Detection of Inexpensive Sweetener Addition to Grapefruit Juice by HPLC-PAD

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Fingerprint oligosaccharide analysis by high-performance liquid chromatography with pulsed amperomeric detection was used to detect the adulteration of grapefruit juice. Thirty pure grapefruit juice samples were analyzed, 15 fresh-pressed/pasteurized and 15 concentrates. Individual inexpensive sweeteners (high-fructose corn syrup, beet medium invert sugar, and beet invert sugar) were intentionally added to pure grapefruit juice samples at levels of 5, 10, and 20%. Each of these materials could be readily detected (at 5% levels) when added to grapefruit juice. The origin of the oligosaccharides in each of these inexpensive sweeteners has been elucidated. In addition, the elution program was shortened to 115 min (total time between sample analysis), which makes this method more conducive to routine sample analysis.

INTRODUCTION

The adulteration of pure citrus juice is a significant worldwide problem. In 1990–1991 world grapefruit production was approximately 3.5×10^6 metric tons. Approximately 45% of this fruit was further processed into juice and had a commercial value of more than $250 million (Florida Department of Agriculture, 1991). The adulteration of this processed product by the undeclared addition of inexpensive sweeteners is fraud. Adulteration at any level will have a serious detrimental economic impact on the citrus industry.

The literature clearly shows that as methods to detect adulteration are discovered and introduced, so are means to circumvent these authenticity tests (Nagy et al., 1988).

Debasing pure fruit juice with carbohydrate solutions is one of the easiest methods of fruit juice adulteration. As the major soluble solids present in fruit juice are carbohydrates, their addition is necessary to maintain both the correct °Brix value and the major carbohydrate profile of the fruit juice. Sweeteners derived from cane, beet, and corn can be added to fruit juice because they meet these two requirements. They also have the added benefit of being inexpensive.

The major carbohydrate in beet sugar is sucrose (Hardinge et al., 1965). Two commercially available beet sugar hydrolysates are beet medium invert sugar (BMIS; 50% hydrolyzed beet sucrose) and beet invert sugar (BIS; 92–95% hydrolyzed beet sucrose). These hydrolysates can be produced either by acid (hydrochloric) or by enzymes.

High-fructose corn syrup (HFCS) is produced commercially by the enzymatic hydrolysis of corn starch followed by enzymatic isomerization of a portion of the resulting glucose to fructose (Linko, 1987). Commercial corn syrup products include HFCS 42 (42% fructose, with the remainder being mainly glucose) and HFCS 55 (55% fructose).

The major carbohydrates present in grapefruit juice (and orange juice) are glucose, fructose, and sucrose. The approximate ratio of these major carbohydrates in these juices is 1:1:2 (Dillon, 1991; Curl and Veldhuis, 1948).

A number of commercially available inexpensive sweeteners are available for the production of fraudulent juice. BMIS, BIS plus beet sucrose, and HFCS (42 or 55) plus beet sucrose could be used to adulterate grapefruit juice. The addition of these materials would maintain the °Brix value, the correct major carbohydrate content, and the glucose/fructose/sucrose ratio. Addition of these materials at low levels (<20%) would also result in an acceptable °Brix/acid ratio. The °Brix/acid ratio may also be maintained by the addition of pure citric acid (Brause, 1992).

Methods have been developed to detect the addition of inexpensive sweeteners to citrus juices. Doner (1988) utilized the differences in the $^{13}C/^{12}C$ ratio of the carbohydrates present in pure citrus juice and cane or corn sugars as a method to detect the addition of these materials to pure citrus juices. This method suffers from a fairly wide natural $^{13}C/^{12}C$ isotope ratio, which results in detection limits of approximately 20% (Winters et al., 1988). Adulteration with beet sugar cannot be detected by this method as it has $^{13}C/^{12}C$ ratios in the same range as the carbohydrate present in citrus fruits (Nissenbaum et al., 1974).

Bricout and Merlivat (1971) and Brause et al. (1984) examined the $^2H/^1H$ and $^{18}O/^{16}O$ ratios of naturally occurring water in citrus fruits (juices) and of the groundwater in citrus- and beet-growing regions. These data have been used to detect the addition of beet sugar to fruit juices. Single-strength orange juice (produced from concentrate with local water) and the utilization of the water isolated from citrus juice concentrate production have limited the usefulness of this adulteration detection method.

Doner et al. (1987) developed a method to detect beet sugar addition to orange juice by correlating both the $^2H/^1H$ and $^{18}O/^{16}O$ ratios derived from these materials. Bricout and Koziet (1987) determined the $^2H/^1H$ ratio of nonexchangeable carbon-bound hydrogen in sugar from orange and beet. These authors used the difference in $\delta(^2H)$ of these two sources of sugars as a method of adulteration detection. Unfortunately, due to the wide natural $^2H/^1H$ and $^{18}O/^{16}O$ ranges and the explosive nature of the sugar nitrates (required in the $^2H/^1H$ analysis), these methods have encountered little industry acceptance.

The site-specific natural isotope fractionation NMR (SNIF-NMR) method is currently the best method for the detection of beet sugar/beet sugar hydrolysate addition

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to citrus juices (Martin, 1990). However, to obtain accurate results, the application of this method requires an extensive data base of pure citrus juice samples from each growing region. The method also requires significant capital equipment investment, and to our knowledge only one laboratory (Eurofins, Nice, France) is currently available for sample analysis. It is important to point out that few data have been published regarding the SNIF-NMR criteria which can be used to unequivocally determine if a sample is adulterated.

In our laboratory we have introduced (Swallow et al., 1991) a high-performance liquid chromatographic method to detect the addition of BMIS to orange juice. This method was based on the analysis of oligosaccharide patterns in authentic orange juice(s) and commercial BMIS. We noted the presence of several oligosaccharides that were either unique to BMIS or present in much lower concentrations in authentic juices. These oligosaccharides were then used as "fingerprints" to detect adulteration.

This work represents an extension of this fingerprint method to the adulteration of grapefruit juice. We analyzed 30 pure grapefruit juices and 3 different inexpensive sweeteners (HFCS, BMIS, BIS). Selected authentic samples were adulterated at levels of 5, 10, and 20%. In each case the presence of fingerprint oligosaccharides was used to detect adulteration.

MATERIALS AND METHODS

Samples. Thirty grapefruit samples (15 fresh-squeezed/pasteurized; 15 concentrates) were supplied by the Florida Department of Citrus. Samples were kept frozen (−18 °C) until analyzed. Concentrates were diluted 1:5 with reverse osmosis HPLC grade H_2O prior to chemical analysis. Fresh-squeezed/pasteurized samples were analyzed directly by chemical analysis.

Commercial beet medium invert sugar (73.7 °Brix; Michigan Sugar Corp., MI) and high-fructose corn syrup 55 (72.3 °Brix; CASCO Ltd., London, ON) were used as adulterants in this study.

Beet medium invert and beet invert sugar were produced in our laboratory as follows: 90.0 g of beet sucrose (65.5 °Brix; Alberta Sugar Co., Taber, AB) was heated at 65 °C with 100 μL of concentrated HCl (37% solution; BDH Chemicals Canada Ltd., Edmonton, AB), and aliquots were analyzed by HPLC until either 50 (approximately 60 min) or 94–96% (approximately 90 min for total invert) of the sucrose was hydrolyzed. The reaction was stopped (pH 7.0) by the dropwise addition of a 25% sodium hydroxide solution (Fischer Scientific Co., Fair Lawn, NJ).

All adulterants used in this study were diluted to 4.0 °Brix and were added to the pure juice sample after juice cleanup. A 4.0 °Brix concentration was used as this value corresponded to the total sugars (°Brix value) found in the 30 pure samples used in this study which were diluted and then subjected to the cleanup procedure. The range for these 30 samples was 3.9–4.2 (average of 4.0).

Sample Preparation. Samples (15–20 mL) were diluted with HPLC grade H_2O to a final reading of 5.5 °Brix. The resulting solution was centrifuged (Beckmann Instruments Inc.) at 4 °C for 15 min (2000*g*). The supernatant was passed through 6 cm^3 of AG 50W-X8 cation-exchange resin, 100–200 mesh (Bio-Rad Laboratories, Richmond, CA); the first 5 mL was discarded. Samples were then passed through 6 cm^3 of AG1-X4 anion-exchange resin (formate form), 100–200 mesh (Bio-Rad Laboratories); the first 5 mL was discarded. The sample was then passed through a C_{18} Sep-Pak cartridge and a 0.22-μm syringe filter (both from Waters Associates, Milford, MA). The final °Brix reading for these samples ranged from 3.9 to 4.2. Ninhydrin and Bradford tests were performed on selected samples to ensure the absence of amino acids and proteins (at the detection limits of these tests). All samples were either analyzed immediately or stored at −18 °C until analyzed.

HPLC Oligosaccharide Analysis. The resulting samples were analyzed on a Waters 625 metal-free gradient high-performance liquid chromatograph. Carbohydrates were separated using two Carbo Pac PA1 (Dionex) pellicular anion-exchange columns (4 × 250 mm) connected in series. Injection of 100 μL using a Waters 712 Wisp autosampler was utilized for analysis of the grapefruit juice samples. The flow rate was 0.70 mL/min. The carbohydrates were detected by a pulsed amperometric detector (PAD; Waters Model 464) with a gold electrode and triple-pulsed amperometry at a sensitivity of 50 μA. The electrode was maintained at the following potentials and durations: E_1 = 0.05 V (T_1 = 0.299 s); E_2 = 0.60 V (T_2 = 0.299 s); E_3 = −0.80 V (T_3 = 0.499 s). A postcolumn delivery system (Waters) of 300 mM sodium hydroxide (NaOH) at a flow rate of 0.80 mL/min was used to minimize baseline drift. The linear gradient elution used to achieve separation of the oligosaccharides is shown in Chart I.

Chart I

time, min	composition[a] % A	% B	% C
0.00	100	0	0
6.59	100	0	0
23.00	97	3	0
53.00	0	100	0
63.00	0	100	0
63.10	0	0	100
83.10	0	0	100
83.20	100	0	0
115.00	100	0	0

[a] % A, 100 mM NaOH; % B, 100 mM NaOH/100 mM NaOAc; % C, 300 mM NaOH.

Glucose, Fructose, and Sucrose Analysis. Samples were prepared for analysis by dissolving 200 mg of the sample in a 250-mL volumetric flask and making up to volume with HPLC grade water. The samples were filtered through a 0.22-μm syringe filter (Waters Associates).

The filtered samples were analyzed using the aforementioned equipment with the following modifications: a single Carbo Pac PA1 column was used; elution was accomplished with an isocratic mobile phase of 60 mM NaOH at a flow rate of 1.0 mL/min with no postcolumn addition of NaOH.

Sugar standards were prepared for each carbohydrate both individually and as a mixture. Sample concentrations for each ranged from 0.01 to 0.10 mg/mL. Regression lines from the HPLC analysis of these standards had correlation coefficients of 0.993, 0.994, and 0.983 for glucose, fructose, and sucrose, respectively. The standard carbohydrate mixture had a regression line correlation coefficient of 0.992.

Titratable Acidity. Samples were analyzed using AOAC Method 20.042. All analyses were performed in triplicate.

°Brix. The Brix value for each sample was obtained using a refractometer (Canlab, Edmonton, AB) maintained at 22 °C.

pH. A Fischer Accument (Model 620) pH meter was used to determine sample pH values.

RESULTS AND DISCUSSION

Prior to the work done by Swallow et al. (1991), the use of oligosaccharide profiles to detect citrus juice adulteration had not been extensively examined. Their research provided a reasonably rapid (3 h), facile method to detect the addition of one inexpensive sweetener (beet medium invert sugar; BMIS) to orange juice. Their research was based on the hypothesis that the minor oligosaccharides observed in orange juice and BMIS were present in the raw materials and remained during processing. We have conclusively shown that the minor oligosaccharides detected by HPLC-PAD arose during the acid-catalyzed inversion of pure beet sucrose to BMIS (Figure 1). The formation of complex oligosaccharides via acid treatment of selected carbohydrates (glucose and sucrose) is not new. Krol (1978) and Toth et al. (1968) observed the formation of isomaltose and gentiobiose when glucose was heated in the presence of hydrochloric acid. Although we did not observe either of these two carbohydrates, a number of

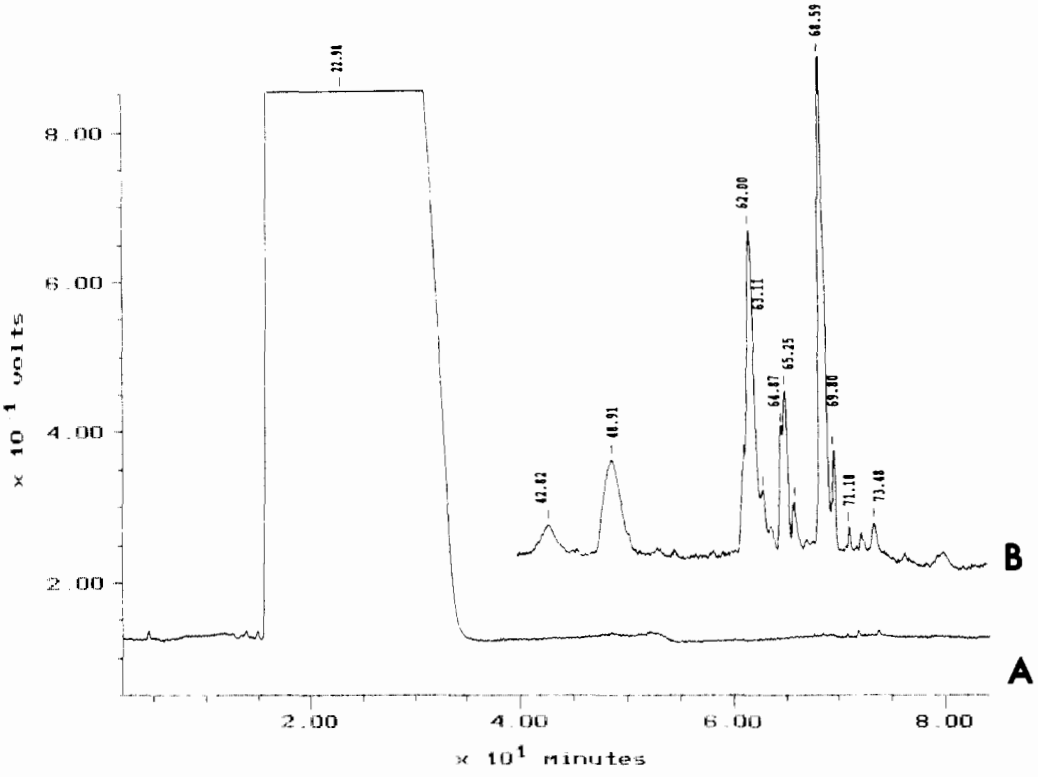

Figure 1. High-performance liquid chromatograph-pulsed amperometric detection (HPLC-PAD) of pure beet sucrose (A) and beet medium invert sugar (BMIS) (B).

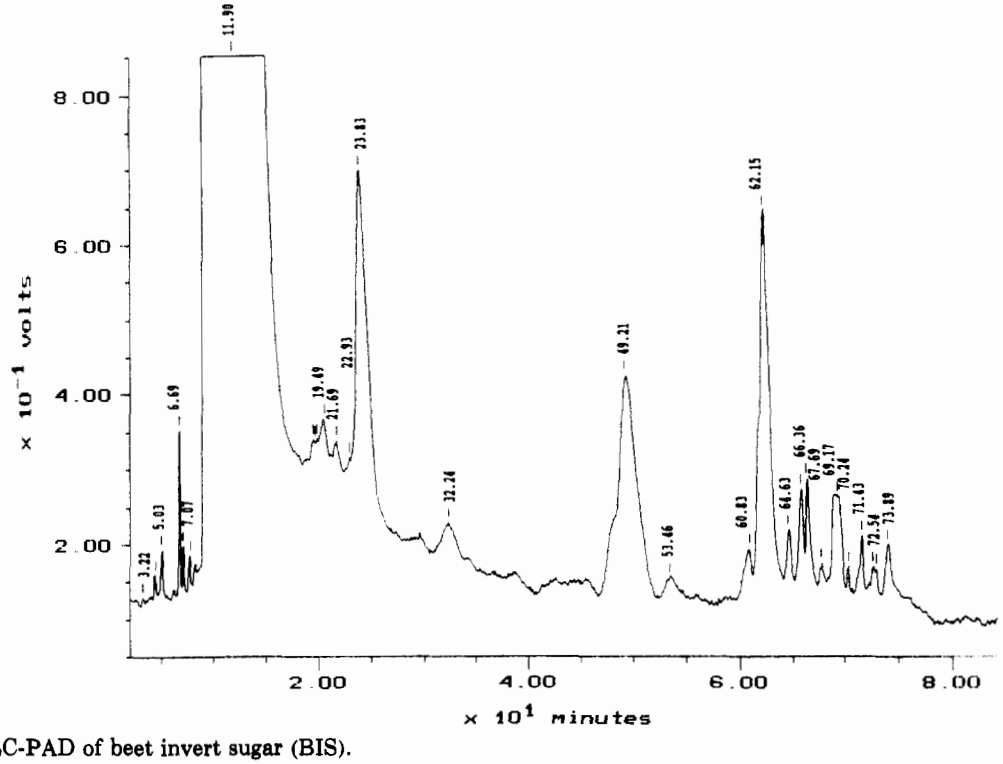

Figure 2. HPLC-PAD of beet invert sugar (BIS).

trisaccharides were formed during the acid-catalyzed hydrolysis of beet sucrose to BMIS. The oligosaccharides present in commercial beet medium invert sugar have recently been isolated and identified in our laboratory, and this information will soon be published.

Although raffinose is a natural constituent of beet sucrose, the absence of this compound in Figure 1 can be explained by the fact that the presence of this compound in "pure" beet sucrose is very low (Tsang and Clarke, 1991). In our laboratory we have noted that raffinose will coelute (under our HPLC conditions) with sucrose when the ratios of sucrose/raffinose are more than 100:1, which is the case for commercial beet sucrose (unpublished results). The raffinose concentration in beet sucrose could be seen when this material was analyzed by capillary gas chromatography (unpublished results).

A number of other inexpensive sweeteners are also commercially available and could be used as fruit juice adulterants. These include the extended acid hydrolysis of beet sucrose to produce beet invert sugar (BIS). This

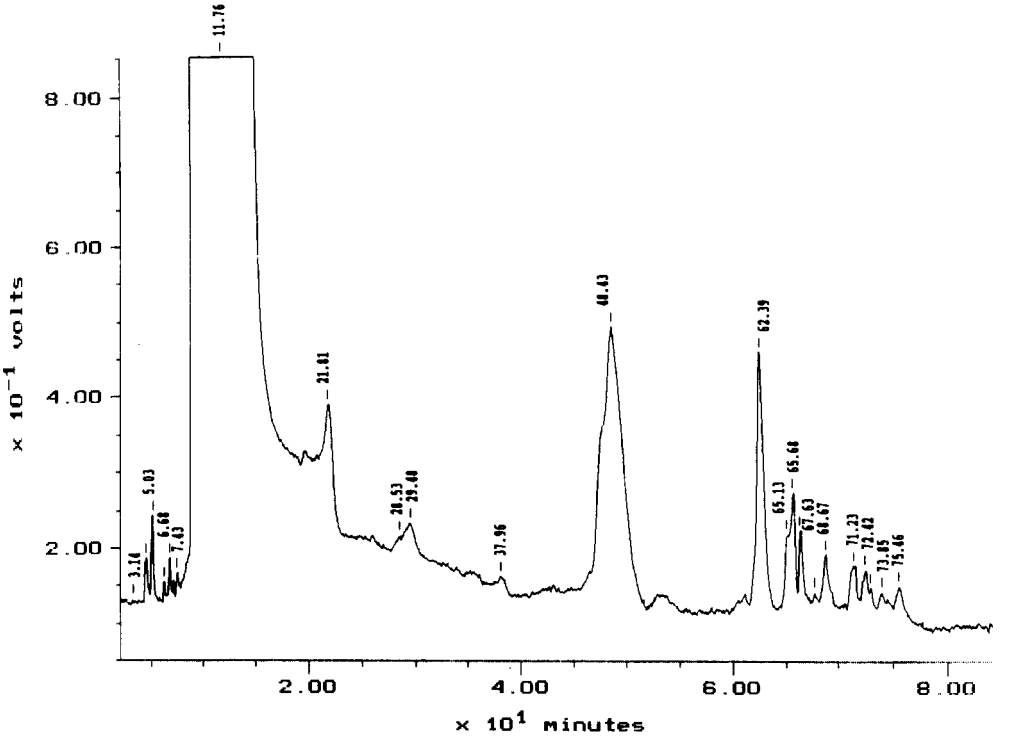

Figure 3. HPLC-PAD of high-fructose corn syrup (HFCS).

Table I. Chemical Composition Data for the 30 Authentic Grapefruit Juice Samples

	mean	range
°Brix	9.8	8.9–10.7
pH	3.3	3.2–3.6
% TTA	1.18	0.96–1.45
°Brix/acid	8.4	7.3–9.4
glucose, g/100 mL	2.30	1.75–2.76
fructose, g/100 mL	2.28	1.87–2.60
sucrose, g/100 mL	2.44	1.88–3.12

hydrolysis also results in the formation of distinct oligosaccharides (Figure 2). The retention times of some of these carbohydrates are significantly different from those which are formed during BMIS production. These BIS oligosaccharides can be used to detect the addition of this material to pure citrus juice. Oligosaccharides are also formed during the enzymatic production of high fructose corn syrup (HFCS). Trace levels of maltose (retention time of ~48 min), maltotriose (~62 min), maltotetraose (~65.5 min), and other dextrose polymers are present in commercial HFCS (Figure 3). These "naturally" occurring oligosaccharides may also be used as fingerprints for the addition of this inexpensive sweetener to fruit juices.

Naturally occurring oligosaccharides present in citrus

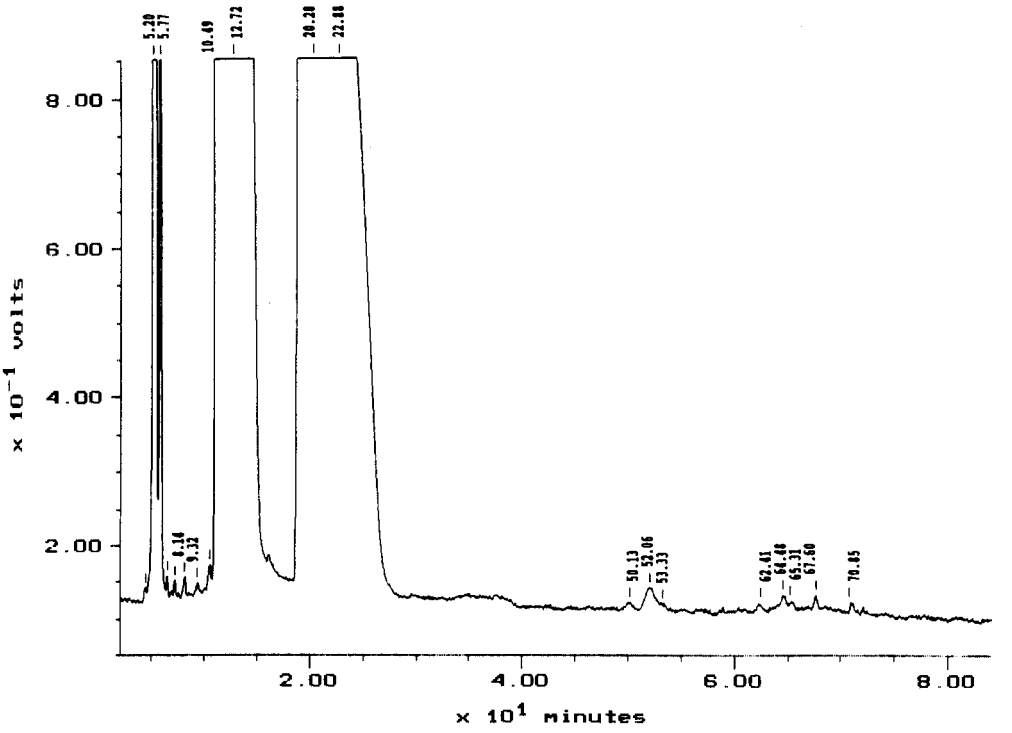

Figure 4. HPLC-PAD of pure single-strength grapefruit juice from concentrate.

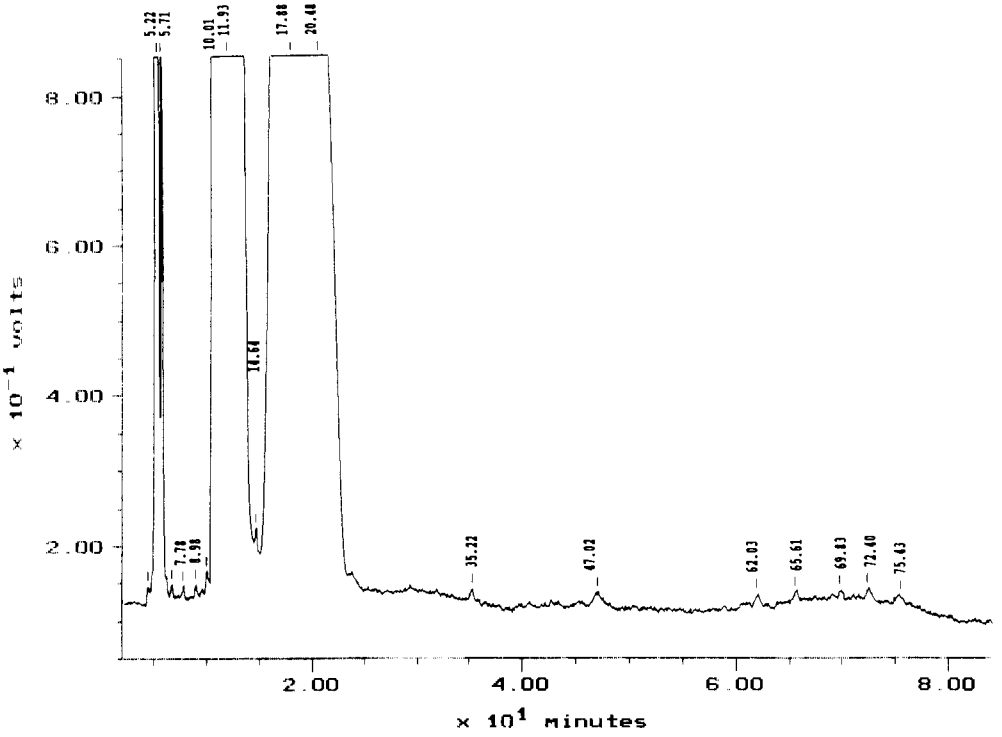

Figure 5. HPLC-PAD of pure fresh-squeezed/pasteurized grapefruit juice.

Table II. Chemical Composition Data for Selected Pure and Adulterated Samples

sample	adulterant	°Brix	pH	% TTA	°Brix/acid	glucose	fructose	sucrose
1	pure	10.5	3.45	1.22	8.6	2.34	2.43	2.34
2	5% BMIS	10.4	3.44	1.18	8.9	1.78	1.79	2.34
3	10% BMIS	10.2	3.43	1.09	9.4	1.88	1.88	2.34
4	20% BMIS	10.0	3.43	0.97	10.3	1.99	1.98	2.74
5	5% BIS	10.3	3.45	1.17	8.8	1.81	1.80	2.33
6	10% BIS	10.1	3.43	1.10	9.2	1.89	1.89	2.41
7	20% BIS	10.0	3.44	1.04	9.6	2.01	2.01	2.64
8	5% HFCS	9.8	3.45	1.08	9.1	1.81	1.87	2.14
9	10% HFCS	8.9	3.33	0.97	9.2	1.84	1.94	2.01
10	20% HFCS	8.3	3.45	0.86	9.7	1.88	1.94	1.89

juices are mainly due to the transferase activity of plant hydrolase enzymes or the transferase enzyme uridine diphosphate D-fructose (which acts as a fructosyl donor) (Whiting, 1970). It has been reported that reversion products, due to citric acid catalyzed hydrolysis of sucrose within the plant, many also be present (Sinclair, 1984). Oligosaccharides may also be formed during grapefruit juice processing (specifically aseptic concentrate production or concentrate pasteurization) by the citric acid catalyzed reaction of sucrose, glucose, and fructose in the concentrate. However, the 30 commercial grapefruit juice samples analyzed in this study all showed very low levels of naturally occurring oligosaccharides.

The monosaccharide profiles of the 30 grapefruit samples analyzed in this study showed an average glucose/fructose/sucrose ratio of 1:1:1.1 (Table I). These values differ from the 1:1:2 ratio reported by Curl and Veldhuis (1948) and Roberts and Gassum (1934), but they were consistent with those reported by Lifshitz et al. (1974). Experimental results (Table I) on pH, total titratable acidity (as citric acid), and the °Brix/acid ratio were well within published literature values for single-strength grapefruit juice (concentrates were diluted to single strength prior to analysis) (Dillon, 1991).

Each of the grapefruit juice samples analyzed had low levels of naturally occurring oligosaccharides. Representative samples are shown in Figure 4 (concentrate) and Figure 5 (fresh-squeezed/pasteurized). The large offscale peaks in these chromatograms occurring in the 10.5–27-min range were glucose, fructose, and sucrose. A number of these pure samples were intentionally adulterated with low levels (5, 10, and 20%) of each of the inexpensive sweeteners previously mentioned. The adulterated samples were each analyzed for °Brix, pH, % TTA, °Brix/acid ratio, oligosaccharide, glucose, fructose, and sucrose. Table II lists the results from the chemical analyses of each of the adulterated samples. These results show that it was impossible to distinguish adulterated and authentic samples by measuring only these parameters. Organic acids, metals, and flavanoids are also routinely measured to determine authenticity. However, each of these chemical constituents has a wide natural range; therefore, the addition of low levels of inexpensive sweeteners could not be readily detected by monitoring these constituents. Sophisticated producers of fradulent juices could easily add metals, salts, and pure citric acid to avoid detection (Brause, 1992).

Oligosaccharide profiles of the intentionally adulterated samples (Figures 6–8) show fingerprint patterns for each of the inexpensive sweeteners. These chromatograms are vastly different from those obtained for the pure juices. The pronounced fingerprint peaks which elute in the region of 48–71 min clearly indicate whether a juice has been adulterated. Results from these analyses indicate that adulteration levels of 10% are easily detected and that 5% detection levels are possible.

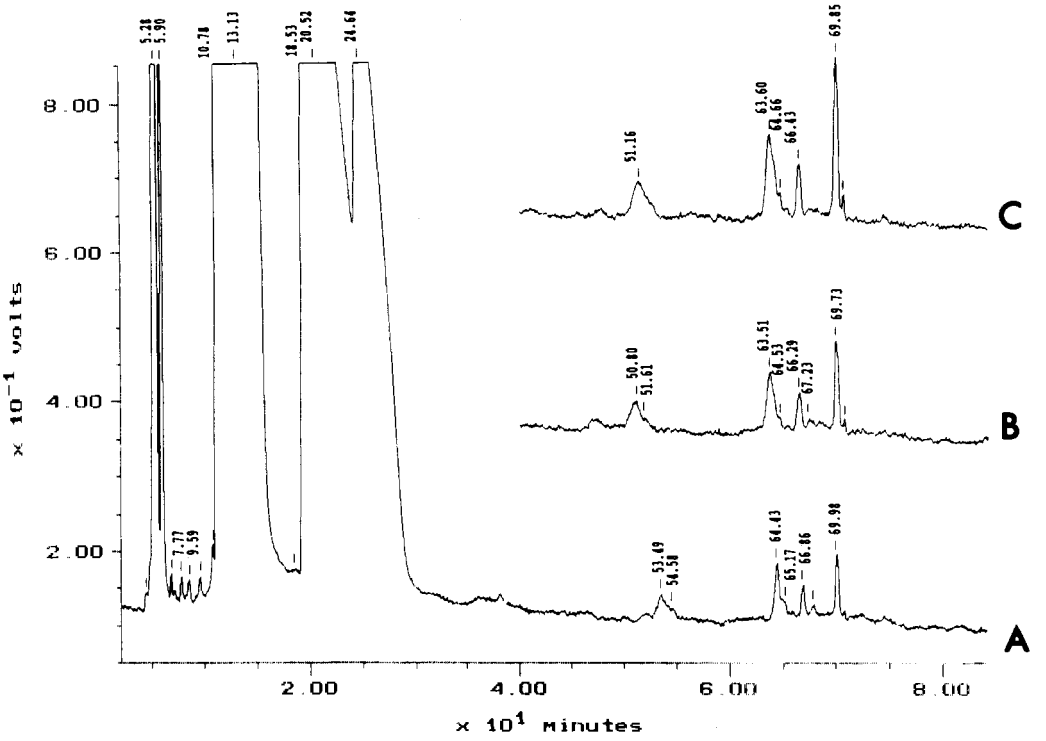

Figure 6. HPLC-PAD of pure grapefruit juice intentionally adulterated with BMIS at levels of (A) 5, (B) 10, and (C) 20%.

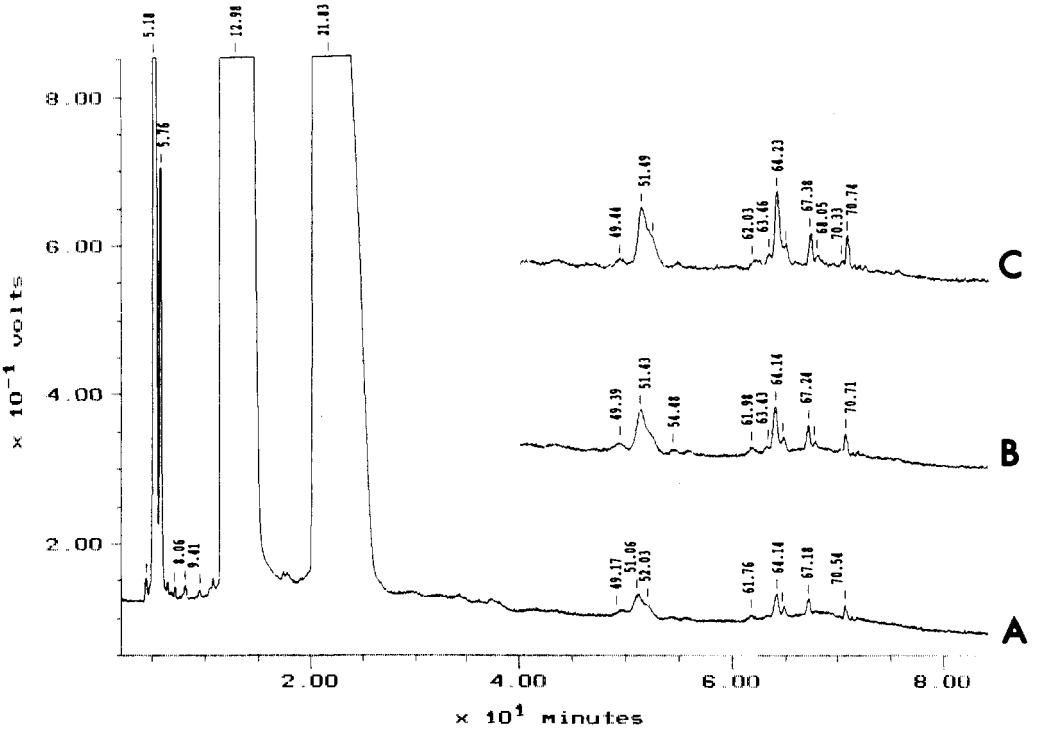

Figure 7. HPLC-PAD of pure grapefruit juice intentionally adulterated with BIS at levels of (A) 5, (B) 10, and (C) 20%.

As the fingerprint carbohydrates present in commercial BMIS and BIS are formed via acid hydrolysis of sucrose, experiments were conducted to examine the effects that processing and storage would have on oligosaccharide formation/hydrolysis. Two aseptically packaged grapefruit juice concentrates were used in this study; one was stored at 22–24 °C (room temperature) for a period of 3 months, the second sample was intentionally adulterated with 15% BMIS and stored at the same temperature. Samples were removed (aseptically) at time 0, 1, 2, and 3 months. Experimental results indicated that there was no formation or hydrolysis of these fingerprint oligosaccharides during this study.

Analytical data for intentionally adulterated grapefruit juice samples are presented in Table II. As can be seen from these data, all of the adulterated samples have values well within the ranges found for the pure juices. Samples which were adulterated with HFCS have lower °Brix and sucrose values due to the fact that HFCS contains no sucrose. Adulteration with HFCS may be supplemented with the addition of pure beet sucrose. This simultaneous addition would result in a higher profit margin while maintaining °Brix and sucrose content, and the adulterated sample would also appear to be authentic by SNIF-NMR. For example, the mean SNIF-NMR value for pure single-strength citrus juice is 103.5 ppm (based on D/H

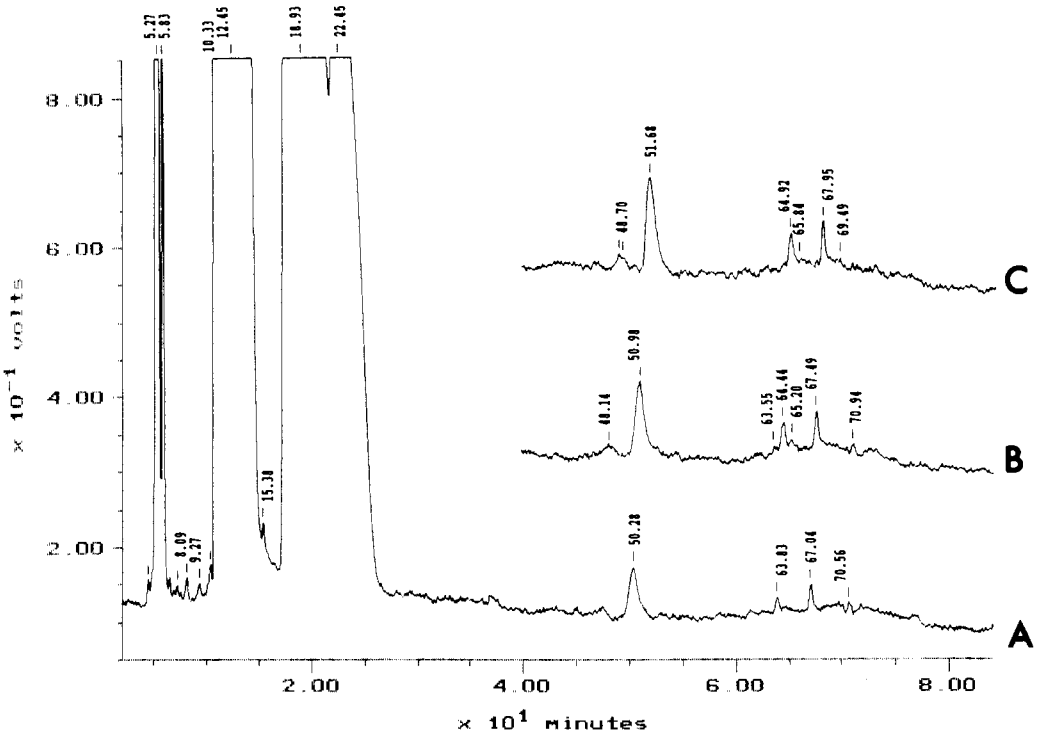

Figure 8. HPLC-PAD of pure grapefruit juice intentionally adulterated with HFCS at levels of (A) 5, (B) 10, and (C) 20%.

NMR determinations), for beet it is 90 ppm, and for corn/cane it is 112 ppm (Martin, 1990). The addition of 10% HFCS and 5% beet sucrose (resulting in a glucose/fructose/sucrose ratio of 1:1:1 for the adulterants) to a typical pure grapefruit juice would give a final SNIF-NMR value of approximately 103.2 ppm [0.85(103.5) + 0.10(112) + 0.05(90)]. Therefore, oligosaccharide data on grapefruit juice samples could be an integral part of an analytical matrix to determine authenticity.

One of the major problems with the work done by Swallow et al. (1991) was the long analysis time (178-min total run time including column equilibration) and cumbersome sample preparation. Experiments were conducted to reduce the sample analysis time while maintaining oligosaccharide resolution. Adjustments to the gradient elution resulted in a more rapid analysis time (115 min including column equilibration) with no significant loss in oligosaccharide separation. During this research we also discovered that the initial passage of the sample through charcoal/Celite was unnecessary. These two refinements of the methodology make it more conducive to routine sample analysis.

CONCLUSIONS

Sophisticated adulteration of grapefruit juice can easily go undetected by conventional analytical techniques. Analysis of the oligosaccharide content of grapefruit juice by high-performance liquid chromatography with pulsed amperometric detection is a useful tool for detecting the addition of a wide range of commercially available inexpensive sweeteners. The methodology previously developed has been modified to result in more facile and rapid sample preparation and analysis.

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