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Effects of Certain Processing Methods, Substrate Level, and Polyphenoloxidase on the Stability of Ascorbic Acid in Kiwi Fruit[1]

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Abstract. **Ascorbic acid content in kiwi fruit (*Actinidia chinensis* Planch, cv. Hayward) decreased by 20% when the pulp was boiled for 2 hours. Frozen samples when left to thaw at room temperature also lost ascorbic acid to an equal degree; whereas, homogenization for an hour resulted in complete oxidation of ascorbic acid to dehydroascorbic acid. Reacting polyphenoloxidase prepared from mature kiwi fruit with extracts of the same revealed that the latter contained low amounts of polyphenols. Since ascorbic acid inhibits the oxidation of many polyphenols, our finding may account for the lack of browning reaction in tissues of bruised fruits.**

Kiwi fruit or the Chinese gooseberry is well-known for its nutritiousness, especially its ascorbic acid content (1, 6). As plantings of this exotic species spread to temperate fruit growing areas outside New Zealand, culinary artists and food processors, including kiwi wine makers, will need more information about the fruit. In preparing this fruit for fresh consumption or as processed food such as jellies, care should be taken to conserve its food value. The following experiments were conducted to assess the stability of ascorbic acid under vigorous treatments which are known to enhance its oxidation (4, 5). Ascorbic acid (AA) and dehydroascorbic acid (DAA) were determined by macerating a known weight of pulp with 20 ml of 5% metaphosphoric acid (MPA) and bringing it to 100 ml with 3% solution of MPA (7). A 2-ml filtered aliquot was analyzed for total AA and DAA using 2,6-dichlorophenol indophenol and 2,4-dinitrophenylhydrazine in 9N H_2SO_4.

Boiling. A 100-g sample of pulp was blended with 400 ml of water for 1 min. A fraction of the homogenate sufficient for the experiment was poured into a hot beaker sitting in a boiling water bath. After the mixture began to boil, 5-ml aliquots were drawn at intervals and homogenized with 35 ml of 5% MPA and brought to 100 ml. Under these conditions, the total AA content remained unchanged for the first 20 min, although there was oxidation of AA to DAA (Fig. 1A). This much oxidation could have occurred during the initial 1-min homogenation period and before the sample began to boil. During the following 100 min, both AA and DAA decreased, indicating that AA was oxidized to DAA which in turn was presumably metabolized to diketo-1-gulonic acid (7).

Blending or homogenation. When a 100-g sample was blended continuously for 2 hr, except for short intervals when a 5-ml aliquot was withdrawn, the AA content decreased very rapidly to a minimum value within 60 min (Fig. 1B). Although blending, which incorporated much air into the mixture, resulted in complete oxidation of AA to DAA, the total AA content did not change.

Freezing and thawing. To simulate a common household practice of temporarily freezing fruits in a deep freezer and serving them later upon thawing, 5-g samples were kept for 5 days at –20°C, after which they were brought to 21°. The samples macerated in 5% MPA were analyzed for AA and DAA hourly for the first 4 hr and then bi-hourly for the next 8 hr. This process resulted in oxidation of AA to DAA but little of the DAA was destroyed (Fig. 1C). The accumulation of DAA rather than its destruction is attributed to the slow gradual warming of the samples to room temperature prior to analysis.

Polyphenoloxidase (PPO) activity and substrate level. To evaluate PPO activity in the presence of tannins and polyphenolic substances in mature kiwi fruit, the enzyme was prepared by blending a known weight of fresh kiwi pulp with about 4 times the weight of acetone (2, 9). The mixture was filtered and the residue washed with ether to remove lipids. A 200-mg sample of dry powder was dissolved and brought to 100 ml with phosphate buffer, pH 6.2. After the protein content of the solution was determined (3), an aliquot equivalent to 1mg of protein diluted to 10 ml was used for the following test.

The tannin extract was prepared from 1 g of freeze-dried material homogenized with 35 ml of 80% ethanol using a tissue grinder and filtered. Air was bubbled through the filtrate for 3 hr and brought to 100 ml. Five-ml aliquots of the yellowish filtrate were evaporated and taken up with 5.0 ml of phosphate buffer, pH 6.2. It was assumed that during the extended aeration period and evaporation, AA was completely oxidized so as not to interfere with PPO activity. Previous trials showed that presence of AA in an extract would lengthen the lag time or if AA is added to the reaction mixture after darkening set in, the solution decolorized immediately but darkened again after AA was oxidized.

The OD initially increased at a rate of about 0.029 OD units/min-mg protein

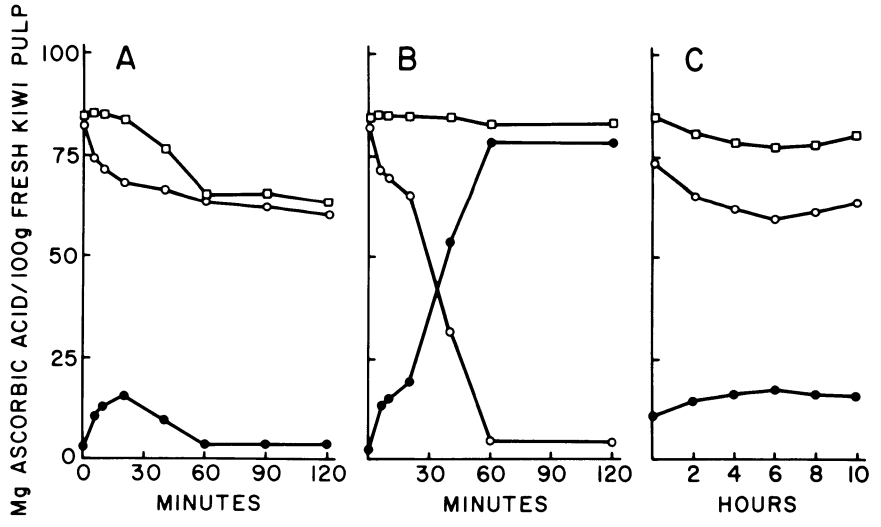

Fig. 1. Curves showing the effects of boiling (A), blending (B), and freezing and thawing (C) of 'Hayward' kiwi fruit pulp on the levels of total ascorbic acid, (□), ascorbic acid (○), and dehydroascorbic acid (●).

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when 10 ml of the kiwi PPO containing 1.0 mg of protein were added to the above kiwi extract in phosphate buffer. While the OD of the catechol solution continued to increase at 0.033 units/min-mg for several minutes, that of the extract quickly decreased to an average of 0.0025 OD units/min-mg (Fig. 2). Experiments with PPO prepared from apple and persimmon fruit revealed that at a fixed enzyme concentration increasing the substrate level delayed the onset of this plateau (unpublished). Therefore, when solid catechol was added to the reaction mixture and the OD immediately increased, it indicated that the PPO was still active and that the leveling of the reaction curve was due to substrate limitation rather than feedback inhibition by end-products.

The yellow pigment in the ethanolic extract was not decolorized by the addition of ascorbic acid as with some tannins (4, 5, 8). A portion of this extract was streaked on Whatman 3M paper and chromatographed using n-butanol:acetic acid:water (4:1:5, v/v/v). The chromatogram revealed 3 fluorescent bands under UV light. These fluorescing substances at Rf 0.19, 0.34, and 0.58 reacted with $FeCl_3$, substantiating that small quantities of phenolic substances are present in fresh kiwi fruit. (I. Okuse and K. Ryugo, in press).

The high ascorbic acid content and low tannin and polyphenolic levels in mature 'Hayward' fruit coupled with a relatively low PPO activity would explain why a bruised fruit develops a water-soaked appearance rather than discoloring and turning brown as in other fruits (8).

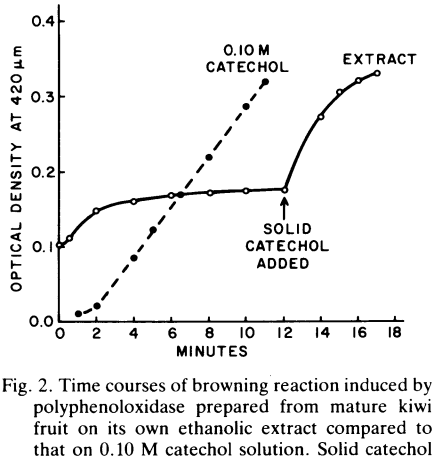

Fig. 2. Time courses of browning reaction induced by polyphenoloxidase prepared from mature kiwi fruit on its own ethanolic extract compared to that on 0.10 M catechol solution. Solid catechol was added to the substrate extract at the twelfth minute. The PPO solution contained an equivalent of 1 mg of bovine albumin per ml.

Literature Cited

1. Buisson-Lefresne, J. 1968. Quelques fruits exotiques sur le marche francais leur valeur nutritionnelle. Alimentation et La Vie. 56:5-16.
2. Conn, E. E. and P. K. Stumpf. 1972. Outlines of biochemistry, 3rd ed. Wiley, New York.
3. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.
4. Ponting, J. D. 1960. The control of enzymatic browning of fruits. In: H. W. Schultz (ed.) Food Enzymes. AVI, Westport, Conn.
5. Ponting, J. D. and M. A. Joslyn. 1948. Ascorbic acid oxidation and browning of apple tissue extracts. Arch. Biochem. 19:47-63.
6. Randoin, L. and J. Boisselot. 1945. Valeur energetique, minerale et vitaminique du fruit comestible d'*Actinidia chinesis*, Planchon. Bul. Soc. Sci, Hyg. Alimen. et d'Alim. Rationnelle. 33:144-153.
7. Roe, J. H., M. B. Mills, M. J. Oesterling, and C. M. Damron. 1948. The determination of diketo-1-gluonic acid, dehydro-1-ascorbic acid and 1-ascorbic acid in the same tissue extract by the 2,4-dinitrophenylhydrazine method. J. Biol. Chem. 174:201-208.
8. Tate, J. N., B. S. Luh, and G. K. York. 1964. Polyphenoloxidase in Bartlett pears. J. Food Sci. 29:829-831.
9. Zocca, A. and K. Ryugo. 1975. Changes in polyphenoloxidase activity and substrate level in maturing 'Golden Delicious' apple and other cultivars. HortScience 10:586-587.

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Effects of Calcium, EDTA, and Oxalic Acid on Respiration of Apple Slices[1]

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Abstract. **Pretreatment or incubation of apple flesh tissue in $CaCl_2$ solutions increased O_2 consumption. Tissue incubated in ethylenediaminetetra-acetic acid (EDTA) and oxalic acid showed an initial respiratory burst with a subsequent decrease in respiration after 2 hours. Pretreatment alone did not induce a respiratory burst, but rather caused respiration to lag behind controls. The increased O_2 consumption induced by $CaCl_2$ was explained by the presumed restoration of membrane integrity in the senescing tissues which allowed a higher rate of respiration than expected.**

Calcium can reduce or prevent many metabolic disorders of fruits and vegetables. Marinos (7) concluded from the ultrastructure study of Ca-deficient plants that Ca is essential for the maintenance of cell membranes on which the functional integrity and cellular metabolism are dependent. This role of Ca has been used to explain its effect on the respiration of fruits (1). Correlations among Ca levels and the respiratory mechanism (2, 4), and the permeability (9, 10) of apple fruit tissue have been examined. All of these studies utilized fruit close to maturity. We investigated the effects of Ca and Ca-chelating agents on the respiration of senescing apple fruit tissue.

Cylinders of flesh tissue were removed from apples using a 6-mm cork borer, sliced with a razor blade into discs about 1 mm thick, and placed into double-distilled water in an ice bath. Tissue slices were then rinsed 5 times with double-distilled water. Samples of 1 g were either weighed and used in the respiration experiments immediately, or incubated in pretreatment solutions of either 1×10^{-1} M $CaCl_2$, 1×10^{-1} M oxalic acid or sodium ethylenediaminetetra-acetic acid (EDTA),or in 0.5 M $KHPO_4$ buffer. Pretreated samples were incubated in the above mentioned solutions for 1 hr, rinsed 5 times with double-distilled water, and placed in 0.5 M $KHPO_4$ buffer during the respiration measurements. All samples were transferred to a double-sidearm Warburg gas-exchange flask which also contained 2 ml of 20% KOH and filter paper in the center well for CO_2 absorption.

The flasks were agitated in a water bath at 25°C on a Gilson Differential Respirometer, and allowed to equilibrate for 20 minutes before O_2 consumption was measured. Of the 3 apple cultivars studied, 'York Imperial' and 'Delicious' had been stored at 1°C for about 9 months, whereas 'Lodi' was stored for only 2 weeks. Each treatment was replicated 9 times.

Respiration rates were similar for all cultivars tested; therefore, 2 representa-

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