Gas Chromatographic Determination of Starch Hydrolyzate Saccharide Distribution through Maltoheptaose

Through the use of short, lightly-loaded columns, the trimethylsilyl ethers of maltooligosaccharides up to the octaose can be eluted. The procedure describes a method for analyzing starch hydrolyzates through maltoheptaose with an accuracy equal to or better than that obtained by paper chromatography. Maltooctaose is severely absorbed on the column which prevents quantitative determination of this component. Over-all analytical time including sample preparation is less than 1 hour.

Since the development of the Sweeley procedure for separating the trimethylsilyl ethers of carbohydrates by gas chromatography (Bentley *et al.*, 1963; Sweeley *et al.*, 1963), numerous applications of the method have appeared. In addition, Sawardeker *et al.* (1965) and Albershein *et al.* (1967) have published methods describing the separation of aldoses and sugar alcohols after acetylation. In the Sweeley procedure, trimethylchlorosilane was used to catalyze the reaction. Ammonium chloride precipitates during the reaction, and reliable sampling of the resulting heterogeneous mixture is difficult. Since the reagents also react with water, the large amount of ammonium chloride formed in reactions involving "wet" syrups causes small but appreciable losses of sample through coprecipitation. These problems were eliminated when Brobst and Lott (1966b) adopted trifluoroacetic acid as the catalyst. The ammonium trifluoroacetate formed is soluble in the reaction mixture, permitting reproducible sampling and accurate dilution of the mixture. The modified procedure was applied to analysis of commercial syrups for dextrose and maltose, and more recently for identification of some of the hydrolysis products from epichlorohydrin-amylose reaction products (Brobst and Lott, 1966b; Brobst *et al.*, 1967).

Paper chromatography has been the preferred method for quantitative determination of the saccharide distribution in starch hydrolyzates in these laboratories. The procedure requires three days' elapsed time and separates the saccharides through maltohexaose. Maltoheptaose and higher saccharides are reported only as a single total value, although this value does serve to give a measure of total carbohydrates present in the sample. Concentrations of all components are calculated as dextrose, and must be corrected for weight increase which occurs upon hydrolysis of the polymers. Relatively little has been reported on the quantitative measurement of polysaccharides by gas chromatography (Brobst and Lott, 1966a, 1966b; Otter and Taylor, 1967). We wish to report a chromatographic method for determining the composition of starch hydrolyzates through maltoheptaose. Maltooctaose can also be eluted, but adsorption problems have precluded accurate quantitation.

EXPERIMENTAL

Apparatus. After preliminary experiments with a variety of equipment, this work was completed on an F & M Model 5750 gas chromatograph containing matched $\frac{1}{8}$-inch × 18-inch stainless steel columns packed with 3% JXR on 80- to 100-mesh Chromosorb W (AW-DMCS). The columns are conditioned at 350° C. for 48 hours at a carrier gas flow rate of 10 to 20 ml. per minute. Incomplete conditioning resulted in partial adsorption of the DP 5 and 6 components, and the DP 7 appeared to be totally adsorbed. Instrument parameters used were:

Column temperature program: Raise the column oven temperature from an initial 90° C. at a rate of 15° C. per minute to a maximum of 400° to 410° C.

Injector temperature: 370° C.

Flame ionization detector temperature: 470° C.

Carrier gas: helium, at a flow rate of 45 to 50 ml. per minute.

Reagents. Hexamethyldisilazane was purchased from Pierce Chemical, Rockford, Ill. Mallinckrodt analytical reagent pyridine was used, and trifluoroacetic acid was obtained from Eastman Kodak.

Calibration Procedure. The pure maltooligosaccharides used as primary standards in this investigation were prepared by column chromatography, with either cellulose or a Sephadex as the absorbent. Synthetic saccharide mixtures, containing 7 to 10 mg. of each component from DP 1 to DP 7 and about 8 mg. of an internal standard, β-phenylglucopyranoside, were prepared in 125-ml. Soxhlet extraction flasks. To each mixture, 3 ml. of pyridine, 3 ml. of hexamethyldisilazane, and 0.3 ml. of trifluoroacetic acid were added, after which the flask was attached to a water-cooled condenser and the contents refluxed for 15 minutes. After cooling to room temperature, the reaction mixture was transferred to a 25-ml. volumetric flask and diluted to volume with pyridine. Chromatograms were run using 5-μl. aliquots of the solution, and peak areas were obtained by means of an Infotronics Model CRS-100 electronic integrator. Sensitivity factors relative to the internal standard were calculated for the seven saccharides. Over-all standard deviation between results for two standard solutions was 0.71% relative; the range of variation was 0.13 to 1.62% relative.

As a basis for preparation of secondary standards, the composition of a corn syrup sample was determined, using the primary sensitivity factors. The gas chromatographic results are compared in Table I with averages of five replicate analyses by paper chromatography. Addition of weighed amounts of

Table I. Comparison of Composition of a Corn Syrup as Analyzed by Paper Chromatography and GLC

Method	DP 1	DP 2	DP 3	DP 4	DP 5	DP 6	DP 7
Per cent by GLC	6.8	7.2	9.2	7.4	6.1	7.6	6.6
Per cent by P.C.	6.7	7.2	8.3	6.8	6.2	8.1	...

dextrose and/or maltose to the syrup produced a series of secondary standards which approximated the composition of various commercial syrups.

Application. Day-to-day reproducibility of the method over the anticipated range of sample types was evaluated through use of samples prepared in the same fashion as were the secondary standards. Comparison with paper chromatography data (and consequent inclusion of unpredictable error) was thereby avoided. Typical results are presented in Table II, with standard errors calculated both on a relative and an absolute basis. A typical example appears in Figure 1.

DISCUSSION

For components of DP 1 and 2, dextrose and maltose, relative error was low and absolute error high by comparison with DP 3 to 6, because average levels of dextrose and maltose are high in the sample types of interest. The largest error generally appeared in maltoheptaose concentration. This resulted from an increasing baseline rise during elution of higher saccharides, which caused error in area measurement through inability of the integrator to correct for drift as well as through ambiguity of other integration methods—e.g., triangulation or planimetry. The rise does not occur because of column bleed, but because the nonvolatile ethers of the higher saccharides appear to retard vaporization of the volatile lower components, which increased the amount of overlap between peaks; the extent of the rise is related to the amount of nonvolatile residue present. We were unable to overcome this problem either by on-column injection or by packing the injector with phase-coated glass beads to serve as a heat sink.

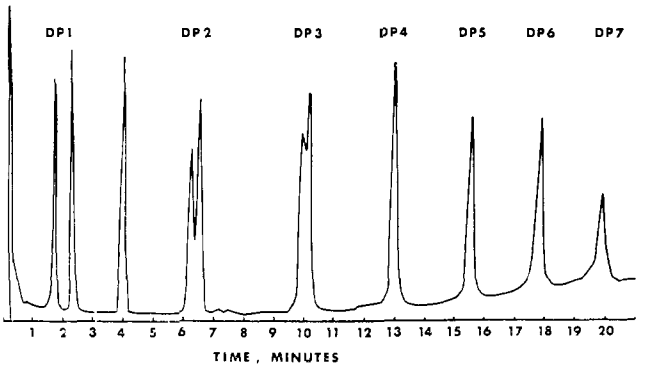

Figure 1. Chromatogram of corn syrup maltooligosaccharides

Third peak represents added β-phenylglucopyranoside

Two other difficulties were encountered during the investigation. Septum bleed presented an interference in the form of a series of closely-spaced peaks eluting between column temperatures of 250° and 325° C. The problem is accentuated when high efficiency injection ports are used: The surface temperature of the septum is higher because of more efficient gas sweep of the port, and the septum decomposition products are more effectively carried onto the column because the gas sweeps across the septum face. The interference was virtually eliminated by placing a 0.02-inch thick Teflon disk, of the same diameter as the septum, between the septum and the metal surface of the injection port. This seal served for four to five injections; when it had to be replaced, contamination of the column head was avoided by maintaining the column at 400° C. during the operation.

Another problem was that of keeping a gas-tight system at the high temperatures required. Expansion and contraction of the fittings during repeated heating cycles ultimately loosens them and causes systematic losses. The effect is especially serious when brass column fittings are mated to the steel fittings normally attached to the chromatograph, but has been observed even when all-steel fittings are used. A daily leak check with an electronic detector has been found advisable. Making duplicate injections of both standards and samples is

Table II. Comparison of Observed Composition by GLC with Known Composition for a Series of Prepared Mixtures

	Per Cent						
Sample	DP 1	DP 2	DP 3	DP 4	DP 5	DP 6	DP 7
I (present)	4.2	49.1	7.0	4.6	3.2	4.1	3.5
(found)	4.2	49.1	7.1	4.7	3.3	4.3	3.7
II (present)	5.2	47.6	8.4	5.7	4.2	5.3	4.5
(found)	5.3	46.6	8.4	5.3	4.0	5.2	4.2
III (present)	6.8	7.2	9.2	7.4	6.1	7.6	6.5
(found)	6.9	7.2	9.2	7.6	5.9	7.3	5.3
IV (present)	6.8	7.2	9.2	7.4	6.1	7.6	6.5
(found)	6.7	7.2	9.2	7.5	6.4	7.7	6.6
V (present)	28.6	24.0	6.0	4.4	3.3	4.2	...
(found)	29.0	24.4	5.8	4.3	3.4	4.5	...
VI (present)	29.1	19.7	7.8	5.8	4.8	6.0	...
(found)	30.0	19.7	7.2	6.1	4.6	5.7	...
VII (present)	30.0	17.6	8.0	6.0	4.9	6.1	5.2
(found)	30.0	17.0	7.3	6.3	4.5	5.9	5.7
VIII (present)	34.1	19.5	5.7	4.2	3.2	4.1	3.5
(found)	32.5	19.6	5.6	4.1	3.1	3.8	3.6
S.d. % relative	2.44	1.65	4.66	4.24	4.93	5.14	10.2
S.d. % absolute	0.68	0.44	0.34	0.23	0.22	0.24	0.55
S.d. All data							
% rel.	4.79						
% abs.	0.41						

recommended, because (in addition to improving accuracy) it provides a clue to location of leaks: When variation is greater at the beginning of the chromatogram, a leak at the front end of the system is indicated, while greater variation in later peaks suggests losses between the column exit and the detector.

An additional precaution should be observed. During a day's operation, a small amount of higher saccharides is adsorbed on the column, and during the night an accumulation of septum decomposition products occurs. The first injection of the day serves to purge the column of these interfering materials; data from this injection are, of course, unreliable, and should be discarded.

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