Total Citrate Content of Orange and Grapefruit Juices

Total citrate in orange and grapefruit juices was determined by high-performance liquid chromatography and by isotachophoresis. Both methods are rapid and simple and afford results that are in close agreement. Estimation of total citrate by an earlier method afforded slightly higher results, probably because other acids known to be present in some citrus juices, e.g., malic and succinic, contributed to that value.

In recent years some commercial bulk orange and grapefruit concentrates have developed a white crystalline precipitate during storage that was identified as mostly potassium citrate. When concentrate is reconstituted to single strength, the potassium citrate dissolves slowly, but its presence in concentrate can result in a lower grade score for that sample (McAllister, 1976). In order to aid processors in trying to prevent the formation of this precipitate, a rapid and simple method for measuring total citrate in citrus juices was needed.

Several methods for measuring total citrate in orange and grapefruit juices have been reported. The method of Pucher et al. (1934) involves oxidation of citric acid to pentabromoacetone, debromination with sodium sulfide, and titration of the liberated bromide with silver nitrate. A correction factor is required since the recovery of pentabromoacetone is not quantitative. Kilburn and Davis (1959) used ion-exchange column chromatography followed by titration with sodium hydroxide solution to determine the salt concentration in grapefruit juice, measuring total salts as an indication of organic acids present. This value plus the total free acid determined by potentiometric titration (Sinclair et al., 1945; Sinclair and Eny, 1946) affords an estimate of total acids plus acid salts present in juice. Since citric is the major acid in most citrus juices, this method would afford an estimate of total citrate present in such juices.

The current study reports two simple and rapid methods for measuring total citrate content of orange and grapefruit juice using either high-performance liquid chromatography (HPLC) or isotachophoresis.

EXPERIMENTAL SECTION

Fresh juice samples were obtained by hand-reaming three to five fruit from a single tree. Processed juice samples were purchased from a local market. All juice samples were clarified by centrifugation at 9000 rpm (Sorvall GSA rotor) for 15 min or prefiltered through a glass-wool plug and then either filtered successively through 1.2- and 0.22-μm Millipore filters (Millipore filtration) or separated by ion-exchange chromatography.

Ion-Exchange Purification. Duplicate 10-mL samples of prefiltered orange juice were each percolated through an 8-mm i.d. column containing 3.5 mL of AG-MP-50 cation-exchange resin (hydrogen form, 50–100 mesh, Bio-Rad Laboratories, Richmond, CA) and then through a similar column containing 3.5 mL of Bio-Rad AG-MP-1 (formate form) anion-exchange resin (Shaw and Wilson, 1981). The anion-exchange resin was washed with 30 mL of water to remove the sugars and then with 20 mL of 6 N formic acid followed by 30 mL of water to remove the acids. The combined 50 mL of eluate containing the organic acids was concentrated to dryness at 65 °C under reduced pressure, the residue was dissolved in 10 mL of water, and the resulting solution was filtered by Millipore filtration as described above. The ion-exchange columns were checked for acid recovery by chromatographing duplicate 10-mL samples of an aqueous solution of 0.20% malic acid and 0.70% citric acid under the above conditions. HPLC analysis showed 98.6% recovery of malic acid and 99.2% recovery of citric acid.

HPLC Analyses. Total citrate was determined by HPLC using a Waters Model 202 LC equipped with a differential refractometer (RI), or an LDC Spectromonitor III variable-wavelength ultraviolet (UV) detector set at 206 nm, an Altex Model 905-42 injector fitted with a 20-μL injection loop, a Waters Model 6000A pump, and a Hewlett-Packard Model 3390A recording integrator. A Waters Model RC-100 radial compression unit fitted with an 8 mm i.d. × 10 cm column (5-μm C-18 packing) and a Waters 10-μm C-18 Guard-PAK precolumn insert were used. The eluting solvent was 2% $NH_4H_2PO_4$ at pH 2.7 at a flow rate of 1.8 mL/min. Alternate runs of a standard citric acid solution 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 4% with the RI detector and less than 9% with the UV detector.

Isotachophoresis. The determination of citrate was performed with an LKB Model 2127 Tachophor, equipped with a thermocouple and a UV detector operated at 254 nm. An LKB Model 2210 two-channel recorder was used to record the thermal and UV traces. The leading electrolyte consisted of 10 mM HCl and 0.4% Methocel K15M adjusted to pH 3.13 with β-alanine. Caproic acid (10 mM) was used as the terminating electrolyte. The juice samples were filtered as described earlier and were diluted 1/10 with distilled water. For each determination a 2-μL sample of the diluted juice was injected. The separation was initiated at 200 μA for 5 min, followed by reduction of the current to 50 μA which was used for detection. The width of the citrate zone was measured on the UV trace with an optical comparator equipped with a scale affording 0.1-mm resolution. The citrate zones obtained from the samples were compared with zone widths of standard citrate solutions for quantitation. Identity of the zones was established by comparing thermal step height of the sample with those of the standard citrate solutions or ultimately with the characteristics of citrate-enriched samples. Malate was clearly separated from citrate under the conditions employed but could not be quantitated due to the dilution of the sample.

RESULTS AND DISCUSSION

Two rapid and simple methods, HPLC and isotachophoresis, have been developed for the determination of total citrate in orange and grapefruit juices. Total combined time for sample preparation and a single analysis was about 30 min by either method. Table I lists the total citrate content of several fresh and processed orange and grapefruit juices determined by using these two methods. As shown in Figure 1, HPLC using the RI detector gave

Table I. Total Citrate in Single-Strength Orange and Grapefruit Juices (Weight Percent)

sample[a]	HPLC RI	HPLC UV	isotachophoresis	total acid[b]	total salt[c]	acid + salt	juice pH
orange							
fresh Hamlin	1.03	0.97	1.04	0.87	N[d]	N	3.80
fresh Hamlin[e]	0.96	0.90	0.90				
canned	1.17	1.08	1.22	1.09	0.21	1.30	3.55
canned[e]	1.14	1.07	1.10				
carton[f]	0.88	0.85	0.81	0.72	0.25	0.97	3.95
grapefruit							
fresh Duncan	1.64	1.53	1.68	1.47	0.20	1.67	3.25
canned	1.24	1.25	1.29	1.15	0.21	1.36	3.45

[a] Values are the average of duplicate runs. [b] Determined by titration to pH 7.8 (Sinclair et al., 1945). [c] Determined by difference in total acid before and after ion-exchange chromatography (Kilburn and Davis, 1959). [d] N = not determined because insufficient sample remained. [e] Sample purified by ion-exchange chromatography. [f] Sample in wax-coated cardboard container, labeled "not made from concentrate".

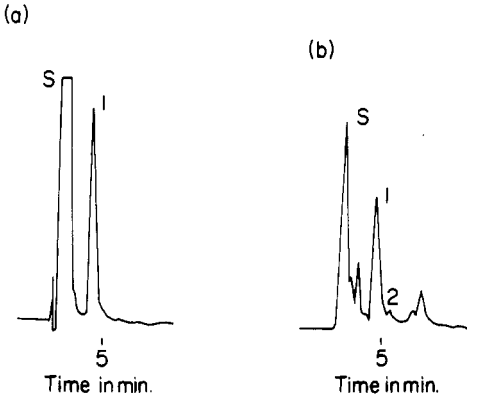

Figure 1. Canned orange juice on a C-18 column (after glass-wool purification only) using (a) an RI detector and (b) a detector at 206 nm. Peak identifications: S, solvent; 1, citric acid; 2, succinic acid.

a less complex chromatogram in which the citric acid peak was easier to integrate than when the UV detector was used. However, succinic acid was observed only with the UV detector. The coefficient of variation for triplicate runs of a given sample with the RI detector was generally less than half that for the UV detector, indicating a more accurate integration with the RI detector. Values determined by HPLC using either detector compared about equally with those determined by isotachophoresis.

An approximate value for total citrate can be determined with earlier methods as shown in Table I. Titration to pH 7.8 was shown by Sinclair et al. (1945) to afford the total free acid present, which they considered as total citric, since that is the major acid in most orange and grapefruit juices. Use of ion-exchange chromatography by the method of Kilburn and Davis (1959) affords a measure of total salts of organic acids present in the juice. The combined value for total acid and total salts (Table I) is generally higher than the value for total citrate determined by either HPLC or isotachophoresis. This is to be expected since significant quantities of malic and succinic acids occur in some orange and grapefruit juice samples (Vandercook, 1977), and their presence would contribute to both the total acid and total salt values determined by the above methods.

Significant quantities of malic acid were seen in the canned orange juice sample which was purified by ion-exchange chromatography (Figure 2). The peak at 2.9 min contains malic acid, but it is not sufficiently resolved from the peak preceding it to be accurately quantified. A trace of succinic acid was probably also present in this juice sample (Figure 1b, succinic acid RT = 5.2 min). Since the area under the citric acid peak was not affected by ion-exchange purification, this step can be omitted.

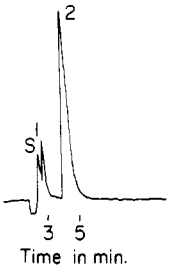

Figure 2. Canned orange juice after purification by ion-exchange chromatography; RI detector. Peak identities: S, solvent; 1, malic acid; 2, citric acid.

Isotachophoresis, a relatively new instrumental method based on differences in ionic mobilities in an applied electric field (Everaerts et al., 1976), can be used to determine ionic constituents in biological extracts. The primary advantage of this method is the minimal amount of preparation required. Under the conditions described earlier, the citrate zone is readily distinguishable from other ionic constituents. Identity of the zone was established by comparing thermal step heights of standard citrate and the suspected zone in the sample, as well as by enrichment. Quantitation was accomplished by measurement of zone width in comparison with that of standard citrate solutions. Table I indicates the results obtained with this method. Analysis time was approximately 10–15 min/sample.

Thus, total citrate in citrus juices can be readily determined by HPLC or isotachophoresis. Values found by the two methods were comparable but were 0–20% higher than the total acid by titration. No sample preparation was necessary for analysis of single-strength juice by isotachophoresis, while filtration, to remove particulate matter, was required for HPLC analysis. These methods will aid citrus processors in monitoring frozen concentrated orange and grapefruit juices to determine reasons for formation of potassium citrate crystals in certain concentrated juices.

Registry No. Citric acid, 77-92-9; malic acid, 6915-15-7; succinic acid, 110-15-6.

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