Application of High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection to Sugar Analysis in Citrus Juices[†]

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The need for rapid and simple analytical techniques for carbohydrates in fruit juices is becoming increasingly critical as more attention is being placed on authenticity and nutritional content. A high-performance anion-exchange chromatographic method that uses an alkaline mobile phase (0.14 M NaOH) on a pellicular quaternary amine resin column (Dionex CarboPac PA1) at ambient temperature, coupled with pulsed amperometric detection, has been applied to the analysis of the three major sugars: glucose, fructose, and sucrose. Data were compared with those obtained from independent analysis by HPLC on an amino-bonded silica column with refractive index detection. Results demonstrate the usefulness of the anion-exchange method for the routine quantitation of sugars in fruit juice. Run times were less than 10 min. The sensitivity of the electrochemical detector was 100 times that of the refractive index detector; limits of detection were estimated to be 45 ng for glucose, 60 ng for fructose, and 300 ng for sucrose. Coefficients of variation were 0.8, 1.6, and 6.7% for the three sugars, respectively.

INTRODUCTION

Analysis of foods for carbohydrates has been a common practice in the food and beverage industry for many years, and the quantitation of sugars in fruits and fruit juices is of special interest. Varietal, geographic, seasonal, and maturity differences can affect the sugar composition of some fruits in dramatic ways. On the other hand, fruits not greatly influenced by these factors exhibit characteristic sugar distribution patterns that permit the composition to be used to determine the authenticity of their juices and concentrates (Wrolstad and Shallenberger, 1981). Furthermore, the expansion of nutritional labeling programs and an increased consumer interest in nutrition have created new demands on the food industry to specify the quantity of individual carbohydrate fractions in fresh and processed foods (Conrad and Palmer, 1976). Therefore, it is essential that analytical methodology for carbohydrates in foods be easily automated or otherwise amenable to routine use.

Gas–liquid chromatography (GLC) has been used for the analysis of sugars and provides for excellent separation (Sweeley et al., 1963); it has been the method of choice where high sensitivity is required. However, GLC requires a time-consuming derivatization step that may result in unreacted materials or the production of side products, making quantitation difficult. Quantitation is also complicated in some instances by the fact that anomeric pairs (e.g., α- and β-glucose) are separated (Birch, 1973) and must be summed.

High-performance liquid chromatography (HPLC) has been widely used for the separation and quantitation of sugars in foods (Shaw, 1988). The method does not require sample vaporization as does GLC; thus, thermal degradation of components is unlikely. The most common separations utilize amino-bonded silica columns (Yang et al., 1981; Linden and Lawhead, 1975); however, it has been noted that this method can suffer from column deteri-

oration due to aminocarbonyl reactions in which Schiff bases are formed, requiring regular column regeneration (Pirisino, 1984). Another method employs ligand exchange on a metal-ion form cation-exchange column at elevated temperature (Fitt et al., 1980), but the high temperatures required can lead to detection difficulties, particularly when refractive index detectors are used (Edwards et al., 1987). Other methods have employed derivatization and separation on reversed-phase columns (McGinnis et al., 1986) or ion exchange as borate complexes (Sinner and Puls, 1978).

Since carbohydrates exhibit only weak absorbance in the UV–vis region, the measurement of refractive index (RI) has been the conventional means of detection. In an analysis of citrus juice (Shaw and Wilson, 1983), refractive index detection (RID) was reported to provide detection limits of 30 μg for fructose and 60 μg for glucose and sucrose with no sample cleanup required. Other workers have reported similar results (Binder, 1980). However, refractive index is a measure of a bulk property of solution and, as such, does not offer selectivity for sugars over other compounds. Low-wavelength UV detection (<200 nm) of sugars has also been applied to fruit juice analyses (Shaw and Wilson, 1983), offering an enhanced sensitivity over RI detection of about 25-fold for fructose and 7-fold for glucose and sucrose. Unfortunately, the method is highly susceptible to interference, requiring significant sample cleanup.

The chromatographic analysis of sugars was substantially improved when the first separation by high-performance anion exchange (HPAE) was demonstrated on a polymeric anion-exchange resin column using sodium hydroxide (pH 12–14) as eluent (Rocklin and Pohl, 1983). The method takes advantage of the affinity between ionized group(s) on the saccharide at alkaline pH and a pellicular quaternary amine stationary phase (Townsend et al., 1988). The anion-exchange affinity, and thus retention time, follows the order sugar alcohols < monosaccharides < disaccharides < oligosaccharides. Resolution depends largely on the pH of the eluent and control of temperature in the 20–45 °C range.

Carbohydrates are electroactive in alkaline solution, and the development of triple-pulse amperometric detection (Hughes and Johnson, 1982) enabled detection of as low as 10 pmol of underivatized carbohydrate, with closely related structures exhibiting similar responses. The usefulness of this detection method for sugars and sugar alcohols in beverages was illustrated by Hughes and Johnson (1982), where separation was accomplished on a calcium-loaded cation-exchange column with postcolumn delivery of 0.10 M NaOH. Rocklin and Pohl (1983), by combining pulsed amperometric detection (PAD) with HPAE separation, demonstrated a highly selective and sensitive method for sugar alcohols and mono-, di-, and oligosaccharides, a method well suited for the analysis of complex samples which contain components that may interfere with detection by RI or UV absorbance. The present study was undertaken to examine the utility of HPAE/PAD for the routine analysis of the major sugars: glucose, fructose, and sucrose in citrus juices. Comparison was made with results obtained from an independent analysis using HPLC on an amino-bonded silica column with refractive index detection.

EXPERIMENTAL PROCEDURES

Reagents. Reagent grade anhydrous dextrose (D-glucose), D-fructose, and sucrose (Fisher Scientific, West Haven, CT) were dried under vacuum at 65 °C for no less than 3 h. A primary stock solution containing approximately 10% (w/v) sugar (sucrose/glucose/fructose in ratio 2:1:1) was prepared by weighing the dried solids into a 100-mL volumetric flask and diluting to volume. This was kept frozen until use. Secondary stock solutions were prepared as needed by dilution of the primary stock. Reagent grade dulcitol (Sigma, St. Louis, MO) and *dl*-threitol (99%, Aldrich, Milwaukee, WI) were employed as internal standards in the HPAE/PAD and the HPLC/RID experiments, respectively. They were added by pipetting 1 mL of their stock solutions (1.0 and 10.0%, respectively) into the primary standard or sample solutions prior to dilution. Sodium hydroxide (50% w/w, Fisher Scientific Co., Fair Lawn, NJ), diluted to 0.14 M (pH 13) with filtered (0.45 μm) HPLC grade carbonate-free water, was used as the mobile phase for analysis by HPAE/PAD. Care was taken to exclude carbon dioxide. HPLC grade acetonitrile (Fisher) and water, mixed in volume ratio 76:24 and filtered through a 0.45-μm filter, was used in the HPLC/RID study.

Instrumentation. *HPAE/PAD.* Anion-exchange chromatography was performed with a Waters (Milford, MA) Model 510 pumping system and a Dionex (Sunnyvale, CA) CarboPac PA1 column (4 × 250 mm) at ambient temperature (23 ± 2 °C). No guard column was used in order to minimize band spreading. The mobile phase (0.14 M NaOH) was filtered on-line prior to the column through a 0.45-μm stainless steel filter (Rheodyne, Inc., Cotati, CA), which was cleaned ultrasonically in methanol and replaced as needed. Tubing and fittings were stainless steel. The pumping rate was 1.0 mL/min, resulting in a head pressure of about 1200 psi. Injections (20 μL) were made by using a Waters WISP 710B autoinjector. The detector (EG&G, Princeton Applied Research, Princeton, NJ) consisted of a single gold working electrode operated at potentials (vs Ag;AgCl) of E1 = +50 mV (167 ms), E2 = +650 mV (167 ms), and E3 = –950 mV (500 ms). Sensitivity was set at 100 μA full-scale, and current was sampled during E1. Data were acquired at a frequency of 2 Hz by using a 20-bit A/D converter (M160 CSI, Autochrom, Inc., Milford, MA) and analyzed by APEX chromatography software (Autochrom, Inc.) using a CompuAdd (Austin, TX) 286 AT computer.

HPLC/RID. Separations were performed on a Du Pont (Wilmington, DE) Zorbax amine column (4.6 × 250 mm) at ambient temperature. A 0.45-μm filter and a 3-cm guard column (Brownlee Labs, Santa Clara, CA) with similar packing material were used on-line prior to the column. The mobile phase (76:24 CH_3CN/H_2O) was pumped at 1.0 mL/min (1500 psi) with a Waters Model 6000A pump. Injections (20 μL) were
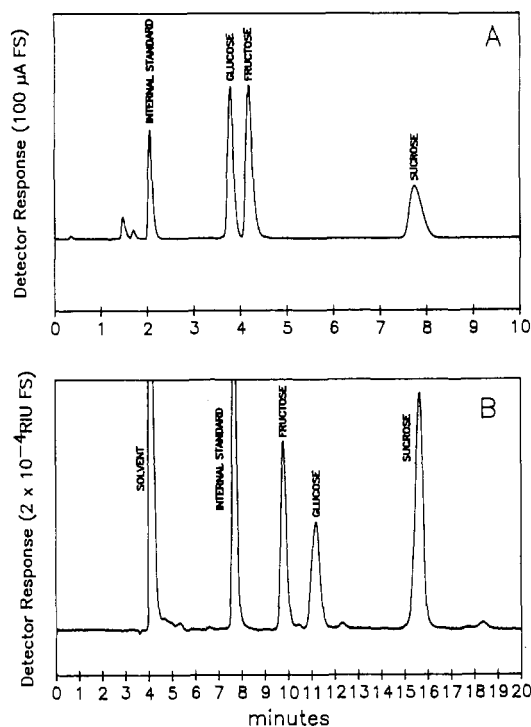
Figure 1. Chromatographic separation of sugars in a commercial sample of orange juice from concentrate (OJFC). Comparison of two methods (A; HPAE/PAD; B, HPLC/RID).

Table I. PAD Response for Three Major Sugars in Citrus Juice: Best-Fit Parameters for the Equation y (Peak Area or Peak Height[a]) $= a + bx + cx^2$ (x = Micrograms of Sugar)

sugar	a, μA·s	b, μA·s μg^{-1}	c, μA·s μg^{-2}	r^2
glucose	5.67	90.7	0	0.9999
	(1.66)	(9.75)	0	0.9998
fructose	1.44	83.2	0	0.9995
	(−0.54)	(7.6)	0	0.9996
sucrose	28.7	30.9	0	0.997
	44.2	22.5	0.768	0.9999
	(1.70)	(1.4)	0	0.996
	(2.56)	(0.98)	(0.042)	0.999

[a] Best-fit parameters when y is the peak height (μA), values in parentheses.

made with a Waters WISP 712 autoinjector. The RI detector was a Waters Model R401 set at an attenuation of 8x. Data were collected at a 2.5-Hz sampling rate by using a Spectra-Physics (San Jose, CA) Winner Model 319 integration system. Data were transferred in ASCII code to a CompuAdd 286 AT computer for analysis with APEX chromatography software.

Sample Preparation. Juice samples were prepared for analysis by either a simple 100-fold dilution with water for HPAE/PAD or a 4-fold dilution with 50% acetonitrile for HPLC/RID. The standard sugar mixture was treated in an identical manner. All samples and standards were filtered through 0.2-(HPAE/PAD) or 0.45-μm (HPLC/RID) nylon syringe-type filters prior to injection. External standard calibration was made within every four to eight sample injections.

RESULTS AND DISCUSSION

Chromatographic results for a typical juice sample analyzed by the two methods are illustrated in Figure 1. Baseline resolution of the three sugars by HPAE/PAD is accomplished in roughly half the time as required by the HPLC/RI method. Note the reversed order of elution of the monosaccharides. Over the course of the experiments, the relative standard deviation of the retention time on the HPAE system was less than 0.6% for glucose and fructose, but was considerably larger (2.2%) for sucrose.

Prior to analysis by HPAE/PAD, the standard sugar solution and juice samples were diluted 100-fold to a

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Table II. Sugar Distribution (% w/v) in Some Commercial Citrus Products As Determined by HPAE/PAD and HPLC/RID Methods

	glucose, %		fructose, %		sucrose, %	
sample	PAD	RID	PAD	RID	PAD	RID
OJFC (1)	2.33 ± 0.02	2.29 ± 0.06	2.54 ± 0.04	2.55 ± 0.07	4.5 ± 0.3	4.3 ± 0.2
OJFC (2)	2.22	2.13	2.46	2.42	4.6	4.4
OJFC (3)	2.18	2.06	2.39	2.31	4.6	4.2
GFJ (1)	2.69	2.74	2.88	2.98	2.5	2.1
GFJ (2)	2.22	2.32	2.35	2.50	3.2	3.1
GFJ (3)	2.37	2.54	2.51	2.74	3.6	3.6
pulpwash (1)	2.29	2.24	2.38	2.36	4.5	4.4
pulpwash (2)	2.11	2.03	2.28	2.24	5.1	4.7
pulpwash (3)	2.23	2.20	2.42	2.49	5.5	5.0

Table III. Sugar Distribution in Fresh-Squeezed Juice from Several Citrus Cultivars

cultivar	glucose, % w/v	fructose, % w/v	sucrose, % w/v	total sugar, % w/w[a]	°Brix	total sugar/°Brix, %
Bearss lemon	1.53	1.49	0.3	3.2	8.5	38
Persian lime	0.91	0.86	0.2	1.9	9.4	20
Page orange	2.50	2.64	5.8	10.5	11.8	89
Hamlin orange	1.94	2.22	5.6	9.4	10.1	93
Orlando tangelo	1.35	1.46	4.0	6.5	8.3	78
Minneola tangelo	1.75	1.97	4.4	7.8	8.7	90
Robinson tangerine	2.47	2.70	5.8	10.6	11.9	89
Star Ruby grapefruit	1.40	1.50	3.0	5.7	7.5	76
Duncan grapefruit	2.09	2.14	3.4	7.3	8.9	82
Marsh grapefruit	1.83	1.90	1.9	5.4	6.4	84

[a] Converted from % w/v to % w/w by using density = 1.04 g mL^{-1}.

concentration range that would ensure no significant loss of resolution due to overloading of the pellicular anion-exchange column (Olechno et al., 1987). A 20-μL injection of the diluted solutions delivered sugar levels to the detector cell in the microgram range. Multilevel calibration using five successive dilutions of the secondary (100-fold dilute) sugar standard exhibited linear responses for the three sugars over the range 0.5–10 μg. Sucrose data fit slightly better to a quadratic equation. Plots of peak area (microampere seconds) vs sugar (micrograms) gave somewhat better results than corresponding plots using peak heights (microamperes). Results are summarized in Table I.

The slopes of plots of peak height (microamperes) vs micrograms of sugar (b values in parentheses, Table I), or calibration sensitivity, are useful for estimating the limit of detection for the PAD. On the basis of an average peak-to-peak baseline noise of about 0.15 μA and by use of the criterion of three-times peak-to-peak noise, the minimum detectable amounts were calculated to be roughly 45, 60, and 300 ng for glucose, fructose, and sucrose, respectively. A similar calculation using calibration data obtained from the RI detector yielded detection limits of about 5 μg for the three sugars. While the baseline noise of the PAD was of a random nature, the RID noise was caused primarily by the pulsation of the pump. The PAD signal-to-noise (S/N) ratio is a function of the sampling potential (E1), and although the signal response can be made to increase by increasing E1, beyond a certain level the noise increases proportionally; thus, no real gain in signal-to-noise (S/N) ratio is achieved. An E1 = +50 mV (vs Ag;AgCl) was found to be optimal under our conditions.

Both RI and PAD detector methods were used to analyze the sugar contents of three samples each of the following: commercial orange juice from concentrate (OJFC), grapefruit juice from concentrate (GJFC), and processing pulpwash. Results are given in Table II. The error estimates are standard deviations calculated from replicate injections ($n \geq 5$) of standards similar in concentration to the samples analyzed. The higher standard deviation for sucrose with the PAD system may be due in part to quantitation errors caused by the shifts in retention time noted

earlier. It has been reported (Rocklin and Pohl, 1983) that PAD sensitivity to sugars gradually increases with use from the time that the electrode is polished, necessitating regular calibration for maximum accuracy. This phenomenon was observed for the three sugars of interest here, yet the response to dulcitol did not vary in an analogous fashion; no improvement in precision was gained over the short term by internal standard calibration. Such calibration would probably be of use in compensating for long-term drift, however, such as the changing base potential of the Ag;AgCl reference electrode. It can be seen by comparison of the results obtained that the two methods agree within experimental error. It should be noted that in spite of the greater sensitivity of the PAD over the RI detector, the precision of the two methods is of the same order of magnitude, primarily due to the significant dilution of samples prior to HPAE/PAD analysis.

The HPAE/PAD method was further used to analyze the sugar content of fresh-squeezed juice from 10 early-season citrus cultivars. Results are given in Table III, along with the °Brix (% w/w total soluble solids) obtained independently by refractometry. The last column of the table lists the total sugars (converted to a w/w basis) as a percentage of the total soluble solids. The values, which range from 76 to 93% for the orange and grapefruit juices, are within the expected range for these cultivars (Nordby and Nagy, 1980).

Conclusion. High-performance anion-exchange chromatography with pulsed amperometric detection can be used routinely to quantitate glucose, fructose, and sucrose in citrus juice. The method is simple and requires no sample preparation other than dilution and filtration. Run times are less than 10 min. The detection system is rugged and extremely selective for sugars and sugar alcohols. The sensitivity is at least 100 times that of RI detectors. The pellicular anion-exchange column easily resolves glucose, fructose, and sucrose under the described conditions. No decrease in column performance was noted after as many as 350 injections.

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