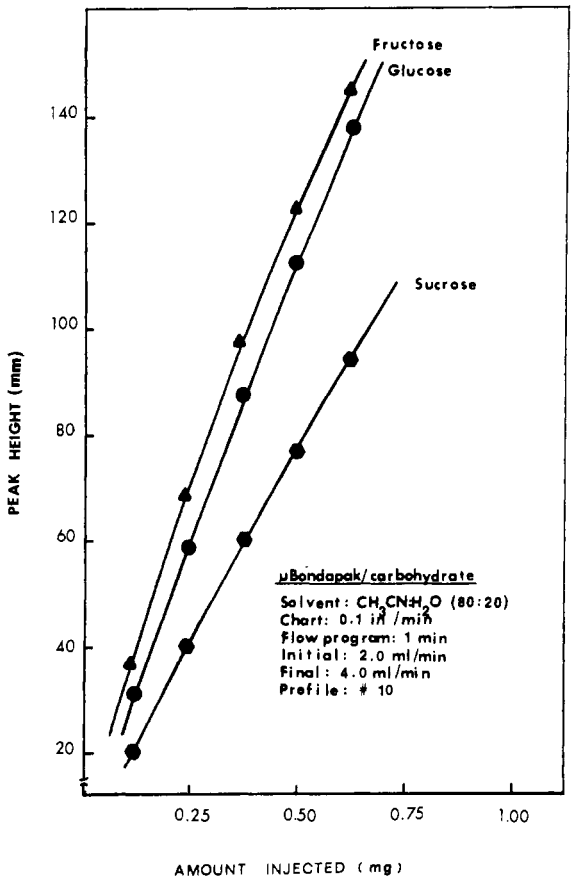

Figure 3. Quadratic models for the calibration of sugars chromatographed on μBondapak/carbohydrate column with flow programming.

resolution. Hence, adequate resolution and complete analysis were obtained in an acceptable time of 11.5 min with the aid of the flow programmer.

Calibration. Peak heights for each sugar were measured for different known injection volumes of the standard mixture. The results obtained were analyzed by least-squares analysis to determine the best model relating peak heights as the dependent variable and milligrams of sugar injected as the independent variable. Quadratic relationships were found between the two variables for each sugar in the mixture:

$$\text{fructose} = 294.006X - 103.076X^2 + 1.731$$

$$\text{glucose} = 243.792X - 39.603X^2 + 1.269$$

$$\text{sucrose} = 176.879X - 39.893X^2 - 0761$$

where X = detector response. A graphic representation of the quadratic model is given in Figure 3.

Analysis of Cane Juice. Diluted cane juice samples were analyzed using the flow-programmed scheme. Elution times and separation of fructose, glucose, and sucrose was identical with that obtained with standards.

CONCLUSION

This high-pressure liquid chromatographic system offers a simple and rapid method for the separation and determination of sugars. Good resolution and reproducibility of results are obtained while achieving the separation in less than 12 min. The main advantage provided by this system is that the true sucrose content is measured in contrast to the apparent sucrose as determined by polarimetric techniques.

One serious consideration about the μBondapak system is that the presence of slower eluting compounds, e.g., raffinose and higher molecular weight compounds such as the dextrans in cane juices, makes it difficult to know when the last peak in a sample is eluted. Also, if left for some time on the shelf, the column requires frequent recalibration when put back into use.

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Analysis of Sugar Cane Saccharides by Liquid Chromatography. 2. Ion-Exchange Resins

J. Wong-Chong* and F. A. Martin

The separation of sugar cane saccharides using Aminex A5 (13 ± 2 μm) column of 7 mm i.d. × 25.4 cm, Aminex Q15S (22 ± 3 μm) column of 7 mm i.d. × 61 cm, and Aminex Q150S (28 ± 7 μm) column of 7 mm i.d. × 61 cm is described. Resolution of sucrose, glucose, and fructose in cane juice samples can be completed in less than 8 min. Products of juice deterioration can also be analyzed on Aminex Q150S, which has been converted into the potassium form. An isocratic elution mode is utilized with water as the only solvent and detection of the sugars with a differential refractometer gives good reproducibility of results.

Some of the earliest reports of separation of carbohydrates by ion-exchange resins were by Khym and Zill (1951, 1952) who separated mixtures of mono- and oligosaccharides on columns packed with Dowex-1 (borate form). The solvent delivery systems employed were by gravity feed. This resulted in a long, tedious process, often requiring more than 60 h, and resolution was incomplete in many cases which rendered the technique inapplicable to most routine carbohydrate analyses. However, as interest in pressurized chromatography intensified, sepa-

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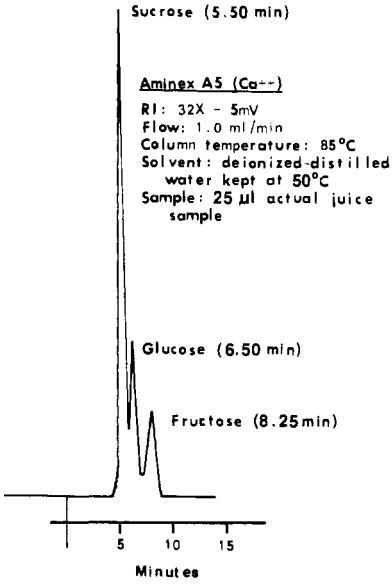

Figure 1. Chromatographic separation of an actual cane juice sample on Aminex A5 (Ca^{2+}).

ration times decreased to hours (Walker and Saunders, 1970; Brobst et al., 1973) and eventually to minutes (Palmer and Brandes, 1974; Scobell et al., 1977).

The work reported herein was initiated to determine the applicability of these recent developments to the analysis of sugar cane saccharides by liquid chromatography.

MATERIALS AND METHODS

A Waters Associates ALC/GPC 244 liquid chromatography instrument was used in this study. Aminex A5, Aminex Q15S, and Aminex Q150S were obtained from Bio-Rad Laboratories. The procedure described by Scobell et al. (1977) for resin preparation and column packing was used. A column water jacket (Wong-Chong, 1978) was constructed to elevate column temperatures.

Preparation of Sugar Cane Juice Samples. Celite was added to the juice sample and centrifuged at 10000*g* for 10 min. The clear supernatant was taken to make a 1:10 v/v dilution. A 10-mL aliquot of the diluted sample was deionized with 0.2 g of Amberlite MB1. The solution was gently shaken for 10 min in a swirling motion and then filtered through a 0.22-μm millipore filter.

Solvent. Degassed, deionized–distilled water held at 50 °C to avoid retake of oxygen was the only solvent used.

RESULTS AND DISCUSSION

A. Analysis of Fresh Juice. On the basis of results of some recent studies (Palmer and Brandes, 1974; Scobell et al., 1977) a series of column systems for high-pressure liquid chromatography was tested for sugar cane juice analysis.

1. Separation of Fresh Juice on Aminex A5 (Calcium Form). Aminex A5 resin converted to the calcium form was used in a 7 mm i.d. × 25.4 cm column held at a constant temperature of 85 °C.

The elution times of a standard sugar mixture containing 20 mg/mL of sucrose, 5 mg/mL of glucose, and 5 mg/mL of fructose, using degassed, deionized–distilled water as the solvent with a flow rate of 1.0 mL/min was 5.5, 6.5, and 8.25 min, respectively. Using samples of fresh cane juice complete fractionation was achieved in 9 min with good resolution obtained among the three sugars concerned (Figure 1). The best resolution was achieved even between glucose and fructose. These results are comparable to those obtained by Scobell et al. (1977) working with a high-fructose corn syrup using a flow rate of 1.2 mL/min.

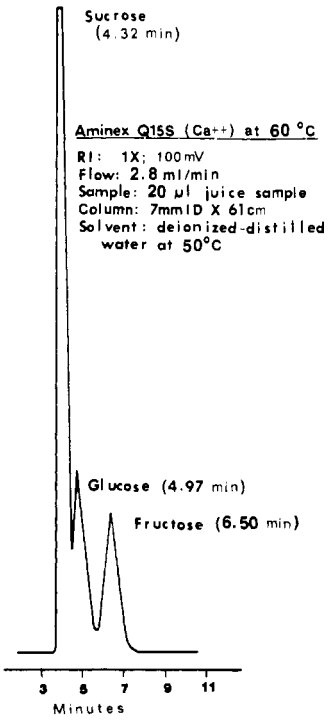

Figure 2. Separation of juice sample on Aminex Q15S (Ca^{2+}).

Increasing the flow rate to 2.0 mL/min lowered the elution times, at the expense of a loss of resolution between sucrose and glucose.

2. Separation on Aminex Q15S (Ca^{2+}). Aminex Q15S was converted to the calcium form and used in a 7 mm i.d. × 61 cm column heated to a constant temperature of 60 °C. Using deionized–distilled water at a flow rate of 2.8 mL/min, sucrose, glucose, and fructose eluted at 4.32, 4.97, and 6.50 min, respectively. The separation of these sugars from a fresh cane juice sample is demonstrated in Figure 2.

Scobell et al. (1977) achieved a separation of high-fructose corn syrup on an Aminex Q15S (Ca^{2+}) column at 85 °C in less than 25 min using a solvent flowing at 0.6 mL/min. We were able to complete one separation in less than 8 min, and with overlap injections it was even possible to carry out the analysis of one sample per 5 min in this all-aqueous column system. Analysis of the chromatogram obtained shows that the separation of sucrose, glucose, and fructose is quite feasible with the Aminex Q15S resin under those conditions mentioned above.

3. Separation on Aminex Q150S (K^+). Maximum speed of separation with maximum resolution and accuracy was attained with yet another column system. Aminex Q150S, a cation-exchange resin converted to the potassium form was the packing material of choice. Again a 7 mm i.d. × 61 cm column kept at a constant temperature of 60 °C was utilized.

Excellent separation of sucrose, glucose, and fructose was obtained with deionized–distilled water at a flow rate of 2.5 mL/min. The elution times obtained for a standard mixture containing sucrose, glucose, and fructose were 5.40, 6.89, and 7.44 min, respectively. A typical chromatogram of a fresh cane juice separated on this column system (Figure 3) shows the high degree of resolution achieved.

The relative retention times for the sugars studied were identical with those reported by Palmer and Brandes (1974) on this type of resin for the analysis of different food extracts. Temperatures higher than 60 °C would only slightly improve the resolution, at the risk of accelerating resin breakdown (Palmer and Brandes, 1974). A flow rate

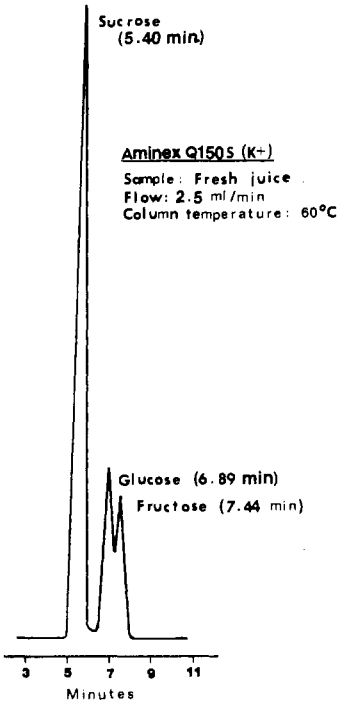

Figure 3. Separation of an actural fresh juice sample on Aminex Q150S (K^+).

Table I. Relative Retention Times of Deterioration Products, Salts, and Sugars Chromotographed on Aminex Q150S

inorganic salts	0.53
dextran	0.53
lactic acid	0.57
acetic acid	0.62
sucrose	0.78
mannitol	0.93
glucose	1.00
fructose	1.08
ethanol	1.15

of 2.5 mL/min appeared to be optimal, yielding separations in about 8.50 min, and with overlap injections samples could be run in less than 6.00 min.

B. Analysis of Deteriorated Cane. Although insignificant amounts of polysaccharides are normally in fresh cane juice, conditions that result in increased polysaccharide content may prevail. The presence of dextran in deteriorated sugar cane juice is of practical importance because it presents serious problems in juice clarification and the crystallization process of the boiling-house (Kamoda et al., 1968; Wong-Chong, 1978). The ability to identify deterioration products on the same column used for mono- and oligosaccharides would be of an immense benefit for analyzing deteriorated cane.

To deal with the problem of separating deterioration products from mono- and disaccharides the Aminex Q150S column system was also evaluated. Excellent separation of dextran, sucrose, glucose, and fructose was obtained at a flow rate of 2.5 mL/min (Figure 4). Results identical with those of the standard mixture were obtained with actual deteriorated juice samples (Wong-Chong, 1978). It should also be pointed out that the separation of sucrose, glucose, and fructose in deteriorated juice samples was as good as the separations obtained in fresh juice samples.

Known products of sugar cane deterioration were run on the Aminex Q150S column. Of these known deterioration products lactic acid and dextran were found to emerge together at 3.58 min. The relative retention times obtained for a mixture containing sugars, inorganic salts,

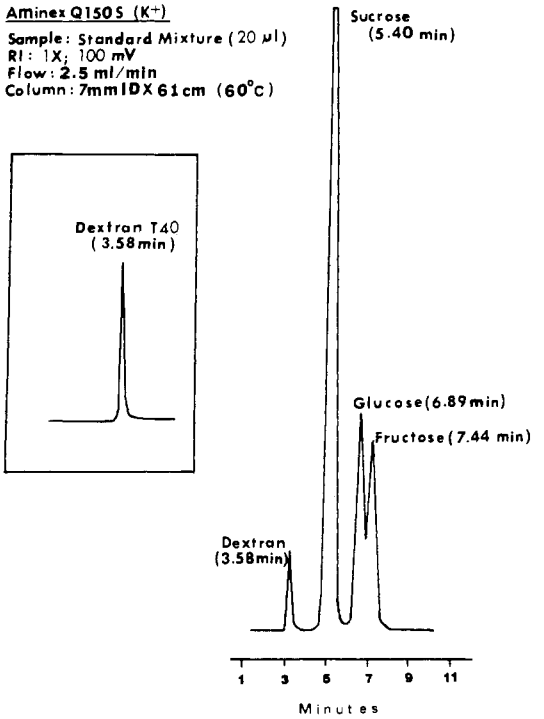

Figure 4. Separation of standard mixture containing 16 mg/mL of sucrose, 4 mg/mL of glucose, 4 mg/mL of fructose, and 0.8 mg/mL of dextran T40 on Aminex Q150S (K^+).

Table II. Best Fit Models Relating Detector Response and Concentration of the Saccharide for Each of the Aminex Resins Tested

	resin		
saccharide (range)	Aminex A5 (Ca^{2+})	Aminex Q15S (Ca^{2+})	Aminex Q150S (K^+)
dextran (0.2-1.0 mg/mL)			linear
sucrose (4-20 mg/mL)	linear	quadratic	quadratic
glucose (1-5 mg/mL)	linear	quadratic	linear
fructose (1-5 mg/mL)	linear	quadratic	linear

and deterioration products are given in Table I. Although the peak emerging at 3.58 min does not represent dextran alone, nevertheless, these salts and products of deterioration are well separated from sucrose, glucose, and fructose.

The separation of dextran from simple sugars was also attempted on the Aminex Q15S column. The dextran peak came out at 3.35 min but was followed by a second peak at 3.86 min (Wong-Chong, 1978). This second peak associated with dextran would overlap the front of the sucrose peak. Due to the overlapping of the smaller dextran peak with that of sucrose, Aminex Q15S is not suited for the analysis of deteriorated juices containing high-molecular-weight polysaccharides.

Quantitative Aspects. For each resin studied, least-squares analysis was used to determine the model that best describes the relationship between detector response and saccharide content (Wong-Chong, 1978). A summary of these relationships is found in Table II. With the Aminex A5 (Ca^{2+}) column a linear relationship was found between content and detector response for each sugar studied. The relationships on the Aminex Q15S (Ca^{2+}) column on the other hand were quadratic. With the Aminex Q150S (K^+) column the relationships were

linear for dextran, glucose, and fructose and quadratic for sucrose.

CONCLUSION

Of the three types of resin tested, the application of Aminex A5 proved to be reliable for the separation of sucrose, glucose, and fructose since reproducible results could be obtained and good linearity response could be achieved as measured by peak heights. Aminex Q15S showed great potential as the method for analyzing juice samples free of dextran as one complete separation can be achieved in 5 min with overlap injections in this all-aqueous column system.

Best resolution between sucrose and reducing sugars was achieved with Aminex Q150S (K^+ form). Also Aminex Q150S is superior for the rapid fractionation of juice samples that have been subjected to a significant degree of microbial attack. We have found that a column packed with this resin can last as long as 4 months without the need of any regeneration.

Samples need only to be clarified by centrifugation and deionized before injection. Samples containing as much as 15% mud have been clarified and analyzed successfully by this procedure without any adverse effects on the column packing material.

The advantage of this system based on Aminex resins over adsorption chromatography is that since the products of deterioration elute first, it is possible to know exactly when the run is over regardless of whether these deterioration products are present or not in the sample. In contrast, adsorption chromatography does not permit this prediction since the deterioration products would be held indefinitely on the column system (Wong-Chong and Martin, 1978).

We have found this all-aqueous system to be well suited for the rapid and accurate analysis of juice samples in research work. The method shows great potential for routine laboratory analysis in the sugar cane industry.

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Determination of Chloramine-T in Foodstuffs

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A gas chromatographic method for the quantitative determination of chloramine-T (C-T) in milk, ice cream, whole egg, mechanically deboned poultry meat, and croquettes has been developed. After hydrolysis of the C-T and precipitation and filtration of proteins and lipids, the hydrolysis products, *p*-toluenesulfonamide (*p*-TS), is extracted with ethyl acetate. Dried and concentrated extract is subjected to gas–liquid chromatography (column: 10% OV-7 on 100–120 mesh Gas-Chrom Q), using a flame ionization detector. This method is capable of detecting 1 ppm of C-T and the average recovery is 80%.

Chloramine-T (C-T) [(*N*-chloro-*p*-toluenesulfonamide) sodium] is a widely used disinfectant. For long it was generally believed to be nonpoisonous, although several cases of death and serious poisoning due to mistaking C-T tablets for headache tablets have been reported (Serin, 1949).

Provided C-T is used as a disinfectant for plant sterilization in food industry in the prescribed concentration (3 g/L), and the cleaned objects are well rinsed, less than 1 ppm of C-T will be found in foodstuffs (Van de Haar and Veenkamp, 1977). However, there are indications that C-T is added to various foodstuffs as a preservative in order to suppress bacterial growth (Brouwer, 1956; Van Gils, 1970). The potential toxic property of C-T led to a preferred level of 1 ppm of C-T in foodstuffs (Van Gils et al., 1975).

Although there are already a number of reports describing a variety of detection procedures for C-T in various food products, they are not specific enough (Van Gils, 1970; Van Gils and Hidskes, 1972), time consuming (Brouwer, 1956), or not sensitive enough (Rondags and Beljaars, 1978) or applicable only for one food product (Van Gils and Hidskes, 1972; Van Gils et al., 1975; Rondags and Beljaars, 1978).

Stavric et al. (1974) reported a GLC/FID method for the ortho isomer of toluenesulfonamide (detection limit 0.05 ppm). Using the same procedure these authors also obtained separation of *p*-TS. This paper describes a method for isolation and determination of C-T as *p*-TS in mechanically deboned poultry meat (MDPM), ice cream, milk, whole egg, and croquette.

EXPERIMENTAL SECTION

Reagents. Carrez I reagent: 10.6 g of potassium ferrocyanide trihydrate (Baker analyzed reagent) in 100 mL of distilled water.

Carrez II reagent: 23.8 g of zinc acetate dihydrate (Baker analyzed reagent) and 3 g of anhydrous acetic acid (Baker analyzed reagent) in 100 mL of distilled water.

Carrez II solution: a 2.5% (v/v) dilution of Carrez II reagent with distilled water. 35% (w/v) basic lead acetate (Baker analyzed reagent) in distilled water; 2% (w/v) sodium sulfite heptahydrate (p.a., Merck) in distilled

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