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On the Anaerobic Degradation of Ascorbic Acid in Dehydrated Tomato Juice

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During a study aimed at developing methods for computer-aided simulation of ascorbic acid loss in dehydrated tomato juice, we investigated the influence of environmental variables. Previous studies on dehydrated products showed an aerobic reaction in some cases, but no oxygen effect in others. To establish definitively the existence of anaerobic reaction in our system, reaction rates in a catalytically deoxygenated system were compared with rates in the presence of oxygen; and an oxygen mass balance experiment was conducted in a system containing a known and limited amount of oxygen. The results definitively establish that ascorbic acid degradation in dehydrated tomato juice is largely anaerobic.

During storage of dehydrated foods in permeable packages, many changes occur that lead to quality deterioration. An important index in deterioration of dehydrated juices is the loss of vitamin C. Environmental factors and package properties affect the rate of loss of this vitamin, and environmental conditions may change during storage. Moisture is transferred into the package, resulting in the increase of moisture content and equilibrium relative humidity; oxygen is transferred into the package where it may accumulate in the headspace or react with the food. We have recently conducted a study directed at developing methods for computer-aided simulation of ascorbic acid degradation during storage. Dehydrated tomato juice powder was the food chosen for the study, and oxygen pressure was one of the storage variables to be studied. In the course of the study it became apparent that the effect of this variable was negligible. The present paper reports the results of experiments undertaken to establish whether degradation of the vitamin was truly anaerobic in this system.

Vitamin C losses in several dehydrated foods had been reported to be the result of aerobic reaction and, in other foods, an anaerobic degradation reaction. Heberlein and Clifcorn (1944) found that an inert atmosphere favored the retention of ascorbic acid in dehydrated fruits and vegetables at room temperature. Miers et al. (1958) monitored ascorbic acid retention of spray-dried tomatoes during storage and found that high moisture levels, presence of oxygen, and storage temperature above 90 °F were factors detrimental to the storage stability.

On the other hand, the retention of ascorbic acid in storage of tomato flakes was found to be independent of the package atmosphere (Continental Can Co., 1944, 1945), and Karel and Nickerson (1964) found that in stored dehydrated orange juice ascorbic acid degradation occurred at the same rate in air and in vacuum. Lempka and Prominski (1967) and Lempka et al. (1969, 1970) studied the changes in the ascorbic acid content of dehydrated fruits and vegetables and have shown that storage of freeze-dried products in air had little effect on ascorbic acid. In a study of various tomato products during storage, Hummel et al. (1950) found that the amount of oxygen present in the headspace (not considering potential occluded air) could not account for the resulting ascorbic acid degradation and that the reduction of dissolved air by increasing processing time did not improve ascorbic acid retention.

In our studies the effects of various oxygen levels (21, 7.2, 3.5, and 0.2%) on the rate of ascorbic acid degradation in dehydrated tomato juice were found to be insignificant. These observations necessitated further investigation of the following hypotheses: (a) degradation of ascorbic acid in this system is an anaerobic reaction or oxygen is available in the product in quantities sufficient for aerobic degradation; (b) reaction is aerobic, i.e., oxygen is available in the system in quantities sufficient for aerobic degradation; (c) degradation occurs both aerobically and anaerobically. These alternatives are schematically illustrated in Figure 1. In this study we describe the approaches used in determining which of the alternatives is the correct one.

EXPERIMENTAL SECTION

The Food System. Commercially available frozen tomato concentrate (Vitality Brand, Lykes Pasco Co., Dade City, Fla.) was used for preparing a stock of freeze-dried tomato juice powder. The concentrate was diluted 3:1 with distilled water, a point at which it had a 6.7° Brix and a pH value of 4.1. It was frozen (slow freezing, −25 °F) and freeze-dried (Vacudyne freeze-drier) for 72 h. The dehydrated tomato juice was kept in desiccators under vacuum at −25 °F in the dark.

Ascorbic Acid Assay. L-Ascorbic acid was determined by a 2,6-dichlorophenolindophenol titration, an AOAC (1975) official method. Since the investigated samples were

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Table I. First-Order Reaction Characteristics of Ascorbic Acid Loss in Dehydrated Tomato Juice

Temperature	Water activity	Storage condition	Type of dehydrated tomato juice	k, rate constant (day^{-1})	r, correlation coefficient
37 °C	0.32	O_2-"free"	New	0.06	0.96
			Original	0.06	0.99
		Air	New	0.08	0.98
			Original	0.07	0.99
	0.75	O_2-"free"	New	0.54	0.99
			Original	0.51	0.98
		Air	New	0.56	0.99
			Original	0.58	0.99

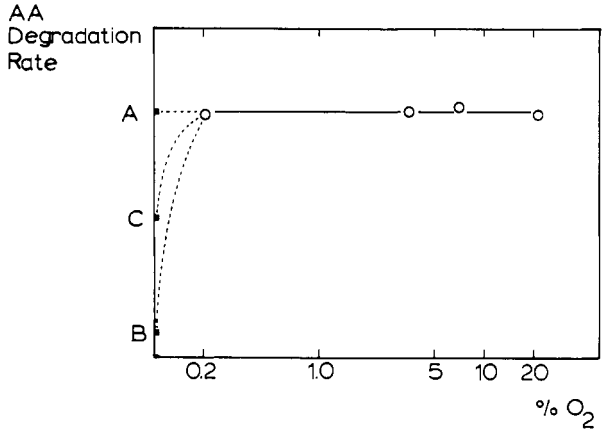

Figure 1. Effects of O_2 on rate of ascorbic acid degradation.

colored (pink), the visual titration method was insufficient to determine accurately the end point. A potentiometric titration method was employed to overcome this problem (Spaeth et al., 1962). Using a Redox (platinum combination) electrode, the titrations were followed to their end point with acceptable precision. In addition the use of an automatic burette and a recorder aided in minimizing technical errors. The following equipment was used: Corning pH meter Model 12; Fisher Platinum Combination Electrode (No. 13-639-82); Autoburette ABU 12 Radiometer, Copenhagen; Sargent Recorder Model DSRG.

O_2-"Free" Experiment. The elimination of oxygen both during preparation of dehydrated tomato juice and during storage studies was achieved by a reaction with H_2 on palladium catalyst, a technique used by Bishov et al. (1971). Dehydrated tomato juice was newly prepared in a Virtis freeze-drier. Powder was transferred under vacuum to a 95% N_2–5% H_2 controlled environment (glove compartment) with Pd catalyst where all handling took place. Storage at O_2-"free" conditions was carried out in desiccators containing 95% N_2–5% H_2 with Pd catalyst in constant temperature cabinets in the dark, at specified temperatures and water activities. Water activities were controlled by equilibrium over saturated salt maintaining constant humidity (Karel and Nickerson, 1964). According to a predetermined schedule, samples were withdrawn from storage for ascorbic acid content determination.

O_2 Mass Balance Experiment. The system developed for the O_2 mass balance experiment is illustrated in Figure 2. Thirty grams of dehydrated tomato juice were placed in a triple-neck flask (250 mL) equipped with two Oxygen Probes (lead–silver electrode probes as described by Quast and Karel, 1972). The probes were positioned to monitor the headspace of the flask (A) and the bulk of the powder (B). The system was evacuated and flushed with nitrogen several times until the oxygen level was lower than 0.01%. At that point 105 mL of deaerated, distilled water was added to the system, turning the powder into tomato juice.

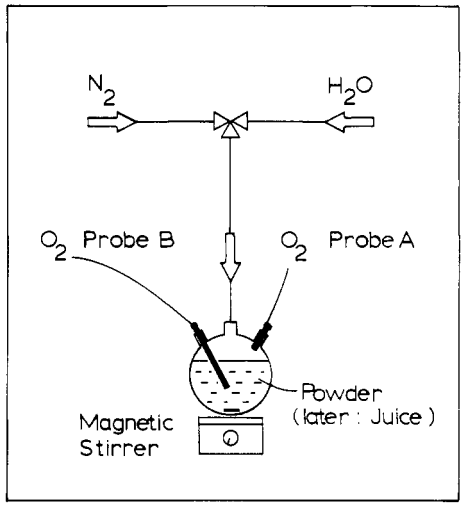

Figure 2. O_2 mass balance experiment system.

Oxygen levels were measured by probes A and B at their respective positions before and after the addition of water. The system was then monitored for 6 days. Ascorbic acid assays of dehydrated tomato powder at time zero and of the juice at the end of the experiment (time = 6 days) were carried out and were used for analyzing the O_2 mass balance calculations. The experiment was carried out in a constant temperature room (25 °C).

RESULTS AND DISCUSSION

O_2-"Free" Experiment. The first approach toward determining which of the hypotheses is the correct one was carried out through the O_2-"free" experiment. The experimental design aimed at measuring retention of ascorbic acid of both new powder (prepared under O_2-"free" conditions) and the original powder (used in the previously mentioned 21–0.2% oxygen level experiments) at both storage conditions (in O_2-"free" atmosphere and in air). Results are the average of two samples and initial ascorbic acid content of new and original samples was 128 and 160 mg/100 g, respectively.

When results of ascorbic acid retention were plotted against time in semilogarithmic coordinates, straight lines were obtained, indicating that the degradation of ascorbic acid in dehydrated tomato juice follows first-order reaction kinetics. Figures 3 and 4 illustrate this behavior under conditions of either exposure to air or O_2-"free" environment. These figures present only data obtained at a water activity of 0.75. Subsequent papers will deal with the effects of water activity on degradation kinetics. This first-order kinetics of the system served as a useful means for comparing the behavior of the various powders and storage conditions. Table I summarizes the first-order reaction characteristics under all the investigated conditions. The correlation coefficients in all cases are high and indicative of how well the kinetics model fits the data.

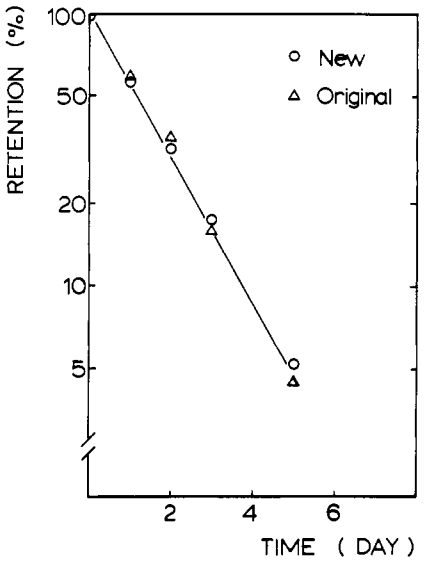

Figure 3. Ascorbic acid retention in dehydrated tomato juice stored in air; water activity = 0.75. ("New" and "original" refer to two different preparations of dehydrated tomato juice.)

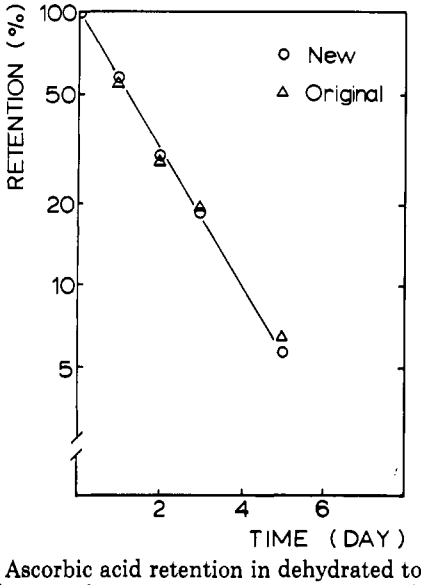

Figure 4. Ascorbic acid retention in dehydrated tomato juice stored in absence of oxygen; water activity = 0.75. ("New" and "original" refer to two different preparations of dehydrated tomato juice.)

Observing the rate constants, little difference is noticeable in the methods used to prepare the dehydrated tomato juice; the slopes obtained from the new powder are very similar to those from the original powder. No significant difference can be determined from the values of the effect of air and O_2-"free" storage.

The results of the O_2-"free" experiment are in accordance with hypothesis A as illustrated in Figure 1. This means that the degradation of ascorbic acid in this system may be an anaerobic reaction or oxygen is available in the product in quantities sufficient for aerobic degradation. Assaying for total ascorbic acid (total = reduced + dehydroascorbic acid) concurrently with the O_2-"free" experiment gave information on patterns of dehydroascorbic acid degradation in dehydrated tomato juice. Initial dehydroascorbic acid content in dehydrated tomato juice was about 23% of the total ascorbic acid. The reaction order and the rates of degradation of dehydroascorbic acid were found to be similar to those of ascorbic, as can be observed in Figures 5 and 6. No significant difference

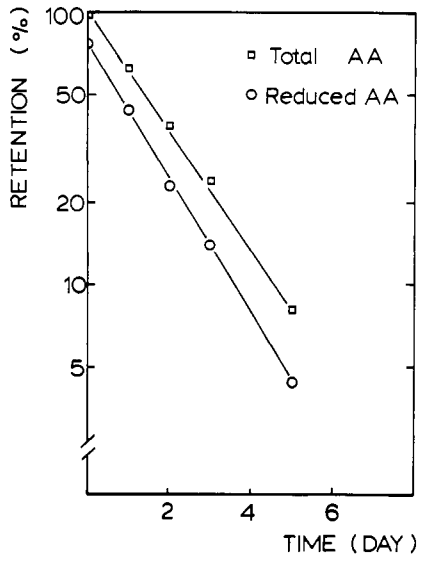

Figure 5. Retention of total and reduced ascorbic acid in dehydrated tomato juice stored in air; water activity = 0.75.

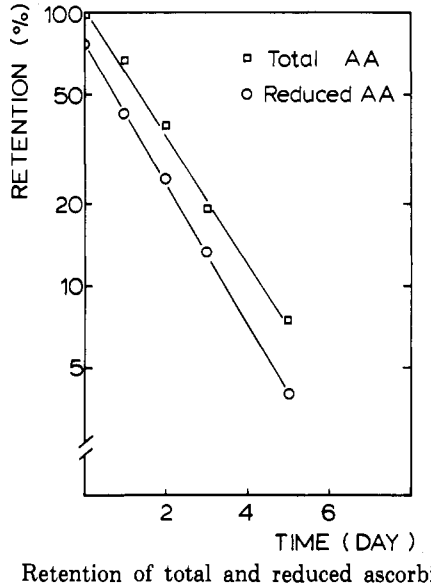

Figure 6. Retention of total and reduced ascorbic acid in dehydrated tomato juice stored in absence of oxygen; water activity = 0.75.

between samples stored in an O_2-"free" environment and samples stored in air could be observed. Further investigation of hypothesis A was attempted in the O_2 mass balance experiment.

O_2 Mass Balance Experiment. This experiment was designed to determine whether dehydrated tomato juice prepared under O_2-"free" conditions contained entrapped oxygen in quantities sufficient for aerobic degradation. The system used in this experiment is described in Figure 2, and the following are the system's parameters upon which oxygen mass balance calculations were based: volume of flask, 240 mL; true volume of dehydrated tomato juice (as measured by Beckman 930 Air Pycnometer), 42 mL; volume of added deaerated water, 105 mL; volume of juice (powder + H_2O), 140 mL; O_2 content of deaerated water, 0.15×10^{-3} mL of O_2/mL of H_2O. The results of the O_2 mass balance experiment are presented in Tables II and III.

a. Entrapped Oxygen. The value 4 μmol of O_2 of system condition I (O_2 of water plus O_2 of evacuated, N_2-flushed flask) does not include the oxygen that had been entrapped in the powder. That entrapped quantity

Table II. Oxygen and Ascorbic Acid Levels in the System

System condition	Probe A headspace O_2, %	Probe B bulk O_2, %	Ascorbic acid, mg AA/100 g
I. Evacuated and N_2-flushed flask	<0.01	<0.01	130.6[a]
II. After addition of water	0.5	0.02×10^{-3} [b]	
III. After 6 days	0.4	0.05×10^{-3} [b]	107.1[a]

[a] Results are average of three samples. [b] Expressed in (mL of O_2/mL of H_2O); calculation was based on solubility of oxygen in water at 26 °C.

Table III. O_2 Mass Balance Calculations

System condition	Total O_2 content
I. Deaerated water; evacuated and N_2-flushed flask	105 mL(0.15×10^{-3}) + 198 mL(0.01%) = 3.6×10^{-2} mL of O_2; equals ca. 1.5 μmol of O_2 (Water; Flask)
II. After addition of water	140 mL(0.02×10^{-3}) + 100 mL(0.5%) = 0.5 mL of O_2; equals ca. 23 μmol of O_2 (Juice; Headspace)
III. After 6 days	140 mL(0.05×10^{-3}) + 100 mL(0.4%) = 0.4 mL of O_2; equals ca. 18 μmol of O_2 (Juice; Headspace)

of oxygen was released and became measurable when water was added in condition II. The difference between the total oxygen of conditions II and I (23 – 4 = 19 μmol of O_2) represents, therefore, the oxygen entrapped in the powder.

b. Support of Hypothesis A. Thirty grams of dehydrated tomato juice with initial ascorbic acid concentration of 130.6 mg of ascorbic acid/100 g represents a pool of approximately 225 μmol of ascorbic acid. Maximum oxygen available in the dry system (at which this investigation is aimed) is approximately 23 μmol. This ratio, close to 10:1, in a system in which losses of over 17% of the original ascorbic acid are measured raises significant doubts as to the possibility of aerobic degradation. It complements the observations of the O_2-"free" experiment, leading to the conclusion that ascorbic acid degradation in this dry system is anaerobic.

c. Ascorbic Acid Degradation in Solution. In solution ascorbic acid degradation was anaerobic. Oxygen consumed in the system may account for less than 15% of the measured ascorbic acid degradation. (Monitoring the system for 6 days, a loss of about 5 μmol of O_2 was observed compared to a loss of approximately 41 μmol of ascorbic acid.)

Various studies in model systems of the anaerobic degradation of ascorbic acid exhibited similar characteristics to our investigated system. Huelin (1953) studied the anaerobic decomposition of ascorbic acid in buffered solutions of pH 2.2–6.0 and the effect of substances known to be present in foods. He found that first-order reaction was evident down to 25% retention of the original ascorbic acid and that the anaerobic decomposition passed through a maximum at pH 3–4. A more rapid destruction was promoted by fructose and related substances. Products with higher concentration of fructose and related substances were found to lose their ascorbic acid even more rapidly. Reaction products at pH 4.0 and at 30 °C were mainly carbon dioxide and a small amount of furfural. Cier et al. (1959) studied the degradation of ascorbic acid in aqueous solutions under inert atmosphere. Ranges of temperature were 60–100 °C and of pH 2.6–5.4. First-order reaction was observed, and the main reaction products were carbon dioxide, furfural, and xylose. Kyzlink et al. (1970) studied the influence of sucrose concentration on the course of ascorbic acid oxidation in liquid medium and found that increasing the concentration of sucrose led to the retardation of the degradation process even under the conditions of sufficient and excessive oxygen. The dehydrated food product used in this study originated from a juice of pH 4.1. It is high in sugar content, and as observed in Figures 3–5, it displayed first-order reaction kinetics.

In conclusion, the effects of oxygen on the deterioration of ascorbic acid in dehydrated tomato juice were negligible. Two independent experimental studies have shown that the degradation proceeded at similar rates under conditions of oxygen excess as well as under conditions in which oxygen content was well below the amount required for oxidation of the vitamin lost during the experiment. The anaerobic degradation of ascorbic acid in this food system was similar to that reported earlier in model systems.

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