than the immediately following extract. Although the ninth extract did show ninhydrin activity when tested directly against the ninhydrin reagent (*8*), ion exchange chromatography experiments, using the Moore and Stein procedure (*7*), showed no definite peaks in the effluent curve.

The fourth and fifth extracts were estimated to contain very few acid and neutral amino acids, these having been essentially removed in the first three extracts. Only four peaks caused by this group were found in the fourth extract. The largest of these, the asparagine peak (which may contain glutamine and possibly serine) accounted for only 0.43% of the original nitrogen in the potatoes or about 0.75% of the extracted nitrogen. (As seen from Table III, this peak accounts for most of the extractable nitrogen.) In terms of leucine equivalents, the other three peaks amounted to only 22% of the asparagine peak. In the fifth extract, the asparagine peak amounted to only 0.006% of the original nitrogen and only two other smaller peaks were found in this group. The sixth (first Soxhlet) extract contained only slightly more of the acid and neutral amino acids than did the fifth. Thus essentially all of the acid and neutral amino acids are removed when 40 to 50% of the lysine and 30 to 40% of the arginine are still unextracted.

Fortunately, the acid and neutral amino acids are easily removed, as one of these, glutamine, would be converted by heat into pyroglutamic acid (*14*) if it were not essentially removed before Soxhlet extraction is begun. While asparagine is not completely stable to heat (*12*), small amounts of it remaining until the Soxhlet extraction would not significantly change the results. Other members of the acid and neutral fractions are probably more stable than asparagine.

The results of the second batch extraction (Table I, column 5 closely duplicated the first except that the Soxhlet extraction was not quite as efficient as before. Table III gives the amino acid estimations (*7*, *8*) for the first extract and for the combined aliquots of all the extracts except number eight, which was lost. Some of the other individual extracts were also checked, though the results are not included in the table. Expressed as percentage of amino acids found in the combined extracts, the seventh and ninth extracts contained 9.5 and 3.8%, respectively, of the lysine, and 5.4 and 2.0% of the arginine, while the fourth and fifth extracts contained 0.14 and 0.10% of the acid and neutral amino acids. In Table III, the relative amounts of the amino acids do not differ greatly in the two columns except in the case of lysine and arginine. In a few cases—i.e., threonine—the amount estimated in the combined aliquots was less than in the first extract—probably because of poor separation between peaks and the resultant error in deciding the correct amount to allot to each peak.

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ASCORBIC ACID OXIDATION

Hydrogen Peroxide–Induced Oxidation of Ascorbic Acid in Passion Fruit Juice

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Passion fruit juice is particularly suited for study of the hydrogen peroxide–induced oxidation of ascorbic acid. Although hydrogen peroxide is decomposed in the fresh juice, concurrent oxidation of ascorbic acid occurs. The catalatic activity is destroyed by heat. The aerobic, nonenzymic, peroxidatic-like oxidation is first order with respect to ascorbic acid, exhibiting a rate constant between 0.048 and 0.060 min.$^{-1}$ in natural juice at pH 3.0. The rate of the anaerobic reaction decreases rapidly after 15 minutes. In this reaction, the peroxide is apparently consumed stoichiometrically, while, in the aerobic reaction, it acts as a catalyst for the autoxidation of ascorbic acid. Significance of the results is discussed on the basis of induced reactions of hydrogen peroxide in the presence of ferrous ions.

Ascorbic acid is catalytically oxidized in the presence of hydrogen peroxide by the peroxidase system and by metal ions and their nonenzymic compounds. Both systems are present in the higher plants and have been individually demonstrated in several fruit juices. Neither has been investigated in passion fruit (*Passiflora edulis*). Routine analyses at the Food Processing Laboratory indicated that juice of the yellow passion fruit (*38*) variety (*P. edulis f. flavicarpa*) is comparable to citrus juices (*37*), as regards stability of ascorbic acid. Thus, hydrogen peroxide–induced oxidation of ascorbic acid in passion fruit juice may be measured with negligible interference from concurrent aerobic oxidation.

Peroxidase acts through a number of different oxidoreduction intermediaries to bring about the oxidation of ascorbic acid (*16*). Results by Doskocil (*11*) may indicate a direct oxidation of zero order, while Keilin and Hartree (*17*) found it necessary to eliminate all trace of metal contaminants in order to demonstrate the direct oxidation of ascorbic acid in a coupled reaction.

The catalytic activity of iron salts and nonenzymic iron compounds has been known for a long time. Since the review by Oppenheimer and Stern (*27*), further investigations have led to the free radical concept in accounting for both catalatic (peroxide decomposition) and peroxidatic (substrate oxidation) activities (*40*). The mechanisms for the decomposition of hydrogen peroxide by catalysts have been extensively reviewed by Baxendale (*4*). Competition between organic substrate and hydrogen peroxide for the free radical, or an active intermediary, determines the relative extent of catalatic and peroxidatic reactions. Weiss (*40*) points out the great complexity of the role of the ferrous ion–hydrogen peroxide system in the oxidation of organic substrate. Of special interest in the present study is the work of Kolthoff and Medalia (*19, 20*), which considers the different mechanisms for hydrogen peroxide–induced reactions in the presence and absence of oxygen.

Although the presence of hydrogen peroxide in fruit products is possible (*3, 8, 13, 28, 29, 34*), its significance in ascorbic acid oxidation and quality changes has been considered negligible (*15*). Nevertheless, hydrogen peroxide–induced reactions are of potential concern in food processing because of their catalytic nature and factors which influence the relative extent of catalatic and peroxidatic activities. This viewpoint is developed further in the discussion of results.

The objective of this investigation was to determine the kinetics of oxidation of ascorbic acid catalyzed by the highly reactive combination of passion fruit juice and hydrogen peroxide.

Experimental

Passion fruit juice exhibits an average pH of 3.0 and a titratable acidity of 4.0%, expressed as citric acid (*9*). All oxidative reactions were studied with diluted juice without alteration, except for added reagents and the presence of 0.07*M* citrate–citric acid buffer at a pH of 3.0, which maintained this pH in experimental reactions ±0.1 pH unit. Solutions and dilutions were made with double-distilled water (once through all-glass apparatus). Stock ascorbic acid solutions were prepared in 0.2*M* citrate–citric acid buffer of pH 3.0, in which it was stable during the period of experiments. Except where noted otherwise, the added ascorbic acid substrate was 5.7 millimolar (m*M*) in the reaction volume. Other reaction concentrations were 4.2m*M* hydrogen peroxide, 0.3m*M* catechol, and 0.1m*M* chlorogenic acid as mediators in tests for polyphenolase and peroxidase activity. The experiments were initially patterned after those described by Arthur and McLemore (*1*) and modified as described in this and later sections.

The rate and extent of oxidation of ascorbic acid was measured manometrically in Warburg microrespirometers (*36*). Initial and final concentrations of ascorbic acid were determined by direct titration of aliquots from the reaction mixture with 2,6-dichlorobenzenoneindophenol. The Warburg apparatus was used to determine oxygen evolution in studies of catalatic activity. Filter paper rolls and 0.2 ml. of 10% potassium hydroxide were added to the center wells of the Warburg vessels for all runs.

The anaerobic oxidation of ascorbic acid was carried out in Thunberg tubes, evacuated under a 29-inch vacuum and flushed twice with nitrogen (*36*). Determinations of ascorbic acid were made before and after the anaerobic reaction. Tests were made by the Thunberg technique for dehydroascorbic acid reductase activity (*9*), using glutathione added to diluted juice as the hydrogen donor.

All reactions were carried out at 25.0° C. Reactions were initiated by adding one reactant from the sidearm, and stopped by adding 2.5% oxalic acid in the proportion of 0.5 to 3.0 ml. of reaction mixture in the aerobic experiments and 1.0 to 6.0 ml. in the anaerobic ones.

Determinations of ascorbic acid were made on weekly receipts of passion fruit at the Food Processing Laboratory, employing both visual titration and photometric methods of Rubin, Jahns, and Bauernfield (*33*). Formalin treatments were used for reductone corrections of hydrogen sulfide–reduced ascorbic acid. The determinations were made on free-flowing juice strained from random samples of fruit, and on samples after centrifugal extraction and two paddle pulping and finishing operations. Juice was handled throughout in stainless steel equipment.

Results

Ascorbic Acid and Dehydroascorbic Acid in Passion Fruit Juice. The proportion of dehydroascorbic acid in fresh passion fruit juice was found to be small, averaging 5% of the total ascorbic acid in the five weekly samples. No difference in this proportion was observed between samples of hand-extracted and machine-extracted juice. Ascorbic acid values varied irregularly throughout the season, ranging from a low of approximately 10 mg. per 100 ml. to a high of 15 mg. per 100 ml. An increase of ascorbic acid content after mechanical extraction was found to vary between 30 and 40% of the content in hand-extracted juice.

Tests for Oxidative Enzyme Activity. Diluted passion fruit juice was found to catalyze the decomposition of hydrogen peroxide as measured by oxygen evolution in Warburg manometers. The rate of evolution was constant over an 8-minute reaction period and the rate per minute between 2 and 10 minutes was taken as a measure of the catalatic activity in the juice. The activity was destroyed by boiling the juice for 5 minutes. In unboiled juice the activity was directly proportional to concentration of juice from $^1/_{48}$ to $^1/_6$ dilutions. The initial rate of oxygen evolution was independent of hydrogen peroxide concentration between 2.1 and 8.3m*M*, in the juice dilutions used.

The magnitude of the catalatic activity in micromoles of hydrogen peroxide decomposed was calculated from oxygen evolution measurements. This value averaged 2.0 μmole of hydrogen peroxide decomposed per minute per ml. of juice for 14 determinations. There was no significant difference in activity between hand- and machine-extracted juice, nor between the presence and absence of added citrate buffer.

Practically zero oxygen consumption and 100% retention of ascorbic acid were observed during a reaction period of 1 hour with $^1/_6$ diluted juice and 5.7m*M* added ascorbic acid. This same result was obtained in the presence of 0.3m*M* catechol and 0.1m*M* chlorogenic acid as oxidoreduction intermediaries. However, a rapid oxidation of ascorbic acid did occur in the presence of 4.2m*M* hydrogen peroxide, both with and without added catechol or chlorogenic acid. There was no appreciable difference in the initial rate of oxidation with and without these polyphenols. The tests for dehydroascorbic acid reductase were negative.

Peroxidatic-like Oxidation of Ascorbic Acid in Diluted Passion Fruit Juice. The addition of passion fruit juice, in the tests for peroxidase activity, was found to increase the initial rate of oxidation of ascorbic acid in the presence of dilute hydrogen peroxide from a few tenths of 1% oxidized per minute in citrate buffer to approximately 3% per minute in $^1/_6$ diluted juice. The reaction was not enzymatic, as the activity was not reduced by boiling juice for 5 minutes prior to dilution. The initial rate of the reaction was dependent on ascorbic acid concentrations between 2.1 and 8.3m*M*. That the ascorbic acid was reversibly oxidized to dehydroascorbic acid was shown by reduction with hydrogen sulfide and determination of total ascorbic acid after a hydrogen peroxide–induced reaction had been allowed to proceed for 40 minutes.

Fresh orange, pineapple, and guava

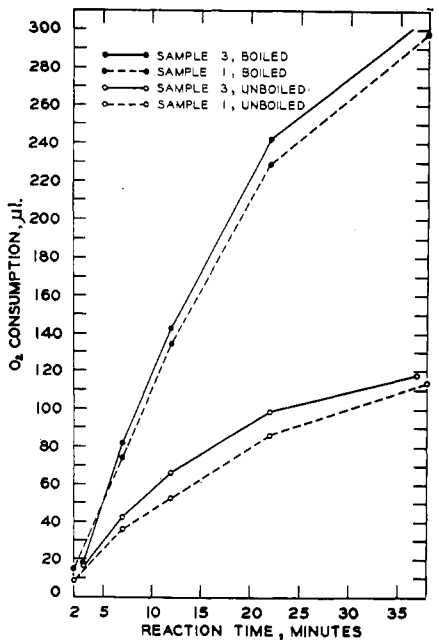

Figure 1. Oxygen consumption of $^1/_3$ diluted passion fruit juice during a 40-minute reaction at 25° C.

Initial concentrations. 5.8mM ascorbic acid and 4.2mM H_2O_2
Sample 1. Thawed, frozen juice from random sample of fresh fruit
Sample 3. Thawed, frozen juice after centrifugal extraction and pulping operations

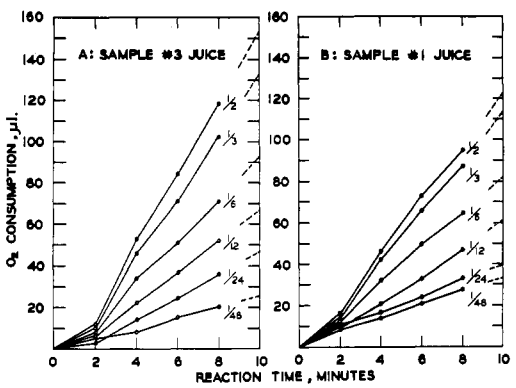

Figure 2. Oxygen consumption at various dilutions of passion fruit juice during an 8-minute reaction, extrapolated to 10 minutes when ascorbic acid determinations were made

Sample 1. Thawed, frozen juice from random sample of fresh fruit
Sample 3. Thawed, frozen juice after centrifugal extraction and pulping operations

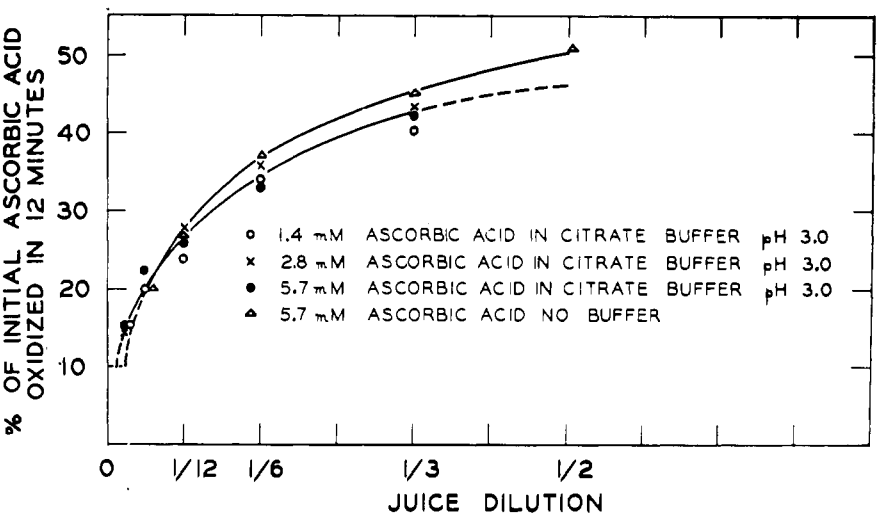

Figure 3. Percentage initial ascorbic acid oxidized in 12 minutes at various concentrations of added ascorbic acid for passion fruit juice dilutions from $^1/_2$ to $^1/_{48}$, in the presence of 4.2mM H_2O_2 initially

juices, commerical bottled apple juice, and the water fraction of coconut milk all exhibited this peroxidatic-like activity. Except for orange juice, the other products exhibited enzymic oxidations in addition to the nonenzymic reaction. The latter reaction was greatest in passion fruit juice and the present investigation of the kinetics of the reaction furnished the following results.

Oxygen Consumption. Results plotted in Figure 1 show that a rapid aerobic oxidation occurs in diluted passion fruit juice in the presence of added ascorbic acid and hydrogen peroxide. The rate of oxygen consumption is practically constant during an initial period of approximately 10 minutes. The initial rate of oxygen consumption in unboiled juice is lower than that in boiled juice by an amount equivalent to the initial rate of oxygen evolution owing to catalatic activity in releasing oxygen from the hydrogen peroxide. As the reaction period progresses, the oxygen consumption in unboiled juice falls off rapidly—presumably because of decomposition of the peroxide.

The procedure of disconnecting vessels and adding oxalic acid required an additional 2 minutes to stop the reaction. In Figure 2, the oxygen consumption rates for various juice dilutions are extrapolated from an 8-minute measurement period to 10 minutes when ascorbic acid determinations were made. There is an induction period of 2 minutes at the beginning of the measurements. Figures 1 and 2 show that machine-extracted juice exhibits a slightly higher activity than hand-extracted, as measured by oxygen consumption.

Ascorbic Acid Oxidation. The added ascorbic acid was normally prepared in citrate buffer adjusted to pH 3.0. In a run to check the effect of added citrate ions, no buffer was used. The results as shown in Figure 3 indicate that there is a slight lowering of oxidation rate in buffered juice dilutions less than $^1/_{12}$. The same general relationship of initial oxidation rate and juice dilution was observed, however, and citrate buffer of pH 3.0 was used in all experiments as a desirable constant factor. Stock solutions of ascorbic acid were stable in this buffer throughout all experimental periods.

The substrate for the oxygen consumption reported in Figure 1 is indicated by the concurrent oxidation of ascorbic acid as shown in Figure 4. These results also show the more active aerobic oxidative activity in machine-extracted juice compared to hand-extraced juice. However, the rate and extent of anaerobic reactions in the two juices are practically the same for the two different boiled juices. During an initial period of 10 minutes, the rates of oxidation of ascorbic acid in hand-extracted juice are practically constant and equal for boiled and unboiled juice under both aerobic and anaerobic conditions. After 15 minutes, the anaerobic oxidation falls off much more rapidly than the aerobic. In machine-extracted juice, aerobic oxidation is greater during the initial period than that under anaerobic conditions. Initial anaerobic conditions would not continue in unboiled juice owing to evolution of oxygen from hydrogen peroxide. This would not be a factor in hand-extracted juice because initial rates are the same for aerobic and anaerobic oxidations.

The plot of logarithm of juice content (as per cent juice) against initial rate of oxidation was found to be linear and this allowed extrapolation to 100% juice to obtain values which could be used for comparing oxidative activity of various juices. Full strength juice could not be studied of course because of dilution by buffer and added reagents.

The relationships for the initial rates shown in Figure 4 for $^1/_3$ diluted passion fruit juice were found for other juice dilutions from $^1/_{48}$ to $^1/_2$ as shown by Figure 5. These results indicate no appreciable difference between aerobic and anaerobic oxidations in hand-extracted juice and a slightly greater aerobic oxidative activity than anaerobic in machine-extracted juice. No differences between boiled and

unboiled juices at any of the dilutions gave further evidence for the nonenzymatic nature of this oxidation in hand-extracted juice. Catalatic activity in unboiled machine-extracted juice under initially anaerobic conditions would indirectly account for this increased oxidation over that in boiled juice because of liberation of oxygen from hydrogen peroxide.

Rate Constants. When the log of the percentage initial ascorbic acid oxidized is plotted against time for the aerobic oxidation of boiled juices, a first order–type reaction is indicated by the straight line. Extrapolated to 1 hour, 90% of the initial ascorbic acid would be oxidized in machine-extracted juice and 85% in hand-extracted juice, with calculated rate constants (k) of 0.038 and 0.032 min.$^{-1}$, respectively, for $^1/_3$ diluted juice. These constants were calculated from the slopes on the basis of the first order relationship:

$$k = \frac{2.3}{t} \log \frac{100}{P}$$

where $P = \frac{a - x}{a} \times 100\%$ when a is initial amount of ascorbic acid and x is the amount oxidized in t minutes.

Catalatic action in unboiled juice decreases the concentration of hydrogen peroxide sufficiently so that after 15 minutes the aerobic reaction is no longer first order with respect to ascorbic acid concentration.

Further evidence for the first order nature of the aerobic reaction is given by the results in Figure 3. The initial percentage oxidation rates were independent of ascorbic acid concentration over a fourfold concentration range. This fact was observed for juice dilutions from $^1/_{48}$ to $^1/_2$ and added ascorbic acid concentrations from 1.42 to 5.68mM.

The semilogarithmic plots in Figure 5 were extrapolated to 100% juice to give intercept values for comparative purposes. This activity amounts to oxidation rate during the first 10 minutes of 38% of the initial ascorbic acid, with a reaction rate constant of 0.048 min.$^{-1}$ for all four treatments of hand-extracted juice. A nearly equal value of k, 0.046 min.$^{-1}$, was determined for the anaerobic reaction in boiled machine-extracted juice. The rate constant increases to 0.060 min.$^{-1}$ under aerobic conditions.

Mole Ratios. From oxygen consumption data reported in Figure 2 and concurrent ascorbic acid oxidation, molar ratios of oxygen consumed to ascorbic acid oxidized were calculated and tabulated in Table I. At $^1/_3$ and $^1/_2$ juice dilutions, the aerobic oxidation of ascorbic acid in the presence of added ascorbic acid and hydrogen peroxide appeared to be the same type of reaction for hand- and machine-extracted juice—1 mole of oxygen being used per mole of ascorbic acid oxidized. At greater than $^1/_3$ dilutions, the ratio decreased, becoming slightly less for machine-extracted juice than for hand-extracted juice as the dilutions were increased.

The oxygen to ascorbic acid ratios were also calculated for the rate experiment described in Figure 1 and tabulated in Table II. Ratios here increased from 0.7 during an initial 5 minutes of reaction to 1.1 between 25 and 40 minutes, with no appreciable difference between hand-extracted and machine-extracted juice.

Hydrogen peroxide to ascorbic ratios may be calculated from data shown in Figure 4, assuming stoichiometric utilization of the added hydrogen peroxide. That the peroxide is consumed, or its catalytic activity for the aerobic reaction otherwise lost, during the first part of the anaerobic reaction was indicated by an experiment in which the anaerobic conditions were broken after 25 minutes. No further oxidation occurred when oxygen was bubbled through the solution for 15 minutes following the anaerobic reaction period. The reaction was allowed to proceed anaerobically in another tube for 40 minutes when the degree of oxidation was the same as that at 25 minutes—i.e., approximately 50% of the initial concentration. If all of the hydrogen peroxide from 4.2mM solution

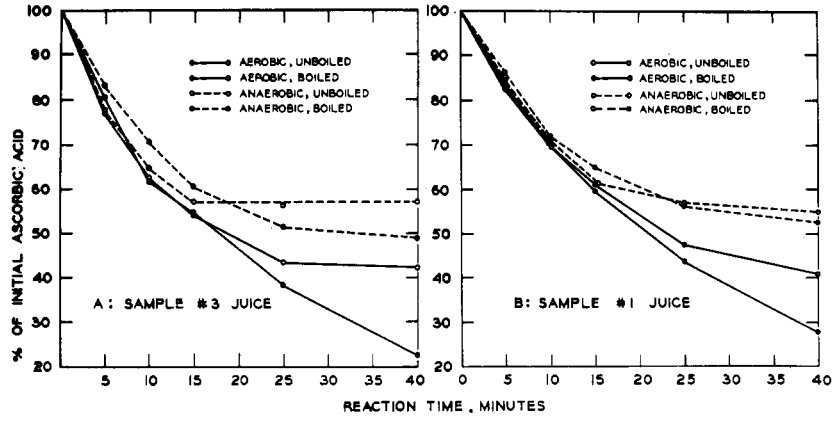

Figure 4. Oxidation of ascorbic acid during a 40-minute reaction at 25° C. for $^1/_3$ diluted passion fruit juice

Initial concentrations. 5.8mM ascorbic acid and 4.2mM H_2O_2
Sample 1. Hand-extracted juice from random sample of fruit used in preparation of machine-extracted juice (sample 3)

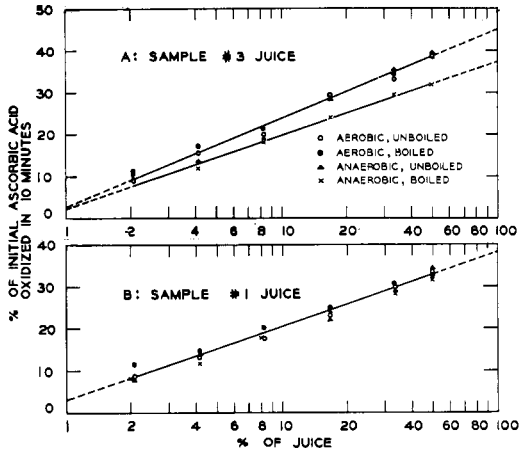

Figure 5. Semilogarithmic relationship of initial rates of ascorbic acid oxidation and dilutions of passion fruit juice

Initial concentrations. 5.8mM ascorbic acid and 4.2mM H_2O_2
Sample 1. Hand-extracted juice from random sample of fruit used in preparation of machine-extracted juice (sample 3)

Table I. Molar Ratios Oxygen Consumed to Ascorbic Acid Oxidized during 10-Minute Reaction

(Initial concentrations: 5.68mM added ascorbic acid and 4.17mM hydrogen peroxide in passion fruit juice)

Juice Dilution	Hand-Extracted			Machine-Extracted		
	O_2, μmole	Ascorbic acid, μmole	Molar ratio	O_2, μmole	Ascorbic acid, μmole	Molar ratio
1/48	1.48	1.96	0.76	1.13	1.93	0.59
1/24	1.80	2.23	0.81	2.12	2.99	0.71
1/12	2.75	3.18	0.87	2.97	3.73	0.80
1/6	3.69	3.89	0.95	4.14	5.04	0.82
1/3	5.13	5.22	0.98	5.94	5.78	1.03
1/2	5.53	6.00	0.92	6.85	7.01	0.98

Table II. Molar Ratios Oxygen Consumed to Ascorbic Acid Oxidized during 40-Minute Reaction in 1/3 Diluted Passion Fruit Juice (Boiled)

(Initial concentrations: 5.68mM ascorbic acid added and 4.17mM hydrogen peroxide)

Reaction Period, Min.	Hand-Extracted			Machine-Extracted		
	O_2, μmole	Ascorbic acid, μmole	Molar ratio	O_2, μmole	Ascorbic acid, μmole	Molar ratio
5	2.25	3.39	0.66	2.25	3.19	0.71
10	4.91	5.53	0.89	5.27	5.98	0.88
15	7.25	7.69	0.94	7.65	8.33	0.92
25	10.8	10.5	1.03	11.3	10.9	1.04
40	13.9	13.2	1.05	14.3	13.8	1.04

were consumed in this oxidation of initially 5.7mM ascorbic acid, the molar ratio would be 1.5.

Metallic Ion Catalysis. Evidence for metallic ion catalysts in the hydrogen peroxide–induced oxidation of ascorbic acid in passion fruit juice was obtained from experiments on the effect of a chelating agent. Disodium ethylenedinitrilotetraacetate in excess of calculated metal ion content of juice caused 90 to 100% inhibition of the aerobic oxidation in boiled juice, and 80 to 90% inhibition in unboiled juice. Limiting amounts of this chelating agent to cause slightly more than 50% inhibition were found to be between 20 and 30 p.p.m. on the basis of full strength juice.

Several metallic ions were tested as oxidative catalysts in the presence of hydrogen peroxide. Salts of nickel sulfate, manganese sulfate(ous), chromium sulfate(ic), cobalt sulfate(ous), copper sulfate(ic), ferrous and ferric sulfates were used. Only copper and iron ions showed appreciable activity during a 10-minute reaction period. Iron ions alone, in the range of 0.25 to 2.5 p.p.m., catalyzed the aerobic reaction at an initial rate comparable to that observed with juice. Results of these experiments are given in Table III.

Discussion

In a review of mechanisms for the decomposition of the hydrogen peroxide, Baxendale (*4*) discussed also the "ready oxidation of many organic substances by solutions containing ferrous iron and hydrogen peroxide." A suitable hydrogen donor competes for a free radical to replace the catalatic reaction, resulting in rapid oxidation of the organic substrate. In the absence of oxygen, the mechanism discussed by Kolthoff and Medalia (*19*) accounts for consumption of hydrogen peroxide in the over-all induced oxidation, as was indicated in the anaerobic experiments with passion fruit juice. This is not shown stoichiometrically from the calculation of a mole ratio (H_2O_2 to AH_2) of 1.5 (rather than 1.0) from data shown in Figure 4. However, in aerobic experiments, 1mM hydrogen peroxide was the low concentration limit for inducing the reaction. Thus, only 75% of the initial 4.2mM hydrogen peroxide may need be consumed to bring about termination of the reaction. If this is the case, the calculated ratio is 1.1 moles of hydrogen peroxide consumed per mole of ascorbic acid oxidized.

Kolthoff and Medalia (*20*) have also investigated hydrogen peroxide–induced reactions of ferrous iron in the presence of oxygen. Here hydrogen peroxide serves principally to initiate a reaction which may result in chain autoxidation of activated hydrogen donor. The mole ratio (O_2 to AH_2) expected in this autoxidation is 0.5, as in the ascorbase-catalyzed oxidation of ascorbic acid (*35*). The mechanism proposed by Nord (*26*) for copper-catalyzed aerobic oxidation also predicts this ratio. The ratio for the hydrogen peroxide–induced oxidation in passion fruit juice is variable (Tables I and II) and greater than 0.5. A shift of the reaction from autoxidation of the activated hydrogen donor to autoxidation of ferrous ion and regeneration of hydrogen peroxide may be involved (*20*, *35*).

Very few workers have reported investigations of the kinetics of a nonenzymic, hydrogen peroxide–induced oxidation of the specific substrate, ascorbic acid. Doskocil (*10*) investigated the polarographic reduction of hydrogen peroxide, catalyzed by the iron complex of ascorbic acid. The complex is considered to act by free radical formation from the peroxide. Kiese (*18*) describes the reduction of hemoglobin by ascorbic acid as the first order breakdown, a hemoglobin–ascorbic acid complex following a course of reduction of the iron. Hemoglobin was found (*23*) to break down to choleglobin anaerobically in the presence of ascorbic acid and hydrogen peroxide more rapidly than aerobically with ascorbic acid alone. This reaction is described by Lemberg and Legge (*24*) as an oxidation of ascorbic acid by a chain mechanism.

A somewhat different viewpoint of the role of ascorbic acid in peroxidatic-like reactions is presented by Bezssonoff and Leroux (*6*). They consider the action of ascorbic acid to be "peroxidasic" in the transfer of hydrogen from substrates, such as cresols, tyrosine, benzidine, and polyphenols (*7*). Copper and iron salts were required for maximum activity with benzidine and monophenols, but not for the polyphenols. Leroux (*25*) continued the work with tyrosine, varying concentrations of each of the reactants. The extent of the reaction is pronounced in 10 minutes with concentrations of 2.5μM ascorbic acid, 5 to 20μM tyrosine, and iron (from Fe_2Cl_6) from 2 to 4 p.p.m. As the concentration of ascorbic acid is increased above 2.5μM, the extent of the oxidations of phenols drops off rapidly. The influence of copper in the reaction appears to be dependent on ascorbic acid concentration. Results given in the report indicate that the reaction does not proceed in the absence of ascorbic acid.

The nearly explosive nature of hydrogen peroxide–induced oxidation of ascorbic acid in fruit juices is potentially of concern to food technologists. In passion fruit juice, calculation from the rate constant for this first order reaction in heat-treated juice shows a 94% aerobic oxidation in 1 hour in the presence of 65 p.p.m. peroxide. Without added hydrogen peroxide, no measurable oxidation occurs in 1 hour. As the mechanism of the reaction likely involves free radical formation, it is basically related to the deleterious effects of ionizing radiations on foods (*2*, *22*, *30*).

Although dissolved oxygen disappears from canned orange juice shortly after canning (*31*), oxidation of ascorbic acid continues under this anaerobic condition during storage, especially at elevated temperatures (*32*). This is true for other canned fruit juices, including passion fruit juice. Huelin (*14*) relates this anaerobic decomposition of ascorbic acid to furfural formation. A possible explanation may lie in mechanisms for oxidative reactions in the absence of oxygen as discussed here. Recent work (*17*, *21*) showing peroxidatic reactions resulting from the gradual generation or addition of very small amounts of hydrogen peroxide places a new light on the properties of certain iron compounds in this respect.

The concurrent catalatic and peroxidatic-like activities in fresh passion fruit juice demand more study. The fact that the decomposition of hydrogen peroxide does not occur in boiled juice indicates that this catalatic activity is enzymatic. Whether this is catalase activity or not,

Table III. Catalytic Effect of Copper and Iron Ions on Initial Oxidation Rate of Ascorbic Acid in Presence of 4.2mM Hydrogen Peroxide

Ion Concn., P.P.M.	Ion Species and Source		
	$Cu(CuSO_4)$	$Fe(FeCl_3)$	$Fe(FeSO_4)$
% Initial Ascorbic Acid (5.7mM) Oxidized in 10 Minutes			
25	...	79.1	...
20	21.8	...	...
10	15.1	79.4	...
5.0	9.6	75.1	80.3
2.5	...	61.7	70.7
2.0	6.9	...	...
1.0	5.4	35.9	49.8
0.50	...	...	32.4
0.25	...	...	21.6

cannot be concluded from the manometric measurements alone (*5*). This study shows that the hydrogen peroxide–induced oxidation of ascorbic acid practically ceases after the added peroxide has been decomposed by this activity in fresh juice. Consequently, this induced oxidation is of more concern in heat-treated passion fruit juice than in fresh or frozen juice. The magnitude of this "protective" action was shown by an experiment in which 100 ml. each of boiled and unboiled juice containing 1m*M* hydrogen peroxide was aerated by sintered glass aerators for 10 minutes. Forty-seven per cent of the initial ascorbic acid content remained in the boiled juice and 78% in the unboiled juice.

The iron content of fresh passion fruit juice (*12*) is in the range (2 to 5 p.p.m.) found for most common fruit juices (*39*). As iron from salts in this concentration range is much more active (Table III) than passion fruit juice in promoting the hydrogen peroxide–induced oxidation of ascorbic acid, an inhibitor of the reaction is suspected to be present in the juice. Another indication of this possibility is the rapid leveling off of initial rates for the greater juice concentrations (Figure 3) in a semilogarithmic relationship (Figure 5). For the iron salt solutions, the increase of initial rates with increasing metal ion concentration (Table III) is steep and linear over the range of rates observed for juice dilutions from $^1/_{48}$ to $^1/_2$. Exploratory experiments do show a pronounced inhibitory action of passion fruit juice on the initial aerobic rate of oxidation of ascorbic acid in solutions containing ferrous ion and hydrogen peroxide.

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PROTEIN FRACTIONATION

Fractionation of Commercial Papain by Ion Exchange

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THE PRACTICALITY OF FRACTIONATION of relatively low molecular weight and basic proteins by ion exchange chromatography is well established. Such functionally diverse proteins as cytochrome c (*9*), ribonuclease (*4, 5*), lysozyme (*5, 11*) egg white fraction (*10*), and calf thymus histone (*2*) have been fractionated.