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ARTICLES

Analysis of Simple Sugars and Sorbitol in Fruit by High-Performance Liquid Chromatography

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The application of a high-performance liquid chromatographic (LC) procedure for the determination of sugars and sorbitol in fresh fruits is described. This system combines the use of two bonded phase carbohydrate columns, joined in tandem; a ternary mobile phase (acetonitrile–water–ethanol) and a differential refractometer to accurately and precisely separate fructose, glucose, sorbitol, sucrose, and maltose. Total analysis time was 20 min for the five-sugar mixture. Twenty-four fruits were analyzed including eleven from the family Rosaceae, which often contain sorbitol. Sample recoveries ranged from 98% for fructose to 102% for maltose.

Recently, Lee (1978) reviewed the many methods available for determining carbohydrates in foods. Carbohydrate analysis may be separated into the following categories: physical, chemical, colorimetric, and enzymatic. Of the different techniques available, enzymatic and chromatographic (a physical method) procedures are most commonly used. The various chromatographic procedures include paper, thin-layer (TLC), gas–liquid (GLC), ion-exchange (IE), and more recently high-performance liquid chromatography (LC). Automated enzyme assays are also being used to determine carbohydrate content.

Huntington (1978) described the use of an enzymatic analyzer for determining glucose, sucrose, and lactose. Immobilized enzymes are used for the sugar assays and

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they have only a 2-week life span. Also, for each sugar assayed a specific enzyme kit must be used. More recently, Prager and Miskiewicz (1979) reported a GLC procedure for separating and quantifying sucrose, lactose, maltose, and glucose in commercial confectionary products. They quantified the trimethylsilyl (Me_3Si) derivatives of the sugars; chromatographic separations, recoveries, and reproducibility were all very good.

Significant advances in carbohydrate analysis by high-performance LC have led to many different procedures that are considered fast, simple, accurate, and reproducible. Further, samples need not be altered and they may be collected for additional analyses, if desired. Linden and Lawhead (1975) reported that the analysis of saccharides by high-performance LC equals the precision and accuracy of GLC. They also describe a number of applications and problems that are likely to be encountered when doing carbohydrate analyses by high-performance LC. Conrad and Palmer (1976) used high-performance LC to rapidly analyze carbohydrate mixtures in various food and beverage matrices; they also discussed briefly the separation of certain sugar alcohols. Moreover, these authors list numerous high-performance LC advantages and GC disadvantages. Wong-Chong and Martin (1979a) described a rapid method for determining fructose, glucose, sucrose, and raffinose in sugar cane juice by adsorption chromatography. They were able to resolve these carbohydrates in less than 27 min using high-performance LC. In another article (Wong-Chong and Martin, 1979b) these same authors used ion-exchange (IE) chromatography for the separation of sugar cane saccharides. They were able to resolve sucrose, glucose, and fructose in less than 8 min using water as the only solvent. For attainment of adequate resolution of samples by IE, the column must be jacketed to maintain the elevated operating temperatures which are required. Wong-Chong and Martin (1979b) evaluated Aminex A5, Q15S and Q150S ion-exchange resins for their ability to effectively and reproducibily separate saccharides in sugar cane juice.

Recently, Dunmire and Otto (1979) determined the carbohydrate contents of various food products via high-performance LC. Their method is reported to be fast, simple, specific, and reliable over a wide concentration range. They were able to resolve fructose, glucose, sucrose, maltose, lactose, melibiose, raffinose, and stachyose in less than 45 min. Using this procedure, they examined cereals, protein products, processed fruits, chocolate products, baby foods, and health bars. The authors also describe a "minicolumn" sample cleanup procedure to increase column life. Woidich et al. (1978) described two different procedures for determining simple sugars and sorbitol (D-glucitol) in food. They used a modified silica gel column (Lichrosorb-NH_2) for the determination of fructose and glucose in the presence of various disaccharides. Also, by using a strongly basic cation exchanger (Bondapak-AX-Corasil), they were able to separate fructose, glucose, and sorbitol.

As with carbohydrate analyses, many different procedures are described in the literature for the determination of sorbitol and other sugar alcohols. Lara and Yabiku (1974) described a TLC method for the identification of sorbitol. Boehringer Mannehim GmbH, Biochemica (1979), in their manual of new applications, details the enzymatic determination of D-sorbitol in foodstuffs. Finally, Makinen and Soderling (1980) dscussed the quantitative analysis of various sugar alcohols in wild berries and commercial fruits. They made polyacetyl ester derivatives of the sugar alcohols and then used GC to determine polyol concentrations.

Frattali (1980) recently reviewed the regulatory and nutritional aspects of fructose and sugar alcohols in foods. A major point of concern in this article was directed to the nutritional needs of the diabetic. By providing qualitative and quantitative values for simple carbohydrates (including sorbitol) in food, the diabetic, in consultation with a professional, would be able to select from a much broader range of products. Sorbitol occurs naturally in many fruits and is frequently found in fruits of the family Rosaceae. Some fruits in this family include apples, pears, and plums. In the apple, sorbitol apparently plays a major role in the translocation of carbohydrates to the developing fruit, and during low-temperature storage it is believed that fructose is reduced to sorbitol (Bollard, 1970).

Because of concerns for labeling dietetic and other foods containing sorbitol in the presence of glucose and other saccharides, and because of ripening and storage changes involving sorbitol and other simple sugars, a multiple-component high-performance LC assay was developed in this laboratory (Brandao et al., 1980). Fresh fruit from various families were assayed for their simple sugars and sorbitol content in order to show application of this technique. Elution order is fructose, glucose, sorbitol, sucrose, and maltose. Total analysis time is only 18 min for the five-saccharide mixture.

EXPERIMENTAL SECTION

Preparation of Fruit Extracts. One to two kilograms of fruit samples was obtained from a local farmer's market. From several ripe, sound fruits, slices of edible tissue weighing a total of 20–40 g were excised and placed in a Waring blender. The fruits were covered with sufficient 100% ethanol to make the final concentration of ethanol 80%. Fruit and ethanol were then blended at high speed for 2–3 min (depending on tissue softness). The resulting slurry was refluxed under stirring for 2 h with a condenser. The extract was then filtered through Whatman No. 54 paper; the residue and flat-bottom flask were washed with additional 80% ethanol (~200 mL). The extract plus washings were then reduced to a volume less then 25 mL by using a rotary vacuum evaporator. Samples were concentrated until the ethanol odor was completely gone. Finally, the fruit concentrate was made to 25 mL with distilled water and filtered through Whatman No. 42 paper.

All of the fruit sample concentrates were deeply pigmented and would thus severly reduce analytical column life if they were to be injected directly into the system. Therefore, Sep-Pak C_{18} cartridges (Waters Associates, Inc., 1979) were used to retain these varied and colorful pigments. Resultant solutions were water clear with all the coloring material being retained on these small columns.

The Sep-Pak was easily placed at the end of a 10-mL graduated syringe. The C_{18} cartridge was first prewet with 2 mL of acetonitrile and then flushed with 5 mL of distilled water. After this, the cartridge was flushed with 2–3 volumes of air before the sample was placed into the syringe. The first 2 mL of sample was discarded, while the second 2 mL of sample was collected for high-performance LC analysis. Before the injection, however, the samples were filtered through a 0.45-μm Metrical membrane (Gelmann Fitration Products, Ann Arbor, MI) to further ensure removal of any particulate impurities that might be present.

High-Performance LC Analysis of Extracts. Sugar analyses were carried out as previously described by Brandao et al. (1980). Isocratic separations of the various sugars were made on two bonded phase carbohydrate

Table I. High-Performance LC Analysis of Simple Sugars in Some Common Fruits

fruit	family	sugar content, % fresh weight			
		fructose	glucose	sucrose	maltose
avocado	Lauraceae	–[a]	0.15	–	–
banana	Musaceae	2.41	2.58	14.0	–
blueberry	Vaccinium	3.21	2.99	0.25	–
cherry tomato	Solanaceae	1.94	0.87	0.09	–
grape	Ampelidaceae	7.33	8.05	4.65	0.05
honey dew melon	Cucurbitaceae	2.66	1.91	12.09	0.20
lime	Rutaceae	0.32	0.33	0.03	–
mango	Anacardiaceae	3.18	0.49	9.86	–
orange	Rutaceae	3.02	2.93	7.02	0.32
papaya	Caricaceae	2.34	2.48	4.43	–
pineapple	Bromeliaceae	2.32	1.65	9.50	–
strawberry	Fragaria	2.59	2.41	1.64	0.10
watermelon	Cucurbitaceae	2.98	1.32	7.39	0.49

[a] Not detected.

Table II. High-Performance LC Analysis of Simple Sugars and Sorbitol in Fruits of the Rosaceae Family

fruit	sugar and sorbitol content, % fresh weight				
	fructose	glucose	sorbitol	sucrose	maltose
apple (Golden delicious)	7.87	1.65	0.26	1.11	–[a]
apple (Red delicious)	7.96	3.46	0.24	0.51	–
pear (cv. Bartlett)	9.03	0.90	1.66	1.24	–
pomegrante	6.05	4.71	0.30	0.70	–
red plum	0.83	1.26	–	4.24	0.05
prune plum	3.29	3.08	2.65	4.41	0.11
yellow plum	1.04	2.05	0.26	1.58	–
sour cherry (cv. Montmorency)	3.74	4.06	1.04	–	–
sweet cherry	4.92	4.77	2.10	0.13	–
blackberry	1.55	1.18	–	0.14	–
peach	0.45	0.32	–	3.13	–

[a] Not detected.

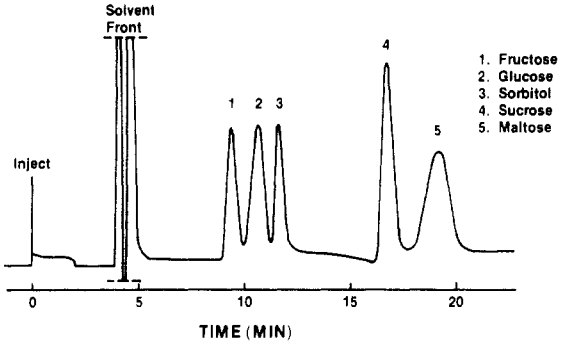

Figure 1. High-performance LC chromatogram of the standard carbohydrate mixture. Dual column arrangement; mobile phase, acetonitrile–water–ethanol (80:15:5 v/v/v); flow rate, 1.8 mL/min; injection volume, 10 μL; attenuation, 8×.

columns connected in tandem with a ternary mobile phase of acetonitrile–water–ethanol (80:15:5 v/v/v). Generally, volumes injected ranged from 2 to 10 μL, and a 25-μL syringe was used. When injection volumes from 10 to 50 μL were necessary, a 100-μL syringe was used. These large injection volumes were sometimes necessary to adequately quantify trace sugars present in the sample. Peak height measurements were used to quantify the free sugars and sorbitol in the fruit, and linear regression equations were established for each compound. All quantitative determinations were made in duplicate (two aliquots from the same fruit macerate).

RESULTS AND DISCUSSION

Recovery experiments were conducted by spiking known quantities of standards into an apple sample and then assaying the sample before and after the addition. Further, the prepared standard solution mixture was also assayed in the same way (Brandao et al., 1980). Spiking was done during initial sample and standard mixture preparation.

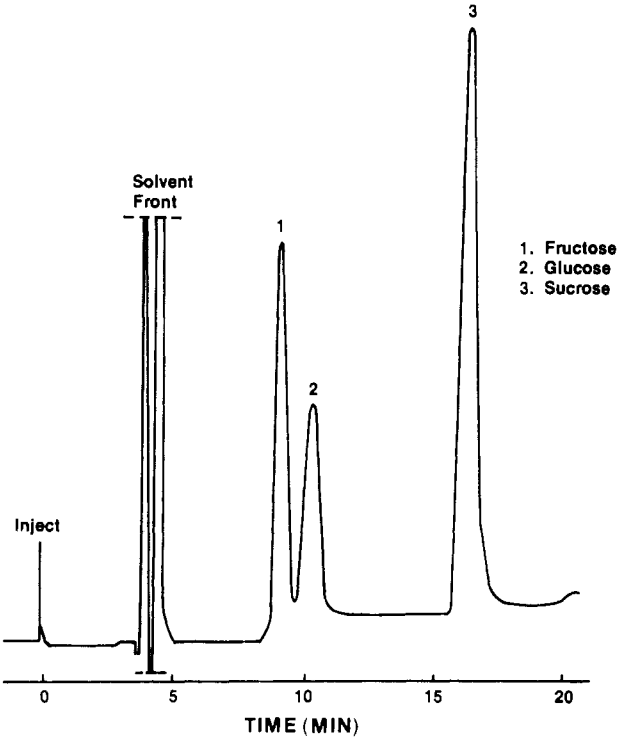

Figure 2. High-performance LC chromatogram of carbohydrates in the orange. Dual column arrangement; mobile phase, acetonitrile–water–ethanol (80:15:5 v/v/v); flow rate, 1.8 mL/min; injection volume, 5 μL; attenuation, 8×.

Sample recovery (%) in the apple was as follows: fructose, 98.3; glucose 101.3; sorbitol, 98.0; sucrose 101.2; maltose, 102.1. These values were also very similar to recoveries in the standard solution mixture. The identification of the sugars and sorbitol was based on high-performance LC

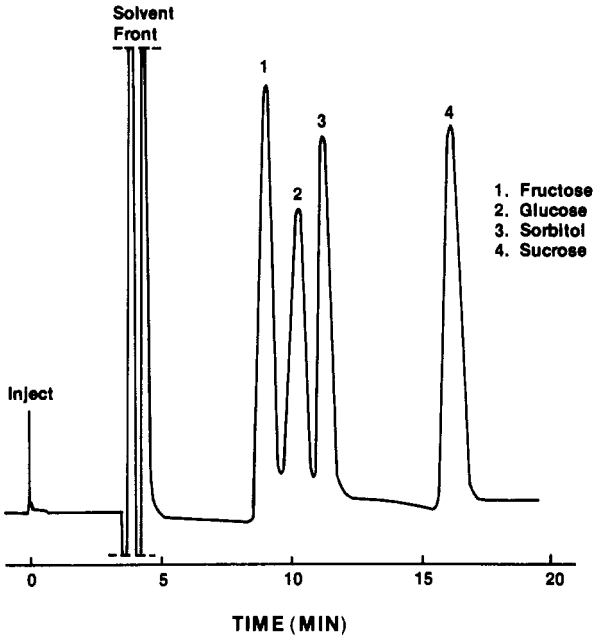

Figure 3. High-performance LC chromatogram of carbohydrates and sorbitol in the purple plum. Dual column arrangement; mobile phase, acetonitrile–water–ethanol (80:15:5 v/v/v); flow rate, 1.8 mL/min; injection volume, 5 μL; attenuation, 8×.

retention times (Figure 1). Regression equations and correlation coefficients were determined for the carbohydrate standards. Correlation coefficients for all standards were nearly identical with values ranging from 0.9990 to 0.9999. These equations and coefficients were true in the concentration range 30–130 μg for fructose, glucose, and sorbitol and 30–180 μg for sucrose and maltose.

In order to be consistent with other literature, our data are presented as percent fresh weight of edible tissue (percent fresh weight). In general, the data in Tables I and II compare favorably with values reported in the literature (Whiting, 1970; Lee et al., 1970). Sugar analyses of fruits from a number of different families are depicted in Table I. None of these fruits contained sorbitol. Even when large volumes were injected, no sorbitol peak was present. An actual chromatogram for the orange is shown in Figure 2. On the other hand, when fruits of the Rosaceae family were examined (Table II), sorbitol was often, but not always, present. Sorbitol was not detected in the red plum, blackberry, or peach. A chromatogram of the purple plum is shown in Figure 3. When large injection volumes were used, maltose was observed in only a few fruits (Tables I and 2). In addition, we have also presented sugar profiles of some novel fruits that are not often reported in the literature.

By use of two carbohydrate columns connected in tandem and a ternary mobile phase of acetonitrile, water and ethanol sorbitol can be adequately and reproducibly separated in one simple procedure from its parent sugar glucose, in systems containing fructose, sucrose, and maltose.

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