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High-Performance Liquid Chromatographic Determination of Ascorbic Acid in Aseptically Packaged Orange Juice Using Ultraviolet and Electrochemical Detectors

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Three different methods were used to measure ascorbic acid values in aseptically packaged orange juice from commercial sources. Values using ultraviolet (UV) and electrochemical (EC) detectors were not significantly different from those obtained by a potentiometric titration method. The EC detector was 100 times more sensitive to ascorbic acid than was the UV detector. Ascorbic acid values generally declined slowly and steadily in samples stored under normal marketing conditions.

Disposable cartons of shelf-stored aseptically packaged citrus juices provide the consumer with a convenient source of individual servings. This new product is the fastest growing segment of the fruit beverage industry (Tillotson, 1984). The single-strength 100% juice product is generally prepared from concentrate and packaged in 250-mL flexible, multilayered cartons. It is reported to be shelf stable for periods of 3–6 months (Kryger, 1985), but there is relatively little information in the literature on ascorbic acid retention in this product. Since citrus juice is considered one of the most reliable sources of dietary ascorbic acid (Rouseff, 1979), there is a need for information on ascorbic acid retention in stored aseptically packaged citrus juices produced commercially.

The standard method for measuring ascorbic acid in foods is by titration with 2,6-dichlorophenol–indophenol (AOAC, 1980). For orange juice, the method can be limited by substances in the juice matrix that obscure end point determination; use of potentiometric titration eliminates this problem in orange juice (Nagy and Smoot, 1977). Toledo (1984) studied the effect of residual hydrogen peroxide on the stability of experimental samples of aseptically packed orange juice and found results similar to those reported by Nagy and Smoot (1977) for stored single-strength juice. High-performance liquid chromatography (HPLC) using strong anion-exchange or reversed-phase columns and ultraviolet (UV) detection has been reported to be a rapid and sensitive method for analyzing citrus juices and other food products for ascorbic acid (Sood et al., 1976; Rouseff, 1979; Carnevale, 1980; Shaw and Wilson, 1982; Haddad and Lau, 1984; Wills et al., 1984). Recoveries of added ascorbic acid determined by HPLC were generally quite good as were comparisons of HPLC values with those obtained by a standard titration method (Haddad and Lau, 1984; Augustin et al., 1981; Carnevale, 1980; Rouseff, 1979).

Detection and quantification of ascorbic acid has been carried out primarily with a UV detector at 254 nm, but there are disadvantages to the method. Other UV-absorbing compounds can either coelute or elute close to ascorbic acid, thereby causing interference in the method (Rouseff, 1979; Wills et al., 1984). Detection of ascorbic acid with an electrochemical (EC) detector, which reduces interference from coeluting compounds because of its specificity and increased sensitivity, has been reported in animal tissue, blood plasma, urine, multivitamins, and foods (Tsao and Salami, 1982; Pachla and Kissinger, 1976). Values for citrus juice concentrate and for whole fruit were within the expected range and were comparable to values determined by titration (Pachla and Kissinger, 1976).

In the current study the abscorbic acid content of aseptically packaged orange juice was determined with EC and UV detectors, and the results were compared to those determined by a potentiometric titration method.

EXPERIMENTAL SECTION

Juice Samples. Samples of aseptically packaged orange juice were obtained from local markets and stored at 5 °C until use.

Sample Preparation. Juice samples (ca. 25 mL) were centrifuged at maximum switch setting for 5 min in an International clinical centrifuge, Model CL (International Equipment Co., Boston, MA), to remove suspended solids. A 2-mL sample of juice was mixed with 2 mL of 6% metaphosphoric acid and then filtered successively through 1.2- and 0.45-μm Acrodisc filters (Gelman Sciences, Inc., Ann Arbor, MI). Filtered samples were stored at 0 °C.

Analytical Methods. The instrument used was a Perkin-Elmer Series 2 pump and Model LC-85B variable-wavelength detector (245-nm maximum sensitivity for ascorbic acid) connected to a Hewlett-Packard Model 3390A recording integrator. Electrochemical detection was accomplished at +0.6 V with an LDC-Milton Roy Chromatronix CMX-20 amperometric detector fitted with a glassy carbon working electrode, an Ag/AgCl reference electrode, and a stainless-steel auxiliary electrode. A Rheodyne Model 7120 injector fitted with a 5-μL loop was used with either a 4.6 mm × 22 cm or a 4.6 mm × 10 cm, 5-μm C18 Brownlee column connected to a 4.6 mm × 3 cm, 5-μm, C18 Brownlee guard column. The mobile phase was aqueous 2% ammonium dihydrogen phosphate adjusted to pH 2.8 with phosphoric acid at flow rates of 0.5 mL/min (10-cm column) or 1.0 mL/min (22-cm column). Ascorbic

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Table I. Ascorbic Acid in Aseptically Packaged Orange Juice

sample[a]	time remaining before expir date[b]	mg ascorbic acid/100 mL titrn	HPLC UV	HPLC EC
A-1	6	43.6	43.7	45.7
B-1	5	36.4	35.1	34.4
A-2	3	34.8	37.6	35.2
A-3	2	36.1	37.6	35.2
C-1	2	33.5	31.6	32.5
A-4	1	33.4	34.2	34.8
sample	**time lapsed after expir date[c]**	**mg ascorbic acid/100 mL titrn**	**HPLC UV**	**HPLC EC**
C-2	1	29.1	28.9	28.4
A-5	1	29.2	31.8	28.6
D-1	3	34.5	33.3	33.2
C-3	3	29.1	28.9	28.4
C-4	3	24.9	24.4	22.9
B-2	6	22.7	23.7	23.0
D-2	7	23.3	24.2	23.9

[a] Samples with identical letters indicate samples from different lots from the same manufacturer; samples C and D were from 100% Valencia juice. [b] Number of months remaining after purchase until sample expiration date. [c] Time lapsed between purchase date and expiration date of sample (months).

acid standards were prepared in 3% metaphosphoric acid solution with ±10% of the peak area integration values for the unknown samples.

Alternate runs of standard and unknown were run in triplicate, and the mean of three values for the same sample was used as one measurement. The coefficient of variation for the three determinations was generally less than 10%. A 2-mL sample of orange juice sample B-1 from Table I was treated with 300 mg of charcoal for 30 min, centrifuged, and filtered as described above to afford an HPLC pattern with no peak eluting at the retention time for ascorbic acid (3.8 min) on the 10-cm column. Analysis of variance was used to compare the two HPLC methods and the titration method. Ascorbic acid values were obtained by the potentiometric method of Spaeth et al. (1962) for comparison with HPLC values.

RESULTS AND DISCUSSION

Ascorbic acid values from 13 samples of aseptically packed orange juice were determined with electrochemical and UV detectors, and the results were compared with values obtained by a potentiometric titration method (Spaeth et al., 1962) (Table I). The values obtained by titration and by the two HPLC methods were in close agreement; by analysis by variance there was no significant difference between values obtained by the various methods.

Ascorbic acid determination with an EC detector offers two main advantages when compared to a UV detector: specificity and sensitivity (Tsao and Salami, 1982; Pachla and Kissinger, 1976). Numerous investigations on the assay of ascorbic acid by HPLC using UV detection appear in the literature, but few of these reports determined whether UV-absorbing compounds coelute with ascorbic acid (Rouseff, 1979; Wills et al., 1984; Dennison et al., 1981). We found no compounds present in orange juice that coeluted with ascorbic acid after treatment with activated charcoal to convert ascorbic acid to dehydroascorbic acid that elutes at a different retention time (Rouseff, 1979). Since orange juice does not contain coeluting or closely eluting compounds that interfere with UV detection at 245 nm, either detector was adequate for accurate determination of ascorbic acid. With regard to relative sensitivity of the two detectors, the EC detector was about 100 times more sensitive than the UV detector. Thus, food products with relatively small amounts of ascorbic acid can readily be assayed with the EC detector. With foods such as citrus that contain nutritionally significant amounts of ascorbic acid, either detector had acceptable sensitivity. However, as with the other two methods, dehydroascorbic acid cannot be measured with the EC detector at the voltage setting used to determine ascorbic acid.

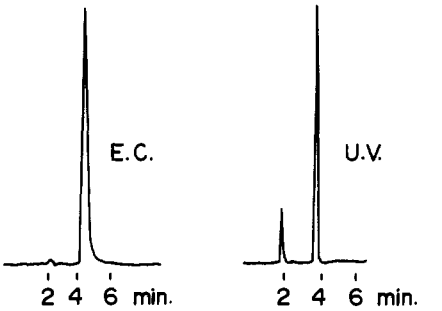

Figure 1. HPLC separation of an orange juice sample diluted 200:1 for the EC detector and 2:1 for the UV detector (245 nm), showing the ascorbic acid peak at about 4 min in each case.

Chromatograms of orange juice (Figure 1) show the separation and response for the two detectors. The two chromatograms are similar, since there are no interfering compounds in the juice sample that respond to either detector.

Ascorbic acid values (Table I) for the 13 samples of aseptically packaged orange juice showed a gradual and steady decline in ascorbic acid as storage time increased, even with samples from four different producers used in this study. Although the number of samples analyzed was insufficient to be statistically significant, ascorbic acid retention for aseptically packaged juice was similar to orange juice packaged in tin cans and glass and cardboard containers and stored at similar temperatures (Bissett and Berry, 1975; Nagy and Smoot, 1977; Nagy, 1980). Ascorbic acid in commercial single-strength Florida chilled orange juice averages 43.5 mg/100 mL (Nagy, 1980).

All samples were obtained directly from supermarket shelves under normal storage conditions of 21 °C. Samples that were beyond their expiration date generally contained the least amounts of ascorbic acid. All but the two, samples stored the longest after their expiration dates (B-2 and D-2, in Table I) contained sufficient ascorbic acid per 250-mL container to meet the 60-mg U.S. RDA minimum daily requirement (Ting, 1980). Those two samples provided almost enough ascorbic acid (57 mg/250 mL) to meet the minimum daily requirement.

In conclusion, use of EC or UV detectors to quantitate ascorbic acid in orange juice by HPLC was equally satisfactory, and the values were not significantly different from those determined by the potentiometric titration method. Aseptically packaged single-strength orange juice stored under actual marketing conditions showed a gradual steady decline in ascorbic acid content. Juices stored up to 3 months past their expiration date retained sufficient ascorbic acid to meet or exceed the minimum U.S. RDA for this vitamin.

Registry No. Ascorbic acid, 50-81-7.

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Binding of Condensed Tannins to Salivary Proline-Rich Glycoproteins: The Role of Carbohydrate

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Salivary proline-rich proteins have a high affinity for tannin and protect against the antinutritional effects of dietary tannins. Several of these proteins are glycosylated so we have investigated the role of the carbohydrate in their binding to tannin. The results suggest that oligosaccharides enhance the affinity and selectivity of binding to tannins and increase the solubility of the resulting tannin/glycoprotein complexes.

Protein/tannin interactions have been widely investigated with respect to the chemical nature of the interactions (Haslam, 1974; Oh et al., 1980). Among the features studied have been the stoichiometry of the complexes (Calderon et al., 1968), the effect of tannin chain length (Porter and Woodruffe, 1984), the ability of tannins to selectively bind certain proteins (Hagerman and Butler, 1981), and the effects of protein size, conformation, and amino acid composition (Hagerman and Butler, 1981). Carbohydrate/tannin interactions have also been examined (Davis and Hoseney, 1979; Deshpande and Salunkhe, 1982; McManus et al., 1985). However, interactions between glycoproteins and tannins have scarcely been studied. Strumeyer and Malin (1970) reported that yeast invertase (a glycoprotein) is resistant to inhibition by tannins and suggested that the carbohydrates of glycosylated enzymes may protect these glycoproteins against binding by tannins. Jones and Mangan (1977) reported that condensed tannin does not precipitate bovine submaxillary mucin at temperatures above 25 °C. They ascribed this lack of precipitation to the carbohydrate on the protein.

Tannins have been reported to be responsible for antinutritional effects including inhibition of digestive enzymes (Griffiths, 1979), formation of relatively less digestible complexes with dietary protein, depressed growth rates, and altered food consumption (Reddy et al., 1985). Rats and mice adapt to dietary tannin by the induced synthesis of several proline-rich (up to 44%) salivary proteins (PRPs) (Mehansho et al., 1983, 1985b). These proteins apparently diminish the antinutritional effects of dietary tannin by strongly binding to it. Hamsters do not produce salivary PRPs in response to dietary tannin and may be killed by tannin-containing diets to which rats and mice readily adapt (Mehansho et al., 1985a).

Many of these salivary PRPs contain carbohydrate in amounts up to 40% by weight (Mehansho and Carlson, 1983; Mehansho et al., 1985b). The strong affinity of these glycoproteins for tannins led us to examine the role of carbohydrate in the binding of tannins to these salivary proline-rich proteins.

MATERIALS AND METHODS

Bovine serum albumin (BSA), hen egg albumin, *Clostridium perfringens* neuraminidase, and SDS were obtained from Sigma (St. Louis, MO). Condensed tannin was purified from *Sorghum bicolor* L. Moench hybrid BR-64 (Hagerman and Butler, 1980) and from quebracho (Asquith and Butler, 1985). Crude quebracho powder was purchased from the Trask Chemical Co. (Marietta, GA)

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