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# Dehydroascorbic Acid Levels in Fresh Fruit and Vegetables in Relation to Total Vitamin C Activity

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Thirteen types of fresh fruit and vegetables were purchased from retail markets and held at 20 °C until they were unacceptable for consumption. Produce were analyzed for dehydroascorbic acid (DHA) and ascorbic acid at frequent intervals. At the time of purchase, DHA was present in only six produce at 1.0–4.6 mg/100 g and contributed less than 10% of total vitamin C in all produce except for celery (about 40%). During storage all produce except banana showed an increase in DHA. Highest levels were in cantaloupe and broccoli (16.0 and 11.3 mg/100 g, respectively) with other produce having a maximum level between 3.0 and 6.0 mg/100 g. The proportion of vitamin C present as DHA was greater than 50% in celery and cucumber, greater than 25% in potato, cantaloupe, and broccoli, between 10 and 20% in Brussels sprouts, silver beet, tomato, lemon, and orange, and less than 5% in banana and parsley.

Fresh fruit and vegetables are significant sources of dietary vitamin C. The principal biologically active form is L-ascorbic acid but an oxidation product, L-dehydroascorbic acid (DHA), is also active. Vitamin C activity is commonly determined by the dye-titration method using 2,6-dichlorophenolindophenol (AOAC, 1980), which, however, only measures ascorbic acid. It is commonly assumed that the level of DHA in fresh fruit and vegetables is low and therefore the error incurred in such analyses is small. There have, however, been few meaningful studies to determine the relative levels of ascorbic acid and DHA in fresh produce. The most comprehensive studies have been by Mills et al. (1949) and Lu and Chou (1955). Mills et al. (1949) purchased 27 types of produce from city markets (presumably in Washington, DC) in a range of physical condition from good to old and withered and found that while 17 samples contained some DHA, the amount was small unless the food had deteriorated considerably. Lu and Chou (1955) obtained 26 types of produce from the markets in Ch'angsha, China, during winter and found that DHA was present in all samples and accounted for >25% of the total vitamin C activity in 10 produce.

These determinations were made by using some modification of the 2,4-dinitrophenylhydrazine colorimetric method of Roe and Oesterling (1944), which essentially measures the two forms of the vitamin by difference following an oxidation or reduction reaction. However, it has been claimed (Davidek et al., 1972) that such methods overestimate DHA due to interfering substances. High-performance liquid chromatographic methods that allow the rapid and simultaneous estimation of ascorbic acid and DHA have recently been developed (Finley and Duang, 1981; Rose and Nahrwold, 1981; Wimalasiri and Wills, 1983), and we have used such a method to determine the levels of ascorbic acid and DHA in a range of fresh fruit and vegetables.

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## MATERIALS AND METHODS

Thirteen types of fresh fruit and vegetables of good commercial quality were obtained from local retail markets in Sydney, Australia. A sample of each type was immediately analyzed for ascorbic acid and DHA, and the remaining produce was stored at 20 °C with analyses being conducted at regular intervals until the produce was considered to be not acceptable for consumption. At each time of analysis, two analytical samples of a produce were prepared, each by blending together the edible portion from at least four pieces. Duplicate estimations were made on each sample.

The method of extraction and analysis was identical with that described by Wimalasiri and Wills (1983). This involved extraction of 10–50 g with 3% citric acid solution, which after filtration through paper was further purified by passage through a membrane/ultrafilter cell (Diaflo ultrafilter, Amicon Corp.) and a short disposable column containing μBondapak $C_{18}$ ($C_{18}$ Sep-PAK, Waters Associates). An aliquot (20 μL) was injected onto a μBondapak/Carbohydrate column (Waters Associates) (30 cm × 4 mm i.d.) installed in a Waters liquid chromatograph (Model ALC/GPC 244) equipped with a 41-mPa pump and U6K injector. The mobile phase was acetonitrile–water (70:30 v/v) containing 0.01 M ammonium dihydrogen phosphate (pH 4.3) at 2 mL/min. Column effluents were monitored by two UV detectors set at 254 nm (Waters Model 440) and 214 nm (Waters Model M441) for estimation of ascorbic acid and DHA, respectively. The amounts of ascorbic acid and DHA present were determined by comparison of peak areas with standard curves produced from solutions of ascorbic acid (Ajax Chemicals, Sydney) and DHA (Pfaltz and Bauer, Stanford, CT).

The specificity of the method to estimate only ascorbic acid and DHA was confirmed for each produce. Ascorbic acid was removed from the purified extracts by oxidation with activated charcoal (AOAC, 1980), and the solutions were reanalyzed to confirm that no peak was present at the retention time of ascorbic acid and that the ascorbic

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**Table I. Changes in Dehydroascorbic Acid and Ascorbic Acid in Fruit and Vegetables during Storage at 20 °C[a]**

| compound | produce | level, mg/100 g, present during time of storage | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 12 |
| | | Time Scale in Days | | | | | | | | | | |
| DHA, mg/100 g | banana | 0.0 | | 0.0 | | 0.0 | | 0.0 | | r | | |
| | broccoli | 4.3 | | 6.5 | | 11.3 | | d | | | | |
| | brussels sprouts | 4.6 | 0.0 | 1.3 | 1.6 | 4.6 | d | | | | | |
| | canteloupe | 1.0 | | 2.5 | | 3.2 | | 6.0 | | 10.2 | 11.0 | 16.0 |
| | cauliflower | 0.0 | 6.0 | 4.2 | 4.8 | r | | | | | | |
| | celery | 4.2 | 4.0 | 4.2 | 5.0 | 4.5 | w | | | | | |
| | cucumber | 1.2 | | 2.8 | | 1.6 | | 6.2 | | 4.4 | 3.2 | w |
| | parsley | 0.0 | 4.5 | 1.0 | 3.5 | w, d | | | | | | |
| | silver beet | 0.0 | 0.0 | 1.8 | 4.1 | w | | | | | | |
| | tomato | 1.5 | | 3.6 | | 1.1 | | 1.4 | | 2.3 | w, d | |
| | | Time Scale in Weeks | | | | | | | | | | |
| DHA, mg/100 g | lemon | 0.0 | 0.0 | 0.0 | 0.0 | 2.3 | 3.1 | 1.0 | 1.2 | r | | |
| | orange | 0.0 | 0.0 | 0.0 | 0.0 | 1.1 | 3.0 | r | | | | |
| | potato | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 | 4.2 | s | | |
| | | Time Scale in Days | | | | | | | | | | |
| ascorbic acid, mg/100 g | banana | 8.0 | | 8.0 | | 5.4 | | 4.0 | | | | |
| | broccoli | 75.7 | | 52.5 | | 32.3 | | | | | | |
| | brussels sprouts | 57.6 | 57.2 | 52.6 | 50.3 | 37.0 | | | | | | |
| | cantaloupe | 62.0 | | 56.5 | | 53.0 | | 44.6 | | 40.0 | 34.5 | 28.2 |
| | cauliflower | 48.6 | 41.5 | 40.0 | 36.5 | | | | | | | |
| | celery | 6.0 | 4.5 | 4.2 | 0.0 | 0.0 | | | | | | |
| | cucumber | 12.3 | | 11.0 | | 8.5 | | 4.5 | | 2.0 | 2.0 | |
| | parsley | 152.0 | 146.5 | 132.0 | 125.0 | | | | | | | |
| | silver beet | 52.0 | 49.7 | 47.8 | 40.3 | | | | | | | |
| | tomato | 18.7 | | 14.3 | | 12.3 | | 10.6 | | 10.0 | | |
| | | Time Scale in Weeks | | | | | | | | | | |
| ascorbic acid, mg/100 g | lemon | 42.4 | 40.7 | 40.6 | 37.1 | 36.3 | 30.7 | 30.4 | 30.1 | | | |
| | orange | 47.5 | 39.9 | 36.5 | 34.2 | 29.5 | 27.3 | | | | | |
| | potato | 13.8 | 13.5 | 13.0 | 11.3 | 10.0 | 9.6 | 5.9 | 4.7 | | | |
| | | Time Scale in Days | | | | | | | | | | |
| ratio of DHA:(ascorbic acid + DHA), % | banana | 0 | | 0 | | 0 | | 0 | | | | |
| | broccoli | 5 | | 11 | | 26 | | | | | | |
| | brussels sprouts | 7 | 0 | 2 | 3 | 11 | | | | | | |
| | cantaloupe | 2 | | 4 | | 6 | | 12 | | 20 | 24 | 36 |
| | cauliflower | 0 | 13 | 9 | 12 | | | | | | | |
| | celery | 41 | 47 | 51 | 100 | 100 | | | | | | |
| | cucumber | 9 | | 20 | | 16 | | 58 | | 69 | 62 | |
| | parsley | 0 | 3 | 1 | 3 | | | | | | | |
| | silver beet | 0 | 0 | 4 | 9 | | | | | | | |
| | tomato | 7 | | 20 | | 8 | | 12 | | 18 | | |
| | | Time Scale in Weeks | | | | | | | | | | |
| ratio of DHA:(ascorbic acid + DHA), % | lemon | 0 | 0 | 0 | 0 | 6 | 9 | 3 | 4 | | | |
| | orange | 0 | 0 | 0 | 0 | 4 | 10 | | | | | |
| | potato | 0 | 0 | 0 | 0 | 0 | 0 | 36 | 47 | | | |

[a] Produce became not acceptable for consumption due to development of rotting (r), wilting or shriveling (w), discoloration, either yellowing or browning (d), or sprouting (s).

acid was quantitatively converted to DHA. DHA was removed by reduction with homocysteine (Hughes, 1956), and the solutions were similarly reanalyzed. Although other compounds may be oxidized by activated charcoal, reduction of the reducible oxidized form enhances the specificity of the method.

## RESULTS AND DISCUSSION

Table I gives the levels of DHA and ascorbic acid and the ratio of DHA:total vitamin C activity (i.e., ascorbic acid plus DHA) in 13 fresh fruit and vegetables during storage at 20 °C from the time of purchase as mature edible produce at retail outlets until they were not acceptable for consumption. At the time of purchase only six produce contained DHA with the levels ranging from 1.0 to 4.6 mg/100 g. The level is celery comprised about 40% of the total vitamin C. During the postpurchase storage period at 20 °C that the produce remained edible, all produce except banana showed an increase in DHA. Cantaloupe and broccoli accumulated the highest levels of 16.0 and 11.3 mg/100 g, respectively, with other produce having a maximum value between 3.0 and 6.0 mg/100 g. The proportion of vitamin C present as DHA became greater than 50% in celery and cucumber, greater than 25% in potato, cantaloupe, and broccoli, between about 10 and 20% in other produce except for parsley and banana where it accounted for less than 5% at all times. While the level of ascorbic acid showed a continuous decrease in all produce during storage, changes in DHA did not always follow a uniform trend. The general trend was for DHA to increase slowly at first and then at a faster rate as the produce neared the end of its edible life. However, Brussels sprouts, cauliflower, cucumber, parsley, tomato, and lemon showed an initial peak value of DHA, which decreased for a short time and then increased continuously with further produce aging.

The overall effect of these changes was that the proportion of vitamin C present as DHA varied not only between produce but also within a produce at different stages of senescence. Thus, DHA can be a significant contributor to total vitamin C activity, and methods of analysis that

do not measure DHA may substantially underestimate vitamin C. However, since the relative contribution of DHA to vitamin C activity is not constant, the error due to nonestimation of DHA will not remain constant for any produce.

**Registry No.** DHA, 490-83-5; ascorbic acid, 50-81-7.

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# Quantitative Study of Volatiles in a Model System by a Headspace Technique

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A test solution was prepared with 16 pure compounds belonging to various chemical classes and representative of those found in fruits and fermented beverages aroma. These volatiles were extracted during 24 h by nitrogen entrainment associated with continuous Freon 11 liquid extraction in an apparatus similar to the one described by Rapp and Knipser. Analysis of the recovered material was done by quantitative gas chromatography. Reproducibility of the method is good; coefficients of variation are below 15%. Polar compounds such as alcohols, 4-ethylphenol, and lactones are not quantitatively recovered (2–10%) as they are badly entrained with nitrogen. Unstripped compounds are recovered by back-extraction of the remaining test solution. Apolar compounds such as terpene hydrocarbons are completely entrained with nitrogen but are partially lost during the extraction procedure. Losses are below 20%.

In the recent years, $N_2$ entrainment associated with trapping on a porous polymer has been widely used to study the aroma of wines and fruits (Jennings et al., 1972; Noble, 1978; Williams and Strauss, 1977; Murray, 1977). Minor components often critically important to aroma have been detected by this method while they could not have been revealed by an equilibrium method such as liquid and piston displacement (Williams et al., 1978).

The porous polymers have the common advantage of possessing minimal retention for ethanol as well as water, but some specific drawbacks have been detected for each of them. Tenax has been extensively used to study wines volatiles (Bertuccioli and Viani, 1976; Noble, 1978; Noble et al., 1980). Because of its high thermal stability, heavy compounds were quickly desorbed but its low specific area could create a fast saturation of the trap (Butler and Burke, 1976; Brown and Purnell, 1979). Porapack and Chromosorbs have higher sampling capacity, but high boiling compound recoveries are incomplete due to their low thermal stability (Williams et al., 1978). These different selectivities of porous resins induce distortion in the composition of collected headspace volatiles. A last common drawback is the necessity of conditioning the trap before and after each sample collection.

To avoid the disadvantages associated with the use of an adsorbent, Rapp and Knipser (1980) have proposed a method that combines $N_2$ entrainment and volatile trapping by Freon 11 continuous extraction. Williams (1982) advocated this method to collect volatiles of fruit or alcoholic beverages, but this procedure has been questioned by Novak (1981, 1982). However, recently Simpson and Miller (1983) and Guichard (1984), respectively, applied this technique with success to the study of Riesling wine and raspberry.

Rapp and Knipser evaluated reproducibility of their method for terpenoid components and others volatiles of interest for Moriot Muscat wine aroma. In this work we have tested this method with a model system of pure compounds belonging to various chemical classes of interest for fruit or fermented beverage aroma. Recovery of the pure compounds and the losses associated with this method were investigated by quantitative estimations of the constituents in the aroma extract and in the remaining test solution. The reproducibility of the techniques was determined for each compound.

## MATERIALS AND METHODS

**Model Solution.** The test solution was made by adding 1 ppm (v/v) of each pure compound to a 10% aqueous ethanol solution. Water was purified on $XAD_2$ resin as described by James et al. (1981).

**Extraction Procedure.** The apparatus as proposed by Rapp and Knipser to collect volatiles was slightly modified: the tip of the collecting funnel was fitted with a fritted disc (porosity 2), which creates a dispersion of solvent in the sample.

Eight-hundred milliliters of test solution was placed in a flask kept in a water bath at 25 °C. Volatiles were entrained by purified nitrogen swept at the rate of 50

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