Improved Flavor of Navel Orange and Grapefruit Juices by Removal of Bitter Components with β-Cyclodextrin Polymer

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Use of β-cyclodextrin polymer at 1 g of polymer/50 mL of juice in a continuous flow fluid-bed or a batch process lowered the major bitter components limonin, nomilin, and naringin in grapefruit juice and limonin and nomilin in navel orange juice by about 50%. The polymer was regenerated by treatment with dilute aqueous alkali or ethanol. Taste panel members preferred the juices with reduced bitterness when compared to the control juice. The polymer treatment did not significantly affect the soluble solids, total acid, or ascorbic acid contents of the juice, but it did lower the oil level about 40%, and several other trace constituents were also partially removed. α-Cyclodextrin polymer was also effective in removing bitter components from these juices.

Excessive bitterness is an undesirable flavor quality of some commercial Navel orange and grapefruit juice products. For grapefruit juice processed in Florida, there is an upper limit on content of the two major bitter components, limonin (5.0 ppm) or naringin (600 ppm by Davis test), for juice processed from Aug 1 to Dec 1 to meet quality standards (Florida Department of Citrus, 1975). So far, no acceptable commercial process has been found for decreasing the levels of these bitter components without affecting desirable components of the juice. A commercial process is needed that removes bitter components without adding anything to the juice, while still maintaining the expected flavor and nutrition of the product.

Many attempts have been made to remove bitter components from navel orange juice [Hasegawa et al., 1973, 1982; reviewed by Maier et al. (1977)]. Fewer attempts have been made to decrease bitterness in grapefruit juice. However, treatment of the juice with enzymes to hydrolyze naringin to nonbitter compounds has been successful [reviewed by Kefford and Chandler (1970)]. Recently, ion-exchange resins were used to remove titratable acids, as well as limonin and naringin, from grapefruit juice (Johnson and Chandler, 1982). β-Cyclodextrin monomer, which is soluble in aqueous solution, was shown to decrease the bitter taste of limonin and naringin in citrus juices (Konno et al., 1981, 1982) although analyses for these components showed them to still be present in the juice medium (Shaw and Wilson, 1983). In an earlier study, we found that β-cyclodextrin polymer reduced the levels of limonin and naringin in clarified citrus juices and that water or organic solvents regenerated the polymer for use in debittering additional juice samples (Shaw and Wilson, 1983).

The current study reports use of α- or β-cyclodextrin polymer with commercial citrus juices in a continuous fluid-bed or batch process to reduce levels of limonin, nomilin, and naringin in the juices. The debittered juices were evaluated by an experienced taste panel.

EXPERIMENTAL SECTION

Samples. Florida navel oranges were purchased at a local market on Oct 21, 1982. The juice from 12 fruit (10.1 °Brix) was heated to 85 °C to convert all limonin precursor to limonin. Frozen concentrated navel orange (65 °Brix) and grapefruit juices (55 °Brix) and single-strength glass-packed grapefruit juice (10.0 °Brix) were obtained from Florida Citrus World, Lake Wales, FL.

Preparation of Cyclodextrin Polymers. A mixture of 25 g of α- or β-cyclodextrin (Sigma Chemical Co., St. Louis, MO) and 11 mL of water was treated with a freshly mixed solution (80 °C) of 13.5 g of sodium hydroxide in 13.5 mL of water. To the magnetically stirred mixture was added 50 mg of sodium borohydride followed by rapid, dropwise addition of 24.6 mL of epichlorohydrin. The resulting pasty mixture underwent a rapid, exothermic reaction in 10–20 min with brief, spontaneous refluxing, and a frothy, hard, glassy reaction product was formed. The mixture was allowed to stand at room temperature overnight and then heated to 50 °C for 5 h (Solms and Egli, 1965). If the spontaneous reflux failed to occur, the mixture was heated to 60 °C for 30 min. The glassy polymeric reaction product was easily broken up and was washed with acetone, 10 × 75 mL of water (until neutral), and 2 × 75 mL of ethanol to remove water, and dried in air overnight to yield 20–30 g of polymer. The ethanol wash gave a polymer that dried and handled easier than when the last wash was with water.

If polymer was added to juice to be used in taste tests, the final ethanol wash was checked for removal of epichlorohydrin by gas chromatography (see below). If epichlorohydrin was still present, the polymer was washed further with ethanol until no epichlorohydrin was detected (<0.5 ppm).

Procedures for Debittering. A laboratory-scale fluid-bed column was used for the continuous-flow debittering studies (Figure 1). Juice was filtered through a 60-mesh screen (Millipore 47-mm stainless screen with glass filter holder) prior to use in the fluid-bed apparatus or batch process. A Masterflux pump with Model 7013-20 pump head was used to pump juice at 2.5 mL/min through the column (40-mL total bed volume) containing 4–6 g of 40–60-mesh β-cyclodextrin polymer. A fluidized bed was maintained either by bubbling nitrogen gas at 200 mL/min through a sintered glass frit at the bottom of the column (Figure 1) or by magnetic stirring with a $^3/_4$ in. diameter Spinfin Teflon stirring bar (Bel-Art Products, Pequannock, NJ). When nitrogen gas was used, Antifoam B (Fisher Scientific Co., FairLawn, NJ) was added to the juice.

After 50 mL of juice/g of polymer was treated, the fluid-bed column was regenerated by successively pumping 200 mL of 2% NaOH solution, 200 mL of water, and 140 mL of ethanol through the column as the polymer was stirred. The dilute alkaline wash was collected in five 40-mL portions for measurement of components removed during regeneration (Table I).

A batch process was used for debittering juices for taste panel evaluation. Thus, 500 mL of juice was stirred

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Table I. Fluid-Bed Process To Reduce Bitterness in Citrus Juices

juice sample, component, polymer	S^e	debittering in column, concn in ppm in fraction no.[a] 1	2	3	4	5	% removed[b]	regenerating column, % recovered[c] in wash no.[a,d] 1	2	3	4	5
navel orange												
limonin												
fresh	10	2	3	4	5	5	62	37	28	13	9	6
regenerated	10	2	2	3	5	4	68	28	18	10	10	5
nomilin												
fresh	4	2	3	2	3	4	30	N[f]	N	N	N	N
regenerated	4	2	2	2	2	2	50	N	N	N	N	N
grapefruit												
limonin												
fresh	16	4	5	6	8	7	62	30	24	9	6	4
once regenerated	16	8	8	9	11	11	41	19	19	8	4	4
twice regenerated	16	9	9	11	11	13	34	25	19	17	14	6
nomilin												
fresh	7	3	4	4	6	5	37	25	19	6	N	N
once regenerated	7	3	4	5	5	5	37	11	11	11	6	6
twice regenerated	7	4	4	5	7	5	29	6	6	3	3	N
naringin												
fresh	702	257	381	414	439	547	42	36	20	11	7	6
once regenerated	702	488	480	526	543	638	24	39	25	11	8	4
twice regenerated	702	427	484	537	521	635	26	20	16	9	7	4
naringinin 7β-rutinoside												
fresh	343	83	110	120	117	146	66	19	10	6	4	3
once regenerated	343	122	138	183	172	204	52	23	17	8	5	3
twice regenerated	343	150	169	191	190	234	46	21	19	10	8	6

[a] Each fraction represents 10 mL of eluent/g of polymer. [b] Average value for five fractions (50 mL of eluent/g of polymer). [c] Percent of adsorbed component recovered (10 mL of wash solution/g of polymer). [d] Dilute alkali washes (2% NaOH) used for navel juices and EtOH washes for grapefruit juices. [e] S = concentration at start of experiment. [f] N = not detected.

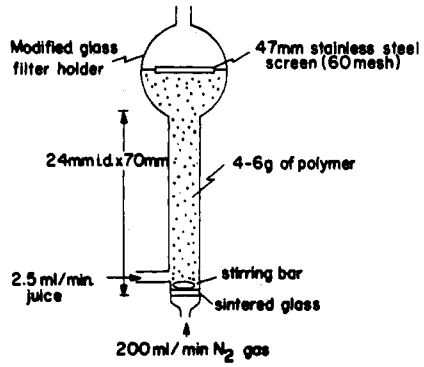

Figure 1. Fluid-bed apparatus for debittering citrus juices using a glass filter holder No. GV050/2, from Schleicher & Schuell, Inc., Keene, NH, custom-modified by Scientific Specialties, Inc., Auburndale, FL.

magnetically for 60 min with 10 g of 20–60-mesh β-cyclodextrin polymer. The polymer was removed by filtration through a 60-mesh screen and regenerated by stirring the polymer for 30 min each time with two or three 200-mL portions of 2% NaOH solution or absolute ethanol. Polymer regenerated with dilute NaOH solution was then washed with 5 × 20 mL of water and 2 × 50 mL of ethanol and dried in air before reuse (Table II).

Analytical Procedures. The total soluble solids (corrected °Brix), total acid, ascorbic acid, and oil content of the citrus juice samples were determined by standard methods used by the citrus industry (Praschan, 1975).

High-performance liquid chromatography (HPLC) separations for quantification of naringin and naringenin 7β-rutinoside in grapefruit juice were carried out on a Perkin-Elmer Series 2 pump and Model LC-85 variable-wavelength ultraviolet detector connected to a Hewlett-Packard 3390A recording integrator. The integrator was interfaced to an Apple 2 plus computer for statistical calculations (Shaw and Miller, 1983). A Perkin-Elmer high-speed 5-μm C-18 column 12.5 cm long with a Brownlee 5-μm C-18 guard column 4 cm long was used with a 6-μL loop. The eluting solvent was 20% acetonitrile–80% water at 1.5 mL/min (Fisher and Wheaton, 1976).

Table II. Batch Process To Reduce Bitterness in Citrus Juices

juice sample,[a] component, polymer	batch debittering, concn in ppm[b] starting	debittered	% removed	col regn, % removed[c] in wash no. 1	2	3
navel orange						
limonin						
fresh A[d]	18	8	56	43	N[e]	N
once regenerated	18	9	50	22	tr	N
twice regenerated	18	7	61	19	2	N
grapefruit						
limonin						
fresh A	9	4	56	20	N	
fresh B[f]	9	4	56	32	N	
naringin						
fresh A	600	377	37	26	14	
fresh B	600	358	40	54	9	
naringenin 7β-rutinoside						
fresh A	286	132	54	6	3	
fresh B	286	130	55	62	10	

[a] Nomilin was not detected in these samples. [b] 50 mL of juice/g of polymer. [c] Percent of adsorbed component recovered. [d] Base washes (2% NaOH) used to regenerate polymer A. [e] N = not detected. tr = trace. [f] Ethanol washes used to regenerate polymer B.

Thin-layer chromatography (TLC) was carried out by an earlier procedure with 10% sulfuric acid in ethanol as the spray reagent (Tatum and Berry, 1973) except the developing solvent was 100:50:50:3 benzene–chloroform–ether–acetic acid. Both limonin (R_f = 0.08) and nomilin (R_f = 0.10) could be quantitatively estimated by visual comparison with standards of 0.1–0.5 μg of each compound spotted on the center five bands of the plate. For identification of sterols, coumarins, psoralens, flavonoids, and

Table III. Samples for Flavor Evaluation of Debittered Navel Orange and Grapefruit Juices

juice sample	°Brix	% acid	% oil	bitter components, ppm: limonin	naringin	Davis test	flavor: preference	% confidence level
navel orange								
control	11.8	1.07	0.0137	18			4	
debittered	11.8	1.10	0.0136[a]	8			24	99.9
grapefruit								
control	10.1	1.07	0.0060	9	592	861	4	
debittered	10.0	1.10	0.0056[a]	4	316	545	26	99.9

[a] Adjusted level for flavor evaluation.

nootkatone, a standard sample of each compound was spotted adjacent to a juice or wash sample on the plate.

Residual epichlorohydrin in the washed polymer was determined by injection of 1 μL of ethanol wash solution into a Hewlett-Packard Model 5840 gas chromatograph equipped with a 0.3 mm i.d. × 30 m fused silica capillary column coated with the nonpolar phase SE-54 (DB-5). Temperature programming was 40 °C for 0.5 min, then increased to 60 °C at 20 °C per min, held for 1.5 min, and increased at 4 °C/min to 100 °C. The carrier gas (H_2) flow was 25 cm/s, the flame ionization detector was at 350 °C, and an injection port splitter at 250 °C was used with a split ratio of 100:1. The lowest detectable amount of epichlorohydrin (RT = 3.40 min) was 0.5 ppm.

Flavor Tests. Navel orange juice used in flavor tests was prepared by first blending 190 μL of Valencia orange oil in 180 g of concentrated juice and then diluting with 5.9 parts by volume of deionized water to afford single-strength juice with 11.8 °Brix and 0.0137% oil content. Debittered navel orange juice had an oil content of 0.0084%. Since oil is added to concentrated rather than single-strength juice to raise the oil level of the juice (Rice et al., 1952), the debittered juice was mixed 95:5 with single-strength juice from the same concentrated navel orange juice, but with an oil level of 0.106%, so that the final oil content of the debittered juice used for taste evaluation was 0.0133%.

Glass-packed single-strength grapefruit juice (0.0063% oil) was mixed 95:5 with grapefruit concentrate with low oil content (0.0001% oil) to give a control juice sample with an initial oil content of 0.0060% oil (Table III). The oil content in the debittered juice sample was 0.0035%. It was blended 95:5 with single-strength juice prepared from the same concentrated juice treated with cold-pressed grapefruit oil to afford a high oil content (0.125% oil) so that an oil content of 0.0056% was present in the debittered juice used for taste evaluation.

A paired comparison test with a trained taste panel of 14–15 members was used (Boggs and Hanson, 1949). Each panelist was given two presentations spaced 15 min apart to minimize effects of lingering bitterness present when multiple samples of bitter juice are tasted.

RESULTS AND DISCUSSION

β-Cyclodextrin polymer reduced levels of the major bitter components in navel orange and grapefruit juices to about half their original values when 1 g of polymer/50 mL of juice was used, either in continuous flow (fluid-bed) or batch processes. Most of the fine pulp in orange and grapefruit juices will flow through a 60-mesh screen, so that polymer beads large enough to be stopped by a 60-mesh screen can be used to debitter these juices. Generally, 20–60-mesh polymer was used in fluid-bed and batch processes.

Fluid-Bed Process. A laboratory-scale continuous-flow fluid-bed process was used to evaluate the efficiency of fresh and regenerated polymer to debitter navel orange and grapefruit juices (Table I). Navel orange juice from fresh fruit had an initial level of the bitter components limonin and nomilin of 10 and 4 ppm, respectively. Use of fresh or regenerated polymer reduced the level of limonin or nomilin to 2 ppm in the first fraction treated (10 mL of eluent/g of polymer). The fifth fraction still had the limonin and nomilin levels reduced to about half those of the starting juice. The polymer was regenerated with 2% sodium hydroxide solution, which removed decreasing amounts of limonin as five fractions were successively collected (Table I). Nomilin was not detected in any fraction during the regeneration process with dilute alkaline wash solution. However, dilute alkali was more effective at removing limonin than ethanol or water used in an earlier study on clarified juice (Shaw and Wilson, 1983). Regenerated polymer was as effective as fresh polymer in reducing the levels of these bitter components. The ascorbic acid content for navel orange juice, although unusually low for a commercial juice sample (about 14 mg/100 mL), was unchanged in the debittered juice (fractions 1–6 combined).

With grapefruit juice in the fluid bed process (Table I), the bitter components limonin, nomilin, and naringin and the isomer of naringin, naringenin 7β-rutinoside, were all reduced by 50% or more in the first fraction. By the fifth fraction, the polymer was still as effective in removing limonin and nomilin as it had been when removing only limonin and nomilin from navel orange juice (about 5 ppm of limonin removed) but was no longer effective in removing naringin and naringenin 7β-rutinoside. The reason for this difference in polymer effectiveness is not known. Regenerated and twice-regenerated polymer was less effective than fresh polymer in removing all components except nomilin. However, the once-regenerated and twice-regenerated polymers were about equally effective in removing bitter components. Ethanol washes were used to regenerate the polymer each time, and the washes removed more limonin, nomilin, and naringin from fresh polymer than from the two regenerated polymers. The ascorbic acid content for grapefruit juice (about 55 mg/100 mL) was unchanged in the debittered juice (fractions 1–5 combined).

When nitrogen gas was used to maintain a fluid bed, considerable foaming resulted unless an antifoaming agent was used, causing polymer to collect on and plug the screen. A portion of the polymer was therefore not in contact with liquid juice during all of the 15-min residence time. A magnetic stirrer was more effective in maintaining a proper fluid bed.

Removal of several other juice components by the polymer treatment was observed by TLC when quantities of limonin and nomilin were being determined. Removal of these components was most pronounced in the first fraction from the fluid bed column; when the column was being regenerated, these compounds appeared in highest concentration in the first wash. In navel orange juice the flavonoid sinensetin was partially removed by the polymer.

In grapefruit juice, β-sitosterol, nootkatone, 7-methoxy-8-(2,3-dihydroxyisopentyl)coumarin, 5-hydroxypsoralen, and 7-hydroxypsoralen were all partially removed from juice by treatment with the polymer. In all cases, the regeneration procedure washed these components from the polymer. Oil components were also removed by the polymer. Thus, the oil content of navel orange or grapefruit juice debittered by this treatment was about 40% lower than that of the starting juice (see below).

Batch Process. A batch process was used to debitter other navel orange and grapefruit juice samples to be used in flavor evaluation studies (Table II). In navel orange juice, the limonin content was reduced about 50% with either fresh or regenerated polymer. Polymer was regenerated with three washes with dilute alkaline solution. Virtually all limonin was removed in the first wash solution.

The ascorbic acid content of 59 mg/100 mL was unchanged in the debittered juice. The oil content of the juice was lowered from 0.014% to 0.0084% by treatment with either fresh or regenerated polymer. Neither navel orange nor grapefruit juice used in these bath processing studies contained a detectable amount of nomilin.

The batch process treatment of grapefruit juice with β-cyclodextrin polymer lowered limonin content to about 50% and the naringin content to about 60% of the original values, when 1 g of polymer/50 mL of juice was treated (Table II). As was found in an earlier study (Shaw and Wilson, 1983) the polymer removed a greater percentage of naringenin 7β-rutinoside than it did naringin. However, the naringin level is higher than that of its isomer in grapefruit juice, so the quantity of naringin removed is actually greater than that of its isomer.

The polymer was regenerated with either dilute alkali or ethanol to compare the efficiency of each solvent in regenerating the polymer. Both solvents were about equally effective in removing limonin from the polymer, and virtually all limonin was removed with the first wash. Ethanol was more more efficient in removing naringin in the first wash, and this solvent was much more effective in removing naringenin 7β-rutinoside. Ascorbic acid content of 43 mg/100 mL was unchanged in the debittered juice. The oil content of the juice was lowered by polymer treatment from 0.0060% to 0.0035%.

α-Cyclodextrin Polymer for Debittering Grapefruit. Equal quantities of α- and β-cyclodextrin polymers were used to debitter glass-packed single-strength grapefruit juice in a batch process to compare efficiencies of the two polymers for debittering citrus juice. The two polymers were about equal in ability to remove limonin from grapefruit juice, but β-cyclodextrin polymer reduced the naringin content and the oil level about 20% more than did α-cyclodextrin polymer. The latter polymer removed some β-sitosterol, nootkatone, and the same coumarin and psoralens that β-cyclodextrin polymer had removed. However, α-cyclodextrin polymer was much less efficient in removing nootkatone than was β-cyclodextrin polymer. Use of α-cyclodextrin polymer did not change the °Brix or acid content of the juice. Ascorbic acid was little changed, from 43.2 to 40.6 mg/100 mL by the polymer treatment. Since α-cyclodextrin is considerably more expensive than β-cyclodextrin, its polymer would have to be much more efficient in removing unwanted components for it to compete economically with β-cyclodextrin polymer in a commercial process.

Flavor Evaluation. A trained taste panel was used to compare the flavors of debittered navel orange and grapefruit juices to those of the starting juices (Table III). Although the °Brix and percent acid were essentially unchanged by treatment with β-cyclodextrin polymer, the oil level was significantly reduced in both navel orange and grapefruit juices. The oil level of the debittered juice sample was adjusted so that the oil levels of the control and experimental juice samples were essentially equal. In this process, the debittered juice samples were diluted with 5% of nondebittered juice of high oil content. This adjustment insured that taste panel members would not judge the preference test based on a difference in oil content. Debittered navel orange and grapefruit juice samples were both preferred over the control juice samples at a confidence level greater than 99.9%. The debittered navel orange juice still contained slightly more limonin (8 ppm) than the reported flavor threshold level in orange juice [6.5 ppm by Guadagni et al. (1973); <5.5 ppm by Tatum and Berry (1973)]. Panel members felt that the debittered navel juice sample was either nonbitter or only slightly bitter and that it tasted sweeter. Debittered grapefruit juice still had some of the bitterness expected of grapefruit juice, but at a greatly reduced level compared to the starting juice. Flavor thresholds and optimum levels of limonin and naringin in grapefruit juice have not been established. However, Guadagni et al. (1974) have shown that subthreshold levels of limonin and naringin in aqueous solution act synergistically to produce a bitter flavor.

Thus, the debittering process with β-cyclodextrin polymer does not adversely affect the flavor of navel orange and grapefruit juices, nor does it decrease the levels of major desirable components—sugars, acids, and ascorbic acid. The decrease in oil content caused by the polymer treatment can be corrected by addition of oil back to the juice.

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Registry No. β-Cyclodextrin polymer, 79647-56-6; limonin, 1180-71-8; nomilin, 1063-77-0; naringin, 10236-47-2; α-cyclodextrin polymer, 90320-92-6; naringenin 7β-rutinoside, 14259-46-2.

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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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