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Note

Determination of sucrose, glucose and fructose in plant tissue by high-performance liquid chromatography

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Increased interest in use of crop biomass for energy has created additional demand for a rapid method to analyse for the common sugars in crop plants, mainly sucrose, glucose and fructose. The method should include capabilities to qualitate and quantify for the sugars and involve a minimum of sample preparation due to large numbers of samples that are often produced from a experiment.

Use of high-performance liquid chromatography (HPLC) is gaining wide acceptance for carbohydrate studies. Development and subsequent use of the silica column[1–7], with various modifications, has greatly increased the ability to detect many of the carbohydrates and their derivatives. Silica columns have also been particularly applicable to resolution of sugars such as the disaccharides. Insufficient resolution of glucose from fructose for quantification is sometimes experienced, however[1]. The cation-exchange resin columns[2,8–13] have further enhanced HPLC analysis of various sugar mediums. Care must be exercised to protect the column[5] with either precolumns or sample pretreatment when large numbers of samples containing contaminants are to be analysed.

This paper describes a rapid procedure to extract, and quantify for sucrose, glucose and fructose in oven-dried ground plant tissue using a cation-exchange resin column (Ca^{2+}) and protective precolumns.

EXPERIMENTAL*

The HPLC equipment used in this study was a Beckman system (Beckman Instruments, Berkeley, CA, U.S.A.) consisting of a Model 421 microprocessor, Model 110A pump, Model 210 sample injector, Model 156 refractive index (RI) detector set at attenuation × 16, and a Scientific Systems (State College, PA, U.S.A.) CH 20-C column heater. Output was recorded on a Hewlett-Packard (HP; Avondale, PA, U.S.A.) 3390 A reporting integrator equipped with an input/output board. An electrical contact fitted on the injector was connected through the microprocessor to

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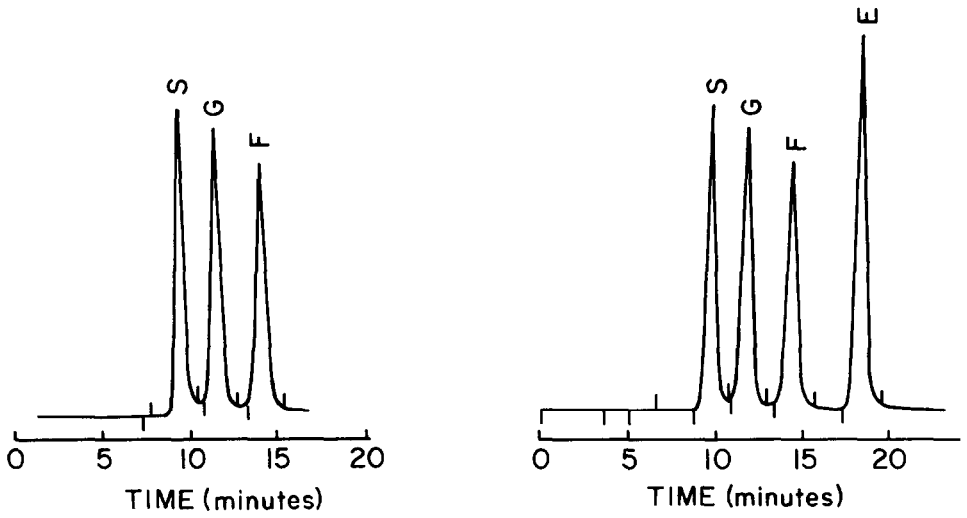

Fig. 1. Example of chromatogram for standard solution of 0.125% sucrose (S), glucose (G), and fructose (F) prepared in water and used for some tissue analyses. Attenuation for RI detector, × 16. Attenuation and threshold for integrator, × 4 and 3, respectively; chart speed, 0.3 cm/min; peak width, 0.16; area reject, 5. Flow-rate, 0.6 ml/min. Eluent, water. Column temperature, 85°C. Sample size, 20 μl. Column, Aminex HPX-87. Precolumns, Aminex HPX-85H, A-25.

Fig. 2. Chromatogram of standard solution of 0.5% sucrose; glucose; fructose and 2% ethanol (E). Attenuation, × 6; threshold, 4 for integrator. See Fig. 1 for other details.

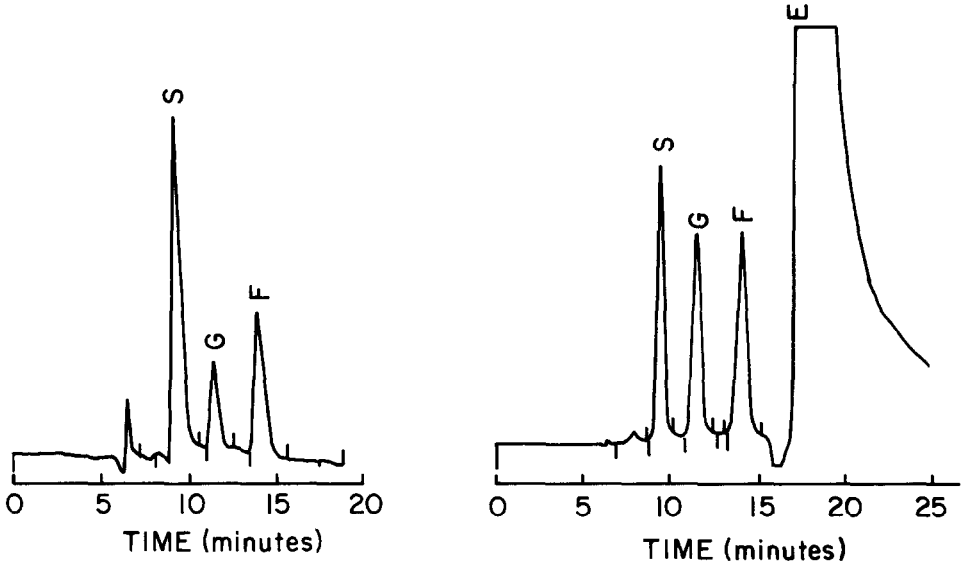

Fig. 3. Typical chromatogram showing quantitative amounts of sucrose, glucose and fructose in low-sugar-type cultivary (37) extracted with warm water (1 g/100 ml). Attenuation, × 2; threshold, 2 for integrator. See Fig. 1 for other details.

Fig. 4. Typical chromatogram for low-sugar-type cultivar (37) with sugars (Table I) added to plant sample before extraction in boiling 95% ethanol (1 g/100 ml). Attenuation, × 3; threshold, 3. See Fig. 1 for other details.

the HP integrator so that the microprocessor and integrator were both started upon injection of the sample. A precolumn arrangement consisted of an in-line series of two precolumn holders (Micro-Guard; Bio-Rad Labs., Richmond, CA, U.S.A.) containing a cation- and anion-exclusion cartridge (Aminex HPX-85H, A-25; Bio-Rad Labs.) in respective sequence and connected to the injector. The precolumns were followed by an HPX-87 carbohydrate column (Bio-Rad Labs., 300 × 7.8 mm I.D.) placed in the column heater. The column heater was maintained at 85°C and degassed water was used as the eluent at a flow-rate of 0.6 ml/min.

Culms from two sorghum cultivars, ATx378 × RTx7000 (31) and ATx623 × R74CS5388 (65), were used which contained relatively low and moderate concentrations of sugar, respectively. A 1-g amount of oven-dried tissue, ground to pass a 1-mm screen, was refluxed for 30 min with boiling 95% ethanol in 50-ml centrifuge tubes with stoppers and fitted capillaries[14]. They were centrifuged after each extraction and the supernatant saved. The process was repeated three times and the collected supernatant was brought to 100-ml volume. Duplicate sets of the plant material were also extracted in deionized, distilled water using the same method with exclusion of the capillaries. The bath for water extraction was set at 60°C and samples extracted for 5 min with periodic stirring. A minor trace of the sugars could only be detected in ethanol extracts and none in water subsequent to four extractions of tissue for the HPLC conditions described. These procedures allow for extraction of a minimum of 32 samples within 4–6-h period.

Three variations of sample preparation were used: (1) sample extracted and pooled extracts brought to 100 ml with extracting solution, (2) sample extracted, measured amount of standard sugar solution (sucrose, glucose, fructose) added to the pooled extractions and resulting solution brought to 100 ml, and (3) prior to extractions, measured amount of standard sugar solution added to oven-dried material and then pooled extracts brought to 100 ml. Subsamples from the extractions

TABLE I

CONCENTRATION OF SUGARS IN CULMS OF TWO SORGHUM CULTIVARS AND PERCENT RECOVERY OF ADDED SUGARS

Refer to legends of Fig. 1, 3 and 4 and Experimental section for conditions. Values are the means ± S.E. of 4 replications and duplicated chromatograms.

Cultivar	*Method of extraction*	*Sucrose*			
		Initial concn. (mg/g)	*Added (mg/ 100 ml)*	*Recovery (%)**	
				Var. 2	*Var. 3*
Low-sugar type	Water	34.06 ± 0.54	12.50	103.02 ± 1.41	101.44 ± 1.11
	Ethanol	29.30 ± 0.26	12.50	99.30 ± 0.89	100.60 ± 2.86
Moderate-sugar type	Water	149.61 ± 0.62	12.50	99.12 ± 0.76	99.47 ± 0.70
	Ethanol	149.08 ± 0.38	12.50	100.40 ± 1.21	101.98 ± 0.09

* Var. 2: standard sugar solution added to extract and brought to volume; Var. 3: standard sugar solution added to oven-dried, ground plant material, extracted and then brought to volume.

were filtered through a 5-μm filter prior to chromatography. A 20-μl injector loop was used to insure precise sample size. All samples were replicated four times and the HPLC runs were duplicated. A silica-based column (Bio-Sil Amino 5S, Bio-Rad Labs.) was initially used to check the plant extractions to ascertain that the sucrose peak was pure and did not contain maltose.

The external standard method (ESTD) of quantification was used for the integrator. Standards for the determinations were prepared from reagent grade sugars. Attenuation and threshold values were varied on the integrator to accommodate the different levels of saccharides. The integrator was programmed so that results were recorded (mg sugar/g oven-dried material) immediately after completing resolution of the sugars for water extracts and upon elution of ethanol for the ethanol extracts.

RESULTS AND DISCUSSION

Values shown in Table I indicate that HPLC can be effectively used to quantify for various mono- and disaccharides in dried, ground plant tissue. The method of extraction is critical because water will remove some of the starch, particularly amylose. Extraction with boiling ethanol has the advantage of separating starch from the sugars of interest. An advantage in use of water is reduction of elution time. Generally, samples extracted in water were eluted and the column equilibrated in approximately 17 min whereas samples extracted in ethanol required approximately 40 min.

A typical chromatogram for the standards is shown in Fig. 1. Standards were rerun and the integrator recalibrated at least twice before analysing and quantifying for the tissue samples. The chromatogram shown in Fig. 2 illustrates this method may also be used to evaluate fermentation broth and potential ethanol production. Values for the extracted oven-dried material without sugar additions and percent

Glucose				*Fructose*			
Initial concn. (mg g)	*Added (mg/ 100 ml)*	*Recovery (%)** *Var. 2*	*Var. 3*	*Initial concn. (mg/g)*	*Added (mg/ 100 ml)*	*Recovery (%)** *Var. 2*	*Var. 3*
10.13 ± 0.54	31.25	101.36 ± 0.26	101.94 ± 4.53	18.83 ± 0.42	31.25	102.14 ± 3.84	98.54 ± 2.45
8.32 ± 0.29	31.25	102.50 ± 5.80	92.00 ± 6.14	15.66 ± 0.55	31.25	98.70 ± 4.64	102.08 ± 3.46
19.07 ± 0.18	31.25	102.99 ± 1.63	101.36 ± 3.73	26.84 ± 0.23	31.25	95.19 ± 1.66	98.16 ± 1.07
17.32 ± 0.31	31.25	103.05 ± 1.90	98.68 ± 2.53	26.26 ± 0.46	31.25	95.40 ± 2.46	94.25 ± 2.73

recovery for both methods of sugar addition are given in Table I. An example of a chromatogram obtained for extraction of the material with water is shown in Fig. 3 and with ethanol in Fig. 4. Sample size for extraction is critical and must be initially determined from experience or by trial. Since 50-ml centrifuge tubes were used for extraction, weights exceeding 1 g of oven-dried material resulted in failure to extract all the sugars from the samples. Weights less than 1 g could have been used for the cultivar with the moderate sugar concentration, but this quantity was necessary to obtain sufficient glucose and fructose concentrations from the low sugar type. An attenuation value of 2 for the integrator with the respective setting of $\times 16$ for the RI detector was near the minimum value that could be used and obtain desirable peaks for quantification.

Minimal sample preparation is required when using this method. In fact, samples may be extracted concurrently while the HPLC analysis is in progress for previous extractions. By use of the cation and anion precolumn cartridges, a range of 300–350 samples were injected before the cartridges required replacement. After injection of several thousand samples, the column appears to be functioning satisfactorily and shows no significant signs of deterioration. This indicates the value of precolumns in preserving longevity of the column and is probably due to removal of contaminants, particularly the salts, that may be present in significant quantities within plant tissue.

This procedure appears to be applicable for accurate analysis of certain types of oven-dried ground plant material that vary over a wide range of sugar concentrations. Since the integrator prints the quantitative values after completion of tracing the chromatogram, results can be obtained immediately. Minimal sample preparation, rapid quantitative analysis and a high degree of repeatability support this HPLC technique as a promising method for analysis of sugars in oven-dried plant tissues.

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