Role of Ascorbic Acid in CO_2 Evolution from Heated Acerola Juice (*Malphigia glabra L.*)

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The role of ascorbic acid in CO_2 evolution from acerola juice at 80° C. under N_2 and O_2 was investigated with L-ascorbic acids-1-^{14}C, -U-^{14}C, and -6-^{14}C. The CO_2 evolved under both anaerobic and oxygenated conditions originated solely from ascorbic acid. The CO_2 contribution from C-1 under N_2 and O_2 was 90 and 80%, respectively. The C-6 contribution to CO_2 was negligible.

ACEROLA (*Malphigia glabra L.*) juice contains between 1.4 to 3.5% ascorbic acid (*1*) and is often used to fortify the natural vitamin C content of other juices. Anthocyanin color loss (*4, 10*), ascorbic acid decomposition (*6*), and swelling of cans due to CO_2 evolution (*9*) are problems in acerola processing. The mechanism for CO_2 evolution in acerola juice has not been established. Because ascorbic acid loss has been correlated with CO_2 evolution in orange juice (*11*) and in model systems (*7, 8*) we investigated the role of ascorbic acid in CO_2 production in acerola juice using L-ascorbic acid-U-^{14}C, -6-^{14}C, and -1-^{14}C. The results reported herein show that CO_2 originates from ascorbic acid and primarily by monodecarboxylation of C-1.

Materials and Methods

Preparation of Acerola Juice. Fully ripened acerola cherries (*Malphigia glabra L.*) were washed, sorted, frozen, and stored at −17.8° C. The cherries were thawed partially and then pulped in a Langsenkamp pulper fitted with a $^3/_{16}$-inch screen. Juice and pulp were separated by centrifuging at 11,700 × G for 30 minutes. The clear supernatant juice (pH 3.05) was decanted into an Erlenmeyer flask, stoppered, and stored at −17.8° C. until used.

Preparation of ^{14}C Ascorbic Acid. L-ascorbic acid-1-^{14}C was obtained commercially and was recrystallized with nonisotopic ascorbic acid. The L-ascorbic acid-6-^{14}C and L-ascorbic acid-U-^{14}C were synthesized from L-sorbose-6-^{14}C and D-glucose-U-^{14}C, respectively, by a modification of the Bothner-By, Gibbs, and Anderson (*3*) procedure. The authors' yields by the published procedure were poor (less than 5%) because of difficulties in crystallizing certain intermediates. However, if the alkaline permanganate oxidation of diacetone-L-sorbose to diacetone-2-keto-L-gulonic acid was carried out until a clear excess of permanganate persisted in the reaction vessel for at least 1 hour, purification of the intermediates by crystallization was unnecessary and the yield was improved significantly. The synthesis was followed by paper chromatographic identification of the intermediates. The spots were located with a radiochromatogram scanner. The R_f values of the intermediates on Whatman No. 1, developed with butanol–acetic acid–water (4:1:5) for 16 hours were D-glucose 0.18, L-sorbitol 0.19, L-sorbose 0.20, monoacetone-L-sorbose 0.77, diacetone-L-sorbose 0.89, diacetone-2-keto-L-gulonic acid 0.86, monoacetone-2-keto-L-gulonic acid 0.66, 2-keto-L-gulonic acid 0.24, and L-ascorbic acid 0.46. Decomposition of ascorbic acid during crystallization was reduced by bubbling H_2S through the solvents prior to their use. The synthesized ascorbic acid cochromatographed with authentic L-ascorbic acid and reacted stoichiometrically with standardized 2,6-dichlorophenolindophenol. The yield from D-glucose was approximately 30%.

Specific activities were determined by filtering a finely ground suspension of ascorbic acid in ether through a tared glass filter paper (Whatman GF/C), drying, and counting on a gas-flow thin-window counter. The specific activities were corrected for self-absorption. The specific activities of the preparations used were for L-ascorbic acids-1^{14}C, -U-^{14}C and -6-^{14}C, 0.91, 5.1, and 2.4 mc. per mmole, respectively.

Heat Treatment of Radioactively Labeled Juice. Samples of the radioactively labeled juice were prepared by adding 1 × 10^5 c.p.m. of ^{14}C ascorbic acid to 25 ml. of juice. A 5-ml. aliquot of the labeled acerola juice, in a 20 × 150 mm. test tube with side arm, was heated in a water bath at 80°C. and continuously bubbled with N_2 or O_2 at 10.5 cc. per minute. Prior to bubbling through the sample the gases were humidified and freed of CO_2 by sparging through water, 2*N* NaOH, and finally water at 80°C. The CO_2 from the juice was collected at 2-hour intervals by traps containing 5 ml. of 2*N* NaOH.

Radioactivity of Evolved CO_2. The trapped CO_2 was precipitated with 5 ml. of 5% $BaCl_2$, collected on a glass filter paper, washed with CO_2-free water, rinsed with acetone, and dried overnight in a vacuum desiccator. A drying tube containing activated molecular sieve 13×, 30 to 60 mesh was used to remove atmospheric CO_2 during filtration. The specific activities were corrected for CO_2 in the reagents.

Results and Discussion

Figures 1 and 2 show the time course contribution from labeled carbons of ascorbic acid to CO_2 evolved from acerola juice heated at 80°C. under N_2 and O_2 atmosphere, respectively, as per cent of maximal specific activity; the maximal specific activity is the value attainable if all the CO_2 evolved were from labeled carbons of ascorbic acid. Calculation of maximal value was based on the total ascorbic acid content of the juice as determined by the Ballantine method (*2*) and the known activity of the added ^{14}C ascorbic acid.

During the first 2 hours, the specific activities of evolved CO_2 were far below the maximal value. Based on results with ascorbic acid-U-^{14}C, ascorbic acid contributed 40 and 58% to the CO_2 evolved under N_2 and O_2, respectively. This low initial specific activity probably was due to ascorbic acid decomposition products or other thermally labile compounds initially present in the juice.

After 4 or more hours' heating with ascorbic acid-U-^{14}C, the maximal specific activity was attained under both N_2 and O_2. This clearly indicates that the origin of CO_2 evolved from heated acerola juice under N_2 or O_2 was exclusively from ascorbic acid. Figure 1 shows that over 90% of the CO_2 from acerola juice heated under N_2 originated from C-1 of ascorbic acid and that the contribution from C-6 was negligible. Figure 2 shows that, under oxygen, about 80% originated from C-1, and that the contribution from C-6 again was negligible. Primary contribution from C-1 of ascorbic acid under either N_2 or O_2 is in agreement with ascorbic acid and dehydroascorbic acid decomposition mechanisms proposed for acid conditions by Woker and Antener (*12*). However,

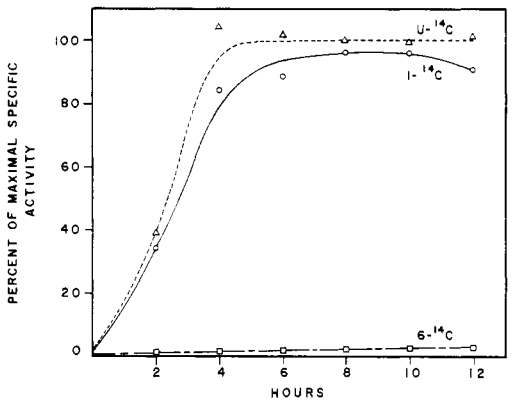

Figure 1. Per cent of maximal specific activity for CO_2 evolved from ^{14}C ascorbic acids heated at 80° C. in N_2

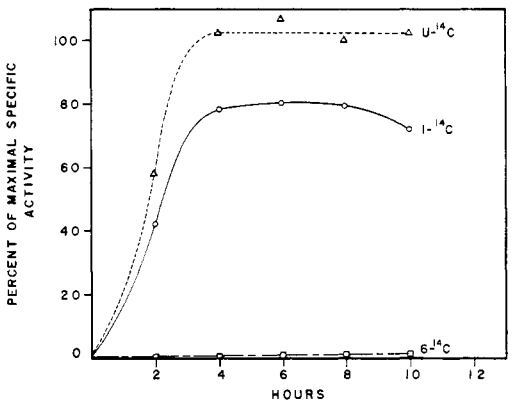

Figure 2. Per cent of maximal specific activity for CO_2 evolved from ^{14}C ascorbic acids heated at 80° C. in O_2

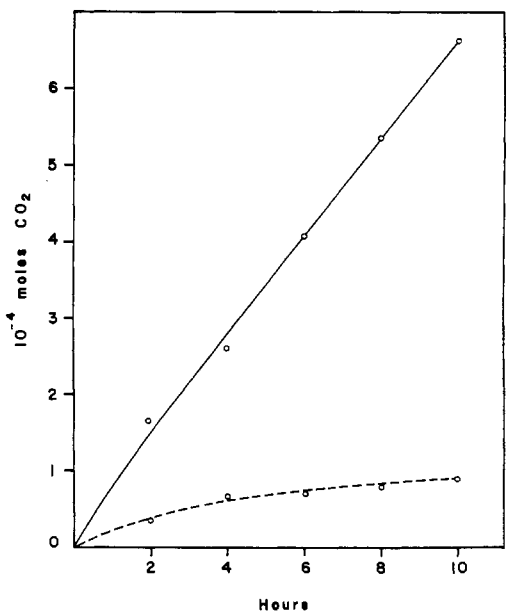

Figure 3. Total CO_2 evolved at 80° C. from 5 ml. of acerola juice containing ascorbic acid-1-^{14}C

a primary but not exclusive contribution of C-1 to CO_2 excludes direct stoichiometry between ascorbic acid lost and CO_2 evolved.

Figure 3 shows that the CO_2 evolution rate from 5 ml. of juice containing L-ascorbic acid-1-^{14}C was appreciably greater under O_2 than under N_2. The total ascorbic acid decomposed after 10 hours was estimated by determining the percentage of the total activity in the juice recovered as $^{14}CO_2$. The values obtained for ascorbic acid decomposition under O_2 and N_2 were 91 and 13%, respectively. However, when the Ballantine method was used to estimate ascorbic acid decomposition on similarly treated samples, values of 25 and 10% decomposition were obtained for O_2 and N_2 conditions, respectively. Ascorbic acid decomposition as estimated by the yield of CO_2 from C-1 of ascorbic acid and by the Ballantine method agree for N_2 but not for O_2 conditions. The $^{14}CO_2$ yield is specific for ascorbic acid but the Ballantine method, which measures gross reducing substances, is not. Furthermore, Euler and Hasselquist (*5*) have shown that oxidative decomposition of ascorbic acid yields reductones. Reductones formed from ascorbic acid decomposition would give false high ascorbic acid values by the Ballantine method and, consequently, false high ascorbic acid retention. Therefore, the 91% ascorbic acid decomposition value obtained from $^{14}CO_2$ yield more correctly represents the extent of ascorbic acid decomposition. This result demonstrates that ascorbic acid methods based on reducing activity alone are inadequate for estimating oxidative ascorbic acid deterioration in foods.

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