High-Performance Liquid Chromatographic Monitoring of Carbohydrate Fractions in Partially Hydrolyzed Corn Starch

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The objective of this paper is to present an integration of three chromatographic methods for the monitoring of saccharides and degradation products in partially hydrolyzed starch. In our method, a single sample preparation is analyzed by two aqueous high-performance liquid chromatography columns and one aqueous size-exclusion chromatography column. In less than 1 h, the quantification of mono- and oligosaccharides as well as the determination of the polysaccharides profile of these hydrolysates is obtained. Simultaneously, the degradation products, mainly levoglucosan and (hydroxymethyl)furfural, are also quantified. This methodology may therefore be applicable for the control of acid or enzymatic processes.

Numerous approaches are available for the analysis of starch hydrolysates and product characterization aimed at either kinetic modeling or process control in both acid and enzymatic processes.

The most commonly used methods are bulk solution chemical assays such as reducing sugar analyses (Somogyi, 1945, 1952; Nelson, 1944). The determination of the monosaccharides present in the hydrolysates can be conducted either by gas chromatography after derivatization (Sweeley et al., 1963; Kennedy et al., 1976) or by liquid chromatography with or without derivatization in organic phase (White et al., 1983), on amino-bonded stationary phase (Binder, 1980), or on cation-exchange resins in aqueous phase (Palmer, 1975).

Quantification of oligosaccharides of degrees of polymerization (DP) from 2 to 20 is less often reported than the reducing sugar or monosaccharide quantification. The common method of separation is through aqueous salt solutions on polyacrylamide gels (Heyraud and Rinaudo, 1978; Kennedy et al., 1980; Atkins and Kennedy, 1985). Silica columns, chemically modified with polyfunctional amine, provide a separation up to DP 20 (White et al., 1980). Reversed-phase high-performance liquid chromatography (HPLC) (Heyraud and Rinaudo, 1980; Verhaar et al., 1984) and HPLC using silver-loaded cation-exchange resins (Scobell and Brobst, 1981; Wharthesen, 1984) are rapid methods and provide improved resolution up to DP 8. A combined HPLC system of different ion-exchange columne with water as eluent is described for complete qualitative separation of mono- and oligosaccharides as well as sugar degradation products (Bonn, 1985).

The higher molecular weight polymers (DP > 20) present in starch hydrolysates are traditionally analyzed by two methods: (a) size-exclusion chromatography (SEC) using an organic solvent [DMSO is often used since it is an appropriate solvent for uncharged polysaccharides and permits the study of native starches that are water insoluble (Salemis and Rinaudo, 1984; Kobayashi et al., 1985)]; (b) aqueous size-exclusion chromatography [relatively complex control of the analytical parameters is needed to eliminate the polymer–solvent–support interactions (Rollings et al., 1982; Gallec et al., 1984)].

During the period of process development for the rapid starch depolymerization via a high-shear jet reactor (Chornet et al., 1986), the large number of samples to be analyzed became critical. Consequently, our main goal was to develop a rapid analytical procedure based on a single sample preparation and water as solvent. The target was the quantitative determination of mono- and oligosaccharides, levoglucosan and (hydroxymethyl)furfural (HMF), as well as the determination of molecular weight distribution (MWD) of polysaccharides for control purposes.

Table I. Conditions of Selected Starch Hydrolysis Experiments

	series 1	series 2
substr	corn starch	corn starch
substr concn (w/w), %	40	40
H_2SO_4 concn (w/w), %	0.03	0.012
temp, °C	190–230	200
residence time, s	2	2–20

EXPERIMENTAL SECTION

Source of Starch Hydrolysates. The starch hydrolysates were produced by our process under the following conditions: preparation of an aqueous suspension (40% w/w) of pearl corn starch; continuous injection as a spray into a chamber heated by addition of live steam; temperature of reaction varying from 150 to 300 °C; nominal residence time of starch droplets and condensed suspension in the chamber ranging from 2 to 20 s; sulfuric acid added to the starch suspension in concentrations ranging from 0 to 0.50% (percent weight acid/suspension); neutralization with sodium hydroxide to pH 4.5–5.5 after the reaction period.

For the purpose of this paper, we selected two series from the experimental grid conducted in our reactor. The rationale for choosing these particular series of experiments is to illustrate the wide range of concentration and molecular weight distributions that can be anaylyzed by the proposed method. Temperature was the parameter varied in the first series for a fixed reaction time while the second series of experiments were conducted at a fixed temperature and a varying reaction time. The experimental conditions for each series are listed in Table I.

Sample Preparation. A 100-mL sample taken from the starch hydrolysate is filtered (Whatman No. 4) to remove insoluble starch. The residue is resuspended in 20 mL of distilled water and refiltered for total collection of the solubles. An aliquot (1 mL) of the soluble fraction is dried at 105 °C for 6 h for total dissolved solids determination. A 5-mL aliquot is diluted with distilled water to reach convenient concentration, filtered (0.45-μm filter), bottled, and stored at 4 °C prior to analysis, which was normally conducted within 24 h. For longer storage pe-

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riods, the samples were kept frozen.

Monosaccharide Analysis. The column is a Pb-loaded cation-exchange stationary phase (HPX-87P, Bio-Rad Laboratories, 300 × 7.8 mm) held in a Bio-Rad column heater at 85 °C. The mobile phase is degassed distilled water pumped at a flow rate of 0.6 mL/min with a Gilson Model 302 pump. The guard system consisted of anion- and cation-exchange cartridges. Sample injection size is 20 μL, and detection is made by differential refractive index (Gilson Model 131). The quantification is made by adding a known concentration of mannitol as internal standard.

Oligosaccharide Analysis. The column was an Ag-loaded stationary phase (HPX-42A, Bio-Rad, 300 × 7.8 mm) held at 85 °C. The conditions used are the same as for the monosaccharide system. The pump used is a Beckman Model 100A, and the differential refractive index is a Waters Model R-401. The quantification of monomer to maltoheptose is made by external calibration curves from pure standards (Sigma Chemical Co.). The quantification of DP ≥8 is derived from the calibration curve of maltoheptaose, assuming that the area response of each saccharide is the same (Scobell et al., 1977).

Size-Exclusion Chromatography. The column is a TSK-PW 3000 (600 × 7.5 mm) (Toyo Soda) held at 30 °C, and the same gel is used in the guard column (75 × 7.5 mm). The mobile phase is filtered, and degassed distilled water containing 0.05 M sodium nitrate and 0.02% sodium azide is added. The flow rate of the Beckman 100A pump is 0.4 mL/min, injection volume is 10 μL, and detection is made by an Altex 156 differential refractive index detector.

Sodium nitrate (0.05 M) is added to the eluent following the observation that our samples generated electrostatic effects when only pure water is used. Reasons for these effects are as follows: (a) the sodium sulfate formed by the neutralization of the sulfuric acid used in the process and (b) the organic acids formed by the degradation of glucose caused the well-known Donnan salt-exclusion effects. Due to these effects, the products eluted more rapidly than expected and interfered with the determination of the MWD of the polysaccharides. The addition of sodium nitrate first proposed by Domard et al. (1979) to eliminate the electrostatic effects in SEC in organic media also eliminates these effects in aqueous SEC. Pullulans of different molecular weights (Shodex Co.) are used to calibrate the size-exclusion column since these polymers have smaller dispersion than dextrans (M_w/M_n <1.14). The range of molecular weight is from 5800 to 853 000. Commercial oligomaltoses (from DP 2 to 7) and glucose are also used. The total volume is determined with injection of heavy water (D_2O).

RESULTS AND DISCUSSION

(a) SEC Calibration and Determination of MWD. The calibration curve is shown in Figure 1. The K_{av} parameter is defined in eq 1 where V_e is the peak retention volume of the sample, V_i is the void volume of the column,

$$K_{av} = (V_e - V_i)/(V_t - V_i) \quad (1)$$

and V_t is the total interstitial volume. A regression analysis of the linear part of the calibration curve (from 100 000 to 200) gave a regression coefficient of 0.9996 and the equation of the straight line is eq 2, with standard deviations of 0.02 and 0.03 for the intercept and the slope, respectively. The addition of $NaNO_3$ did not alter the calibration curve, indicating that there was no interaction between the support and the standard polymers.

$$\log \overline{MW} = 5.21 - 3.56K_{av} \quad (2)$$

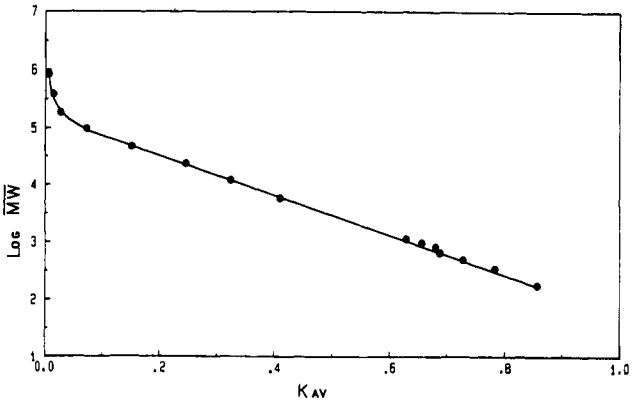

Figure 1. Calibration curve of the TSK-PW 3000 SEC column with pullulans, oligosaccharides, and glucose (conditions defined in text).

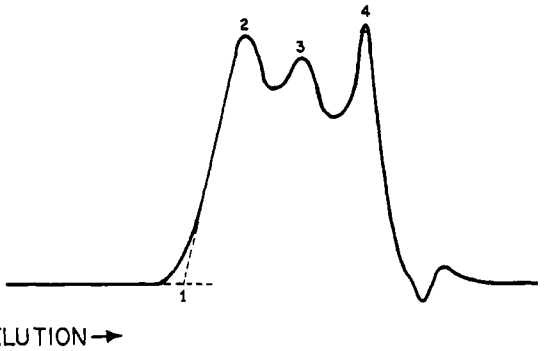

Figure 2. Theoretical SEC chromatogram illustrating the method of determination of MWD (see text for explanation).

The analysis of SEC data is difficult because of the large dispersity and the multimodal MWD of starch polymers in the samples. The average molecular weight in number (M_n) or in weight (M_w) would be meaningless in this case. We decided to use the same approach that Rollings and Thompson (1984) used for the data analysis of the molecular weight distribution of enzymatic starch hydrolysates. This approach implies that the substrate's bonding pattern may be such that a preferential products distribution may result. Consequently, a multimodal MWD should be anticipated like in the case presented here.

A typical theoretical SEC curve is shown in Figure 2 and illustrates the method followed. The molecular weight of the intercept marked as point 1 is calculated from the calibration curve and used to define the maximum molecular weight present in each sample. We have also defined three areas identified by the numbers 2–4 in Figure 2. Each area is defined by determination of the peak-average molecular weight (M_p). Area 2 consists of polymers and large oligomers while area 3 is mainly composed of oligomers. Area 4 is a mixture of di- and monosaccharides, which are quantified by the other two methods. The small negative and/or positive peaks at the end of the elution are caused by the difference in sodium nitrate concentration between the sample and the eluent.

(b) Data Analysis of Series 1 and 2. The size-exclusion, oligosaccharides and monosaccharides, chromatograms of series 1 are shown in Figure 3 for reaction at 200, 210, 220, and 230 °C while their quantification is given in Table II. From Table II and Figure 3 it can be observed that, at the lowest temperature (190 °C), the major products are polysaccharides and high oligosaccharides (DP ≥8). Increasing the temperature to 200–210 °C causes a shift to lower molecular weight products and to oligo-

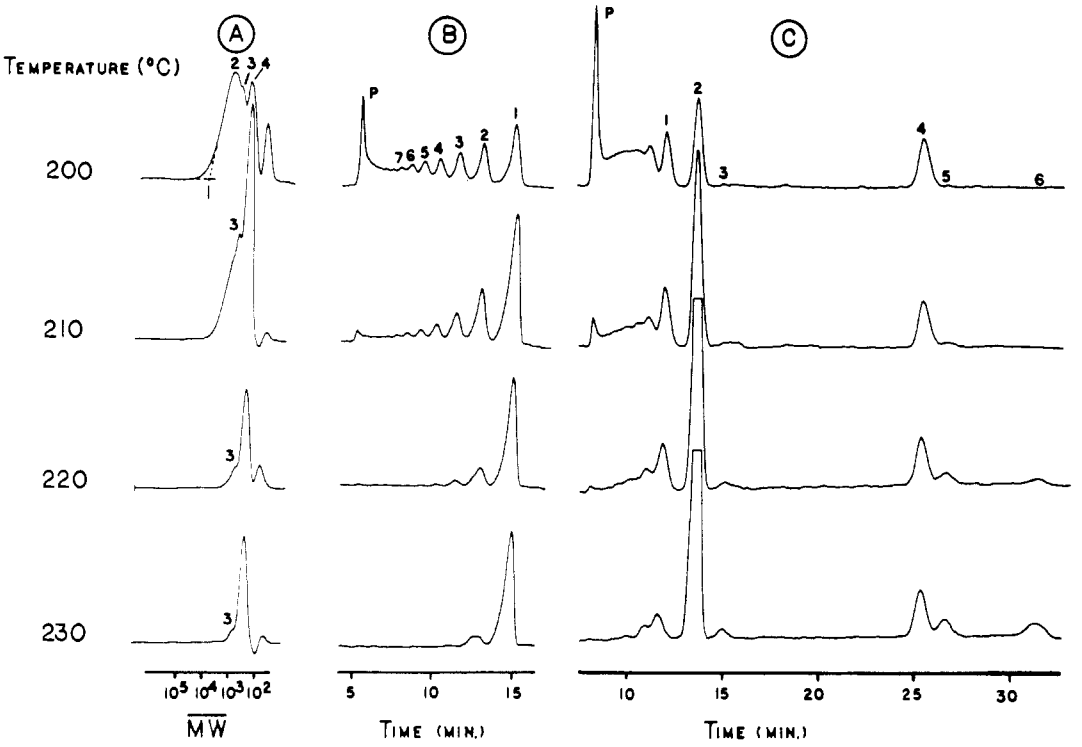

Figure 3. Chromatographic profiles of the series 1 hydrolysates. (A) Size-exclusion chromatograms: numbers correspond to those of Figure 2. (B) Oligosaccharide chromatograms: numbers correspond to DP and P to polysaccharides. (C) Monosaccharide chromatograms: 1, maltose; 2, glucose; 3, mannose; 4, mannitol; 5, levoglucosan; 6, HMF; P, polysaccharides.

Table II. Results of Series 1 Starch Hydrolysis

	temp, °C				
	190	200	210	220	230
Conversion, g/100 g of Starch					
	97.0	97.8	98.1	98.2	98.8
monosaccharides	12.8	19.2	45.2	69.6	89.8
oligosaccharides					
DP 2	11.0	9.3	19.3	15.6	3.8
DP 3	9.5	21.3	17.1	8.6	0.4
DP 4	6.2	13.6	8.6	2.3	0.1
DP 5	3.8	10.3	4.5	0.3	
DP 6	1.8	6.4	2.2		
DP 7	1.0	2.0	0.2		
total (DP 2–7)	33.3	62.9	52.2	26.8	4.3
polysaccharides (DP 8+)	55.9	20.6	2.5	0.4	
levoglucosan			1.7	3.4	5.7
HMF					0.6
MW Distribution					
intercept 1	26100	6900	2700	1200	800
area 2	1400	950			
area 3		470	430	480	480
area 4		190	180	170	160

[a] The conversion is in grams/100 g of starting material, and the saccharides are in grams/100 g of dissolved solids. The molecular weight distribution parameters are explained in the Experimental Section and in Figure 3.

Table III. Results of Series 2 Starch Hydrolysis

	residence time, s				
	2	4	9	16	20
Conversion, g/100 g of Starch					
	77.6	84.0	91.0	95.2	97.6
monosaccharides	7.6	11.2	15.7	30.8	35.9
oligosaccharides					
DP 2	6.5	9.7	13.0	13.4	11.6
DP 3	5.8	6.5	9.4	8.0	6.9
DP 4	4.0	4.5	6.0	3.4	3.9
DP 5	2.9	3.6	5.0	2.0	2.6
DP 6	2.5	2.0	3.4	1.0	1.4
DP 7	1.5	1.1	1.4	0.6	1.0
total (DP 2–7)	23.2	27.4	38.2	28.4	27.4
polysaccharides (DP 8+)	69.2	61.4	46.1	40.8	36.7
MW Distribution					
intercept 1	71000	18700	12100	7400	6400
area 2	1300	1200	900	700	650
area 3				490	440
area 4	250	230	200	210	200

[a] The conversion is in grams/100 g of starting material, and the saccharides are in grams/100 g of dissolved solids. The molecular weight distribution parameters are explained in the Experimental Section and in Figure 3. Note: No levoglucosan and HMF were detected.

saccharides (DP 2–7) as seen on the oligosaccharide chromatograms (Figure 3B). The oligosaccharides are the major product with an important proportion of glucose at 210 °C (45.2%). The thermal degradation product of glucose, i.e. levoglucosan, begins to appear at almost the same time as the glucose. At 220 °C, almost all saccharides over DP 7 have disappeared, and at 230 °C, the monomers (monosaccharides and levoglucosan) represent 95.5% of the soluble material and the HMF is present.

The results for series 2 are shown in Figure 4 and Table III. It can be observed that the molecular weight distributions as a function of reaction time for a given temperature are quite different from the distributions obtained from the series in which the temperature was varied. The net effect of the lower acid concentration (milder conditions) used in series 2 is to lower the conversion relative to that of the first series for a given temperature–time profile. Since increasing conversions (i.e., enhanced saccharification) results in addition of soluble saccharides to the hydrolysates, it follows a slow decrease of the high molecular weight (area 2) and the relatively constant proportions of the oligomers. No degradation products were found in these samples because the temperature of

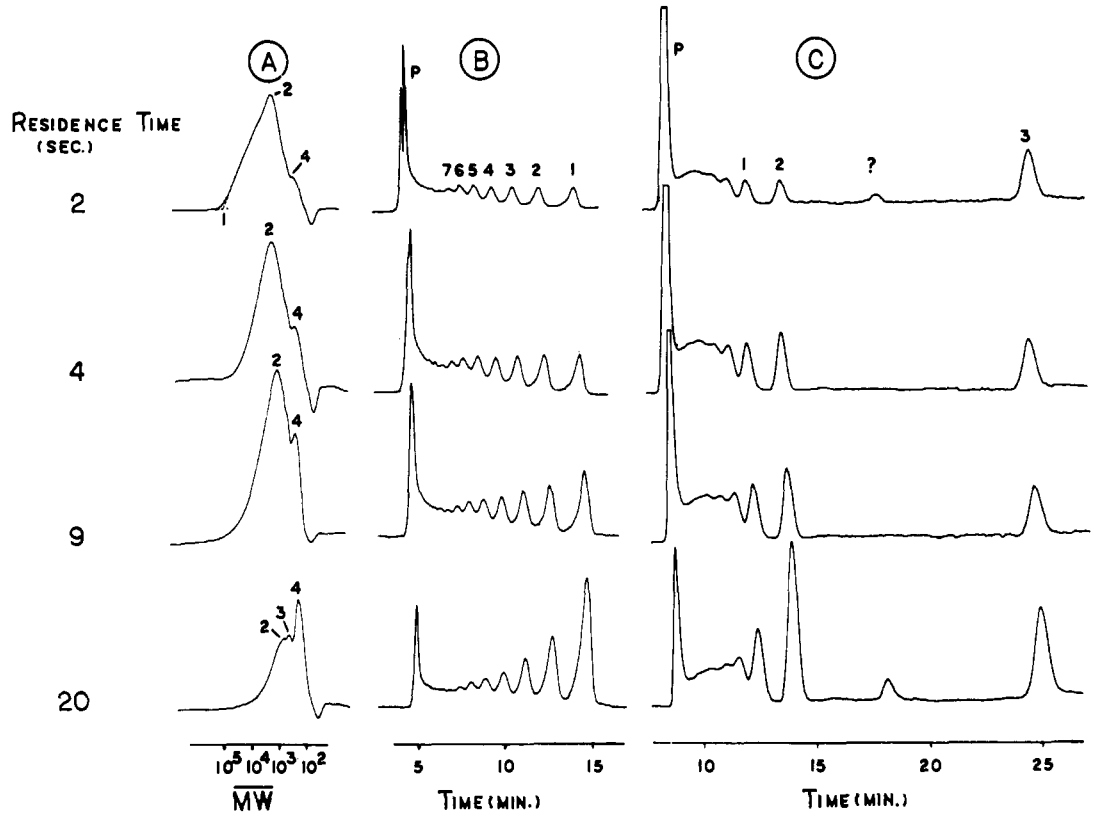

Figure 4. Chromatographic profiles of the series 2 hydrolysates. (A) Size-exclusion chromatograms: numbers correspond to those of Figure 2. (B) Oligosaccharide chromatograms: numbers correspond to DP and P to polysaccharides. (C) Monosaccharide chromatograms: 1, maltose; 2, glucose; 3, mannitol; P, polysaccharides.

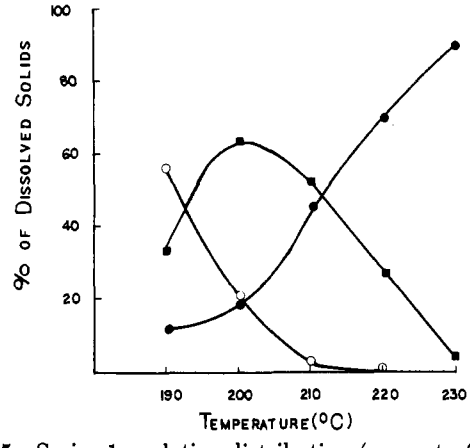

Figure 5. Series 1: relative distribution (percent of soluble material) for monosaccharides (●), oligosaccharides (■), and polysaccharides (○) as a function of the reaction temperature.

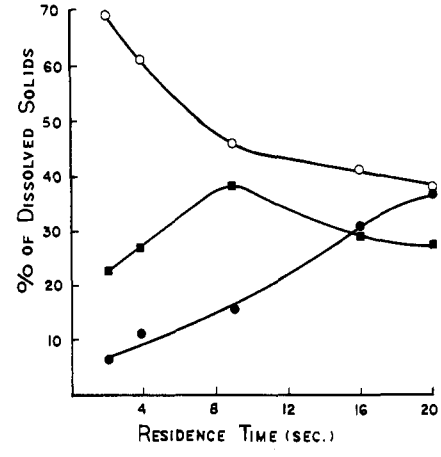

Figure 6. Series 2: relative distribution (percent of soluble material) for monosaccharides (●), oligosaccharides (■), and polysaccharides (○) as a function of residence time.

reaction was below their temperature of formation at the low residence time used in our process.

The distribution of the product yields between monomers, oligomers (DP 2–7), and larger saccharides (DP ≥8) is shown in Figures 5 and 6 for series 1 and 2, respectively. It appears from these results that the starch hydrolysis process used is capable of producing a variety of hydrolysates ranging from high glucose content to solutions with high molecular weight saccharides as major products.

CONCLUSION

The experimental data and the analytical method presented here demonstrate that it is possible to conduct a detailed and rapid analysis of starch hydrolysates involving the determination of polysaccharides, oligosaccharides, and monosaccharides as well as degradation products by a single sample preparation and with water as the eluent. In the present work, three independent analytical systems have been used. It is quite possible to reduce the number of systems to two by connecting the oligosaccharide (HPX-42A) and the monosaccharide (HPX-87P) columns in series with a common guard system.

This method gives sufficiently accurate data to be used in research determinations and is rapid and simple enough for product and quality control.

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LITERATURE CITED

Atkins, D. P.; Kennedy, J. F. "A Comparison of the Susceptibility of Two Commercial Grades of Wheat Starch to Enzymic Hydrolysis and their Resultant Oligosaccharide Product Spectra". *Starch* **1985**, *37*, 421.

Binder, H. "Separation of Monosaccharides by High-Performance Liquid Chromatography: Comparison of Ultraviolet and Refractive Index Detection". *J. Chromatogr.* **1980**, *189*, 414.

Bonn, G. "High-Performance Liquid Chromatographic Elution Behaviour of Oligosaccharides, Monosaccharides and Sugar Degradation Products on Series-Connected Ion-Exchange Resin Columns Using Water as the Mobile Phase". *J. Chromatogr.* **1985**, *322*, 411.

Chornet, E.; Koeberle, P.; Overend, R. P. "Rapid Starch Depolymerization via Spray Reactor". U.S. Patent Application Serial No. 06/930,570, Filing Date Nov 14, 1986.

Domard, A.; Rinaudo, M.; Rochas, C. "Application of Gel Permeation Chromatography to Polyelectrolytes: Salt Rejection Mechanism and Molecular Weight Distribution". *J. Polym. Sci.* **1979**, *17*, 673.

Gallec, G.; Anderson, A. W.; Tsao, G. T.; Rollings, J. E. "System Development for Aqueous Gel Permeation Chromatography". *J. Polym. Sci., Polym. Chem. Ed.* **1984**, *22*, 287.

Heyraud, A.; Rinaudo, M. "Gel Permeation Chromatography of Glucose Oligomers on Polyacrylamide Gels. Thermodynamic and Steric Partition Mechanisms". *J. Chromatogr.* **1978**, *166*, 149.

Heyraud, A.; Rinaudo, M. "Carbohydrate Analysis by High Pressure Liquid Chromatography Using Water as the Eluent". *J. Liq. Chromatogr.* **1980**, *3*, 721.

Kennedy, J. F.; Robertson, S. M.; Stacey, M. "G.L.C. of the O-Trimethylsilyl Derivatives of Hexuronic Acids". *Carbohydr. Res.* **1976**, *43*, 243.

Kennedy, J. F.; Fox, J. E.; Skirrow, J. C. "Automated, Computer Calculated, Qualitative and Quantitative Detailed Analyses of Starch Components and Related Mono- and Oligosaccharides". *Starch* **1980**, *32*, 309.

Kobayashi, S.; Schwartz, S. J.; Lineback, D. R. "Rapid Analysis of Starch, Amylose and Amylopectin by High-Performance Size-Exclusion Chromatography". *J. Chromatogr.* **1985**, *319*, 205.

Nelson, N. "A Photometric Adaptation of the Somogyi Method for the Determination of glucose". *J. Biol. Chem.* **1944**, *153*, 375.

Palmer, J. K. "Liquid Chromatography for Monitoring the Conversion of Cellulosic Wastes to Sugars". *Appl. Polym. Symp.* **1975**, *28*, 237.

Rollings, J. E.; Thompson, R. W. "Kinetics of Enzymatic Starch Liquefaction: Simulation of the High-Molecular-Weight Product Distribution". *Biotech. Bioeng.* **1984**, *26*, 1475.

Rollings, J. E.; Okos, R. M.; Tsao, G. T.; Bose, A. "System Development for Sixe Exclusion Chromatography of Starch Hydrolysates". *J. Appl. Polym. Sci.* **1982**, *27*, 2281.

Salemis, Ph.; Rinaudo, M. "Gel Permeation Chromatography of Starch and Other Uncharged Polysaccharides". *Polym. Bull.* **1984**, *11*, 397.

Scobell, H. D.; Brobst, K. M. "Rapid High-Resolution of Oligosaccharides on Silver Form Cation-Exchange Resins". *J. Chromatogr.* **1981**, *212*, 51.

Scobell, H. D.; Brobst, K. M.; Steele, E. M. "Automated Liquid Chromatographic System for Analysis of Carbohydrate Mixtures". *Cereal Chem.* **1977**, *54*, 905.

Somogyi, M. "A New Reagent for the Determination of Sugars". *J. Biol. Chem.* **1945**, *160*, 61.

Somogyi, M. "Notes on Sugar Determination". *J. Biol. Chem.* **1952**, *195*, 19.

Sweeley, C. C.; Bentley, R.; Makita, M.; Wells, W. W. "Gas–Liquid Chromatography of Trimethylsilyl Derivatives of Sugars and Related Substances". *J. Am. Chem. Soc.* **1963**, *85*, 2497.

Verhaar, L. A. Th.; Kuster, B. F. M.; Claessens, H. A. "Retention Behaviour of Carbohydrate Oligomers in Reversed-Phase Chromatography". *J. Chromatogr.* **1984**, *284*, 1.

Wharthesen, J. J. "Analysis of Saccharides in Low-Dextrose Equivalent Starch Hydrolysates Using High-Performance Liquid Chromatography". *Cereal Chem.* **1984**, *61*, 194.

White, C. A.; Corran, P. H.; Kennedy, J. F. "Analysis of Underivatised d-Gluco-Oligosaccharides (d.p.2-20) by High-Pressure Liquid Chromatography". *Carbohydr. Res.* **1980**, *87*, 165.

White, C. A.; Vass, S. W.; Kennedy, J. F.; Large, D. G. "High-Pressure Liquid Chromatography of Dimethylphenylsilyl Derivatives of Some Monosaccharides". *Carbohydr. Res.* **1983**, *119*, 241.

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