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Note

Rapid determination of sugars in cantaloupe melon juice by high-performance liquid chromatography

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Recent developments promise to make high-performance liquid chromatography (HPLC) a convenient method for analysis of sugars in crude extracts of plant tissues. One such development is the use of silica columns with an aqueous acetonitrile mobile phase to which an amine modifier has been added[1]. This technique avoids the use of chemically bonded amine columns, which are expensive and deteriorate with use, especially when crude extracts containing non-sugars are analysed[1,2]. The use of tetraethylenepentamine (TEPA) as an amine modifier was proposed by Wheals and White after an extensive survey[2], and a recent study on the use of TEPA with radially compressed silica shows that high resolution and good column life can be readily obtained with this amine[3].

Another development is the use of water as a mobile phase for sugar separations, in conjunction with cation-exchange[4] or reversed-phase C_{18} columns[5]. The risk of components in a crude aqueous sample matrix precipitating upon introduction to the mobile phase is avoided where water is the eluent.

In the course of a study on the accumulation of sugars in the developing cantaloupe melon fruit (*Cucumis melo* L. var *reticulatus* Naud.), we have used two HPLC methods for sugar determination. The first method uses a proprietary stationary phase with water as eluent, and the second method uses radially compressed silica as the stationary phase, and an aqueous acetonitrile mobile phase containing the amine modifier TEPA. This paper describes these methods, which require minimal sample preparation, and permit rapid analysis of each sugar constituent.

EXPERIMENTAL

Sample preparation

Cantaloupe melons were harvested at several stages of maturity and juice recovered from the edible flesh with a domestic juice extractor. Sodium azide (0.1 %, w/v) was added as a preservative, and suspended solids were removed by centrifu-

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gation (2000 *g*, 5 min). The supernatant was filtered prior to analysis by drawing it through a 2-μm porosity filter (Supelco, Bellefonte, PA, U.S.A.), fitted onto the polypropylene tip of an automatic pipettor. In one experiment, juice was extracted in the presence of boiling 80% (v/v) ethanol, followed by centrifugation and removal of ethanol at 40°C *in vacuo*.

Reagents

Water for elution was distilled, and then passed through a Millipore Milli-Q purifier (Bedford, MA, U.S.A.). Acetonitrile and methanol were of HPLC grade (Waters Assoc., Milford, MA, U.S.A.). TEPA (technical grade) was supplied by Waters Assoc., Chippendale, Australia. Eluents were degassed before use by vacuum filtration through a 0.5-μm membrane filter. Glucose, fructose, sucrose, ethanol, and sodium azide were of analytical reagent grade.

Apparatus

A Waters liquid chromatograph was used, which consisted of an M45 pump, U6K injector, RCM-100 radial compression module, and R401 differential refractometer connected to a 10-mV potentiometric recorder. The columns were Waters Radial-Pak cartridges of Dextropak and silica (both 100 × 8 mm I.D.).

Operating conditions

The Dextropak column was washed with 30 ml of methanol before each use, and then equilibrated with water. This took about 45 min at a flow-rate of 2 ml/min.

The silica column was initially pretreated with 500 ml of a solution of 75% (v/v) aqueous acetonitrile containing 0.1% TEPA. A mobile phase of 75% (v/v) aqueous acetonitrile containing 0.01% (v/v) TEPA was prepared, and 250 ml of this solution was pumped through the column to waste before each use. The effluent was then placed in the eluent supply reservoir, which was stirred continuously, and this solvent was recirculated overnight at 2 ml/min. Recirculation was maintained during the subsequent analysis. This procedure improved resolution and baseline stability, and conserved solvent.

RESULTS AND DISCUSSION

The separation of melon juice sugars on the Dextropak column with water as eluent is shown in Fig. 1. Salts and acids eluted first as a single peak (a). Glucose and fructose eluted next as a single peak (b), with a retention time of 1.5 min, followed by sucrose (c), with a retention time of 1.9 min. Identification of sugars was based on their retention times relative to standards. Juice from immature melons contained mostly monosaccharides (Fig. 1A), whereas sucrose was the major component in juice from mature melons (Fig. 1B).

When another sample of juice from a mature melon was fractionated on the silica column with aqueous acetonitrile containing TEPA as eluent, fructose (b), glucose (c), and sucrose (d) were resolved with retention times of 2.2, 2.7 and 4.4 min, respectively (Fig. 2). Two unknown peaks (e, f) were found in this separation. Baseline stability using 0.01% (v/v) TEPA was excellent (Fig. 2). Hendrix *et al.*[3] concluded, however, that the baseline could be improved by increasing the concentration of TEPA to 0.02% (v/v).

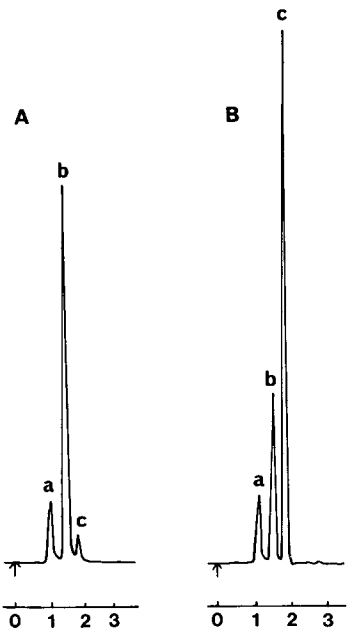

Fig. 1. Separation of juice extracts from (A) immature cantaloupes and (B) mature cantaloupes. Salts and acids (a), total monosaccharides (b) and sucrose (c). Column: Dextropak; eluent: water. Sample size, 10 μl. Flow-rate, 2 ml/min. Detector attenuation, $\times$ 32.

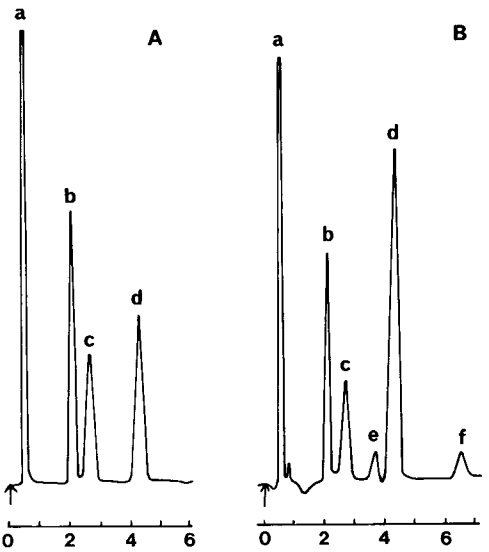

Fig. 2. Separation of (A) a standard solution containing fructose, glucose, and sucrose (each at 2%, w/v) and (B) a juice extract from a mature cantaloupe. Solvent peak (a), fructose (b), glucose (c), and sucrose (d). Peaks (e) and (f) are unknowns. Column: Radial-Pak silica; eluent: acetonitrile–water (75:25), 0.01% (v/v) TEPA. Sample size, 15 μl. Flow-rate, 3 ml/min. Detector attenuation, $\times$ 16.

Carbohydrates in plant tissue extracts are subject to enzymic degradation[6]. Sucrose in particular is liable to undergo hydrolysis, so the stability of sucrose in melon juice was examined. The recovery of sucrose from freshly prepared juice was 102 ± 1% relative to the recovery from a boiling 80% (v/v) ethanol extract. Since 80% ethanol inactivates sugar-degrading enzymes[6], this result is evidence that sucrose was not being lost during juice preparation.

In a second test, freshly prepared juice was incubated for 24 h with and without added sucrose and added azide. Added sucrose was recovered quantitatively immediately after addition (Table I). After incubation for 24 h, only about 95% of the sucrose originally present could be recovered from the samples without azide. Addition of sodium azide (0.1%, w/v), which is an antimicrobial preservative, prevented this loss of sucrose, and did not interfere with the separation. The analysis of crude melon juice samples is, therefore, valid.

TABLE I

EFFECT OF AZIDE ON STABILITY OF SUCROSE IN JUICE

Juice with or without added azide or sucrose was analysed by the HPLC method of Fig. 1 immediately after expression, or after incubation at 30°C for 24 h.

Additives		*Sucrose recovered (%, w/v)*	
Azide (0.1%, w/v)	*Sucrose (0.5%, w/v)*	*0 h*	*24 h*
−	−	3.6 ± 0.05	3.4 ± 0.03
+	−	3.6 ± 0.06	3.6 ± 0.06
−	+	4.1 ± 0.06	3.9 ± 0.05
+	+	4.1 ± 0.04	4.1 ± 0.05

Using the methods described, we have been able to make several hundred injections onto each column. Frequent cleaning of pre-column filters, and back-flushing of the columns is, however, necessary, and the columns ultimately fail from particulate blockage. Where maximum column life is sought, samples should be freed of high-molecular-weight material by methods such as ethanol precipitation or ultrafiltration, and a guard column should be placed in the system.

A feature of both methods is the speed of analysis, which compares very favourably with other published procedures. The need for minimal sample preparation is a further advantage. The Dextropak column gives the same analytical data as two colorimetric reducing sugar assays performed before and after inversion (*i.e.* total reducing sugars and sucrose). The amine-treated silica column gives additional data which, if obtained by colorimetry or enzyme assay, would require specific assays for both glucose and fructose. Near-baseline resolution of glucose and fructose can be obtained routinely within 3 min. Both columns are robust and relatively economical to use. The use of water or of recirculated aqueous acetonitrile as mobile phases also reduces the cost of each assay.

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