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Analysis of Oxalic Acid in Carambola (*Averrhoa carambola* L.) and Spinach by High-Performance Liquid Chromatography

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Oxalic acid was quantified in 15 Florida-grown carambola cultivars by a simple and rapid high-performance liquid chromatographic (HPLC) method using a propylamine column and aqueous sodium dihydrogen phosphate as the eluant. Oxalic acid levels varied almost 10-fold (from 0.08 to 0.73 g/100 g of fruit) among the cultivars tested. The generality of the method for determining oxalic acid in other food products was shown by analysis of fresh spinach.

The carambola is grown in South Florida primarily as an ornamental dooryard tree that produces fruit of unique flavor and relatively high ascorgic acid content. Smaller amounts of carambola are grown for the fresh fruit market and for export to Europe (Wagner et al., 1975). Commercial acceptance has been limited because the fruit is susceptible to shipping damage, it requires storage below 70 °F to maintain optimum quality during shipment (Grierson and Vines, 1965; Vines and Grierson, 1966), and it has an oxalic acid content that is comparable to that of spinach, rhubarb, and other foods known to have relatively high levels of this acid (Wagner et al., 1975; Singh, 1973; Zarembski and Hodgkinson, 1962b). There is a need for more information on the oxalic acid content of different carambola cultivars to see if certain cultivars might combine low oxalic acid content with other favorable marketing characteristics.

Several lengthy methods for determining oxalic acids in foods have been reported. These methods all involve the same basic steps [summarized by Zarembski and Hodgkinson (1962a)]: extraction of oxalic acid from the plant material with hydrochloric acid (HCl) or with sodium carbonate and overnight precipitation of calcium oxalate, followed by treatment of the precipitate with dilute sulfuric acid to form a solution of oxalic acid. The oxalic acid was then quantified by one of the following methods: potentiometric with a quinhydrone electrode (Pucher et al., 1934), titration with standard potassium permanganate (Baker, 1952), or colorimetric by conversion of oxalic acid to glycolic acid and derivitization with 3,6-dihydroxynaphthalene-2,7-disulfonic acid (Zarembski and Hodgkinson, 1962a).

In the above methods conflicting results for complete oxalic acid extraction were found, depending on whether hot or cold HCl was used to extract plant material and whether fresh plant material or dry finely divided sample was extracted. Baker (1952) used hot 1 N HCl to extract oxalic acid from plant material, and he confirmed a previous observation by Hoover and Karunairatnam (1945) that oxalic acid was stable for several hours in hot 1.5 N HCl. He also concluded that drying plant material at 100 °C for 24 h resulted in appreciable loss of oxalic acid. Conversely, Zarembski and Hodgkinson (1962a) reported abnormally high oxalic acid values from foods extracted with hot rather than cold HCl and proposed the major source for increased oxalic acid was carbohydrate degradation. These authors recommended extraction with cold HCl and showed that at least 3 N HCl was required for complete extraction of oxalic acid from most foods. They also reported oxalic acid recoveries of 96–100% from dry finely divided foods with negligible conversion of carbohydrates and other components to oxalic acid.

High-performance liquid chromatography (HPLC) using reverse-phase or anion-exchange columns with organic buffers affords a rapid and sensitive method for analyzing certain mixtures of organic acids in foods (Buslig et al., 1982; Shaw and Wilson, 1981). Oxalic acid, in particular, was well separated from other organic acids normally found in foods on a propylamine column with an aqueous buffer (Buslig et al., 1982).

The current study reports a rapid and simple method for quantification of oxalic acid in foods using HPLC with a propylamine column and sodium dihydrogen phosphate buffer as the eluent. The method was used to determine oxalic acid content in 15 carambola cultivars and in one fresh spinach sample as well.

EXPERIMENTAL SECTION

Carambolas were obtained from the U.S. Subtropical Horticultural Research Station, Miami, FL. Samples were picked in Sept 1981, and the fruit were ripened for 1 week to a uniform yellow color at 21 °C and then kept at −18 °C until extracted. Fresh spinach was purchased at a local market.

Preparation of Samples. Carambola fruit or spinach leaves (500–600 g from five to six fruit) were cut into small pieces and the pieces thoroughly mixed. Then, duplicate 100-g portions were removed from the composite sample, and each 100-g sample was mixed in a blendor with 100

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mL of water or 3 N HCl for 1 min. Carambolas were extracted with water, and spinach was extracted separately with either water or 3 N HCl. The mixture was filtered through a filter aid with a Büchner funnel, and the filter cake was extracted twice by stirring each time with 50 mL of water or 3 N HCl filtering the mixture through the filter aid. The 200 mL of total filtrate was concentrated to 100 mL at 30 °C under reduced pressure (20 mmHg).

For HPLC analysis, a 7-mL portion of each of the above duplicate extracts was filtered first through a C-18 Sep-PAK cartridge (Waters Associates, Milford, MA) pretreated with 2 mL of acetonitrile and 5 mL of water. The first 2 mL of eluate was discarded and the remaining eluate was filtered through a 0.45-μm Millipore filter.

Some samples were separated by ion exchange prior to HPLC analysis. For ion-exchange separations a 25-mL portion of each extract was added to a column containing 3.5 mL of cation-exchange resin (AG50W-X8, 100–200 mesh, hydrogen form, Bio-Rad Laboratories, Richmond, CA) and the column was washed with 25 mL of water. The 50 mL of total filtrate was added to a column containing 3.5 mL of anion-exchange resin (Bio-Rad AGMP-1, 50–100 mesh, granular chloride form), and the column was washed with 75 mL of water. The acids were eluted with 50 mL of 6 N hydrochloric acid followed by 75 mL of water. The combined 125 mL of eluate was evaporated to dryness at 55 °C and reduced pressure (36 mmHg). The residue was dissolved in 25 mL of buffer solution (see below) for HPLC analysis. Oxalic acid recovery from the ion-exchange columns was determined by ion-exchange chromatography of duplicate 25-mL portions of 0.73% aqueous oxalic acid solution by the above procedure. HPLC analysis showed 97 ± 3.3% recovery of oxalic acid.

Oxalic Acid Determination by $KMnO_4$ Titration. The procedure of Baker (1952) was modified as follows. Duplicate 12-mL portions of the above 100-mL aqueous extracts from carambola were acidified with 2.5 mL of 6 N HCl and diluted to 100 mL with water in a 100-mL volumetric flask. With spinach, the 6 N HCl was omitted, since the solutions were already strongly acidic from extraction with 3 N HCl. Each solution was filtered through dry Whatman No. 2 filter paper, and a 25-mL portion was pipetted into a 50-mL centrifuge tube and 5 mL of phosphoric-tungstate reagent was added. The resulting solution was kept for 5 h. The carambola extracts contained no precipitate, but spinach required centrifugation at 2300 rpm (relative centrifugal force of 976) for 5 min to remove the protein precipitate. Then, 25 mL of each solution was pipetted into a 50-mL centrifuge tube, and 5 mL of calcium chloride buffer solution was added. The resulting solution was adjusted to pH 4.6 with concentrated NH_4OH solution by using a pH meter and kept at 5 °C overnight. The mixture was centrifuged at 2300 rpm for 5 min, the supernatant was carefully decanted, the precipitate was stirred with 15 mL of 5% acetic acid solution saturated with $CaCl_2$, and the mixture was centrifuged at 2300 rpm for 5 min. The supernatant was carefully decanted, the precipitate was dissolved in 6 mL of 10% H_2SO_4, and the resulting solution was titrated with 0.02 N $KMnO_4$ solution that had been filtered through a sintered glass funnel and standardized by tittration with 0.0199 N oxalic acid in 10% H_2SO_4 solution. The end point was reached when the pink color of permanganate persisted for 30 s.

HPLC Analyses. Extracts were analyzed on a Waters Model 202LC equipped with an LDC Spectromonitor III variable-wavelength detector set at 206 nm, a 4.6 mm × 25 cm Zorbax amine (Du Pont, Wilmington, DE) column with 7-μm packing and an amino guard column with 10-μm packing (Brownlee Laboratories, Inc., Santa Clara, CA). The instrument was modified with an Altex Model 905-42 injector fitted with a 20-μL loop, a Waters Model 6000A pump, and a Hewlett-Packard Model 3380A recording integrator. The mobil phase was aqueous 0.15 M NaH_2PO_4 at pH 2.4 (buffer solution) at a flow rate of 1.1 mL/min. Oxalic acid was identified by comparison of its retention time with that for an authentic sample. All other organic acids identified earlier as components of carambola (ascorbic, tartaric, malic, citric, α-ketoglutaric, succinic, and fumaric) by Vines and Grierson (1966) had shorter retention times on this column under these conditions. Quantitative determinations were made by comparison of alternate injections of a standard oxalic acid solution and each of the duplicate extracts. Three runs of standard oxalic acid solution and each of the duplicate natural product extracts were carried out. The standards were prepared so that area of the oxalic acid peak (RT = 5–6 min) was within ±10% of the area under that peak in the natural product extract. The coefficient of variation for the three runs of each sample was generally less than 6%.

Table I. Oxalic Acid Content of Some Carambola Cultivars and of Spinach

carambola cultivar	oxalic acid, g/100 g HPLC	ion-exc-HPLC	titration
Fwang Tung	0.08		
Demak (A)[a]	0.09	0.09	
Demak (B)[a]	0.21; 0.16[b]		
Dah Pon (M17734)	0.13		
Arkin (M25157)	0.13		
WA3-24-13	0.17		
Tean Ma (M17737)	0.20		
M18960	0.26	0.26	0.26
M23007	0.28		0.33
M8921	0.36		
WA3-24-23	0.39		
Golden Star (M21024)	0.43		
M9304	0.53		
Robert Newcomb (M27767)	0.57		
WA3-24-37	0.73	0.70	0.66
WA3-24-14	0.73		
spinach			
H_2O extract	0.09		
acidic extract[b]	1.37		1.18

[a] Samples obtained 1 year apart. [b] Extraction with 3 N HCl.

RESULTS AND DISCUSSION

An HPLC method for analysis of oxalic acid in foods has been developed that requires a minimum of sample preparation. This method avoids time-consuming precipitation steps inherent in previously reported methods (Zarembski and Hodgkinson, 1962a) and also uses extraction with water or HCl at ambient temperature to minimize errors due to thermal degradation.

The oxalic acid contents of 15 cultivars of carambola and 1 spinach sample determined by using this HPLC method are listed in Table I. Also listed are oxalic acid values determined on several of the same samples for comparison by using a more time-consuming method described earlier (titration method in Table I; Baker, 1952). In all cases the values determined by the two methods on the same samples were in good agreement.

In most carambola samples, the oxalic acid peak was base line resolved from the peaks that eluted earlier in the

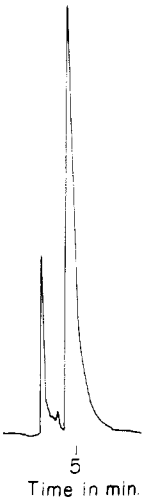

Figure 1. HPLC determination of carambola cultivar WA3-24-23 on the propylamine column using 0.15 M NaH_2PO_4 (pH 2.4) at 1.1 mL/min with the UV detector at 206 nm; oxalic acid eluted at 5.36 min.

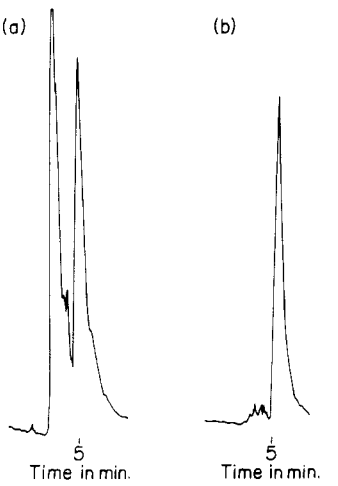

Figure 2. HPLC analysis of carambola cultivar M18960 on the propylamine column with 0.15 M NaH_2PO_4 at (pH 2.4) at 1.1 mL/min; oxalic acid eluted at 5.5 min. (a) is for crude extract. (b) is for extract purified by ion-exchange separation prior to HPLC analysis.

chromatogram under the HPLC conditions used (Figure 1). Peaks that eluted earlier than oxalic acid included other organic acids known to be present in carambola (Vines and Grierson, 1966) and sugars. In three carambola samples (Demak, M18960, and WA3-24-37) the oxalic acid was not completely resolved from the earlier eluting peaks, as shown in Figure 2a for the cultivar M18960. In those cases the organic acid fraction was purified through anion- and cation-exchange resins, to remove amino acids and sugars (Shaw and Wilson, 1981), and oxalic acid was determined on the purified fraction by HPLC (Figure 2b). Comparison of results before and after ion-exchange purification showed virtually the same value for oxalic acid in each case (Table I), indicating the purification step was not necessary for accurate quantification of oxalic acid.

Our procedure for extraction of oxalic acid from carambola used water extraction of macerated fruit at room temperature. Since 3 N HCl was recommended by Zarembski and Hodgkinson (1962a) for complete extraction of oxalic acid from plant material, we compared extraction with 3 N HCl to extraction with water on one sample of Demak fruit (Table I) from the same lot of carambolas used for HPLC analysis. The water extraction gave slightly more oxalic acid than the HCl extraction, but within experimental error (standard deviation = 0.02), the two extracts gave comparable values.

There was almost a 10-fold difference in the oxalic acid content among the 15 cultivars studied. The Fwang Tung had the lowest oxalic acid content at 0.08 g/100 g of fruit. The sample of the Demak (A) cultivar analyzed by ion-exchange–HPLC had an oxalic acid content almost as low as that of Fwang Tung, at 0.09 g/100 g. Additional samples of the same variety obtained 1 year later had more than twice as much oxalic acid (0.21 g/100 g) even though both samples were analyzed at the yellow ripe stage. These differences are probably due to fruit variation rather than maturity. Arkin and Robert Newcomb are recognized as two of the sweeter cultivars of carambola, but the oxalic acid contents were not consistent with relative sweetness. Arkin had one of the lower oxalic acid contents, but Robert Newcomb had one of the highest oxalic acid levels of the cultivars studied. The relative sugar contents of these cultivars are not known, however. Two experimental cultivars, WA-24-37 and WA-24-14 had the highest oxalic acid levels at 0.73 g/100 g of fruit of all cultivars studied. This relatively high level for carambola is still only about half the level of oxalic acid we found in spinach (Table I).

The oxalic acid content of fresh spinach was determined to check the applicability of the method to other food products. Values obtained by HPLC (Table I) were comparable to those reported earlier (Zarembski and Hodgkinson, 1962b; Singh, 1973). Spinach was extracted with water and with 3 N HCl, and oxalic acid was not quantitatively extracted with water alone. Thus, quantitative recovery of oxalic acid from nonacidic foods such as leafy vegetables probably requires extraction with at least 3 N HCl as noted by Zarembski and Hodgkinson (1962a). Acidic food products, such as most fruits, probably do not require extraction with HCl for quantitative recovery of oxalic acid.

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