NOTE

Analysis of Saccharides in Low-Dextrose Equivalent Starch Hydrolysates Using High-Performance Liquid Chromatography[1]

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The saccharide profile of corn syrups and maltodextrins is an important characteristic that affects the functional properties of these starch hydrolysates. The analysis of the level and distribution of saccharides has been improved with advances in high-performance liquid chromatography (HPLC). Separation of the saccharides on the basis of chain length (degree of polymerization in which DP 1 is glucose, DP 2 is disaccharides, etc.) can be performed on amine-type columns on which DP 1 is the saccharide that elutes first. Increases in chain length increase the elution time. Alternatively, resin-based columns can be used where the high molecular weight material elutes first and DP 1 (glucose) elutes last (Conrad and Palmer 1976). Using various cation-exchange resins, Scobell et al (1977) described the separation and quantitation procedures for carbohydrate mixtures. Engel and Olinger (1979) demonstrated the value of resin columns for corn-syrup analysis, although quantitation above DP 2 was limited by the types of column materials used. Fitt et al (1980) used various types of resins to examine corn syrups and obtained resolution and quantitation up to DP 5. Later improvements in column materials have allowed greater resolution of the saccharides in the DP 6–11 range. In 1981, Scobell and Brobst prepared silver forms of cation-exchange resins that gave efficient and rapid separation of the saccharides in starch hydrolysates.

Commercially available prepacked resin columns for separation of higher oligosaccharides have recently become available. The objective of this study was to evaluate this type of HPLC column for measuring the saccharides in corn-syrup solids and maltodextrins. The analysis of low-dextrose equivalent (DE) corn-starch products was emphasized.

MATERIALS AND METHODS

Sample Preparation

Commercially available corn-syrup solids (DE greater than 20) and maltodextrins (DE less than 20) were obtained from the Grain Processing Corporation. Unless otherwise indicated, 1-g samples were quantitatively weighed to the nearest milligram and transferred to 10-ml volumetric flasks. About 6 ml of distilled water was added, and the samples were warmed to 40°C to improve dissolution. After stirring for several minutes with a magnetic stir-bar, the samples were cooled to 20°C, the bar was removed with rinsing, and each flask was made to volume with water. Most samples were injected into the HPLC at this point without further handling. Duplicate samples were prepared for analysis, and mean values were reported.

When mixed with water, samples with a DE less than 10 showed turbidity due to insolubility and could not be injected directly into the HPLC. These turbid samples were transferred from the volumetric flasks to centrifuge tubes and centrifuged at 1,000 × *g* for 20 min. The clear supernatant was removed and used for HPLC analysis. This cleanup procedure allowed only the water-soluble portion of the sample to be injected for HPLC analysis. Moisture content (5–6%) and DE values for the corn syrup solids and maltodextrin samples were supplied by the manufacturer.

HPLC

The chromatographic system consisted of a Waters Associates 6000A pump, a Rheodyne 7010 fixed-loop injector, a Waters 401R differential refractive index (RI) detector, and a Hewlett Packard 3380A recorder integrator. The column was an HPX-42A from Bio-Rad Laboratories (300 × 7.8 mm) held in a water bath at 85°C. The mobile phase was degassed distilled water pumped at a flow rate of 0.5 ml/min. Two Micro-Guard precolumns from Bio-Rad Laboratories, each 40 × 4.6 mm, were used in front of the analytical column. One contained a cation-exchange cartridge, and the other contained an anion-exchange cartridge. Sample injection size was 20 μl.

Quantitation

Sugar standards for DP 1, 2, and 3 were prepared from glucose, maltose, and maltotriose obtained from the Sigma Chemical Co. Dry sugars were stored in a desiccator. HPLC analysis was used to determine the presence of contaminating sugars in the standards, and corrections were made for the purity of the sugar standards. Solutions containing 5–10 mg/ml of each sugar were used to determine retention time and integrator area response. From the integrated area for each saccharide in the samples, the quantities of the saccharides were calculated using the area response factor for known amounts of the three standards.

To determine the reproducibility of the HPLC analysis, six replicate samples of corn-syrup solids were prepared and quantified. The standard deviation and coefficient of variation for each saccharide through DP 10 were calculated.

RESULTS AND DISCUSSION

A chromatogram for the HPLC analysis of a 15.7 DE maltodextrin on the HPX-42A column is shown in Fig. 1. Peaks

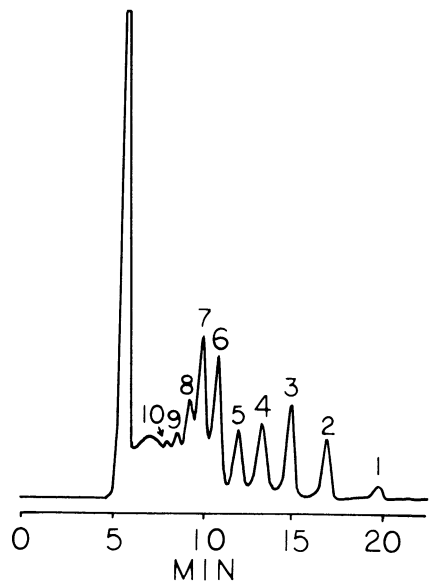

Fig. 1. Chromatogram of a 15.7 dextrose-equivalent maltodextrin using an HPX-42A column. Numbers refer to the degree of polymerization of the saccharides.

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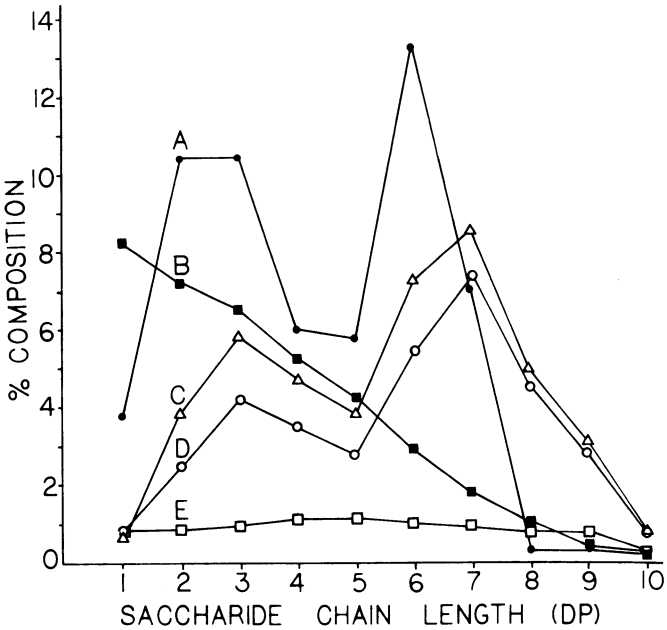

Fig. 2. Saccharide levels in various samples of corn syrup solids and maltodextrins. Dextrose-equivalent values were as follows: A = 25.2; B = 25.3 (acid hydrolysis); C = 15.7; D = 9.0; and E = 6.4.

were obtained for DP 1 through DP 10. At a flow rate of 0.5 ml/min, the chromatographic analysis was completed in 21 min. The large off-scale peak at 5.8 min represents material of high molecular weight. The method of quantitation generally used for determining the amount of material represented by each peak eluting from resin columns is area normalization. Since the area response of each of the saccharides is assumed to be the same, and because all the carbohydrate in the sample elutes from the column, area of each peak divided by the total area of the peaks can be expressed directly as weight percent of the sample on a dry basis (Scobell et al 1977). The advantages of quantifying by area normalization are numerous, and this approach has been used successfully on higher DE corn syrups (Fitt et al 1980).

Some problems were encountered, however, in attempting to use area normalization for samples with low DE. A major consideration is that insoluble carbohydrate material was present in some of the samples. The material would not elute from the column, and the injection of insoluble material on the HPLC is not recommended. To be used as a quantitative technique, area normalization requires that all of the sample be injected and eluted. Because this was not possible, centrifugation was used to clarify the sample before injection. A second problem encountered in low-DE products is that the first peak representing high molecular weight material was very large when the samples contained approximately 10% solids. When varied concentrations of a 10-DE maltodextrin were injected, the area response for the high molecular weight peak became nonlinear above a sample concentration of 5% solids. This nonlinearity was probably due to excessive material passing through the RI detector. Dilution of maltodextrins below 10% solids is not a good alternative; when further diluted, the saccharides become more difficult to detect because they each represent only a small fraction of the solids. To overcome the problems of insolubility and nonlinearity of the large molecular weight peak, the quantitation used external standards instead of area normalization.

External standards of glucose, maltose, and maltotriose were used to establish the area response for the saccharides. The mean area response from the standards was used to determine the weight percent for each saccharide through DP 10 in the corn syrup and maltodextrin samples. This approach requires that the samples be prepared quantitatively, that the sample injection volume be accurate, and that the moisture content of the samples be considered to convert to a dry basis. Because the saccharides have the same response factor (Scobell et al 1977) quantitative determinations for DP 4 through 10 can be made in the absence of specific standards for these saccharides.

The lowest detectable level for each saccharide was about 2 μg injected or 0.1% expressed on a dry weight basis. The practical limit for resolution on this column appears to be DP 10. Slower flow rates did not improve the upper limit of resolution. Measurement of saccharides above DP 10 would also be more difficult because of the extremely low levels found in maltodextrins. Precision of the analysis on replicated samples, as measured by a coefficient of variation, was 1% or less for each saccharide. This variability is similar to the results of previous studies in which variability of analysis on resin columns was measured for higher DE samples (Engel and Olinger 1979, Fitt et al 1980).

The value of using HPLC with the HPX-42A column lies in the ability to rapidly characterize the saccharides in starch hydrolysates through DP 10. Figure 2 shows the saccharide profiles that represent the types of corn syrup solids and maltodextrin samples analyzed. Except for one sample that was hydrolyzed entirely by acid, all samples had been processed by an initial acid treatment followed by an enzyme treatment. The profile for the products that were enzymatically hydrolyzed generally show high levels of DP 2 and 3 and DP 6 and 7. Sample B, hydrolyzed only by acid, shows DP 1 as the major saccharide with a steady decline in concentration as chain length up to DP 10 increased.

The saccharide profile of corn syrup solids and maltodextrins can be determined up to DP 10 within 21 min when a commercially available, prepacked column is used. Problems with quantitation by area normalization may be encountered in low-DE samples, but standards for DP 1, 2, and 3 can be used to improve saccharide measurement.

ACKNOWLEDGMENTS

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