 2,6-dichlorophenolindophenol reagent (AOAC, 1984).

RESULTS AND DISCUSSION

Theoretical Considerations. The oxidation of ascorbic acid in a closed system was assumed to follow a second-order reaction (Singh et al., 1976). The rate of reaction can be expressed as

$$-\mathrm{d}[\mathrm{O}]/\mathrm{d}t = k[\mathrm{A}][\mathrm{O}] \quad (1)$$

where [A] is the concentration of ascorbic acid, [O] is the concentration of dissolved oxygen, t is the time, and k is the second-order rate constant.

If the initial concentration of ascorbic acid (2 g/300 mL) is much greater than the initial concentration of oxygen (9.2 ppm), the concentration of ascorbic acid [A] remains nearly constant during the course of oxidation. Solving eq 1 with the assumption of [A] being a constant yields

$$\ln([\mathrm{O}]_t/[\mathrm{O}]_0) = -k[\mathrm{A}]t \quad (2)$$

where $[\mathrm{O}]_t$ is the concentration of oxygen at time t and $[\mathrm{O}]_0$ is the initial concentration of oxygen. Let $k[\mathrm{A}] = ka$.

Equation 2 is a pseudo-first-order rate equation, and $k[\mathrm{A}]$ is the pseudo-first-order rate constant (K_a). The second-order rate constant k can be calculated from K_a. A plot of log $([\mathrm{O}]_t/[\mathrm{O}]_0)$ vs t gives a straight line of slope $-ka/2.303$.

Effect of pH. The semilogarithmic plots of dissolved oxygen vs time for the oxidation of ascorbic acid in water and acetate buffers in the presence and absence of 10% sucrose at 30 °C are shown in Figure 1. The oxidation of ascorbic acid fits pseudo-first-order kinetics fairly well in all of the solutions. The rate constants for each solution were computed from the plots of log (dissolved oxygen concentration) vs time (Table I). The effects of sucrose on the rate of ascorbic acid oxygen uptake were quite different in the pH range tested. In the vicinity of pH 3 the rate of ascorbic acid oxidation was greater in the presence of 10% sucrose than that in the absence of sucrose both in unbuffered and in buffered solutions. However, in the acetate-buffered solutions in the pH range 4.2–5.0 the rate of oxygen uptake was decreased by the addition of 10% sucrose.

Effect of Temperature. The rate constant increased as the temperature increased in all cases (Table I). Although this temperature range appeared to be narrow, it represented a remarkable increase of the reaction rate constant of ascorbic acid oxidation. The apparent activation energy of the reactions in different solutions can be calculated using the Arrhenius equation

$$K_a = k_0 \exp(-E_a/RT) \quad (3)$$

where K_a is the pseudo-first-order rate constant, k_0 is the Arrhenius constant, R is the gas constant (1.987 kcal/mol), T is the absolute temperature, and E_a is the activation energy. A plot of log K_a vs the reciprocal of the absolute temperature is presented in Figure 2. From each Arrhenius plot, the corresponding activation energy was computed (Table II). The activation energies of ascorbic acid oxidation in buffered and nonbuffered solutions were all higher in the presence of 10% sucrose than in their non-sucrose controls, indicating that the addition of sucrose elevated the activation energy of ascorbic acid oxidation, which reduced the rate of reaction.

In buffered solutions containing no sucrose, the calculated activation energy decreased from 18.719 to 12.786 kcal/mol as the pH of the solution increased from 3.2 to 5.0. This trend was also observed in the study of Blaug and Harjratwala (1972). As a diprotic acid, the relative distribution of ascorbic acid species H_2A and HA^- in solutions varies at different pH levels: H_2A is dominant at low pH, and HA^- is dominant at high pH levels. The decrease of the activation energy toward higher pH levels in the unbuffered solutions implied that the activation energy of the HA^- was lower than that of the H_2A oxidation. However, this pH effect on the activation energy of ascorbic acid oxidation was not manifested when 10% sucrose was present.

There are apparently two major effects of sucrose on the oxidation of ascorbic acid in solutions. The first one is a catalytic effect which probably resulted from the metal impurities contained in sucrose (up to 2 ppm in the analytical grade reagent). The second one is a physical effect of sucrose molecules in solution which hindered the rate at which two reactant molecules could move through the solvent to encounter each other in fast reactions (Levine, 1983). The catalytic effect of metal impurities is influenced by the complexing power of acetate in solution and is pH dependent (Hsieh and Harris, 1987b). The complexing power of acetate is proportional to the ratio of unprotonated and protonated acetate molecules in a solution. In the vicinity of pH 3, only a very small fraction (approximately 2%) of acetate molecules is unprotonated; therefore, the complexing power is much weaker than that in the vicinity of pH 4.7 when approximately 50% of the acetate molecules are unprotonated (Hsieh and Harris, 1987b). For this reason the catalytic effect of metal impurities in an acetate buffer solution decreases sharply as the pH of the solution increases. However, the physical hindrance effect of sucrose in a solution is pH independent as can be shown from the almost uniform increase in activation energy between a pair of sucrose and non-sucrose solutions at different pH levels (Table II). Therefore, in the vicinity of pH 3, the catalytic effect of metal impurities surpassed the physical hindrance effect of sucrose. The overall result is a higher rate of oxidation in the presence

of sucrose at pH 3. In the pH range 4.2–5.0, the catalytic effect of metal impurities dropped sharply, while the hindrance effect of sucrose remained unchanged. The net effect is a lower oxidation rate of ascorbic acid in the presence of sucrose than in the non-sucrose counterpart at pH 4.2–5.0.

Molar Ratio between Oxygen and Ascorbic Acid. There are diverse opinions on the number of moles of oxygen used per mole of ascorbic acid in the oxidation of ascorbic acid (Mushran and Agrawal, 1977). To better understand the reaction mechanism of ascorbic acid oxidation in noncatalyzed sucrose solutions, an experiment was designed to measure the molar ratio between oxygen and ascorbic acid reacted. Results are presented in Table III. Although the duration of the experiment for each sample varied considerably, from approximately 6 to 30 h, the molar ratios of oxygen and ascorbic acid were consistent in different solutions. Approximately 1 mol of oxygen was used per mole of ascorbic acid. This result agreed with the previous findings of Weissberger and LuValle (1944) and Ogata et al. (1986) for copper-catalyzed reactions. According to the equation

$$AH_2 + O_2 \rightarrow A + H_2O_2 \quad (4)$$

theoretically 1 mol of oxygen will be taken up per mole of ascorbic acid during the early stages of oxidation, provided the hydrogen peroxide neither reacts with ascorbic acid nor decomposes to oxygen and water. In the later reactions, the decomposition of hydrogen peroxide or its decomposition products will finally lower the total oxygen uptake to 0.5 mol/mol of ascorbic acid. The reaction measured in this experiment was confined to the simple mechanism involved in the early stages of ascorbic acid oxidation. The data suggested that eq 4 was appropriate not only for copper-catalyzed reactions but also for noncatalyzed ascorbic acid oxidation.

Summary. In a homogeneous closed aqueous system with limited dissolved oxygen, sucrose (10% w/v) accelerated the rate of ascorbic acid oxidation in unbuffered and 0.1 M acetate-buffered solutions in the vicinity of pH 3, in the temperature range from 26.5 to 33 °C. This destructive effect of sucrose on ascorbic acid was attributed to the catalytic power of metal impurities of sucrose at lower pH levels. However, sucrose (10%) retarded the rate of oxygen uptake of ascorbic acid in the acetate-buffered solutions at pH 4.2–5.0. The overall rate of ascorbic acid oxidation increased as pH and temperature increased. The reactions agreed with the pseudo-first-order law fairly well. The activation energies of ascorbic acid oxidation in 10% sucrose solutions were greater than in solutions containing no sucrose at all pH levels tested. Approximately 1 mol of oxygen was used per mole of ascorbic acid in the early stage of the noncatalyzed oxidation of ascorbic acid in various solutions. Two major effects of sucrose on the ascorbic acid in the closed homogeneous aqueous system were suggested: one is a pH-independent physical effect which retards ascorbic acid oxidation, and the other is a pH-dependent catalytic effect which accelerates the ascorbic acid oxidation by the presence of metal impurities in the sucrose. At pH 3 the catalytic effect of sucrose surpassed the physical effect and resulted in an accelerated effect on ascorbic acid oxidation, while in the pH range 4.2–5.0 the physical protective action of sucrose becomes dominant.

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