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Ascorbic Acid Content of Some Tropical Fruit Products Determined by High-Performance Liquid Chromatography

Some guava, mango, papaya, and orange products were analyzed for ascorbic acid by using high-performance liquid chromatography. Many of the products contained relatively low levels as compared with those of fresh fruit. Extraction procedures using methanol–water were compared with those using methanol–6% metaphosphoric acid, and the latter more completely extracted ascorbic acid. Canned orange shells were devoid of ascorbic acid. It may have been removed by extraction during a debittering process.

Fruit and vegetables are the major dietary sources for ascorbic acid in humans, and many fresh tropical and subtropical fruit are particularly rich in this vitamin (Nagy and Shaw, 1980). Retention of ascorbic acid in products from fruit processed in high volume, such as citrus, is well documented (Ting, 1977). However, retention of ascorbic acid in products from other tropical fruit processed in relatively small volume has received much less study, even though many tropical fruits are widely consumed as fresh fruit (Nagy and Shaw, 1980). With increasing concern for the nutritional content in foods and potential increasing development and marketing of processed products from tropical fruit, there is need for basic information on a principal vitamin, ascorbic acid, in processed tropical fruit products.

Processed products from tropical fruit such as guavas, mangos, and papayas have been studied for storage stability, and in some cases ascorbic acid retention has been monitored. Ascorbic acid retention in stored canned guavas was increased by addition of citric acid to increase acidity and sucrose to increase Brix (Gauhar and Durrani, 1972). A canned guava puree concentrate retained 65% of the original ascorbic acid after 5 months at −18 °C, but virtually all ascorbic acid was lost after 1 month at 7 °C (Brekke et al., 1970). Frozen and unfrozen bottled guava juices retained >70% of their ascorbic acid after 11 months (Orr and Miller, 1954). Freeze-dried and drum-dried mango powders were prepared that contained >86% of the original ascorbic acid, but several workers showed that in canned mango pulp ascorbic acid retention was poor during storage at room temperature (Lakshminarayana, 1980). Papaya puree with an initial ascorbic acid content of 50–90 mg/100 g has been prepared in both the canned and frozen forms. Heat treatment used during the process can reduce the ascorbic acid level in the canned product (de Arriola et al., 1980). Papaya puree concentrate and freeze-dried papaya each contained 15–20% less ascorbic acid than the fresh material. No studies on loss of ascorbic acid during storage of papaya products have been documented (de Arriola et al., 1980).

Table I. Ascorbic Acid (AA) Content of Some Tropical Fruit Products

product	ascorbic acid,[a] mg/100 g	% U.S. RDA[b]
guava		
shells[c]	6.0	
shells	13.8	30
shells + 6.8 mg % AA	21.3	
shells + 1000 mg % AA[c]	724	
shells + 1000 mg % AA	826	
paste	1.7	4
marmalade	1.7	4
jelly[d]	40.6	90
jelly[e]	2.9	6
mango		
slices	7.5	15
marmalade	7.9	20
papaya chunks (green)	0.6	2
orange		
shells	N[f]	
syrup from shells	0.1	0.2

[a] Average of two values determined on duplicate 50-g samples. [b] Based on a 100-g serving and a recommended U.S. RDA of 45 mg (Ting, 1977). [c] Ascorbic acid extracted with methanol-water instead of the methanol-6% HPO_3 solution. [d] Guava juice listed as the first ingredient. [e] Guava juice listed as the second ingredient. [f] N = not detected at a level of 0.075 mg % or greater.

These previous studies have provided little information for the consumer about the levels of ascorbic acid in processed products from tropical fruit reported to be good sources of this vitamin in the fresh state. We studied ascorbic acid levels in guava, mango, and papaya products available to the consumer as determined by high-performance liquid chromatography (HPLC). In addition, we studied the ascorbic acid content in a specialty citrus

product we expected to be a good source of ascorbic acid.

EXPERIMENTAL SECTION

Fruit Samples. All tropical fruit products were purchased from a local market and stored at 21 °C until used. The products (listed in Table I) were canned except the two guava jelly samples which were in metal-capped glass jars. Samples for analyses were taken directly from the containers except for those packed in syrup (guava shells, mango slices, papaya chunks, and orange shells). In those samples, the syrup was drained from the fruit pieces prior to sampling.

Extraction Methods. Duplicate 50-g samples of product were each blended with 50 mL of methanol for 1 min and filtered through filter-aid, and the filter cake was blended with two 50-mL portions of either a 6% metaphosphoric acid solution (HPO_3) or water, with filtration through filter-aid each time. When a known amount of ascorbic acid was added to the product, it was dissolved in 5 mL of 6% HPO_3 solution or water and blended with the product for 1 min prior to blending with 50 mL of methanol. In those cases, 95 mL of 6% HPO_3 or water was used in two portions in the subsequent step. The combined filtrate (about 150 mL) was concentrated to 100-mL volume on a rotary evaporator at 2-mmHg pressure and 28 °C. A 7-mL portion of the concentrated extract was purified by percolation through a C-18 Sep-Pak (Waters Associates, Milford, MA) which had been pretreated with 2 mL of acetonitrile followed by 5 mL of water. The first 2 mL of filtrate was discarded and the remaining 5 mL was filtered through a 1.2 μ Millipore filter prior to HPLC analysis.

Analytical Methods. The instrument used was a Waters Model 202 ALC equipped with an LDC Spectromonitor III variable-wavelength ultraviolet detector set at 245 nm, an Altex Model 905-42 injector fitted with a 20-μL injection loop, a Waters Model 6000A pump, and a Hewlett-Packard Model 3380A recording integrator. A 4-cm guard column packed with 10-μm RP-18 packing (Brownlee Laboratories, Inc., Santa Clara, CA) and an 8 mm × 10 cm column packed with 10-μm C-18 packing in a Waters Model RC-100 radial compression unit were used. The eluting solvent was aqueous 2% $NH_4H_2PO_4$ at pH 2.8 at a flow rate of 1.1 mL/min. Alternate runs of a standard solution of ascorbic acid and each of two duplicate extracts were made. Three such runs were carried out for each sample. The coefficient of variation for the three runs of each sample was generally less than 5%.

RESULTS AND DISCUSSION

The method of extraction that would be applied to all products was studied before making final analyses. Generally, aqueous metaphosphoric acid alone, or with acetic acid, has been used to extract ascorbic acid from foodstuffs (Cooke, 1974), but oxalic acid, ethanol, or methanol–water extracts have also been used successfully (Cooke, 1974; Randall et al., 1975; Wilson and Shaw, 1981). We determined the recovery of ascorbic acid at relatively low and relatively high levels in guava shells by methanol–water and methanol–6% HPO_3 extraction procedures (Table I). At a relatively low level (13.8 mg/100 g), more than twice as much ascorbic acid was present in the methanol–6% HPO_3 extract as was present in the methanol–water extract. When 6.8 mg/100 g ascorbic acid was added to the guava shells, virtually all of it was recovered by extraction with methanol–6% HPO_3. At a relatively high level of added ascorbic acid (1000 mg/100 g), where the natural quantity present in guava shells (13.8 mg/100 g) was less than experimental error, 82.6% of the ascorbic acid was recovered by methanol–6% HPO_3 extraction and only 72.4% by methanol–water extraction. Thus, the methanol–6% HPO_3 extraction procedure was used in all subsequent extractions. Since virtually all ascorbic acid was removed at relatively low ascorbic acid levels (with that extraction procedure), no correction factor was necessary for the levels reported in Table I.

Guavas are among the richest natural sources of ascorbic acid, averaging 337 mg/100 g (Wilson, 1980), and several guava products were available in local markets for analysis. Of the guava products analyzed, the shells might be expected to contain the highest level of ascorbic acid. Previous studies have shown high amounts in guava flesh (shells). In addition, the other ingredient, sugar syrup, was largely removed by draining the shells prior to analysis. The shells contained 13.8 mg/100 g ascorbic acid, or 30% of the U.S. RDA for this vitamin, based on a 100-g serving (Ting, 1977). This was a higher level of ascorbic acid than in all other guava products except for one guava jelly sample that listed guava juice as the first ingredient. That jelly sample contained 3 times as much ascorbic acid as did guava shells, and the label did not list ascorbic acid as an added ingredient. This is 90% of the U.S. RDA for ascorbic acid, based on a 100-g sample, but a 16-g sample (1 level tbs, 15% U.S. RDA) would more nearly approximate a typical serving size. The second sample of guava jelly had sugar listed as the first ingredient and guava juice as the second ingredient. Its ascorbic acid content was 15 times less than that of the first guava jelly analyzed. Guava marmalade listed guava pulp as the first ingredient and guava paste listed pulp as the second ingredient. However, both contained about equal but relatively low amounts of ascorbic acid (Table I). Two of the five guava products contained at least 10% of the U.S. RDA and could be considered significant sources for ascorbic acid (Ting, 1977).

Fresh mangos generally contain 25–50 mg of ascorbic acid/100 g of pulp (Lakshminarayana, 1980), and thus a significant quantity might be anticipated in mango products. The two products analyzed, mango slices and marmalade, both contained comparable amounts of ascorbic acid (7.5–7.9 mg/100 g). These products would be considered significant sources based on a 100-g serving.

Fresh papaya contains from 42 mg/100 g ascorbic acid when immature to 55 mg/100 g when ripe (de Arriola et al., 1980). The one sample of processed papaya available in a local market, green papaya chunks, contained the lowest level of ascorbic acid of any tropical fruit product studied (0.6 mg/100 g) except orange shells (see below). This amounts to 2% of the U.S. RDA and is the minimum nutrient level declarable on nutrient labeling (Ting, 1980).

One canned orange product was included in this study because most fresh and processed orange products previously studied had a reasonably consistent level of ascorbic acid (Ting, 1977). This product, orange shells, was devoid of ascorbic acid, within the limits of analysis (Table I). The sugar syrup drained from the shells did, however, contain a trace of ascorbic acid. The label described the orange shells as "bitterless orange peels with skin removed". This product appeared to be orange albedo, with the bitter principles probably removed by exhaustive extraction with water. The ascorbic acid was probably removed in the same process, so that albedo, which normally contains 3 times as much ascorbic acid as juice vesicles (Ting, 1977), lost all of its ascorbic acid by the debittering process.

Dehydroascorbic acid was not measured in this study, because fruit generally have been reported to contain little dehydroascorbic acid. It is rapidly converted to other

degradation products when it is formed from ascorbic acid (Gresswell, 1974).

The results of this study emphasize the need for nutritional labeling to aid the consumer in choice of foods. Most of the processed fruit products analyzed were relatively poor sources of vitamin C compared with levels which might be expected, based on the vitamin C content of the corresponding fresh fruit. As more tropical fruit are processed to open new markets (de Arriola et al., 1980), additional information will be needed on the vitamin content of such products.

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Synthesis and Mass Spectra of Hexachlorocyclopentadiene-$^{37}Cl_6$ and Derived Organochlorine Insecticides

The chemical syntheses of heptachlor-*4,5,6,7,8,8*-$^{37}Cl_6$ (1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene-*4,5,6,7,8,8*-$^{37}Cl_6$), heptachlor-*4,5,6,7,8,8*-$^{37}Cl_6$ epoxide (1,4,5,6,7,8,8-heptachloro-2,3-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindene-*4,5,6,7,8,8*-$^{37}Cl_6$), and *trans*-nonachlor-*4,5,6,7,8,8*-$^{37}Cl_6$ (1-*exo*,2-*endo*,3-*exo*,4,5,6,7,8,8-nonachloro-3a,4,7,7a-tetrahydro-4,7-methanoindan-*4,5,6,7,8,8*-$^{37}Cl_6$) are described. The key intermediate in the synthesis is hexachlorocyclopentadiene-$^{37}Cl_6$, enriched to the >92% ^{37}Cl level. The mass spectra of these compounds show simplified molecular ion clusters which are clearly resolvable from those of the unlabeled analogues, thus illustrating their potential value as stable isotope labeled internal standards for analysis.

In recent years stable isotope labeled compounds have seen increasingly wide use as internal standards for mass spectrometric analysis in biomedical and environmental areas (Klein and Klein, 1979; Pohl and Nelson, 1977). In many cases advances in the use of such analytical techniques are limited by the availability of appropriately labeled standards. The most useful such compounds are labeled in nonexchangeable positions in such a way that they give clearly defined mass spectral molecular ions or fragment ions distinctly separate from those of the natural abundance species. For compounds containing multiple chlorine atoms, the latter requirement takes on unusual importance because the natural isotopic distribution of chlorine gives rise to complicated mass spectral ion patterns. *Substantial* enrichment of such compounds with ^{37}Cl would in many cases provide extremely useful labeled standard materials.

Among the organochlorine insecticides which are important environmental contaminants are a large number derived from hexachlorocyclopentadiene (Brooks, 1974). These include mirex (1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1,3,4-metheno-1*H*-cyclobuta[*cd*]pentalene), Kepone (1,1a,3,3a,4,5,5,5a,5b,6-decachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalen-2-one), heptachlor epoxide, *trans*-chlordane (1-*exo*,2-*endo*,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane), aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo*,*exo*-5,8-5,8-dimethanonaphthalene), dieldrin (1,2,3,4,10,10-hexachloro-6,7-*exo*-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo*,*exo*-5,8-dimethanonaphthalene), endrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-*endo*,*endo*-5,8-dimethanonaphthalene), Kelevan [ethyl 5-(1,1a,3,3a,4,5,5,5a,5b,6-decachlorooctahydro-2-hydroxy-1,3,4-metheno-1*H*-cyclobuta[*cd*]pentalen-2-yl)levulinate], and endosulfan (isomers of 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide).

The present paper describes the synthesis of hexachlorocyclopentadiene-$^{37}Cl_6$ of 92.9% ^{37}Cl isotopic abundance, illustrates its use in the synthesis of some ^{37}Cl-la-

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