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Note

Rapid separation of malto-, xylo- and cello-oligosaccharides (DP 2–9) on cation-exchange resin using water as eluent

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Current studies of enzymic degradation of arabinoxylan, starch and cellulose have made it necessary to introduce a rapid method for the separation and quantitation of different mono- and oligosaccharides in a single analysis.

Efficient separations of oligosaccharides have been achieved by partition chromatography on bonded-phase silica[1,2] and ion-exchange resins[3] using aqueous mixtures of acetonitrile or ethanol as the eluent. However, the solubility of oligomers in aqueous acetonitrile or ethanol mixtures decreases as the degree of polymerization (DP) increases. Gel chromatography on Bio-Gel P-2 with water as eluent[4] gives effective separations of oligosaccharides up to DP 18 but is time-consuming and does not yield sufficient separations of monosaccharides. Partition chromatography on cation-exchange resins in the potassium or calcium form has been successfully used for the separation of mono- and oligosaccharide mixtures[5–7]. Using the cation-exchange resin AG 50W-X4 (Ca^{2+}), cellodextrins were separated up to DP 7 with water as the mobile phase[6].

In this report we describe a simple and rapid method for the analysis of hydrolyzates deriving from the enzymic degradation of different polysaccharides. The separations were performed with Aminex HPX-42, a cation-exchange resin in the calcium form, and with water as eluent. Different monosaccharides and oligosaccharides up to DP 9 were resolved within 60 min in a single run. The columns are suitable for routine sugar analysis over prolonged periods.

EXPERIMENTAL

Materials

Arabinoxylan was obtained from Serva (Heidelberg, G.F.R.). Xylan and maltotriose were from Sigma (St. Louis, MO, U.S.A.). Maltotetraose was prepared as described previously[8]. Maltodextrins (G5–G11) were prepared by hydrolysis of amylose with α-amylase from *Bacillus subtilis* and separated by gel chromatography on Bio-Gel P-2[4]. Cellodextrins were obtained by acid hydrolysis of cellulose MN300 (Macherey, Nagel & Co., Düren, G.F.R.) by the procedure of Miller *et al.*[9] and separated on Bio-Gel P-2. Xylan was partially hydrolyzed at 80°C in 0.25 *M* H_2SO_4 for 6 h. The hydrolyzate was neutralized with $BaCO_3$, centrifuged and lyophilized.

Acid hydrolyzates of starch were commercial preparations and were reduced

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with $NaBH_4$ as described previously[1]. All other carbohydrates and sugar alcohols were from Merck (Darmstadt, G.F.R.). The xylanase was purified from the culture medium of *Trichoderma lignorum* by a procedure of gel and hydroxyapatite chromatography[10].

Apparatus

Liquid chromatography (LC) was performed using a Hewlett-Packard 1084 B chromatograph with a Knauer differential refractometer for monitoring the column effluent. The detector was thermostated to 35°C with a Haake Type F3M constant temperature circulator. Chromatography was carried out with three 30 × 0.78 cm stainless-steel columns connected in series and packed with Aminex HPX-42 (Bio-Rad Labs., Richmond, CA, U.S.A.). Aminex HPX-42 is a 4% cross-linked sulfonic acid type cation-exchange resin in the calcium form with a particle size of 25 μm. A guard column (4 × 0.46 cm) containing Aminex Q 150 S (Bio-Rad) was fitted between pump and injector. Double-distilled water was used as eluent.

Procedure

The column was operated at a temperature of 85°C with degassed water (80°C) as eluent. At a flow-rate of 0.5 ml/min (≈23 bar), the approximate run time was 60 min. In a typical run, 60 μl of a 0.5–2% aqueous solution were applied to the column. An intermediate sensitivity was selected for detection. The refractometer attenuation was × 64. Peak areas were measured with an integrator. The separated carbohydrates were identified on the basis of retention times and co-chromatography. Oligosaccharides obtained from the enzymic degradation of arabinoxylan were identified as described previously[11].

RESULTS AND DISCUSSION

The chromatogram in Fig. 1 shows the separation of a synthetic mixture of fourteen carbohydrates on Aminex HPX-42 with deionized water as the mobile phase. The oligosaccharides (maltoheptaose to maltose) are eluted from the resin bed in order of decreasing molecular size, which is analogous to the fractionation of oligosaccharides by gel chromatography. Monosaccharides and sugar alcohols are resolved in a manner analogous to partition chromatography. Polysaccharides elute immediately rather than binding irreversibly to the column as can be observed with reversed-phase LC. The resolution shown in Fig. 1 and the following figures can only be obtained by utilizing three coupled columns, each 30 × 0.78 cm. After daily use for one year these columns showed no detectable change in resolution.

Using partition chromatography on cation-exchange resins, it is possible to define the action patterns of polysaccharide-degrading enzymes much more simply than had been possible with other chromatographic methods before. Fig. 2 shows the action of a purified *endo*-xylanase from *Trichoderma lignorum* on arabinoxylan. The xylanase degraded arabinoxylan mainly to xylobiose (X_2) and arabinoxylotetraose (AX_4), with smaller amounts of xylotriose (X_3) and arabinoxylopentaose (AX_5) and traces of xylose (X_1), xylotetraose (X_4) and branched arabinose-xylose oligosaccharides from DP 7–DP 9 (A_2X_5–A_2X_7). The xylanase from *T. Lignorum* is unable to liberate arabinose either from the arabinoxylan backbone or from the arabinoxylo-

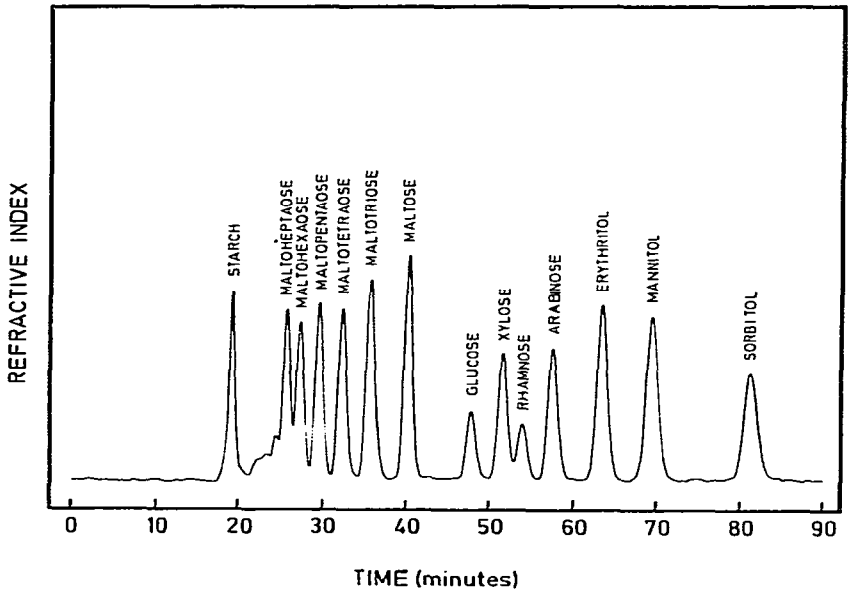


Fig. 1. Partition chromatography of a synthetic carbohydrate mixture. Resin bed, 90 × 0.78 cm; Aminex HPX-42. Column temperature, 85°C; eluent, water; flow-rate, 0.5 ml/min; sample size, 60 µl. Attenuation, 7; refractive index, ×64.

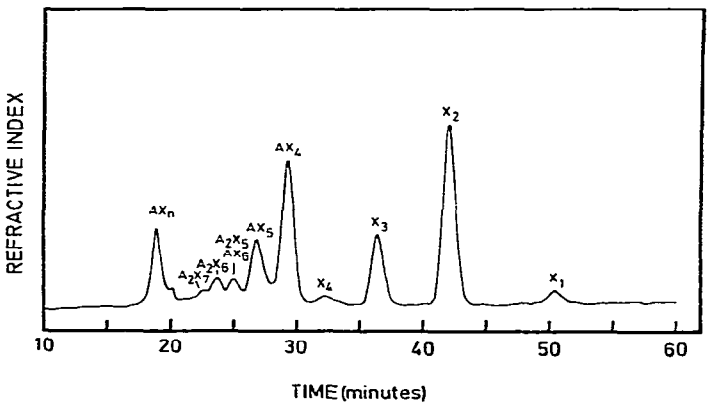


Fig. 2. Chromatography of the mono- and oligosaccharides produced by the action of a xylanase from *Trichoderma lignorum* on arabinoxylan. Identification of peaks was carried out as described[11]. Peak identity: X_1, X_2, X_3 and X_4 denote xylose, xylobiose, xylotriose and xylotetraose, respectively; AX_4 = arabinoxylotetraose; AX_5 = arabinoxylopentaose; AX_6 = arabinoxylohexaose; A_2X_5–A_2X_7 = hepta- to nonasaccharides with two arabinose units; AX_n = oligosaccharides, DP > 15. Sample size, 60 µl of a 2% solution. For other details see Fig. 1.

dextrins, so that the branched arabinose-containing xylo-oligosaccharides accumulate in the digest[10].

The resolution of the carbohydrates in brewing wort is shown in Fig. 3. The separation of maltose (68 mg/ml) and the minor amount of sucrose (3 mg/ml) proved difficult on the Aminex HPX-42 column, but it was possible to separate the other

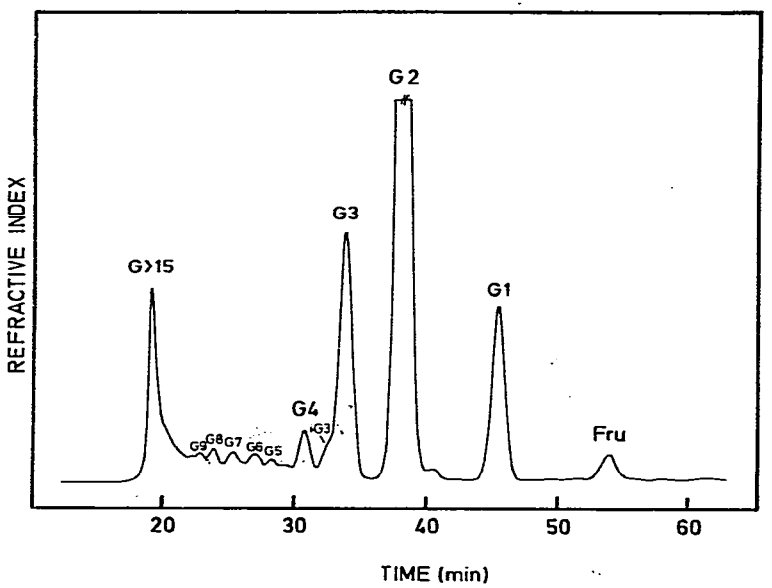
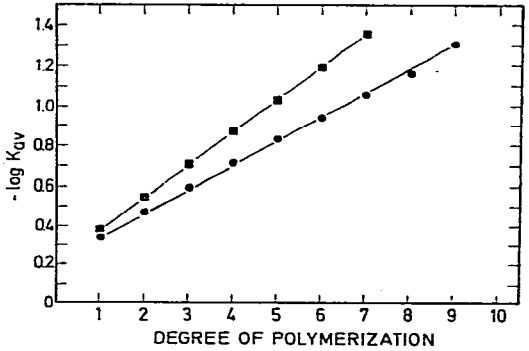


Fig. 3. Chromatography of brewing wort. Injection size: 60 μl of 1:1 dilution with water. Peak identity: Fru = fructose; G1 = glucose; G2 = sucrose and maltose; G3 = maltotriose; i-G3 = raffinose and panose. G4–G9 denote tetrasaccharide to nonasaccharide; G > 15 = oligosaccharides, DP > 15. For other details see Fig. 1.

fermentable sugars, *e.g.*, fructose, glucose and maltotriose in less than 60 min. A good resolution of branched dextrins up to DP 9 was achieved. Because of the high maltose concentration deriving from the combined action of α- and β-amylase on starch, we were unable to obtain a comparable resolution of brewing wort with reversed-phase high-performance LC on a μBondapak carbohydrate column. Chromatography of beer on the cation-exchange resin yielded the non-fermentable branched dextrins and



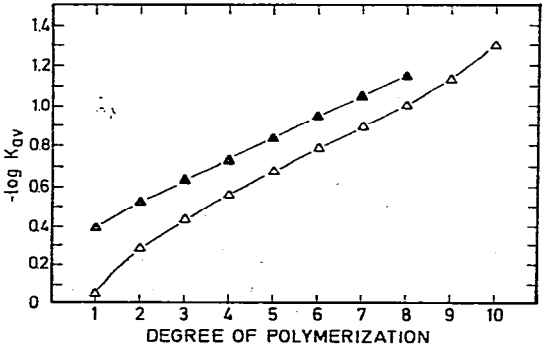


Fig. 4. Plot of $-\log K_{av}$ versus DP of xylo-oligosaccharides, ●, and cello-oligosaccharides, ■. The distribution coefficient K_{av} is defined as $(V_e - V_0)/(V_t - V_0)$, where V_e, V_0 and V_t are the solute elution volume, void volume and total volume, respectively, of the resin mass in the column. V_0 = 9.6 ml was determined by chromatography of starch and xylan. Conditions as in Fig. 1.

Fig. 5. Relationship between $-\log K_{av}$ and DP of malto-oligosaccharides (▲) and their corresponding alditols (△). Conditions as in Fig. 1.

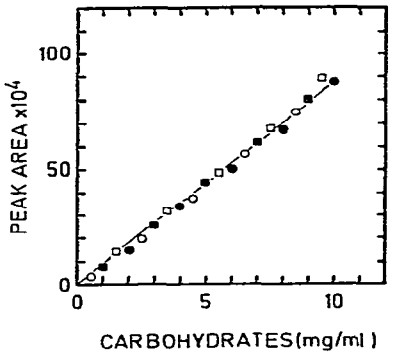


Fig. 6. Plot of peak area as a function of carbohydrate concentration. ● = Glucose; ■ = maltose; ○ = maltotriose; □ = maltotetraose. Sample size, 60 μl; attenuation, 7; refractometer, ×64.

peaks of glycerol and ethanol after 70 min.

A plot of the negative logarithm of the distribution coefficient (K_{av}) *versus* DP of xylo- and cello-oligosaccharides yields a linear relationship (Fig. 4). Both series of homologous oligosaccharides have different slopes. This may be related to their different structures.

A strong increase in retention time was observed for malto-oligosaccharides after reduction with sodium borohydride. Fig. 5 shows a plot of $-\log K_{av}$ as a function of DP for homologous series of malto-oligosaccharides and their corresponding alditols. A linear function between $-\log K_{av}$ and DP was obtained for maltodextrins and their alditols between DP 3–8. In this region the two lines have almost the same slope with an almost constant increment from the maltodextrin series to the corresponding alditol series of $\Delta - \log K_{av} \approx 0.17$.

The calibration curve in Fig. 6 shows that glucose and malto-oligosaccharides fall on the same line when the peak area is plotted as a function of carbohydrate concentration. As the glucose and maltodextrins have the same area response on a mass basis, the column may be calibrated with glucose or maltose. The same linear relationship has been reported previously for glucose and cellobiose using partition chromatography on AG 50W-X4 (Ca^{2+}) with water as eluent and refractive index detection[6].

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